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Cyklodextriny – syntéza derivátů a jejich aplikace

Habilitační práce

(obor organická chemie)

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Prohlášení:

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Podpis

Poděkování

Poděkování všem, kteří přispěli k současnému bodu mé kariéry, kdy předkládám habilitační práci, by jistě vydalo na druh výsledku typu B v databázi RIV. Vzpomenu zde tudíž pouze nejhlavnější činitele přispěvší k mému chemickému postupu.

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Abstrakt

Předkládaná habilitační práce se na základě zkušeností autora a studiu literatury zabývá možnostmi syntézy derivátů cyklodextrinů, a to zejména takových, které mohou sloužit jako prekurzory pro další syntézu. Na vybraných nejpoužívanějších postupech ukazuje současné možnosti cyklodextrinové chemie při syntéze jednotlivých derivátů, přičemž zároveň upozorňuje na její možná úskalí. Závěrem se práce věnuje některým konkrétním aplikacím derivátů cyklodextrinů a pootvírá tak dveře porozumění a jejich dalšímu možnému použití.

Abstract

Based on the author's experience and literature findings, the thesis is focused on the possibilities of synthesis of cyclodextrin derivatives, especially those which can serve as precursors for further synthesis. The thesis shows the current possibilities of cyclodextrin chemistry in the synthesis of individual derivatives using the most favourable procedures. At the same time, the thesis draws attention to possible pitfalls in the synthesis. Finally, the thesis deals with some specific applications of cyclodextrin derivatives and opens the door to better understanding and their further possible uses.

Obsah

Seznam zkratek

Ac	acetyl
Bn	benzyl
CD	cyklodextrin
DIBAL	diisobutylaluminiumhydrid
DIPEA	*N*,*N*-diisopropylethylamin
DMAP	4-(dimethylamino)pyridin
DMF	*N*,*N*-dimethylformamid
DMSO	dimethylsulfoxid
EDC	1-ethyl-3-(3-dimethylaminopropyl)karbodiimid
Et	ethyl
HA	hexanová kyselina
HOBt	hydroxybenztriazol
2KM	2^{A}-*O*-karboxymethyl
3KM	3^{A}-*O*-karboxymethyl
6KM	6^{A}-*O*-karboxymethyl
Me	methyl
PCL	polykaprolakton
Ph	fenyl
PPy	polypyrrol
PyBOP	(benzotriazol-1-yloxy)tripyrrolidinofosfonium hexafluorofosfát
TB	Trögerova báze
TBDMS	*tert*-butyldimethylsilyl
TBTA	tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amin
TIPS	triisopropylsilyl
Tr	trifenylmethyl
Ts	*p*-toluensulfonyl

1. Úvod

Cyklodextriny – cyklické oligosacharidy – se zdají být v současné době, často až nesmyslně posedlé po všem ekologickém, tou nejlepší volbou pro organického chemika. Takováto přírodní látka z obnovitelných zdrojů, netoxická a biologicky odbouratelná je rajským snem každého ekologa. Tentýž cyklodextrin je však zlým snem každého organického chemika, protože tři sady šesti až osmi hydroxylových skupin dokážou potrápit nejednoho zkušeného syntetika.

Tato habilitační práce si klade za cíl shrnout syntetické postupy pro přípravu jednotlivých derivátů cyklodextrinů a tím udělat zlé sny o něco snesitelnější. Zároveň budou v této práci ukázány i příklady použití derivátů cyklodextrinů jako základ snů o nadějné budoucnosti.

Pro přehlednost jsou v habilitační práci odkazy na články, na kterých má autor podíl, uvedeny **tučně**.

2. Cyklodextriny

Tento rok je tomu již 129 let, co byly cyklodextriny objeveny a tento výzkum byl poprvé publikován (Villiers 1891). Zasloužil se o to v červnu roku 1891 francouzský chemik Antoine Villiers. Jeho objev vycházel z experimentu, kdy pomocí mikrobiálního rozkladu 1 kg škrobu mimo jiné obdržel také 3 g neznámé nové látky. Pro rozklad použil kmen *Bacillus amylobacter*. Jak ale ukázaly pozdější výzkumy, tento kmen byl pravděpodobně kontaminován i kmenem *Bacillus macerans* (Crini 2014). Zřejmě tudíž náhoda a následná zvědavost vědce (stejně jak tomu bývá i u dalších objevů) byla příčinou objevu cyklodextrinů. Villiers nově objevenou sloučeninu pojmenoval jako „cellulosine" vzhledem k podobným fyzikálně-chemickým vlastnostem s celulózou.

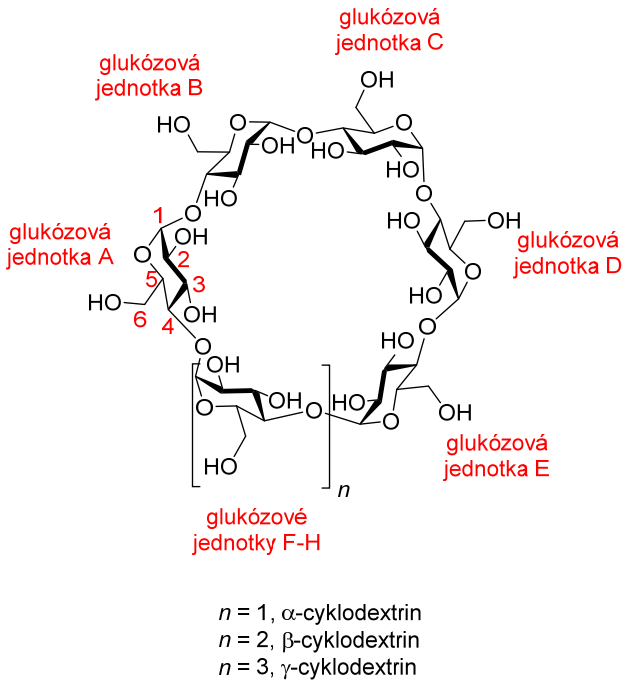
Franz Schardinger, původem Rakušan, v roce 1903 přípravu cyklodextrinů zopakoval, když studoval vysoce tepelně odolné mikroorganismy, jejichž působení vedlo k otravám z jídla (Schardinger 1903; Crini 2014). Rozkladem škrobu pomocí těchto mikroorganismů se mu povedlo připravit vedlejší produkty, které byly až nápadně podobné s cellulosiny popsanými Villiersem (Villiers 1891). Schardinger svým dalším výzkumem položil základy chemie cyklodextrinů.

Výzkum cyklodextrinů během dalších let pokračoval a zájem o ně neutuchá. Vědci se pravidelně scházejí na cyklodextrinových konferencích od roku 1981, kde prezentují a diskutují nejnovější poznatky. Příslib pro velké rozšíření cyklodextrinů je obrovský. Ukazuje se, že mají velký potenciál při použití jako nosiče léčiv (Yi et al. 2018a; Allahyari et al. 2019; Topuz and Uyar 2019; Higashi 2019; Yao et al. 2019; Tian et al. 2020), v dalších biomedicínských aplikacích (Conceicao et al. 2018; Menezes et al. 2019; Zhang et al. 2020), v potravinářství (Szente and Fenyvesi 2018; Uekaji and Terao 2019; Arora et al. 2019), v kosmetice a toaletních potřebách (Sharma and Baldi 2016), jako sorbenty (Sikder et al. 2019; Morin-Crini et al. 2019; Gentili 2020), jako součásti senzorových systémů (Niu et al. 2018; Bae et al. 2019), v chemické katalýze (Dalal et al. 2018), v molekulární elektronice (Masai and Terao 2019) nebo v analytických separačních technikách povětšinou pro separaci enantiomerů (**Řezanka et al. 2014a**; Wang et al. 2019a).

Cyklodextriny však již pronikly i do našich každodenních životů. Jsou používány jako pomocné látky v léčivech, můžeme je nalézt v potravinách a na obalu si o nich přečíst jako o jednom z dnes veřejností nenáviděných „éček“ (E459). Možná méně známou aplikací je také použití sodné soli oktakis[6-(2-karboxyethylthio)-6-deoxy]-γ-cyklodextrinu (sugammadexu) v přípravku s obchodním názvem Bridion®. Je používán pro zrušení neuromuskulární blokády při anestezii tím, že váže svalová relaxancia aminosteriodního typu do své kavity. Podání sugammadexu je tak výhodnější než prosté čekání na farmakokinetiku anestetika či inhibici acetylcholinesterázy. Tímto se však dostáváme již ke struktuře a vlastnostem cyklodextrinů.

2.1 Struktura

Cyklodextriny jsou cyklické oligosacharidy složené z glukózových jednotek. Jedná se o D-enantiomery v pyranózové formě, které jsou mezi sebou spojeny α(1→4) glykosidickou vazbou. Cyklodextriny nejčastěji obsahují 6, 7, nebo 8 glukózových jednotek a jsou po řadě označeny jako α-, β- a γ-CD (obrázek 1).



Obrázek 1. – Struktura cyklodextrinů.

Kromě výše uvedených cyklodextrinů existují další s odlišným počtem glukózových jednotek: pre-α-CD s pěti glukózovými jednotkami (Nakagawa et

al. 1994) a v minulém roce nově syntetizované cyklodextriny „CD3“ a „CD4“ se třemi a čtyřmi glukózovými jednotkami (Ikuta et al. 2019). Existují také tzv. „large-ring“ cyklodextriny, které mají od devíti až po několik desítek jednotek (Endo 2011). Jejich využití (Sonnendecker et al. 2019) je však omezené vzhledem k jejich prozatím špatné dostupnosti.

α-, β- a γ-CD mají tvar dutého komolého kužele, přičemž vnitřní prostor cyklodextrinu (kavita) je vzhledem k vazbám směřujícím dovnitř kavity nepolární. Oba okraje komolého kužele jsou pak zaplněny polárními –OH skupinami jednak v polohách 2 a 3 (sekundární okraj) a jednak v polohách 6 (primární okraj).

2.2 Příprava a výroba

Cyklodextriny s 3, 4 a 5 glukózovými jednotkami se zatím podařilo připravit pouze metodami organické syntézy za využití sofistikovaných metod chemie sacharidů (Nakagawa et al. 1994; Ikuta et al. 2019).

Cyklodextriny s šesti a více glukózovými jednotkami jsou připravovány a vyráběny metodou degradace škrobu a cyklizace kratších řetězců pomocí enzymu cyklomaltodextrin glukanotransferázy (EC 2.4.1.19). Mikroorganismy, které mají tento enzym, jsou v evoluční výhodě oproti ostatním, protože využívají odvěkou strategii vyhladovění nepřítele. Dostupný škrob si uloží ve formě cyklodextrinů a mikroorganismy bez výše uvedeného enzymu uhynou následkem nedostatku zdroje energie (Leemhuis et al. 2010).

Výroba cyklodextrinů začíná výběrem vhodného zdroje glukózy (většinou škrobu, ideálně pouze amylózy) a izolací či úpravou cyklomaltodextrin glukanotrasferázy. Po enzymatické reakci následuje krok čištění, při kterém jsou odstraněny enzymy a výsledný cyklodextrin je vyčištěn. Každý z těchto kroků lze vylepšit, často s využitím metod genetického inženýrství (Biwer et al. 2002; Li et al. 2007; Li et al. 2014).

2.3 Vlastnosti

Cyklodextriny jsou bílé krystalické látky, které jsou poměrně teplotně stabilní. Jejich rozklad začíná až za teplot okolo 270 °C (Hadaruga et al. 2019).

V bazických podmínkách dochází pouze k deprotonaci –OH skupin (viz níže), jinak u nich nedochází k žádným změnám. Dokonce jsou cyklodextriny také částečně odolné vůči kyselé hydrolýze, která nastává až při pH nižším než 3,5 a za teploty zvýšené nad 60 °C (Li and Purdy 1992).

Cyklodextriny jsou vyjma β-CD (1,85 g/100 ml) dobře rozpustné ve vodě i při laboratorní teplotě (α-CD 14,5 g/100 ml a γ-CD 23,2 g/100 ml). Za běžných laboratorních podmínek obsahují přibližně 10–15 % krystalové vody (Szejtli 1998). Pro reakce v bezvodém prostředí je tak nutné cyklodextriny předem vysušit.

Ve vodném prostředí cyklodextriny vytvářejí přechodné agregáty, nano- a mikročástice, pro které je kritická agregační koncentrace 25, 8 a 9 mg/ml pro, po řadě, α-, β- a γ-CD. Při tvorbě mikročásticových agregátů pak již dochází k zakalení roztoku, které lze přechodně odstranit zahřátím a opětovným zchlazením roztoku (Loftsson et al. 2019).

Cyklodextriny jsou po požití netoxické (Irie and Uekama 1997) (samozřejmě s přihlédnutím k Paracelsově filosofii). Cyklodextriny též nedráždí ani pokožku ani sliznice (Cal and Centkowska 2008). Při orálním podání se cyklodextriny vzhledem k jejich velikosti vstřebávají pouze v množství pod 1 % a vstřebané cyklodextriny jsou vyloučeny močí, bez toho aniž by došlo k jejich rozkladu v organismu. α- a β-CD procházejí gastrointestinálním traktem téměř bez strávení, zatímco γ-CD je částečně natráven amylázami (Stella and He 2008).

2.3.1 Tvorba komplexů

Jak již bylo popsáno výše, klíčovým strukturním prvkem cyklodextrinů je jejich lipofilní kavita. Ta umožňuje inkluzi různorodých látek (Rekharsky and Inoue 1998). Tyto sloučeniny musí být schopny interakce s kavitou nebo alespoň interakce s jejím okrajem. Asociační konstanta pak závisí na povaze a množství interakcí. Těmi jsou zejména van der Waalsovy, elektrostatické a hydrofobní interakce a vodíkové vazby (Liu and Guo 2002). U derivátů cyklodextrinů pak mohou do hry vstupovat i další interakce, jako jsou například π-π.

Kavita cyklodextrinu většinou bývá hostitelem pro látky nepolárního charakteru, přičemž poměr hosta a hostitele bývá většinou 1:1. Může ale nabývat hodnot rozličných, až extrémních, jako například v případě poly(pseoudo)rotaxanů (Hashidzume et al. 2019) či supramolekulárních polymerů (Wang et al. 2016).

Jak již bylo zmíněno výše, v případě derivátů cyklodextrinů je situace o mnoho složitější a substituenty přítomné na cyklodextrinovém skeletu mohou mít výrazný vliv na tvorbu komplexu. Jako příklad může sloužit výše zmíněný sugammadex. Jeho komplex s rukoroniumbromidem nabývá hodnoty asociační konstanty 10^7 dm^3/mol (Bom et al. 2002), zatímco cyklodextrin má s uvedenou sloučeninou asociační konstantu o tři řády nižší (Cameron et al. 2002).

2.3.2 Reaktivita

Reaktivita cyklodextrinů souvisí především s přítomnými hydroxylovými skupinami. Tři různé hydroxylové skupiny na každé z glukózových jednotek způsobují, že selektivní modifikace cyklodextrinů je povětšinou obtížná, protože hydroxylové skupiny si navzájem konkurují. Jedinou útěchou organického chemika tak zůstávají přece jen některé odlišnosti. Kromě rozdílu mezi primárními hydroxylovými skupinami v polohách 6 a sekundárními v polohách 2 a 3 jsou mezi nimi ještě rozdíly v kyselosti. Hydroxylové skupiny v polohách 6 jsou nejméně kyselé, zatímco v polohách 2 jsou nejkyselejší. Selektivní modifikace hydroxylových skupin v polohách 3 je tak nejobtížnější (Khan et al. 1998).

Při deprotonaci všech hydroxylových skupin nadbytkem báze, obecně probíhá reakce přednostně v poloze 6 na primární hydroxylové skupině. Zřejmě nejznámějším příkladem tohoto přístupu je syntéza 6^A-*O*-(*p*-toluensulfonyl)-cyklodextrinu (**Řezanka 2016**).

Jelikož hydroxylové skupiny v poloze 2 jsou nejkyselejší (pK_a = 12,2), jsou přídavkem malého množství báze deprotonovány jako první (Sallas and Darcy 2008). Vytvořený anion je pak mnohem nukleofilnější než ostatní nedeprotonované –OH skupiny a reakce je proto poměrně selektivní. Příkladem budiž použití příslušného množství silné báze, jako například NaH nebo LiH, kdy

je substituce přednostně vedena do polohy dva na cyklodextrinovém skeletu (**Řezanka 2016**).

Substituce v poloze 3 je Popelkou mezi přítomnými hydroxylovými skupinami. Netěší se ani jedné z výhod popisovaných výše a selektivní substituce v této poloze je mistrným kouskem v cyklodextrinové chemii. Za dobu zkoumání vlastností cyklodextrinů však bylo objeveno několik metod tuto substituci umožňujících. Například, cinnamylbromid vytváří s β-CD komplex a umožňuje tak selektivní natočení reakčního centra, a tím přednostní substituci v poloze 3 (Jindřich and Tišlerová 2005). Tvorba komplexu činidla s cyklodextrinem nemusí však být vždy jen prospěšná a může zhatit plány organického chemika. Je proto vždy dobré mít tuto eventualitu na zřeteli.

V reaktivitě cyklodextrinů, stejně jako kdekoliv jinde v organické chemii, hrají klíčovou roli rozpouštědla použitá pro reakce. Mohou ovlivnit jak nukleofilitu oxyaniontů, tak i tvorbu komplexu s použitým činidlem. Vzhledem k výše uvedené reaktivitě jsou často výtěžky dosahované při syntéze cyklodextrinových derivátů jednotky, nebo nízké desítky procent.

3. Deriváty cyklodextrinů

Deriváty cyklodextrinů jsou syntetizovány za účelem rozšíření možností jejich použití. Příkladem může být dramatická změna rozpustnosti modifikovaného cyklodextrinu oproti nemodifikovanému. Jak již bylo uvedeno výše, β-CD má rozpustnost za laboratorní teploty 1,85 g/100 ml vody (Szejtli 1998). Avšak per-*O*-methyl-β-cyklodextrin za stejných podmínek téměř 24 g/100 ml (Szente and Szejtli 1999). Navíc, pokud je roztok zahříván, rozpustnost β-CD stoupá, kdežto rozpustnost permethylovaného cyklodextrinu klesá. Mimo to je permethylovaný cyklodextrin (na rozdíl od β-CD) velmi dobře rozpustný ve většině organických rozpouštědel.

Pro různé aplikace je daný derivát volen na základě kompromisu mezi jednoduchostí reakční cesty a plánovaným způsobem užití. Například pokud je požadován více rozpustný derivát β-CD, může být část z hydroxylových skupin neregiospecificky převedena na sulfáty. Tato jednoduchá cesta s sebou však nese úskalí – výsledná sloučenina není jedním izomerem, ale směsí mnoha regioizomerů s různým stupněm substituce. Takováto konverze pak může vést k problémům, že šarže od šarže se odlišuje v přesném zastoupení jednotlivých entit, i když celkový stupeň substituce zůstává totožný (Estrada III and Vigh 2012).

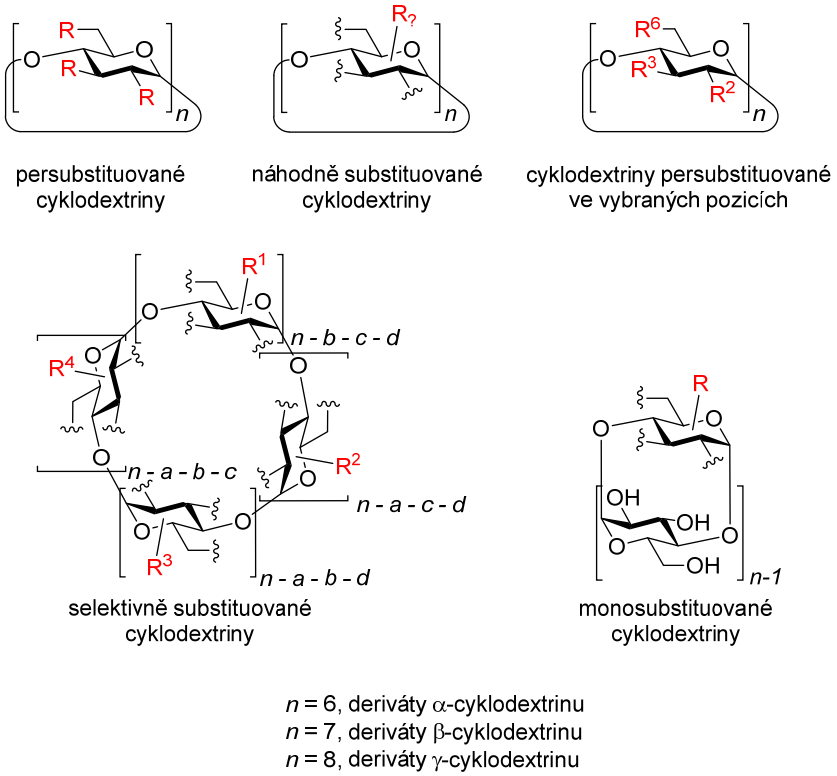
Řešením tohoto problému je použití persubstituovaných derivátů, které jsou nejsnáze připravitelnými deriváty. Nemusí mít však optimální vlastnosti a v případě některých (např. objemných) substituentů je tento způsob nemožný. Syntéza jakýchkoliv jiných derivátů s sebou přináší v drtivé většině případů problémy s tvorbou izomerních produktů. Monosubstituované deriváty mohou být tři, disubstituovaných desítky, trisubstituovaných přes 100 a v některých případech stupně substituce (okolo poloviny z celkového počtu hydroxylových skupin) jich mohou být až desetitisíce (Wenz 1994).

Celkový počet popsaných derivátů α-, β- a γ-CD je řádu tisíců (podle vyhledání substruktur v databázi SciFinder®). U cyklodextrinů s více než osmi glukózovými jednotkami jsou známy pouze jednotky derivátů (Teranishi et al.

2003), přičemž reaktivita se zdá být podobná klasickým cyklodextrinům (α-, β- a γ-CD).

Výčet syntéz všech těchto derivátů přesahuje rámec této habilitační (i jakékoliv jiné) práce. Zaměřme se proto pouze na přípravu derivátů, které mají potenciál být dále jednoduše modifikovány celou škálou reakcí. Tento přístup přináší nesporné výhody. Je nesrovnatelně snazší pro nový derivát využít syntézu přes již známý derivát s vhodnou funkční skupinou ve vhodné poloze, než pro každou novou látku optimalizovat reakční postup, aby substituce proběhla do požadované polohy.

Deriváty cyklodextrinů můžeme podle typu substituce rozdělit do následujících skupin (obrázek 2): persubstituované cyklodextriny, náhodně substituované cyklodextriny, cyklodextriny persubstituované ve vybraných pozicích, selektivně substituované cyklodextriny a monosubstituované cyklodextriny. Pro účely této práce se monosubstituovanými nebo selektivně substituovanými cyklodextriny též rozumí deriváty, kde jsou všechny ostatní hydroxylové skupiny ochráněny chránící skupinou (například všechny ostatní hydroxylové skupiny jsou acetylovány, benzylovány atp.).



Obrázek 2. – Schématické znázornění skupin derivátů cyklodextrinů.

Přístupy pro syntézu derivátů cyklodextrinů mohou být rozděleny do dvou kategorií: přímá syntéza a nepřímá syntéza (**Řezanka 2016**).

Jak již název napovídá, přímá syntéza využívá přímé reakce činidla s cyklodextrinem. Může být dále rozdělena na tři přístupy:

p1) přímá metoda – neregioselektivní syntéza a následná separace derivátů.

p2) chytrá přímá metoda – regioselektivní syntéza, kdy jsou unikátní fyzikálně-chemické vlastnosti činidla využity ve prospěch jednokrokové reakce s vysokým výtěžkem.

p3) „bio“ metoda – kdy jsou deriváty cyklodextrinů syntetizovány pomocí enzymatické reakce.

Nepřímé metody vycházejí z chránění hydroxylových skupin a umožňují tak pro substituční krok dosažení vysokého výtěžku, popřípadě využívají jiné okliky. Tyto metody mohou být dále rozděleny následovně:

n1) nepřímá metoda – používá sérii chránících a odchraňovacích kroků tak, aby klíčový krok substituce byl proveden s vysokým výtěžkem.

n2) chytrá nepřímá metoda – při které je použito selektivní odchránění persubstituovaného derivátu, které probíhá s vysokým výtěžkem.

n3) cyklizační nepřímá metoda – cyklodextrinový derivát je syntetizován *de novo* pomocí cyklizace vhodného oligosacharidu.

3.1 Persubstituované deriváty cyklodextrinů

V současné době je velká řada persubstituovaných derivátů komerčně dostupných. Nicméně stále jsou v laboratořích často syntetizovány a to zejména vzhledem k jednoduchosti přípravy. Příprava typicky zahrnuje reakci příslušného činidla (například methyljodidu, acetanhydridu, benzylchloridu atd.) v bazickém roztoku cyklodextrinu (**Řezanka et al. 2015**; **Řezanka 2019**).

Pokud je místo obyčejné chránící skupiny použita pro persubstituci skupina allylová, je možné tento derivát (a další z něj vycházející) použít jako

výchozí prekurzor pro celou řadu dalších derivátů cyklodextrinů (schéma 1) (Kraus et al. 2001).

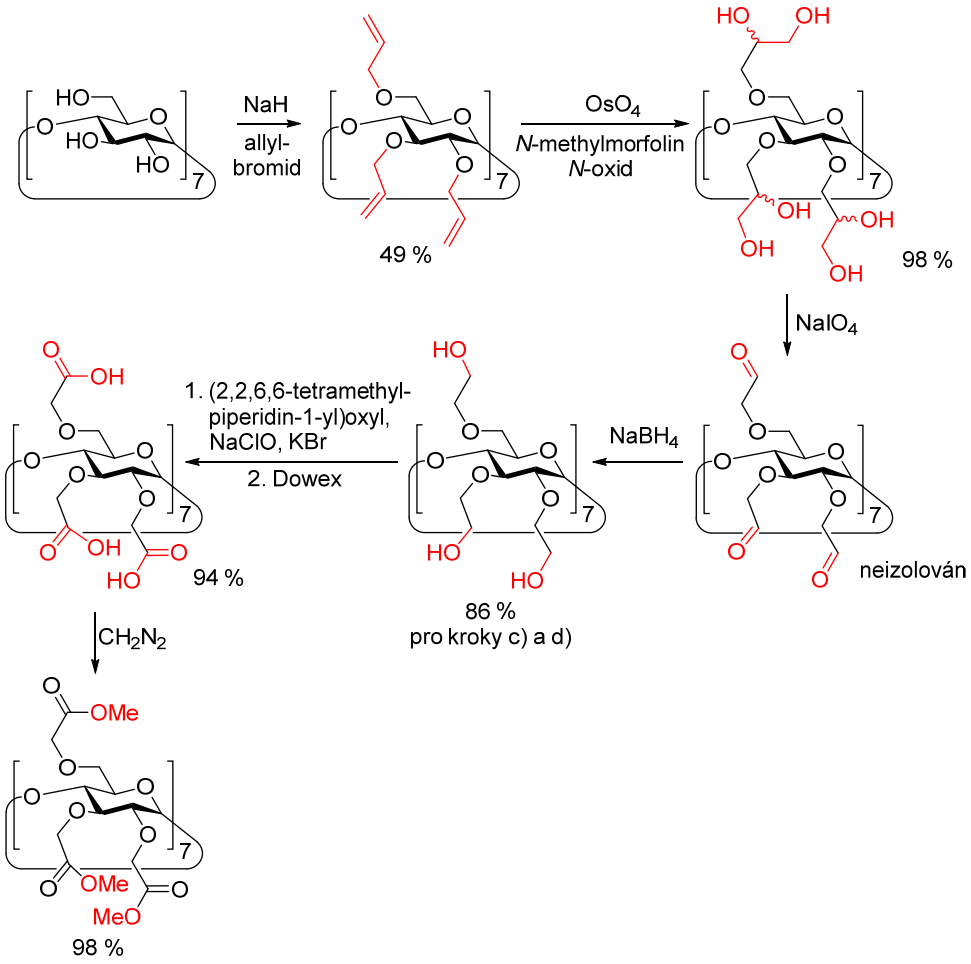


Schéma 1. Příprava pro další syntézu zajímavých persubstituovaných derivátů cyklodextrinů.

3.2 Náhodně substituované deriváty cyklodextrinů

Náhodně substituované deriváty vynikají svou snadností přípravy a jsou velmi často (kromě typu přítomného substituentu) charakterizovány pouze stupněm substituce, který udává průměrný počet zavedených skupin na cyklodextrinu. Stejně jako v případě persubstituovaných derivátů jsou i tyto komerčně dostupné a velmi často používané ve farmaceutických aplikacích (Conceicao et al. 2018). Náhodně substituované deriváty lze samozřejmě připravit i v laboratoři. Stačí přídavek menšího počtu ekvivalentů činidla, než je celkový počet hydroxylových skupin přítomných na modifikovaném cyklodextrinu.

Jak již bylo zmíněno výše, pouhý stupeň substituce nezaručuje stejné zastoupení izomerů ve směsi v případě zakoupení produktu od stejného (natož pak odlišného) dodavatele (Estrada III and Vigh 2012), a tudíž ani stejné chování produktu v následné aplikaci. Studium jednotlivých izomerů poukázalo na jejich velmi výrazné odlišnosti (viz kapitola 4.1.1) (**Navrátilová et al. 2013**; **Řezanka et al. 2014b**; **Řezanka et al. 2016b**).

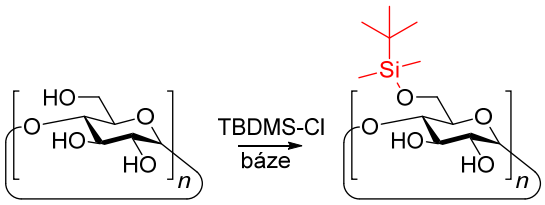
I přes tyto nevýhody jsou však náhodně substituované cyklodextriny hojně využívány pro výše zmíněnou značnou jednoduchost přípravy. Do kategorie náhodně substituovaných derivátů cyklodextrinů patří i příprava cyklodextrinových polymerů, kdy je k cyklodextrinu přidáno vhodné síťovací činidlo (**Řezanka 2019**). Takto lze připravit například i zajímavé nanomateriály – cyklodextrinové nanohouby (z angl. nanosponges) (Krabicová et al. 2020).

3.3 Cyklodextriny persubstituované ve vybraných pozicích

Termín cyklodextriny persubstituované ve vybraných pozicí označuje ty deriváty, které v polohách 2, 3 nebo 6 mají stejný substituent (obrázek 2). Syntéza těchto derivátů využívá rozdílnou reaktivitu hydroxylových skupin, která byla popsána v kapitole 2.3.2, a často také metod nepřímé syntézy popsané též výše.

Nejčastěji používaná je modifikace v poloze 6, jelikož ta obsahuje nejpřístupnější primární hydroxylovou skupinu. Této modifikaci pak dominují substituce pomocí TBDMS nebo halogenu. Tyto deriváty jsou například velmi vhodnými prekurzory pro přípravu rozličných amfifilních derivátů cyklodextrinů (Sallas and Darcy 2008).

Syntéza per-6-*O*-(*tert*-butyldimethylsilyl)-α-, β- a γ-cyklodextrinů je prováděna za standardních podmínek (schéma 2) pomocí TBDMS-Cl a rozličných bází (**Řezanka 2019**), přičemž pyridin poskytuje nejvyšší výtěžky (Ashton et al. 1996).



až 95 %

n = 6, derivát α-cyklodextrinu
n = 7, derivát β-cyklodextrinu
n = 8, derivát γ-cyklodextrinu

Schéma 2. Syntéza per-6-*O*-(*tert*-butyldimethylsilyl)-α-, β- a γ-cyklodextrinu.

Pomocí dalších chránících a odchraňujících kroků, případně s využitím rozdílné reaktivity hydroxylových skupin v polohách 2 a 3, lze dospět i k dalším sériím zajímavých derivátů, které mohou sloužit jako prekurzory pro další syntézu (Tutu and Vigh 2011; **Řezanka 2019**).

Další oblíbenou skupinou výchozích látek pro další syntézu jsou cyklodextriny perhalogenované v poloze 6. Halogenderiváty jsou pak výchozí látkou pro syntézu univerzálních prekurzorů persubstituovaných v polohách 6 azido, amino (Ashton et al. 1996; Gorin et al. 1996) či sulfanylovou (Rojas et al. 1995; Gorin et al. 1996) skupinou (schéma 3).

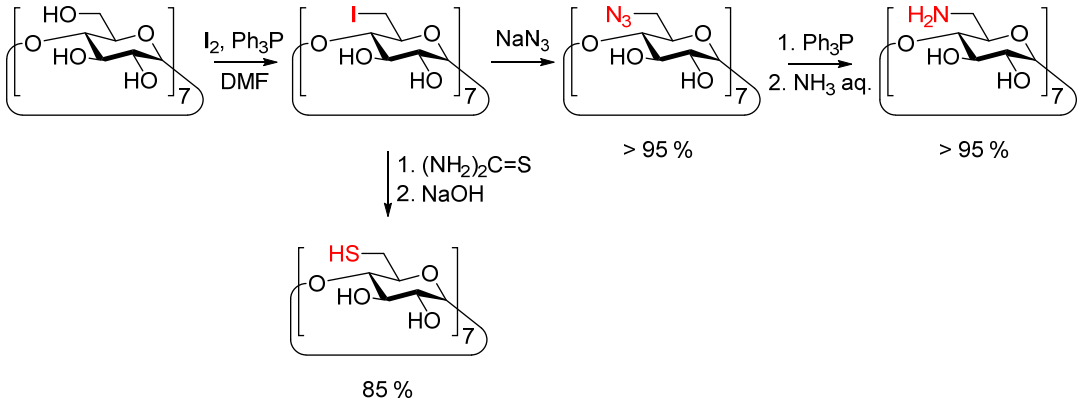
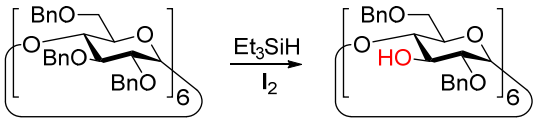


Schéma 3. Syntéza per-6-substituovaných derivátů β-CD.

Pro přípravu derivátů persubstituovaných v poloze 3 lze využít prekurzoru získaného selektivní debenzylací perbenzylovaného α-CD pomocí triethylsilanu a jódu (Guitet et al. 2012). Tato metoda byla původně vyvinuta pro debenzylaci mono- a disacharidů (Pastore et al. 2011) a v případě α-CD probíhá v poloze 3, která je jinak obtížně dostupná (schéma 4).



58 %

Schéma 4. Selektivní debenzylace v poloze 3.

3.4 Selektivně substituované deriváty cyklodextrinů

Selektivně substituované deriváty cyklodextrinů jsou takové jednotlivé sloučeniny, kde je poloha všech substituentů přesně známa. Do této skupiny mohou být zařazeny i monosubstituované cyklodextriny, ale pro účely této habilitační práce jsou vyčleněny do samostatné kapitoly. V této habilitační práci se selektivně substituovanými deriváty rozumí i ty, které obsahují volné hydroxylové skupiny v definovaných pozicích, zatímco všechny ostatní –OH skupiny jsou ochráněny (acetylovány, benzylovány, methylovány atp.).

Syntéza selektivně substituovaných derivátů cyklodextrinů je nejnáročnější disciplína v chemii cyklodextrinů. Uvažme jen, že v případě substituce různými deriváty je počet možných permutací nestejných derivátů určen faktoriálem a dále je třeba započítat počet *n*-prvkových množin pozic substituce, který byl již diskutován výše. Naštěstí byly v průběhu let objeveny reakce, které umožňují selektivní vícesubstituci a to ve výtěžcích v řádu desítek procent. Tyto reakce jsou předmětem zájmu následujících dvou kapitol.

3.4.1 Metody přímé syntézy

Jedním z prvních pokusů, jak selektivně připravit disubstituované deriváty, byly reakce β-CD s disulfonylchloridy, které probíhaly v polohách 6. $6^A,6^B$ derivát vzniká v případě reakce s 4,6-dimethoxy-1,3-disulfonylchloridem (Breslow et al. 1990). Benzofenon-3,3´-disulfonylchlorid poskytuje při reakci $6^A,6^C$ derivát (Tabushi et al. 1981), zatímco *trans*-stilben-4,4´-disulfonylchlorid (Tabushi et al. 1981) nebo difenylmethan-4,4´-disulfonylchlorid (Tabushi et al. 1976) vedou k $6^A,6^D$ derivátu (schéma 5).

Pro přípravu $6^A,6^D$ derivátu lze také použít bifenyl-4,4´-disulfonylchlorid (Tabushi et al. 1984). Produkt vzniká v přibližně 50% výtěžku spolu s příměsí malého množství $6^A,6^C$ derivátu. Je tak s podivem, že někteří autoři popisují

výtěžek až 80 % (Ding et al. 2018). Neklid mysli též probouzí článek popisující stejnou reakci (Kulkarni et al. 2018), avšak autoři se odkazují na článek o přípravě $6^A,6^C$ derivátu pomocí benzofenon-3,3´-disulfonylchloridu (Tabushi and Kuroda 1984). Obecně lze říci, že neobvykle vysoké výtěžky bez důkazů čistoty či struktury a stížnosti dalších autorů (včetně autora této habilitační práce) na reprodukovatelnost daných postupů, určení správného izomeru a dosáhnutí vysokého výtěžku (Martina et al. 2007; Popr et al. 2014; **Řezanka 2016**) se táhnou jako červená nit chemií cyklodextrinů.

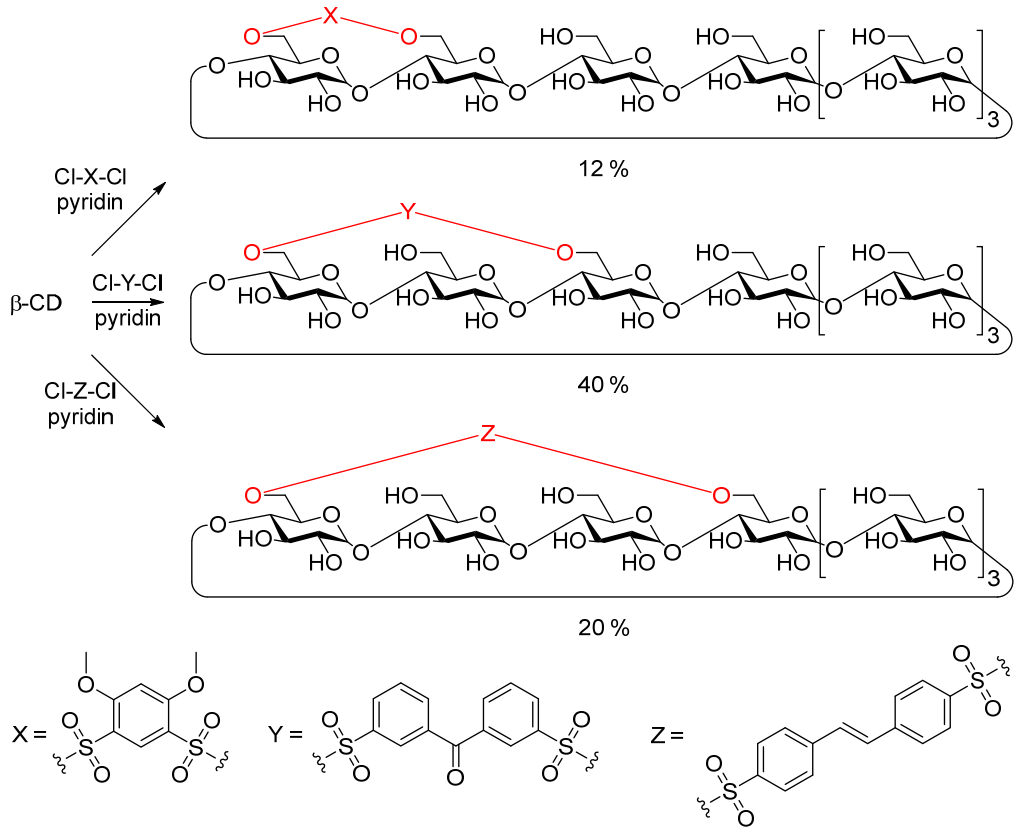


Schéma 5. Regioselektivní syntéza disubstituovaných derivátů.

Syntéza symetricky trisubstituovaného α-CD využívá objemného substituentu pro přednostní tvorbu $6^A,6^C,6^E$ derivátu (Boger et al. 1979) (Schéma 6). Kinetika trifenylmethylace byla důkladně studována později, přičemž byl potvrzen i vznik dalších trisubstituovaných derivátů (Yoshikiyo et al. 2015).

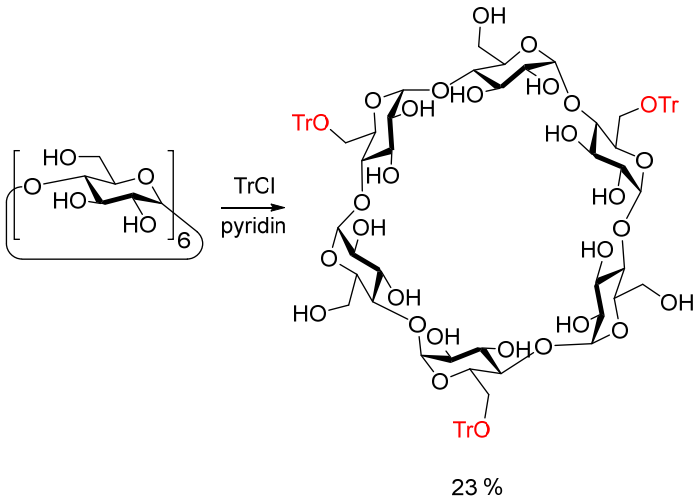


Schéma 6. Syntéza symetricky trisubstituovaného α-CD v polohách 6.

3.4.2 Metody nepřímé syntézy

Pro přípravu selektivně substituovaných derivátů cyklodextrinů jsou popsány dvě hlavní metody. Selektivní bis-*O*-debenzylace perbenzylovaného cyklodextrinu a selektivní bis-*O*-demethylace permethylovaného cyklodextrinu (**Řezanka 2019**).

Selektivní bis-*O*-debenzylace (schéma 7) probíhá za působení DIBAL na perbenzylovaném α-, β- nebo γ-CD a poskytuje i výtěžky přesahující 80 % (Pearce and Sinaÿ 2000). Benzylové skupiny jsou odchraňovány v polohách 6^A,6^D s výjimkou γ-CD, kdy také vzniká 6^A,6^E derivát.

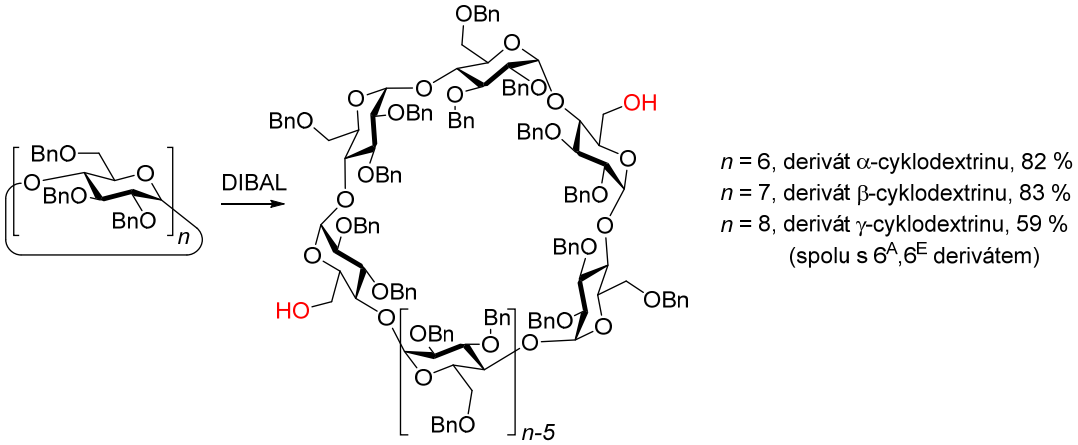
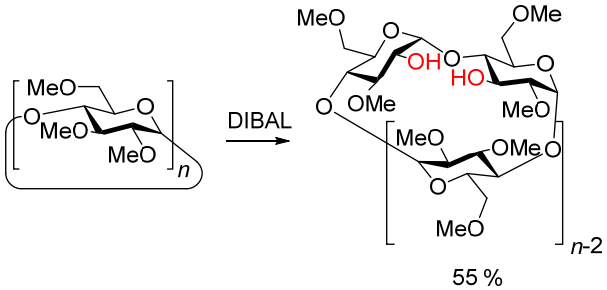


Schéma 7. Selektivní bis-*O*-debenzylace.

Návrh reakčního mechanismu zahrnuje nejméně dvě molekuly DIBAL a reakce probíhá postupně – nejdříve dochází k odstranění jedné a pak teprve

druhé benzylové skupiny (Sollogoub 2013). Tento postupný mechanismus doprovázený substitucí hydroxylových skupin pak umožňuje syntézu i poměrně komplikovaných struktur – trisbstituovaných (Guieu and Sollogoub 2008; Rawal et al. 2010), tetrasubstituovaných (Rawal et al. 2010; Sollogoub 2013), pentasubstituovaných (Guieu and Sollogoub 2008) nebo dokonce hexasubstituovaných derivátů se šesti odlišnými skupinami v polohách 6 na α-CD (Wang et al. 2014). Navíc společně s výše popsaným odchráněním benzylových skupin v poloze 3 lze připravit zajímavé cyklodextrinové deriváty selektivně substituované současně na primárním a sekundárním okraji (Guitet et al. 2012).

Dva roky po objevu selektivní bis-*O*-debenzylace byla popsána selektivní bis-*O*-demethylace, která též využívá DIBAL k odstraňování substituentů (du Roizel et al. 2002). Na rozdíl od debenzylace však tato demethylace odstraňuje methylové skupiny v polohách 2^A a 3^B (schéma 8) a byla popsána na α- a β-CD.



n = 6, derivát α-cyklodextrinu
n = 7, derivát β-cyklodextrinu

Schéma 8. Selektivní bis-*O*-demethylace.

3.5 Monosubstituované deriváty cyklodextrinů

Cyklodextriny mohou být monosubstituovány v polohách 2, 3, nebo 6 a pro jejich přípravu lze využít jak metod přímé, tak i metod nepřímé syntézy.

3.5.1 Metody přímé syntézy

Přímé metody využívají rozdílné reaktivity hydroxylových skupin popsaných výše. Dosahované výtěžky závisí na rozpouštědle, bázi, typu cyklodextrinu a struktuře činidla použitého pro substituci (Masurier et al. 2006; Martina et al. 2010; **Bláhová et al. 2013**; **Řezanka 2016**).

Jak již bylo popsáno výše, i syntézy monosubstituovaných derivátů popisované v literatuře trpí řadou nedostatků a hojně využívané vysrážení reakční směsi acetonem jako jediná čistící operace je nedostatečné. V reakční směsi bývá většinou také přítomen nezreagovaný cyklodextrin a vícesubstituované produkty (Popr et al. 2014).

Přímá substituce v poloze 2 bývá selektivní za použití LiH jako báze, DMSO jako rozpouštědla, katalýze LiI a přidáním alkylačního činidla – například často používaného allyl bromidu. Výtěžky dosahují až 40 % (**Řezanka 2016**).

Substituce do polohy 3 je obtížná. Pro β-CD existuje metoda za použití cinnamylbromidu jako alkylačního činidla (Jindřich and Tišlerová 2005). Reakce je prováděna ve směsi acetonitrilu a vody a použitím NaOH jako báze. Produkt je možné vyčistit pouze rekrystalizací a výtěžek činí velmi dobrých 43 % (schéma 9).

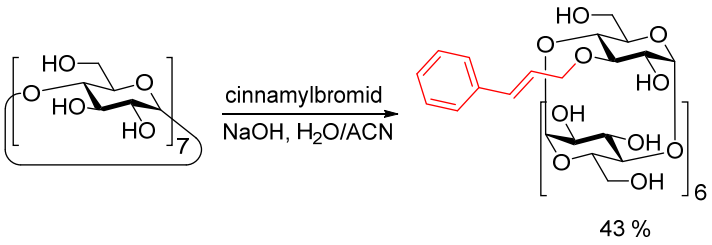


Schéma 9. Syntéza 3^{A}-*O*-cinnamyl-β-cyklodextrinu.

Použití stejných podmínek v případě allyl- či propargylbromidu na γ-CD vede ke směsi monosubstituovaných produktů v polohách 2 a 3 (**Bláhová et al. 2013**). Monosubstituované deriváty mohou být ze směsi separovány a po peracetylaci všech zbývajících hydroxylových skupin od sebe navzájem odděleny (schéma 10). Acetylace s sebou zároveň přináší možnost funkční skupiny dále modifikovat bez obav o osud hydroxylových skupin. Například allyl může být dále oxidován pomocí $NaIO_4/RuCl_3$ na široce uplatnitelnou karboxymethylovou skupinu (**Navrátilová et al. 2013**; **Řezanka et al. 2014b**; **Řezanka et al. 2016b**).

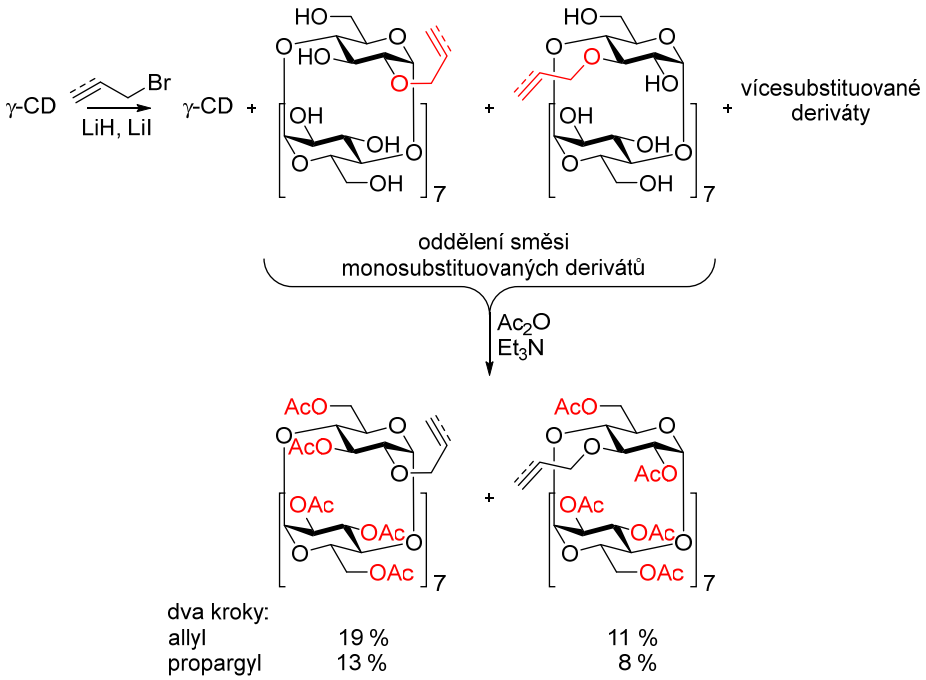


Schéma 10. Syntéza monosubstituovaných derivátů allyl a propargyl γ-CD.

Jednou z metod přímé alkylace je i použití nadbytku báze (například NaOH) a vody jako rozpouštědla. S allyl- či propargylbromidem je možno dosáhnout selektivní substituce v poloze 6 ve výtěžcích většinou mezi 10 a 20 % (**Bláhová et al. 2013**; **Řezanka 2016**).

V poloze 6 je ale nejvíce používaná metoda monosubstituce tosylovou (*p*-toluensulfonovou) skupinou (**Řezanka 2016**). Ta může být následně převedena na několik užitečných prekurzorů pro další syntézu (**Řezanka 2019**): například sulfanyl, azido či amino deriváty (schéma 11).

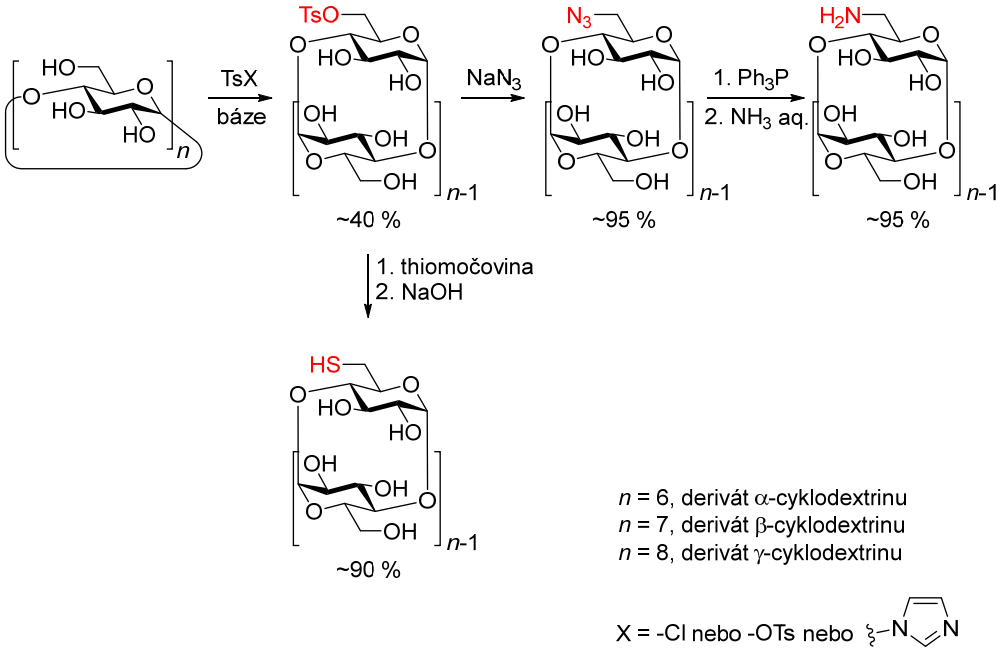


Schéma 11. Syntéza monotosylovaných cyklodextrinů a z nich vycházející další syntézy zajímavých prekurzorů.

Azidoderiváty mohou být dále použity pro měďnatými solemi katalyzovanou 1,3-dipolární (Huisgenovu (Huisgen 1963)) cykloadici (**Lukášek et al. 2018**) a aminoderiváty například pro syntézu amidů (schéma 12) (**Řezanka et al. 2016a**; **Lukášek et al. 2018**).

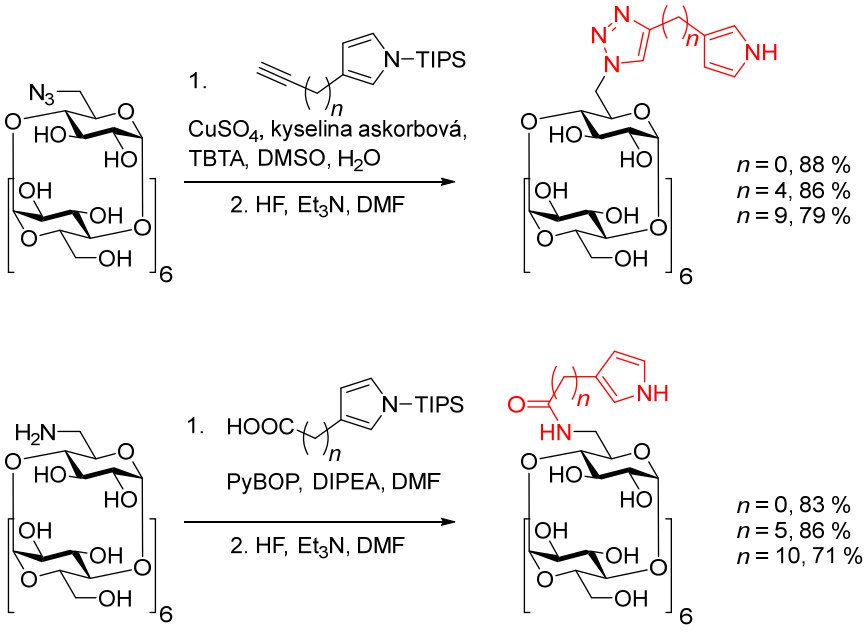


Schéma 12. Příklad syntézy monosubstituovaných derivátů β-CD z azido a aminoderivátu.

3.5.2 Metody nepřímé syntézy

Pro syntézu monosubstituovaných derivátů cyklodextrinů se obzvláště hodí metody, které již byly popsány výše. Selektivní demethylace a selektivní debenzylace. Obě tyto metody dokáží poskytnout volnou hydroxylovou skupiny v definované poloze, zatímco jsou všechny ostatní hydroxylové skupiny chráněny. Poskytují tak v rozumných výtěžcích vynikající prekurzory pro další syntézu, kdy alkylace může probíhat pouze na jednom místě a nehrozí tak vznik regioizomerů. Tato metoda je tudíž univerzálnější než přímá syntéza.

Selektivní bis-*O*-demethylací lze získat permethylovaný 2^A,3^B dihydroxy derivát (schéma 8). Kromě něj při reakci též vzniká 20% podíl permethylovaného 6^A-hydroxy derivátu (du Roizel et al. 2002). Spolu s využitím přednostní reakce 2^A hydroxylové skupiny v případě 2^A,3^B derivátu a následné methylace hydroxylové skupiny v poloze 3^B či opačném způsobu (nejdříve methylace v poloze 2^A) (schéma 13) lze takto získat kompletní sadu permethylovaných monosubstituovaných 2, 3, nebo 6 derivátů (**Řezanka 2016**).

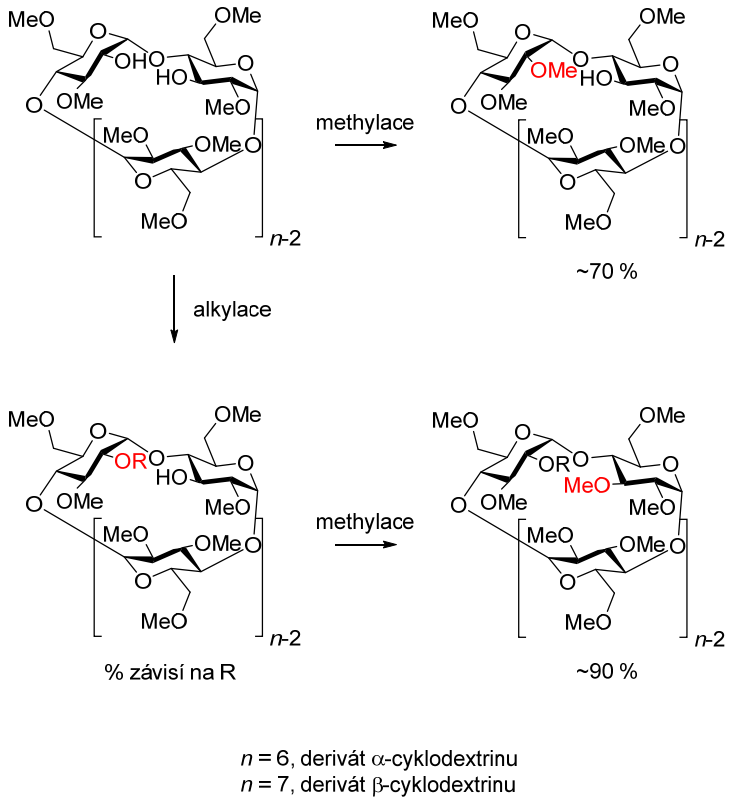


Schéma 13. Syntéza permethylovaných derivátů monosubstituovaných v poloze 2 nebo 3.

Příkladem využití tohoto přístupu může být až syntéza rotaxanu, který má monosubstituované permethylované cyklodextriny jako použité jako zarážky na ose (**Řezanka et al. 2015**). Syntéza probíhá podobně jako na schématu 13 a substituent je zaveden do polohy 2. Následnou dimerizací cyklodextrinu (přípravou osy se zarážkami) a zavedením prstence dojde ke vzniku rotaxanu (schéma 14).

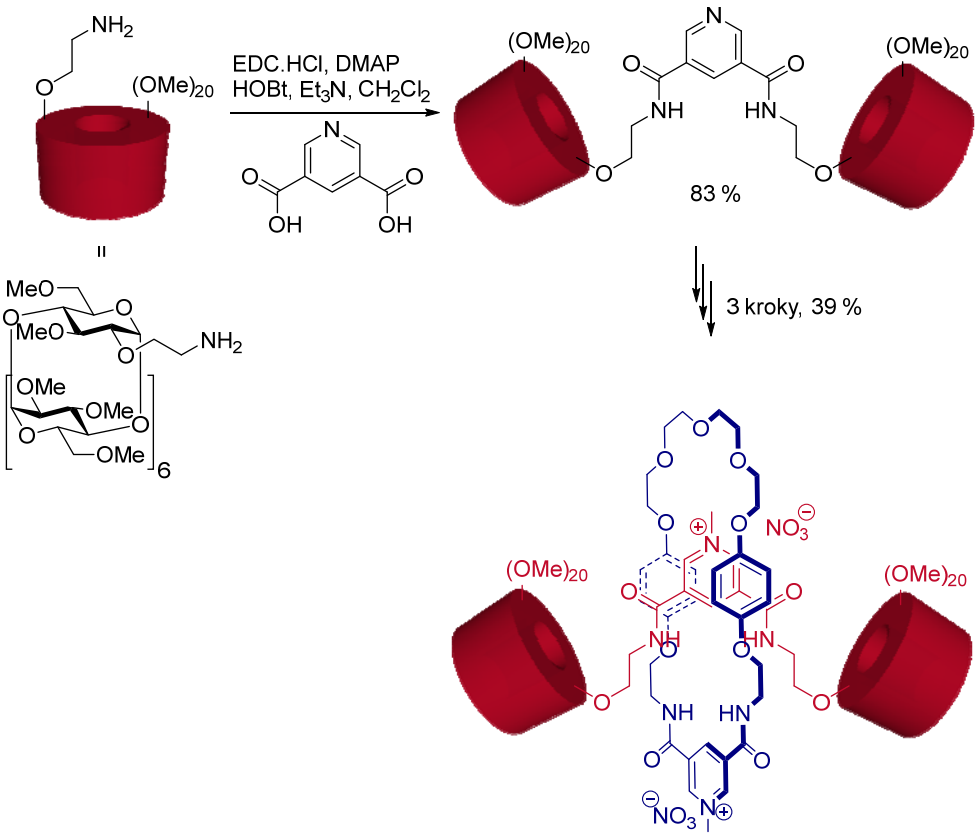
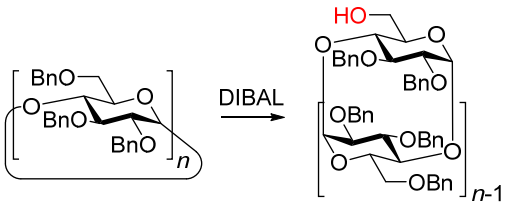


Schéma 14. Příklad syntézy rotaxanu založené na alkylaci částečně demethylovaného β-CD.

V úvodu této kapitoly byla kromě selektivní demethylace použité pro syntézu monosubstituovaných derivátů cyklodextrinů zmíněna i selektivní debenzylace (Pearce and Sinaÿ 2000). Jak již bylo zmíněno výše, reakční mechanismus je postupný a umožňuje tedy i odchránění pouze i jedné hydroxylové skupiny v poloze 6 (schéma 15). Vzhledem k možnosti po reakci volné hydroxylové skupiny odchránit i zbylé benzylové skupiny (Lindbäck et al. 2012), je tato metoda vhodným řešením pro přípravu derivátů, které mají ostatní –OH skupiny volné.



n = 6, derivát α-cyklodextrinu, 64 %
n = 7, derivát β-cyklodextrinu, 60 %
n = 8, derivát γ-cyklodextrinu, 47 %

Schéma 15. Selektivní debenzylace v poloze 6.

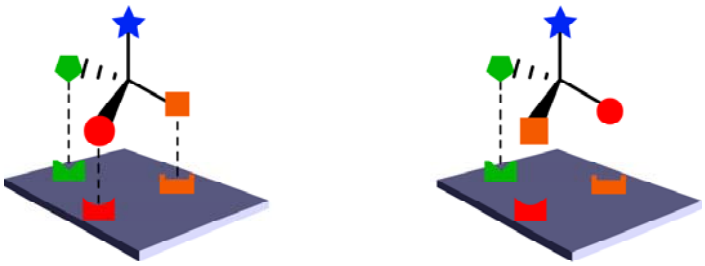
4. Využití derivátů cyklodextrinů

Jak již bylo napsáno na začátku této habilitační práce, cyklodextriny mají velký potenciál uplatnění v různých odvětvích. Další kapitoly na konkrétních příkladech ukáží, kterak deriváty cyklodextrinů, syntetizované podle postupů uvedených výše, mohou nabýt nějaké „hmatatelné“ funkce.

4.1 Cyklodextriny v chirálním rozpoznávání

Chiralita, odvozená od řeckého χειρ (do češtiny přeložitelného jako ruka), je vlastností asymetrických objektů, které nejsou ztotožnitelné se svými zrcadlovými obrazy. Chirální molekuly jsou klíčovou součástí chemických a zvláště pak biologických procesů, které začínají například již u samého embrya (Levin 2005). Proto chirální rozpoznávání hraje v našem světě klíčovou roli.

Chirální rozpoznávání je poměrně složitá úloha, jelikož chirální molekuly (enantiomery) mají stejné chemické a fyzikální vlastnosti v achirálním prostředí. Pokud je však použit chirální selektor, může dojít k typické tříbodové interakci (obrázek 3) (**Hobbs et al. 2020**), poprvé formulované Ogstonem (Ogston 1948).



dochází k rozpoznání nedochází k rozpoznání

Obrázek 3. Chirální rozpoznání založené na tříbodové interakci

Cyklodextriny obsahují velké množství chirálních center a spolu s chirální kavitou a vlastnostmi popsanými výše se jeví jako ideální chirální selektory, přičemž jejich první použití se datuje do roku 1977 (MacNicol and Rycroft 1977). Cyklodextriny jsou tak používány například v nukleární magnetické rezonanci jako chirální posunová činidla (Dalvano and Wenzel 2017), v separačních technikách jako chirální selektory (Xiao et al. 2012; Yu et al. 2013;

Řezanka et al. 2014a; Schurig 2015; Li and Wang 2019), nebo v senzorových systémech (Shahgaldian and Pieles 2006; Wang et al. 2019a).

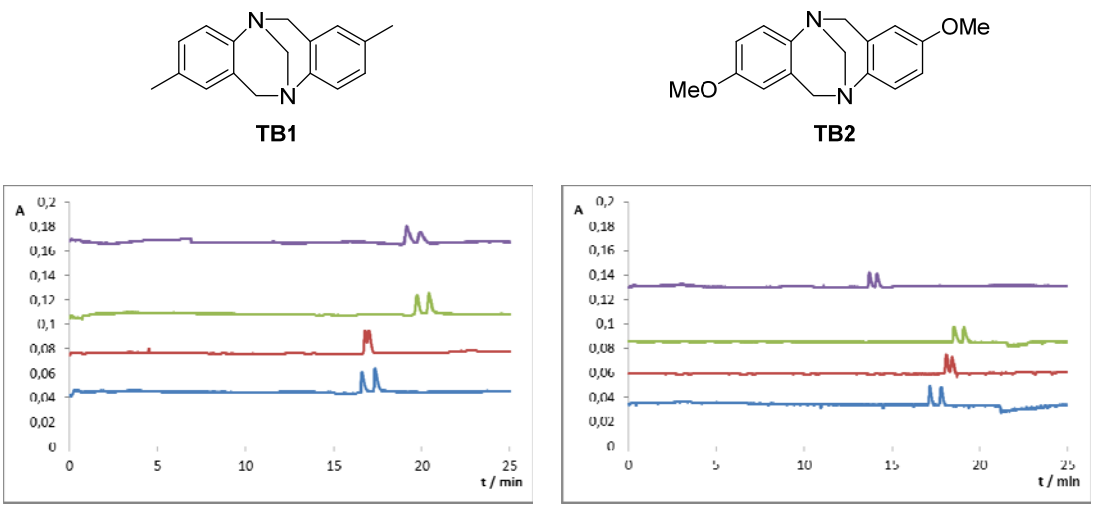
Cyklodextriny mohou chirálně rozpoznávat velké množství různých typů sloučenin, ať už se jedná o léčiva (Guo et al. 2016; Salido-Fortuna et al. 2019), aminokyseliny (Kitagawa and Otsuka 2011; Wang et al. 2019b), alkaloidy (Yatsimirsky 2012; Li et al. 2017), herbicidy (Lubomirsky et al. 2017) nebo dokonce i enantiomery nanoklastrů zlata (Zhu et al. 2018).

4.1.1 Cyklodextriny jako chirální selektory v kapilární elektroforéze

První chirální separace kapilární elektroforézou byla popsána roku 1985 (Gassmann et al. 1985) jako výsledek předchozího bádání na poli enantioseparací (Chankvetadze 2007). Jako chirální selektor zde byl použit měďnatý komplex L-histidinu. První použití cyklodextrinů jako chirálních selektorů v kapilární elektroforéze pak proběhlo o tři roky později (Jelínek et al. 1988). Od té doby se použití cyklodextrinů a jejich derivátů v této metodě značně rozšířilo.

Nejvíce jsou používány náhodně substituované deriváty (**Řezanka et al. 2014a**). Avšak, jak již bylo podotknuto výše, tyto směsi látek mají velkou nevýhodu v neurčitém složení, které může působit problémy při opakování separace. Demonstrujme nyní odlišné chování jednotlivých izomerů na monokarboxymethyl cyklodextrinech.

Za tímto účelem byly metodami popsanými výše syntetizovány jednotlivé 2^A-, 3^A- nebo 6^A-*O*-karboxymethyl-β-cyklodextriny (**Navrátilová et al. 2013**). Jejich jednotlivé porovnání spolu se srovnáním s jejich ekvimolární směsí přineslo zajímavá zjištění (obrázek 4, tabulka 1). Při separaci Trögerových bází jsou patrné jednoznačné rozdíly mezi jednotlivými izomery, přičemž regioizomer v poloze 3 má nejmenší enantioseparační schopnosti, zatímco regioizomer v poloze 2 má tyto schopnosti největší. Není také bez zajímavosti, že ekvimolární směs se v rámci experimentální chyby chová jako průměr všech tří derivátů. Znamená to tedy, že zde neprobíhá žádné synergické působení jednotlivých derivátů.

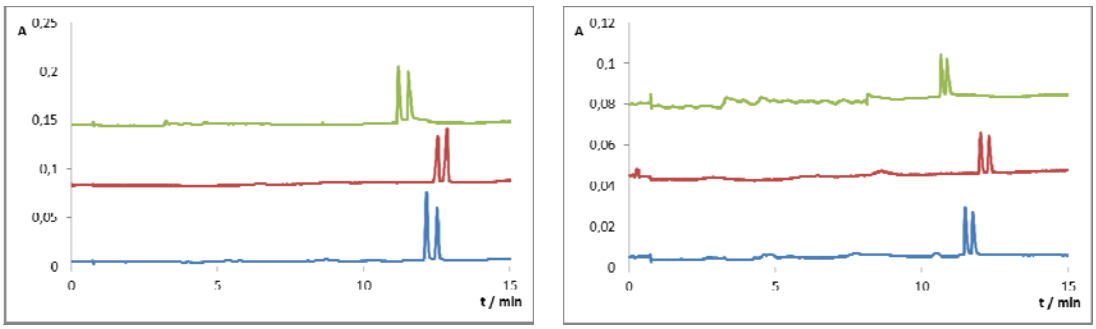


Obrázek 4. Separace Trögerových bází TB1 a TB2 v kapilární elektroforéze při použití 2^A-*O*-karboxymethyl-β-cyklodextrinu (modrá); 3^A-*O*-karboxymethyl-β-cyklodextrinu (červená); 6^A-*O*-karboxymethyl-β-cyklodextrinu (zelená); nebo jejich ekvimolární směsi (fialová).

Tabulka 1. Porovnání rozlišení Trögerových bází v kapilární elektroforéze při použití jednotlivých monokarboxymethylderivátů β-CD nebo jejich ekvimolární směsi.

	2KM-β-CD	3KM-β-CD	6KM-β-CD	směs
TB1	2,8	0,7	2,1	1,8
TB2	2,5	1,3	2,0	1,9

V případě 2^A-, 3^A- nebo 6^A-*O*-karboxymethyl-γ-cyklodextrinu je situace obdobná (**Řezanka et al. 2014b**). Rozlišení jednotlivých enantiomerů opět závisí na poloze karboxymethylového substituentu a 2^A-*O*-karboxymethyl-γ-cyklodextrin je pro uvedené báze nejlepším chirálním selektorem (obrázek 5, tabulka 2).



a b

Obrázek 5. Separace Trögerových bází TB1 (a) a TB2 (b) v kapilární elektroforéze při použití 2^{A}-*O*-karboxymethyl-γ-cyklodextrinu (modrá); 3^{A}-*O*-karboxymethyl-γ-cyklodextrinu (červená); 6^{A}-*O*-karboxymethyl-γ-cyklodextrinu (zelená).

Tabulka 2. Porovnání rozlišení Trögerových bází v kapilární elektroforéze při použití jednotlivých monokarboxymethylderivátů γ-CD.

	2KM-γ-CD	3KM-γ-CD	6KM-γ-CD
TB1	2,6	1,9	2,4
TB2	2,0	2,0	1,5

Po úspěšném ověření teorie, že monosubstituované karboxymethylové deriváty cyklodextrinů mají rozdílné enantioseparační vlastnosti, byla poprvé provedena studie kompletního porovnání asociačních konstant pro všech 9 monosubstituovaných derivátů od α-, β- a γ-CD (**Řezanka et al. 2016b**) s TB1.

Výpočet asociačních konstant byl založen na určení migračních časů a následně efektivní mobilitě při různých koncentracích monokarboxymethylcyklodextrinů, přičemž bylo nutné se vypořádat s faktory, jako jsou tvar píků, měnící se viskozita a iontová síla základního elektrolytu.

Tabulka 3 pak přehledně shrnuje až řádové rozdíly mezi asociačními konstantami jednotlivých monokarboxymethylcyklodextrinů. Výsledky přehledně ukazují významný vliv jak pozice substituentu na cyklodextrinovém skeletu, tak i typu cyklodextrinu (tzn. velikosti kavity) na asociační konstanty a

enantioseparační schopnosti. Obzvláště je tento vliv patrný u derivátů γ-CD kde je mezi jednotlivými regioizomery rozdíl až téměř dva řády. Tento výsledek pak koresponduje s výše popsanými problémy s použitím náhodně substituovaných cyklodextrinů (Estrada III and Vigh 2012).

Tabulka 3. Porovnání asociačních konstant Trögerovy báze se všemi monokarboxymethyl deriváty od α-, β- a γ-CD.

	$K_{[+]\text{-TB1-CD}}$ [dm^3/mol]	$K_{[-]\text{-TB1-CD}}$ [dm^3/mol]
2KM-α-CD	33±3	40±3
3KM-α-CD	85±5	122±7
6KM-α-CD	44±5	50±6
2KM-β-CD	271±12	439±15
3KM-β-CD	230±11	276±11
6KM-β-CD	269±17	372±20
2KM-γ-CD	45±6	53±6
3KM-γ-CD	928±21	948±33
6KM-γ-CD	28±3	36±3

4.1.2 Elektrochemické cyklodextrinové senzory pro chirální rozpoznávání aminokyselin

Elektrochemické senzory mají bohatou historii v aplikacích pro stanovování biologicky aktivních látek. Zřejmě jedním z nejznámějších takových senzorů je osobní glukózový test, který využívá glukóza-oxidázu nebo glukóza-1-dehydrogenázu, kofaktor flavinadenindinukleotid nebo nikotinamidadenindinukleotid a elektrody přítomné na proužku (čipu) ke generování elektrochemického signálu. Ten je pak převeden na koncentraci glukózy v krvi (Yoo and Lee 2010).

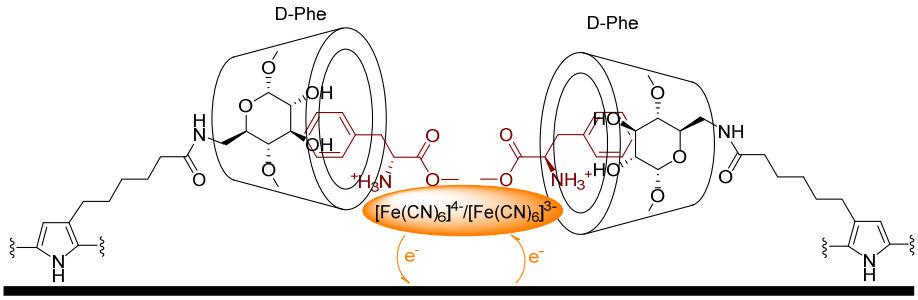
Cyklodextriny v oblasti elektrochemických senzorových systémů nalézají uplatnění také. Zde slouží po navázání na elektrodu jako chirální selektor způsobující odlišení elektrochemických vlastností analyzovaných enantiomerů,

obzvláště pak prekoncentrací jednoho z analytů blízko vodivého povrchu (Lenik 2017; Niu et al. 2018; Bae et al. 2019; Casulli et al. 2019).

V současné době je také velmi populární použití vodivých nanomateriálů (majících vysoký poměr povrchu ku objemu) v uvedených senzorech. Kombinace nanomateriálu s cyklodextriny tak často vede k vysoce citlivé detekci širokého spektra látek (**Hobbs et al. 2020**).

Možností, jak imobilizovat cyklodextrin na povrchu elektrody, je mnoho a jednou z nich je též polymerace vhodného cyklodextrinového derivátu – například konjugátu 6-[pyrrol-3-yl]hexanové kyseliny a 6^{A}-deoxy-6^{A}-amino-β-cyklodextrinu (schéma 12) – na skleněné uhlíkové elektrodě elektrochemickou depozicí (**Shishkanova et al. 2020**). Takto připravený senzorový systém byl použit pro detekci fenylalaninu (resp. hydrochloridu jeho methylesteru). Měření ukázala, že připravený senzorový systém je o dva řády citlivější pro D-formu než L-formu.

Možné vysvětlení pro tuto selektivitu zahrnuje $[Fe(CN)_6]^{4-}$ a $[Fe(CN)_6]^{3-}$ (Peter et al. 1976; Blackwood and Pons 1988), kdy tento negativně nabitý redoxní pár kompenzuje pozitivní náboj přítomný na aminokyselině. V případě L-formy je tento kation ukryt uvnitř kavity cyklodextrinu a je tudíž nedostupný pro redoxní pár (Niu et al. 2019). Zatímco u D-formy je tento kation přístupný pro kompenzaci náboje blízko vodivého povrchu (obrázek 6).



Obrázek 6. Navrhnutý mechanismus chirálního rozpoznávání.

4.2 Cyklodextriny v tkáňovém inženýrství

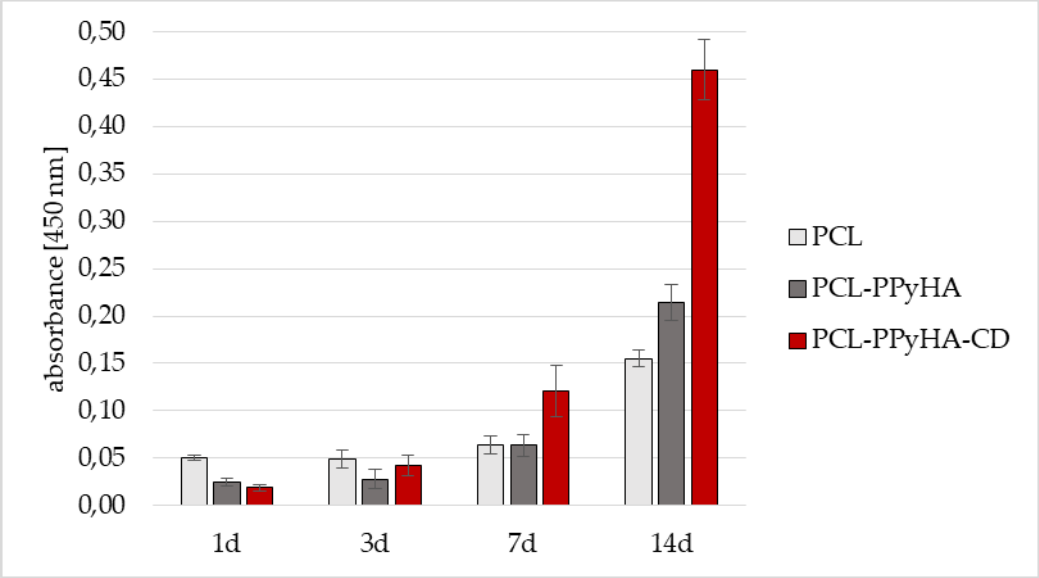
Tkáňové inženýrství (Place et al. 2009; Liu et al. 2017) je složitá disciplína, která mezi sebou propojuje různé oblasti vědy. Klade si za cíl podpořit obnovu tkání a tak napodobovat děje, které dnes a denně probíhají v živých

organismech. K dosažení cíle pak většinou využívá tkáňových nosičů z rozličných materiálů. Jedním takovým je i polypyrrol (Huang et al. 2014).

Polypyrrol je jedním z nejzkoumanějších vodivých polymerů v tkáňovém inženýrství, protože je stabilní, biokompatibilní, podporuje růst buněk a je snadno připravitelný či modifikovatelný (Huang et al. 2014; Balint et al. 2014; Guo and Ma 2018).

Cyklodextriny jsou vzhledem ke svým vlastnostem v tkáňovém inženýrství používány též a ukazuje se, že jejich přítomnost je velmi prospěšná (Garcia-Rio et al. 2014; Venuti et al. 2017; Alvarez-Lorenzo et al. 2017; Yi et al. 2018b). β-CD může být dále používán pro uvolňování růstových faktorů (Grier et al. 2018), pro zvýšení koncentrace kyslíku v tkáňových nosičích (Deluzio et al. 2014) nebo pro regulaci samoskladby kolagenu (Majumdar et al. 2018). Cyklodextriny jsou také díky své kavitě schopné uvolňovat léčiva během kultivace buněk (Prabaharan and Jayakumar 2009; Lee et al. 2016a; Lee et al. 2016b).

Spojením cyklodextrinu a polypyrrolu by tak mohl být připraven zajímavý nosič pro tkáňové inženýrství. Jako výchozí substrát byl vybrán polykaprolakton, který byl následně pokryt vrstvou polypyrrolu modifikovaného v poloze 3 hexanovou kyselinou. Následně byl pak pomocí amidické vazby připojen 6^A-deoxy-6^A-amino-β-cyklodextrin (**Lukášek et al. 2019**). Všechny tři připravené tkáňové nosiče byly podrobeny buněčnému testování, přičemž se ukázalo, že použití cyklodextrinu má významně pozitivní vliv na růst buněk (obrázek 7). Je to pravděpodobně způsobeno adsorpcí proteinů v nativním stavu na povrchu cyklodextrinem funkcionalizovaného tkáňového nosiče.



Obrázek 7. Buněčná proliferace normálních lidských dermálních fibroblastů na tkáňových nosičích připravených z PCL (bílá); z PCL pokrytého vrstvou poly[6-(pyrrol-3-yl)hexanové kyseliny] (šedá); a z PCL pokrytého vrstvou poly[6-(pyrrol-3-yl)hexanové kyseliny] s připojeným 6^{A}-deoxy-6^{A}-amino-β-cyklodextrinem (červená.)

5. Závěr

Tato habilitační práce shrnuje dosavadní poznatky v syntéze derivátů cyklodextrinů: persubstituovaných cyklodextrinů, náhodně substituovaných cyklodextrinů, cyklodextrinů persubstituovaných ve vybraných pozicích, selektivně substituovaných cyklodextrinů a monosubstituovaných cyklodextrinů, přičemž přístupy syntézy rozděluje na přímé a nepřímé, u kterých je využito chránění hydroxylových skupin.

Historie ukazuje, že aby se daná syntéza mohla stát široce použitelnou (alespoň tedy v základním výzkumu), musí se jednat o nekomplikovanou (ideálně jednokrokovou) reakci bez složitých čistících operací. Skvělým příkladem je syntéza 6^{A}-*O*-(*p*-toluensulfonyl)-β-cyklodextrinu. Cyklodextrinové deriváty, které aspirují na to být neméně úspěšné, musí splňovat výše uvedené.

Autor dále představuje též možnosti aplikací. Jedná se jednak o použití cyklodextrinů v chirálním rozpoznávání enantiomerů, kdy byla zejména potvrzena rozdílnost jednotlivých regioizomerů a poprvé byly monostubstituované regioizomery od α-, β- a γ-CD srovnány navzájem. Jako příklad jednoho z témat, kterým se momentálně autor zaobírá, nabízí použití cyklodextrinů v tkáňovém inženýrství.

V habilitační práci jsou uvedeny pouze základní informace a autor přikládá k nahlédnutí plné texty citovaných vlastních prací, kde lze dohledat další podrobnosti. V následující kapitole je vymezen autorův přínos k řešené problematice a jsou uvedeny jeho podíly na publikačních výstupech.

6. Autorův přínos k chemii cyklodextrinů

Autor se v průběhu uplynulých let věnoval syntéze substituovaných derivátů cyklodextrinů a jejich aplikacím v různých oblastech chemie a biologie. V posledních letech se jeho zaměření mění z oblasti organicko-chemické do materiálově-chemické oblasti.

Autorův výzkum byl zaměřen na selektivní syntézu monokarboxymethyl cyklodextrinů a analýzu jejich separačních vlastností v kapilární elektroforéze (kapitoly 3.5.1 a 4.1.1). Největším přínosem je pak srovnání všech devíti (2^A-, 3^A- a 6^A-*O*-karboxymethyl-α, β a γ-cyklodextrinů) monosubstituovaných derivátů cyklodextrinů, které ještě nikdy nebylo provedeno.

Další výzkum směřoval na nově vyvinutou syntézu konjugátů pyrrolu a cyklodextrinu. Tato syntéza umožňuje velkou variabilitu připojení, co se týče délky připojovacího řetězce a jednak samotného způsobu připojení na cyklodextrin (kapitola 3.5.1). Cyklodextrin-pyrrolové konjugáty lze využít ve tkáňovém inženýrství (kapitola 4.2) nebo chirálním rozpoznávání aminokyselin (kapitola 4.1.2).

Autor také provedl první syntézu rotaxanu, kde jsou jako zarážky použity cyklodextriny substituované nikoliv na primárním, ale na sekundárním okraji (kapitola 3.5.2). Tento rotaxan se ukázal být zajímavý pro rozpoznávání aniontů ve vodném prostředí.

Závěrem nelze také opomenout autorův přínos v oblasti souhrnných článků, což velmi oceňují vědci zabývající se chemií cyklodextrinů.

6.1 Aktuální výzkum autora v oblasti cyklodextrinové chemie

V současné době se autor této práce zabývá v cyklodextrinové oblasti vývojem polymerů. Jednak náhodně substituovaných – ve spolupráci s prof. Trottou (University of Turin, Itálie) – pro uplatnění při cílené dopravě léčiv; a jednak definovaných pro uplatnění ve tkáňovém inženýrství, kdy je jako monomer použit disubstituovaný cyklodextrin. Při uplatnění výše uvedených polymerů spolupracuje autor také s Dr. Waclawkem (TUL) při řešení projektu

GAČR Nanočástice elementárního železa a cyklodextriny – jejich synergický účinek pro čistění vod (20-17028Y).

Ve spolupráci s Dr. Lai (National Chung Hsing University, Taiwan) autor vyvíjí konjugáty cyklodextrinů a silanů pro přípravu funkcionalizovaných povrchů. Tyto konjugáty mohou být i zajímavými prekurzory pro přípravu enantioselektivních materiálů v rámci projektu GAČR Hybridní organokřemičité nanomateriály pro heterogenní katalýzu enantioselektivních reakcí (18-09824S, doc. Hodačová, VŠCHT Praha), ve kterém je autor spoluřešitelem.

V neposlední řadě se také autor zabývá senzorovými systémy, které na základě interakce derivátu cyklodextrinu s analytem poskytují barevnou změnu a umožňují tak přístrojově nenáročnou detekci látek ve vodných prostředích.

6.2 Seznam publikací autora použitých v rámci této habilitační práce

V níže uvedeném seznamu publikací je vymezen podíl autora na provedeném výzkumu.

Bláhová M, Bednářová E, Řezanka M, Jindřich J (2013) Complete Sets of Monosubstituted γ-Cyclodextrins as Precursors for Further Synthesis. J Org Chem 78(2):697–701. doi: 10.1021/jo301656p

Autor myšlenky doplnit stávající známé monosubstituované deriváty cyklodextrinů o deriváty γ-CD. Laboratorní vedení studentek Bláhové a Bednářové, analýza NMR spekter, autor textu a obrázků.

Hobbs C, Řezanka P, Řezanka M (2020) Cyclodextrin-Functionalised Nanomaterials for Enantiomeric Recognition. ChemPlusChem 85(5):876–888. doi: 10.1002/cplu.202000187

Autor myšlenky review článku, vedení autorského kolektivu, autor části textu a obrázků.

Lukášek J, Hauzerová Š, Havlíčková K, Strnadová K, Mašek K, Stuchlík M, Stibor I, Jenčová V, Řezanka M (2019) Cyclodextrin-Polypyrrole Coatings of Scaffolds for Tissue Engineering. Polymers 11(3):459. doi: 10.3390/polym11030459

Autor myšlenky spojit pyrrolový polymer s cyklodextriny za účelem vylepšení vlastností tkáňových nosičů. Autor části textu a obrázků.

Lukášek J, Řezanková M, Stibor I, Řezanka M (2018) Synthesis of cyclodextrin–pyrrole conjugates possessing tuneable carbon linkers. J Incl Phenom Macrocycl Chem 92(3):339–346. doi: 10.1007/s10847-018-0854-5

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Navrátilová K, Řezanka P, Řezanka M, Sýkora D, Jindřich J, Král V (2013) The study of enantioselectivity of all regioisomers of mono-carboxymethyl-β-cyclodextrin used as chiral selectors in CE. J Sep Sci 36(7):1270–1274. doi: 10.1002/jssc.201201144

Autor myšlenky analyzovat vliv polohy karboxymethylového substiuentu na separačních schopnostech. Autor části textu, syntéza derivátů cyklodextrinů.

Řezanka M (2016) Monosubstituted Cyclodextrins as Precursors for Further Use. Eur J Org Chem 2016(32):5322–5334. doi: 10.1002/ejoc.201600693

Jediný autor. Přínos článku je jednak v zavedení nomenklatury rozdělení syntézy monosubstituovaných derivátů podle metod přípravy. Dále pak ve shrnutí problematiky syntézy a důkazu použitelnosti dříve formulované metody pro určování monosubstituovaných derivátů CD pouze z ^{1}H NMR spekter. Článek byl oceněn titulní stranou.

Řezanka M (2019) Synthesis of substituted cyclodextrins. Environ Chem Lett 17(1):49–63. doi: 10.1007/s10311-018-0779-7

Jediný autor. Přínos článku je v komplexním shrnutí syntézy derivátů cyklodextrinů. Poslední podobné shrnutí bylo provedeno v roce 1998. Článek byl v jednom období oceněn jako „Highly Cited Paper“ (Web of Science).

Řezanka M, Langton MJ, Beer PD (2015) Anion recognition in water by a rotaxane containing a secondary rim functionalised cyclodextrin stoppered axle. Chem Commun 51(21):4499–4502. doi: 10.1039/C5CC00171D

První syntéza rotaxanu tohoto typu, kdy je cyklodextrin připojen přes sekundární okraj. Syntéza rotaxanu a zkoumání jeho rozpoznávacích vlastností. Autor části textu a obrázků.

Řezanka M, Stibor I, Azizoglu M, Turgut Y, Pirinccioglu N (2016a) Enantiomeric recognition of amino acid ester salts by β-cyclodextrin derivatives: An experimental and computational study. Arkivoc 2016(v):249–267. doi: 10.3998/ark.5550190.p009.657

Syntéza cyklodextrinových amidů. Autor části textu a obrázků.

Řezanka P, Navrátilová K, Řezanka M, Král V, Sýkora D (2014a) Application of cyclodextrins in chiral capillary electrophoresis. Electrophoresis 35(19):2701–2721. doi: 10.1002/elps.201400145

Přínos review je v jedinečném spojení metod syntézy derivátů cyklodextrinů a jejich použitím v kapilární elektroforéze. Autor jedné ze tří hlavních kapitol review.

Řezanka P, Řezanková K, Sedláčková H, Mašek J, Rokosová L, Bláhová M, Řezanka M, Jindřich J, Sýkora D, Král V (2016b) Influence of substituent position and cavity size of the regioisomers of monocarboxymethyl-α-, β-,

and γ-cyclodextrins on the apparent stability constants of their complexes with both enantiomers of Tröger's base. J Sep Sci 39(5):980–985. doi: 10.1002/jssc.201500845

Autor myšlenky analyzovat vliv polohy karboxymethylového substiuentu na separačních schopnostech. Autor části textu a obrázků.

Řezanka P, Rokosová L, Řezanková K, Bláhová M, Řezanka M, Sýkora D, Jindřich J, Král V (2014b) The influence of the substituent position in monocarboxymethyl-γ-cyclodextrins on enantioselectivity in capillary electrophoresis. J Sep Sci 37(19):2779–2784. doi: 10.1002/jssc.201400604

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Shishkanova TV, Habanová N, Řezanka M, Broncová G, Fitl P, Vrňata M, Matějka P (2020) Molecular Recognition of Phenylalanine Enantiomers onto a Solid Surface Modified with Electropolymerized Pyrrole-β-Cyclodextrin Conjugate. Electroanalysis 32(4):767–774. doi: 10.1002/elan.201900615

První elektrodepozice selektivně kovalentně spojeného konjugátu cyklodextrinu a pyrrolu. Autor části textu a obrázků.

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Complete Sets of Monosubstituted γ-Cyclodextrins as Precursors for Further Synthesis

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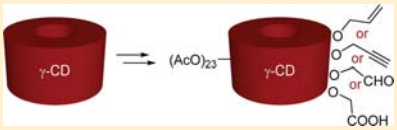
Supporting Information

ABSTRACT: Regioselective alkylation of γ-cyclodextrin with allyl or propargyl bromide, using optimized reaction conditions, followed by peracetylation of the remaining hydroxyl groups and separation of isomers resulted in the set of peracetylated 2^I-*O*-, 3^I-*O*- and 6^I-*O*-alkylated cyclodextrins in up to 19% yields. Ozonolysis or oxidative cleavage of peracetylated allyl derivatives resulted in a complete set of peracetylated 2^I-*O*-, 3^I-*O*-, and 6^I-*O*-formylmethyl or -carboxymethyl derivatives. All of these derivatives are useful precursors for further preparation of regioselectively monosubstituted derivatives of γ-cyclodextrin.



Cyclodextrins[1] (CDs) are macrocyclic compounds with cone-shaped cavity formed by α-1,4-linked D-glucopyranose units. The most widely used CDs are α-, β-, and γ-CD with 6, 7, or 8 glucose units respectively. CDs are very popular building blocks for supramolecular structures, and they are well-known to function as host molecules in aqueous solutions.[2] Chemically modified cyclodextrins allow numerous applications in separation methods,[3] as chemosensors,[4] as artificial enzymes,[5] and in the pharmaceutical industry.[6] CDs can be selectively modified by chemical derivatization. However, this selective modification remains a real challenge, originating from the statistic factors imposed by the large number of hydroxyl groups.[7] The precise structure is essential for the above-mentioned applications because properties of cyclodextrin derivatives highly depend on the position of substituents on the cyclodextrin skeleton.[8]

Preparation of monosubstituted cyclodextrin derivatives could be carried out in two steps: direct monosubstitution of cyclodextrin with a suitable functional group and subsequent transformation of this functional group already regioselectively attached to the cyclodextrin. The advantage of this approach is obvious: it is sufficient to examine reactivity of only a small number of alkylating agents for the preparation of a much larger number of derivatives. In this regard, ideal starting substrates should be selectively allylated, propargylated, formylmethylated, or carboxymethylated cyclodextrins in which the functional group could participate in further reactions, e.g., cross-metathesis,[9] copper(I)-catalyzed azide−alkyne cycloaddition (CuAAC),[10] Wittig reaction,[11] amide formation,[12] etc. Formylmethyl or carboxymethyl derivatives can be prepared from allyl derivatives by oxidative cleavage.[13,14]

As to the currently widely used CuAAC, it should be mentioned that it is mostly carried out with the azide group attached to the CD skeleton and the alkyne group to the other reagent. This approach, however, does not allow easy substitutions in all positions on CD.

Although the majority of the complete sets of 2^I-*O*-, 3^I-*O*-, and 6^I-*O*-allyl, -propargyl, -formylmethyl, and -carboxymethyl-α-CD[9,10,14−16] or -β-CD[10,13,15,17−22] have already been prepared, γ-CD remains almost untouched. Only a few of the mentioned derivatives of γ-CD are known, and the description of their synthesis and properties is often incomplete.

Although Tarver et al. described[23] 2^I-*O*-allyl-γ-CD, it was only briefly mentioned, and no yield or characterizations were given. 2^I-*O*-Propargyl-6^{I-VIII}-octakis-*O*-(*tert*-butyldimethylsilyl)-γ-CD was described by Aime et al.[24] 6^I-*O*-Allyl-γ-CD and per-*O*-acetyl-6^I-*O*-allyl-γ-CD were recently fully characterized by our group.[9] 6^I-*O*-Carboxymethyl-γ-CD was only mentioned in claims of some patents,[25−27] but no synthetic procedures were given.

Herein, we report on the preparation of complete sets of peracetylated 2^I-*O*-, 3^I-*O*-, and 6^I-*O*-allyl, -propargyl, -formylmethyl, and -carboxymehyl derivatives of γ-CD.

At the outset, complete sets of acetylated 2^I-*O*-, 3^I-*O*-, and 6^I-*O*-allyl- and -propargyl-γ-cyclodextrins were prepared (Scheme 1). Our attempts to separate mixtures of 2^I-*O*- and 3^I-*O*- allyl or propargyl isomers (**1a**, **1b** or **2a**, **2b**) were unsuccessful. Column chromatography on silica gel (various ratios of propan-1-ol/water/aqueous ammonia or acetonitrile/water/aqueous ammonia) or on reversed silica gel phase (various ratios of methanol/water) did not lead to separation of single isomers; thus, peracetylation of a mixture of mono-*O*-allyl or propargyl derivatives was carried out. Peracetylated mono-*O*-alkyl derivatives were separated from each other by column chromatography. Peracetylation of remaining hydroxyl groups has another two advantages: (i) signals in NMR spectra are easier to assign and (ii) acetylated hydroxyl groups do not undergo side reactions during the oxidation of the double bond (in the case of allyl derivatives).

Various conditions were used for the preparation of mono-*O*-allyl and propargyl derivatives (Table 1). These conditions were successfully used in our research group as the highest-

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Scheme 1. Preparation of Peracetylated 2^I-*O*-, 3^I-*O*-, and 6^I-*O*-Allyl- and -Propargyl-γ-cyclodextrins

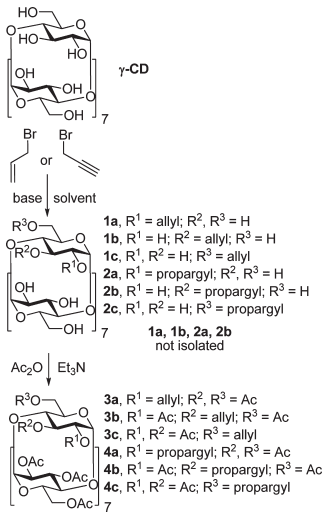


Table 1. Yields of Alkylation (after Acetylation)

			yields (%)		
entry	alk[a]	base, solvent	2^I-*O*-	3^I-*O*-	6^I-*O*-
1	all	LiH, LiI, DMSO	6	1	
2	pro	LiH, LiI, DMSO	4		
3	all	2 equiv of NaOH, H_2O/ACN	19	11	
4	pro	2 equiv of NaOH, H_2O/ACN	13	8	
5	all	30 equiv of NaOH, H_2O			18
6	pro	30 equiv of NaOH, H_2O			12

[a]Alkylation agent: allyl (all) or propargyl (pro) bromide.

yielding alkylation conditions for preparation of 2^I-*O*-, 3^I-*O*-, and 6^I-*O*-allyl or cinnamyl derivatives of α-CD.[14] Yields are stated after alkylation and acetylation so that 2^I-*O*-, 3^I-*O*-, and 6^I-*O*- (which were also isolated in nonacetylated form) derivatives could be compared.

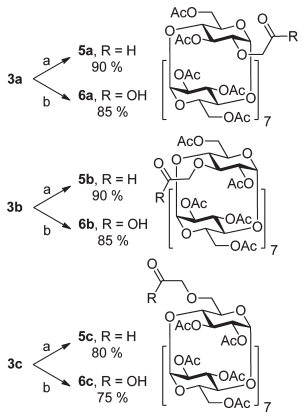
First, the procedure of Hanessian et al.[15] originally used for preparation 2^I-*O*-allyl-α-CD was tried out (entries 1 and 2, Table 1). Yields for this reaction on α- or β-CD usually vary from 24 to 29%.[14,15,28] Surprisingly, these reaction conditions did not lead, in the case of γ-CD, to the same yields. Only 6 and 4% yields were obtained.

Reaction conditions originally developed in our group for 3^I-*O*-cinnamylation of β-CD[13] and successfully applied in the synthesis 2^I-*O*- and 3^I-*O*-allyl derivatives of α-CD[14] were used herein for allylation and propargylation of γ-CD (entries 3 and 4, Table 1). Yields for allyl derivatives **3a** and **3b** (19 and 11%) were similar to those obtained for the corresponding derivatives of α-CD (17 and 10%).[14] In the case of propargyl derivatives **4a** and **4b**, the yields were slightly lower (13 and 8%). In light of these facts, it could be generalized that the reaction conditions consisting of 2 equiv of sodium hydroxide in a mixture of water and acetonitrile (ACN) are very good for preparation of otherwise hardly accessible 3^I-*O* derivatives of cyclodextrins and are the alternative choice when Hanessian's conditions for preparation of 2^I-*O* derivatives have failed.

Entry 5 of Table 1 shows, for comparison, the yield of 6^I-*O* derivative **3c** reported by our group.[9] The same conditions were used for the synthesis of propargyl derivative **4c**, which was obtained in 12% yield. The cooling of the reaction mixture used at the start of this reaction should prevent the eventual overheating and excessive hydrolysis of the alkylation reagent.

The 2^I-*O*-, 3^I-*O*- and 6^I-*O*-peracetylated allyl derivatives of γ-CD (**3a–c**) were used for the synthesis of sets of formylmethyl and carboxymethyl derivatives (Scheme 2). Aldehydes **5a–c**

Scheme 2. Preparation of Peracetylated 2^I-*O*-, 3^I-*O*-, and 6^I-*O*-Formylmethyl- and -Carboxymethyl-γ-cyclodextrins[a]



[a]Key: (a) (1) O_3, (2) Me_2S; (b) $NaIO_4$, $RuCl_3$.

were prepared by ozonolysis of **3a–c** followed by reduction with dimethyl sulfide in 80–90% yields. Carboxylic acids **6a–c** were prepared by oxidative cleavage of the same allyl derivatives by sodium periodate under catalysis of ruthenium(III) chloride in 75–85% yields.

All prepared compounds were characterized by ^1H and ^{13}C NMR spectra, and the recognition between 2^I-*O*, 3^I-*O*, and 6^I-*O* isomers was done with the aid of 2D NMR techniques such as COSY, HSQC, and HMBC. An example of the assignment of the propargyl derivative **4a** is shown in Figure 1. COSY allows

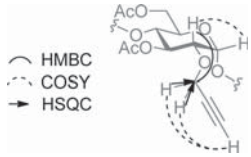


Figure 1. Example of the assignment of the propargyl derivative **4a**.

identification of hydrogen atoms of propargyl group. HMBC cross-peak of the methylene group of the propargyl group makes it possible to find hydrogen atom on cyclodextrin skeleton in the position where the propargyl group is attached. The position of this hydrogen can be deduced from COSY cross-peaks on the glucose unit.

Moreover, our previously suggested method[14] for distinguishing single isomers from each other using only simple ^{1}H NMR spectrum was confirmed on all derivatives **3**–**6**. All peracetylated 2^I-*O* derivatives have one characteristic H-2 NMR signal (dd) shifted out of the usual region for acetylated CDs, 3^I-*O* derivatives have the integral of H-3 atoms reduced to 7, and 6^I-*O* derivatives have one C-6 methylene signal shifted downfiled in APT spectra.

All prepared acetylated cyclodextrins can be easily deprotected by Zemplene deacetylation (e.g., for applications in aqueous environments) as described previously.[8,9,13,14]

EXPERIMENTAL SECTION

I. General Information. Procedures with the highest yields for preparation of monosubstituted allyl and propargyl derivatives are described. All solvents were used as obtained unless otherwise noted. γ-Cyclodextrin was purchased from WAKO Chemicals (Germany) and was used as obtained unless otherwise noted. Other reagents were obtained from common commercial sources. Ozonolysis was performed in a Ozone Tech Systems (Sweden) ACT-3000 apparatus. ^{1}H NMR spectra were recorded at 600.17 MHz and ^{13}C NMR at 150.04 MHz as solutions in deuterated solvents and referenced to residual solvent peak. Chemical shifts are given on δ scale, and coupling constants *J* are given in Hz. Numbering of atoms for NMR spectra transcription was done analogous to Figure 2. The glucose unit

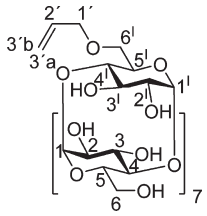


Figure 2. Numbering of atoms in cyclodextrin derivatives.

bearing an alkyl substituent is labeled with "I" where the assignment is unambiguous. All other glucose signals are numbered indiscriminately. The alkyl substituent is labeled with a prime. The assignment of the ^{1}H and ^{13}C signals was based on 2D NMR techniques (^{1}H–^{1}H COSY, HSQC, HMBC) and APT.

Infrared spectra were acquired in KBr by the DRIFT technique, and they are reported in wavenumbers (cm^{-1}). HRMS (MALDI-TOF) spectra were recorded with (*E*)-2-cyano-3-(4-hydroxyphenyl)acrylic acid as a matrix. Silica gel 60 (0.040–0.063 mm, Merck, Germany) was used for chromatography. TLC was performed on silica gel 60 F254-coated aluminum sheets (Merck, Germany). Spots were detected by spraying with 50% aqueous H_2SO_4 solution and carbonization with a heat-gun. All yields below 0.1 g were rounded down to the nearest multiple of 5%.

II. Synthesis of Cyclodextrin Derivatives. *6^I-O-Allyl-γ-cyclodextrin (**1c**).* Compound **1c** was prepared following a previously reported procedure.[9]

*6^I-O-Propargyl-γ-cyclodextrin (**2c**).* γ-Cyclodextrin (2 g, 1.54 mmol) was dissolved in solution of NaOH (3.2 g, 80 mmol) in water (10 mL). The solution was cooled to 0 °C, and propargyl bromide (0.245 mL, 80% solution in toluene, 2.32 mmol) was added dropwise with stirring. The mixture was then stirred for 2 days at room temperature. The reaction was monitored by TLC (propan-1-ol/water/ethyl acetate/aqueous ammonia, 6/3/1/1) and quenched with 50% H_2SO_4 to neutral pH. Products were precipitated with acetone (500 mL) and filtered off. 6^I-*O*-Propargyl-γ-cyclodextrin (**2c**) was separated by chromatography on a silica gel column (propan-1-ol/water/aqueous ammonia, 7/3/1). Na_2SO_4 (product of neutralization) was also removed in this step. Workup afforded 274 mg (13%) of the title compound as a white powder. Mp > 200 °C dec. $[\alpha]^{20}_D$ = +67 (*c* 1.0, H_2O). ^{1}H NMR (600 MHz, D_2O): δ = 5.17 (d, 1 H, H-1), 5.15–5.10 (m, 7 H, 7 × H-1), 4.31 (dd, *J* = 15.8, 1.9 Hz, 1 H, H-1′), 4.25 (dd, *J* = 15.8, 1.8 Hz, 1 H, H-1′), 4.04–3.56 (m, 48 H, 8 × H-2, 8 × H-3, 8 × H-4, 8 × H-5, 16 × H-6), 2.93 (s, 1 H, H-3′) ppm. ^{13}C NMR (150 MHz, D_2O): δ = 103.6–103.5 (6 × C-1), 103.3 (C-1), 103.0 (C-1), 82.4–71.7 (8 × C-2, 8 × C-3, 8 × C-4, 8 × C-5), 81.4 (C-2′), 78.1 (C-3′), 70.3 (C-6^I), 62.21–62.16 (7 × C-6), 60.2 (C-1′) ppm. IR (drift KBr): ν = 1108, 1078, 1027, 614 cm^{-1}. HRMS (MALDI): *m*/*z* calcd for $C_{51}H_{82}O_{40}Na$ [M + Na]$^+$ 1357.4280, found 1357.4275.

*Per-O-acetyl-2^I-O-allyl-γ-cyclodextrin (**3a**).* γ-Cyclodextrin (2 g, 1.54 mmol) was dissolved in a mixture of water (47 mL) and acetonitrile (16 mL). The mixture was cooled to 0 °C. Then solutions of allyl bromide (134 μL, 1.54 mmol) in acetonitrile (1 mL) and NaOH (124 mg, 3.08 mmol) in water (0.33 mL) were added. The mixture was stirred overnight at room temperature. The reaction was monitored by TLC (propan-1-ol/water/ethyl acetate/aqueous ammonia, 6/3/1/1). The reaction was quenched with 50% H_2SO_4 (to neutral pH). Products were precipitated with acetone (550 mL) and filtered off. Mono-*O*-allyl-γ-cyclodextrins were separated by chromatography on silica gel column (propan-1-ol/water/aqueous ammonia, 7/3/1). Na_2SO_4 (product of neutralization) was also removed in this step. The obtained mixture of mono-*O*-allyl-γ-cyclodextrins was then peracetylated. The suspension of mono-*O*-allyl-γ-cyclodextrins in acetic anhydride (4.5 mL) and triethylamine (4.5 mL) was stirred at 80 °C overnight. The reaction mixture was diluted with $CHCl_3$ and washed with 5% HCl, and the organic layer was evaporated in vacuo to give a brown residue that was purified by chromatography on silica gel ($CHCl_3$/MeOH, 70/1). Workup afforded as a main product 547 mg (19% overall yield) of the title compound as a white powder. Mp: 142–145 °C. $[\alpha]^{20}_D$ = +125 (*c* 1.0, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 5.83 (ddt, *J* = 10.8, 5.7, 5.7 Hz, 1 H, H-2′), 5.39–5.16 (m, 10 H, 2 × H-3′, 8 × H-3), 5.15 (d, *J* = 3.8 Hz, 1 H, H-1), 5.13 (d, *J* = 3.3 Hz, 3 H, 3 × H-1), 5.11 (d, *J* = 3.9 Hz, 1 H, H-1), 5.09 (d, *J* = 3.7 Hz, 1 H, H-1), 5.08 (d, *J* = 3.6 Hz, 1 H, H-1), 4.87 (d, *J* = 3.0 Hz, 1 H, H-1^I), 4.80–4.69 (m, 7 H, 7 × H-2), 4.62–3.95 (m, 26 H, 2 × H-1′, 8 × H-5, 16 × H-6), 3.75–3.64 (m, 8 H, 8 × H-4), 3.28 (dd, *J* = 9.9, 3.1 Hz, 1 H, H-2^I), 2.16–1.99 (m, 69 H, 23 × CH_3) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 171.0–169.0 (23 × C═O), 134.4 (C-2′), 117.8 (C-3′), 98.4 (C-1^I), 96.6 (C-1), 96.52 (C-1), 96.45 (C-1), 96.3 (C-1), 96.22 (C-1), 96.16 (C-1), 96.1 (C-1), 78.3–69.1 (7 × C-2, 8 × C-3, 8 × C-4, 8 × C-5), 78.2 (C-2^I), 72.3 (C-1′), 62.9–62.4 (8 × C-6), 21.0–20.8 (23 × CH_3) ppm. IR (drift KBr): ν = 1757, 1368, 1236, 1042 cm^{-1}. HRMS (MALDI): *m*/*z* calcd for $C_{97}H_{130}O_{63}Na$ [M + Na]$^+$ 2325.6866, found 2325.6861.

*Per-O-acetyl-3^I-O-allyl-γ-cyclodextrin (**3b**).* The procedure for preparation of compound **3a** also afforded as a product 319 mg (11% overall yield) of the title compound as a white powder. Mp: 145–148 °C. $[\alpha]^{20}_D$ = +135 (*c* 1.0, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 5.91 (m, 1 H, H-2′), 5.49 (t, *J* = 9.4 Hz, 1 H, H-3), 5.38–5.23 (m, 7 H, H-3′, 6 × H-3), 5.15 (d, *J* = 3.9 Hz, 1 H, H-1), 5.13 (d, *J* = 3.7 Hz, 2 H, 2 × H-1), 5.13–5.07 (m, 5 H, H-3′, 4 × H-1), 5.05 (d, *J* = 3.6 Hz, 1 H, H-1), 4.75–4.62 (m, 8 H, 8 × H-2), 4.56–3.91 (m, 25 H, 2 × H-1′, 7 × H-5, 16 × H-6), 3.80 (d, *J* = 9.3 Hz, 1 H, H-5^I), 3.76–3.60 (m, 8 H, H-3^I, 7 × H-4), 3.53 (t, *J* = 9.2 Hz, 1 H, H-4^I), 2.12–1.99 (m, 69 H, 23 × CH_3) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 170.9–169.3 (23 × C═O), 135.5 (C-2′), 116.0 (C-3′), 97.4 (C-1), 96.6 (C-1), 96.5 (C-1), 96.4 (C-1), 96.3 (2 × C-1), 96.2 (C-1), 96.0 (C-1), 80.5 (C-4^I), 77.3 (C-3^I), 77.2–69.3 (8 × C-2, 7 × C-3, 7 × C-4, 7 × C-5), 74.9 (C-1′), 70.2 (C-5^I), 62.6–62.2 (8 × C-6), 21.0–20.7 (23 × CH_3) ppm. IR (drift KBr): ν = 1751, 1371, 1242, 1048 cm^{-1}. HRMS (MALDI): *m*/*z* calcd for $C_{97}H_{130}O_{63}Na$ [M + Na]$^+$ 2325.6866, found 2325.6861.

*Per-O-acetyl-6^I-O-allyl-γ-cyclodextrin (**3c**).* Compound **3c** was prepared following a previously reported procedure.[9]

*Per-O-acetyl-2^I-O-propargyl-γ-cyclodextrin (**4a**).* γ-Cyclodextrin (1.15 g, 0.77 mmol) was dissolved in a mixture of water (24 mL) and acetonitrile (8 mL). The mixture was cooled to 0 °C. Then solutions of propargyl bromide (85 μL, 80% solution in toluene, 0.77 mmol) in acetonitrile (0.5 mL) and NaOH (56 mg, 1.4 mmol) in

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water (0.2 mL) were added. The mixture was stirred overnight at room temperature. The reaction was monitored by TLC (propan-1-ol/water/ethyl acetate/aqueous ammonia, 6/3/1/1). The reaction was quenched with 50% H_2SO_4 (to neutral pH). Products were precipitated with acetone (500 mL) and filtered off. Mono-*O*-propargyl-γ-cyclodextrins were separated by chromatography on silica gel column (propan-1-ol/water/aqueous ammonia, 7/3/1). Na_2SO_4 (product of neutralization) was also removed in this step. The obtained mixture of mono-*O*-propargyl-γ-cyclodextrins was then peracetylated. A suspension of mono-*O*-propargyl-γ-cyclodextrins in acetic anhydride (1.5 mL) and triethylamine (1.5 mL) was stirred at 80 °C overnight. The reaction mixture was diluted with $CHCl_3$ (100 mL) and washed with 5% HCl (2 × 100 mL), and the organic layer was evaporated in vacuo to give a brown residue that was purified by chromatography on silica gel ($CHCl_3$/MeOH, 100/1). Workup afforded as a main product 256 mg (13% overall yield) of the title compound as a white powder. Mp: 146−148 °C. $[\alpha]^{20}_D$ = +116 (*c* 1.0, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 5.38−5.25 (m, 8 H, 8 × H-3), 5.15 (d, *J* = 3.8 Hz, 1 H, H-1), 5.13 (d, *J* = 3.5 Hz, 2 H, 2 × H-1), 5.12 (d, *J* = 2.5 Hz, 1 H, H-1), 5.11 (d, *J* = 3.8 Hz, 1 H, H-1), 5.09 (d, *J* = 3.8 Hz, 1 H, H-1), 5.08 (d, *J* = 3.7 Hz, 1 H, H-1), 4.99 (d, *J* = 3.4 Hz, 1 H, H-1^I), 4.76−4.68 (m, 7 H, 7 × H-2), 4.58−3.94 (m, 26 H, 2 × H-1′, 8 × H-5, 16 × H-6), 3.74−3.62 (m, 8 H, 8 × H-4), 3.48 (dd, *J* = 10.0, 3.4 Hz, 1 H, H-2^I), 2.44 (t, *J* = 2.3 Hz, 1 H, H-3′), 2.12−1.99 (m, 69 H, 23 × CH_3) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 170.9−169.0 (23 × C═O), 98.5 (C-1^I), 96.7 (C-1), 96.5 (C-1), 96.4 (C-1), 96.3 (C-1), 96.21 (C-1), 96.19 (C-1), 96.1 (C-1), 79.5 (C-2′), 78.4−69.1 (8 × C-2, 8 × C-3, 8 × C-4, 8 × C-5), 75.2 (C-3′), 62.8 (C-6), 62.62 (C-6), 62.61 (C-6), 62.5 (2 × C-6), 62.42 (C-6), 62.39 (C-6), 62.37 (C-6), 58.4 (C-1′), 21.0−20.7 (23 × CH_3) ppm. IR (drift KBr): ν = 1748, 1368, 1239, 1042 cm^{-1}. HRMS (MALDI): *m*/*z* calcd for $C_{97}H_{128}O_{63}Na$ [M + Na]$^+$ 2323.6710, found 2323.6704.

*Per-O-acetyl-3^I-O-propargyl-γ-cyclodextrin (**4b**).* The procedure for preparation of compound **4a** also afforded as a product 154 mg (8% overall yield) of the title compound as a white powder. Mp: 143−146 °C. $[\alpha]^{20}_D$ = +126 (*c* 1.0, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 5.48 (t, *J* = 9.6 Hz, 1 H, H-3), 5.40−5.24 (m, 6 H, 6 × H-3), 5.16 (d, *J* = 3.5 Hz, 1 H, H-1), 5.16 (d, *J* = 3.9 Hz, 1 H, H-1), 5.15 (d, *J* = 4.1 Hz, 1 H, H-1), 5.12 (d, *J* = 3.6 Hz, 1 H, H-1), 5.10 (d, *J* = 3.6 Hz, 2 H, 2 × H-1), 5.09 (d, *J* = 3.5 Hz, 1 H, H-1), 5.05 (d, *J* = 3.5 Hz, 1 H, H-1), 4.76−4.61 (m, 8 H, 8 × H-2), 4.58−3.93 (m, 25 H, 2 × H-1′, 7 × H-5, 16 × H-6), 3.88 (t, *J* = 9.5 Hz, 1 H, H-3^I), 3.82−3.60 (m, 8 H, H-5^I, 7 × H-4), 3.52 (t, *J* = 9.3 Hz, 1 H, H-4^I), 2.63−2.60 (m, 1 H, H-3′), 2.17−2.00 (m, 69 H, 23 × CH_3) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 171.0−169.3 (23 × C═O), 97.64 (C-1), 96.61 (C-1), 96.4 (C-1), 96.3 (C-1, C-1^I), 96.2 (C-1), 96.10 (C-1), 96.08 (C-1), 80.7 (C-4^I), 80.3 (C-2′), 77.2−69.3 (7 × C-2, 7 × C-3, 7 × C-4, 7 × C-5), 76.9 (C-3^I), 75.1 (C-3′), 71.7 (C-2), 70.2 (C-5^I), 62.6 (C-6), 62.53 (C-6), 62.51 (C-6), 62.48 (C-6), 62.4 (C-6^I), 62.28 (C-6), 62.26 (C-6), 62.2 (C-6), 61.2 (C-1′), 21.2−20.7 (23 × CH_3) ppm. IR (drift KBr): ν = 1751, 1368, 1242, 1045 cm^{-1}. HRMS (MALDI): *m*/*z* calcd for $C_{97}H_{128}O_{63}Na$ [M + Na]$^+$ 2323.6710, found 2323.6704.

*Per-O-acetyl-6^I-O-propargyl-γ-cyclodextrin (**4c**).* A suspension of propargyl derivative **2c** (274 mg, 0.21 mmol) in acetic anhydride (5 mL, 53 mmol) and triethylamine (5 mL, 36 mmol) was stirred at 80 °C overnight. The reaction mixture was diluted with $CHCl_3$ and washed with 5% HCl, and the organic layer was evaporated in vacuo to give a brown residue that was purified by chromatography on silica gel ($CHCl_3$/MeOH, 100/1). Workup afforded 448 mg (95%) of the title compound as a white powder. Mp: 147−149 °C. $[\alpha]^{20}_D$ = +135 (*c* 1.0, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 5.38−5.27 (m, 8 H, 8 × H-3), 5.19 (d, *J* = 3.8 Hz, 1 H, H-1), 5.14 (d, *J* = 3.9 Hz, 1 H, H-1), 5.12 (d, *J* = 3.8 Hz, 1 H, H-1), 5.12 (d, *J* = 3.8 Hz, 1 H, H-1), 5.11 (d, *J* = 3.7 Hz, 2 H, 2 × H-1), 5.09 (d, *J* = 3.9 Hz, 1 H, H-1), 5.08 (d, *J* = 3.8 Hz, 1 H, H-1), 4.76−4.69 (m, 8 H, 8 × H-2), 4.59−3.63 (m, 32 H, 8 × H-4, 8 × H-5, 16 × H-6), 4.20 (dd, *J* = 15.9, 2.3 Hz, 1 H, H-1′), 4.16 (dd, *J* = 15.9, 2.3 Hz, 1 H, H-1′), 2.48 (t, *J* = 2.3 Hz, 1 H, H-3′), 2.13−2.01 (m, 69 H, 23 × CH_3) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 170.7−169.3 (23 × C═O), 96.43 (C-1), 96.36 (C-1), 96.33 (C-1), 96.25 (C-1), 96.20 (2 × C-1), 96.18 (C-1), 96.16 (C-1), 79.0 (C-2′), 77.2−69.5 (8 × C-2, 8 × C-3, 8 × C-4, 8 × C-5), 75.4 (C-3′), 67.5 (C-6^I), 62.6 (2 × C-6), 62.53 (C-6), 62.51 (C-6), 62.43 (2 × C-6), 62.40 (C-6), 58.7 (C-1′), 20.8−20.7 (23 × CH_3) ppm. IR (drift KBr): ν = 1748, 1371, 1239, 1042 cm^{-1}. HRMS (MALDI): *m*/*z* calcd for $C_{97}H_{128}O_{63}Na$ [M + Na]$^+$ 2323.6710, found 2323.6704.

General Procedure for Ozonolysis of Peracetylated Mono-O-allyl γ-Cyclodextrins. Ozone was bubbled through a solution of peracetylated mono-*O*-allyl γ-cyclodextrin (100 mg, 43 μmol for **3a** and **3b**; 80 mg, 35 μmol for **3c**) in a mixture of MeOH (1 mL for **3a** and **3b**; 0.85 mL for **3c**) and $CHCl_3$ (1 mL for **3a** and **3b**; 0.85 mL for **3c**) at −78 °C for 10 min. The reaction was quenched by addition of dimethyl sulfide (0.5 mL for **3a** and **3b**; 0.4 mL for **3c**). The reaction mixture was evaporated after reaching laboratory temperature. Purification by chromatography on silica gel ($CHCl_3$/MeOH, 50/1) afforded the desired product.

*Per-O-acetyl-2^I-O-formylmethyl-γ-cyclodextrin (**5a**).* The reaction was run with compound **3a**. Workup afforded 92 mg (90%) of the title compound as a white powder. Mp: 143−146 °C. $[\alpha]^{20}_D$ = +119 (*c* 1.0, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 5.37−5.27 (m, 8 H, 8 × H-3), 5.15 (d, *J* = 3.7 Hz, 1 H, H-1^I), 5.14 (d, *J* = 4.0 Hz, 1 H, H-1), 5.13 (d, *J* = 3.5 Hz, 2 H, 2 × H-1), 5.11−5.07 (m, 4 H, 4 × H-1), 4.76−4.68 (m, 7 H, 7 × H-2), 4.55−3.64 (m, 31 H, 7 × H-4, 8 × H-5, 16 × H-6), 4.21 (s, 2 H, 2 × H-1′), 3.62 (t, *J* = 9.5 Hz, 1 H, H-4^I), 3.30 (dd, *J* = 9.8, 3.2 Hz, 1 H, H-2^I), 2.13−1.93 (m, 69 H, 23 × CH_3) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 198.8 (C-2′), 170.8−169.2 (23 × C═O), 98.2 (C-1), 96.6 (C-1), 96.4 (C-1), 96.32 (C-1), 96.26 (C-1), 96.21 (2 × C-1), 96.15 (C-1), 79.6 (C-2^I), 77.5−69.5 (7 × C-2, 7 × C-3, 8 × C-4, 8 × C-5), 76.6 (C-1′), 72.1 (C-3^I), 62.7−62.4 (8 × C-6), 21.0−20.7 (23 × CH_3) ppm. IR (drift KBr): ν = 1748, 1371, 1239, 1039 cm^{-1}. HRMS (MALDI): *m*/*z* calcd for $C_{96}H_{128}O_{64}Na$ [M + Na]$^+$ 2327.6659, found 2327.6654.

*Per-O-acetyl-3^I-O-formylmethyl-γ-cyclodextrin (**5b**).* The reaction was run with compound **3b**. Workup afforded 90 mg (90%) of the title compound as a white powder. Mp: 145−148 °C. $[\alpha]^{20}_D$ = +131 (*c* 1.0, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 9.68 (s, 1 H, H-2′), 5.48 (t, *J* = 9.6 Hz, 1 H, H-3), 5.39−5.26 (m, 6 H, 6 × H-3), 5.18 (d, *J* = 3.8 Hz, 1 H, H-1^I), 5.16 (d, *J* = 3.9 Hz, 1 H, H-1), 5.15 (d, *J* = 3.9 Hz, 1 H, H-1), 5.13 (d, *J* = 3.3 Hz, 1 H, H-1), 5.12 (d, *J* = 3.8 Hz, 1 H, H-1), 5.11 (d, *J* = 3.6 Hz, 1 H, H-1), 5.10 (d, *J* = 3.7 Hz, 1 H, H-1), 5.07 (d, *J* = 3.7 Hz, 1 H, H-1), 4.79 (d, *J* = 18.4 Hz, 1 H, H-1′), 4.75−4.62 (m, 8 H, 8 × H-2), 4.55−3.91 (m, 24 H, 8 × H-5, 16 × H-6), 4.33 (d, *J* = 18.4 Hz, 1 H, H-1′), 3.80−3.58 (m, 9 H, H-3^I, 8 × H-4), 2.13−1.98 (m, 69 H, 23 × CH_3) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 199.3 (C-2′), 170.7−169.4 (23 × C═O), 97.5 (C-1), 96.7 (C-1), 96.5 (C-1), 96.28 (C-1), 96.25 (C-1), 96.2 (C-1), 96.1 (C-1), 95.9 (C-1), 80.3−69.3 (8 × C-2, 7 × C-3, 8 × C-4, 8 × C-5), 79.8 (C-1′), 79.2 (C-3^I), 62.5−62.2 (8 × C-6), 21.0−20.7 (23 × CH_3) ppm. IR (drift KBr): ν = 1754, 1374, 1236, 1042 cm^{-1}. HRMS (MALDI): *m*/*z* calcd for $C_{96}H_{128}O_{64}Na$ [M + Na]$^+$ 2327.6659, found 2327.6654.

*Per-O-acetyl-6^I-O-formylmethyl-γ-cyclodextrin (**5c**).* The reaction was run with compound **3c**. Workup afforded 67 mg (80%) of the title compound as a white powder. Mp: 144−147 °C. $[\alpha]^{20}_D$ = +121 (*c* 1.0, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 5.38−5.28 (m, 8 H, 8 × H-3), 5.18 (d, *J* = 3.7 Hz, 1 H, H-1), 5.16 (d, *J* = 3.8 Hz, 2 H, 2 × H-1), 5.13−5.11 (m, 2 H, 2 × H-1), 5.11 (d, *J* = 3.9 Hz, 1 H, H-1), 5.10 (d, *J* = 3.9 Hz, 1 H, H-1), 5.09 (d, *J* = 3.8 Hz, 1 H, H-1), 4.75−4.68 (m, 8 H, 8 × H-2), 4.59−3.61 (m, 34 H, 2 × H-1′, 8 × H-4, 8 × H-5, 16 × H-6), 2.11−2.02 (m, 69 H, 23 × CH_3) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 199.7 (C-2′), 170.7−169.3 (23 × C═O), 96.5 (2 × C-1), 96.4 (C-1), 96.34 (C-1), 96.32 (C-1), 96.23 (C-1), 96.20 (2 × C-1), 77.2−69.6 (8 × C-2, 8 × C-3, 8 × C-4, 8 × C-5), 77.1 (C-1′), 69.7 (C-6^I), 62.7−62.4 (7 × C-6), 20.8−20.7 (23 × CH_3) ppm. IR (drift KBr): ν = 1748, 1371, 1236, 1045 cm^{-1}. HRMS (MALDI): *m*/*z* calcd for $C_{96}H_{128}O_{64}Na$ [M + Na]$^+$ 2327.6659, found 2327.6654.

General Procedure for Oxidative Cleavage of Peracetylated Mono-O-allyl γ-Cyclodextrins. Peracetylated mono-*O*-allyl γ-cyclodextrin (100 mg, 43 μmol for **3a** and **3b**; 80 mg, 35 μmol for **3c**) was dissolved in a mixture of acetonitrile (0.9 mL for **3a** and **3b**; 0.7 mL for **3c**) and a saturated solution of sodium periodate (0.9 mL for **3a** and **3b**; 0.7 mL for **3c**). After addition of ruthenium(III) chloride (11 μL,

5% solution in water, 3 μmol for **3a** and **3b**; 9 μL, 5% solution in water, 2 μmol for **3c**), the reaction mixture was stirred 1 h at room temperature. The reaction was monitored by TLC ($CHCl_3$/MeOH, 20/1). The reaction mixture was extracted with chloroform (5 mL) three times, and the collected organic layers were washed with $Na_2S_2O_5$ (5 mL, 2% solution in water) twice, dried with magnesium sulfate, and evaporated. Purification by chromatography on silica gel ($CHCl_3$/MeOH, gradient from 20/1 to 5/1) afforded the desired product.

*Per-O-acetyl-2^I-O-carboxymethyl-γ-cyclodextrin (**6a**).* The reaction was run with the compound **3a**. Workup afforded 87 mg (85%) of the title compound as a white powder: mp 143−146 °C. $[\alpha]^{20}_D$ +118 (*c* 1.0, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 5.37−5.28 (m, 8 H, 8 × H-3), 5.14−5.08 (m, 8 H, 8 × H-1), 4.74−4.68 (m, 7 H, 7 × H-2), 4.52−3.77 (m, 24 H, 8 × H-5, 16 × H-6), 4.17 (s, 2 H, 2 × H-1′), 3.72−3.64 (m, 7 H, 7 × H-4), 3.62 (t, *J* = 8.9 Hz, 1 H, H-4^I), 3.43 (dd, *J* = 9.3, 3.2 Hz, 1 H, H-2^I), 2.13−1.96 (m, 69 H, 23 × CH_3) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 171.4 (C-2′), 170.8−169.4 (23 × C═O), 98.1 (C-1), 96.6 (C-1), 96.34 (C-1), 96.30 (C-1), 96.23 (C-1), 96.15 (3 × C-1), 79.0 (C-2^I), 77.2−69.6 (7 × C-2, 7 × C-3, 8 × C-4, 8 × C-5), 71.9 (C-3^I), 68.1 (C-1′), 62.7−62.4 (8 × C-6), 21.0−20.3 (23 × CH_3) ppm. IR (drift KBr): ν = 1751, 1368, 1242, 1042 cm^{-1}. HRMS (MALDI): *m*/*z* calcd for $C_{96}H_{128}O_{65}Na$ $[M + Na]^+$ 2343.6608, found 2343.6603.

*Per-O-acetyl-3^I-O-carboxymethyl-γ-cyclodextrin (**6b**).* The reaction was run with the compound **3b**. Workup afforded 87 mg (85%) of the title compound as a white powder. Mp: 142−145 °C. $[\alpha]^{20}_D$ = +128 (*c* 1.0, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 5.51 (t, *J* = 9.5 Hz, 1 H, H-3), 5.39−5.26 (m, 6 H, 6 × H-3), 5.20 (d, *J* = 3.8 Hz, 1 H, H-1^I), 5.16 (d, *J* = 3.9 Hz, 1 H, H-1), 5.15 (d, *J* = 4.4 Hz, 1 H, H-1), 5.14 (d, *J* = 4.0 Hz, 1 H, H-1), 5.12 (d, *J* = 4.1 Hz, 1 H, H-1), 5.10 (d, *J* = 4.0 Hz, 1 H, H-1), 5.10 (d, *J* = 3.6 Hz, 1 H, H-1), 5.08 (d, *J* = 3.6 Hz, 1 H, H-1), 4.75−4.62 (m, 9 H, H-1′, 8 × H-2), 4.57−3.62 (m, 33 H, H-1′, H-3^I, 7 × H-4, 8 × H-5, 16 × H-6), 3.60 (t, *J* = 9.4 Hz, 1 H, H-4^I), 2.16−1.99 (m, 69 H, 23 × CH_3) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 171.0−169.4 (C-2′, 23 × C═O), 97.2 (C-1), 96.5 (C-1), 96.4 (C-1), 96.2 (3 × C-1), 96.0 (C-1), 95.9 (C-1), 80.0 (C-4^I), 78.8 (C-3^I), 77.2−69.2 (8 × C-2, 7 × C-3, 7 × C-4, 8 × C-5), 70.9 (C-1′), 62.5−62.2 (8 × C-6), 21.0−20.6 (23 × CH_3) ppm. IR (drift KBr): ν = 1754, 1368, 1245, 1048 cm^{-1}. HRMS (MALDI): *m*/*z* calcd for $C_{96}H_{128}O_{65}Na$ $[M + Na]^+$ 2343.6608, found 2343.6603.

*Per-O-acetyl-6^I-O-carboxymethyl-γ-cyclodextrin (**6c**).* The reaction was run with compound **3c**. Workup afforded 64 mg (75%) of the title compound as a white powder. Mp: 140−143 °C. $[\alpha]^{20}_D$ = +125 (*c* 1.0, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 5.42−5.26 (m, 8 H, 8 × H-3), 5.25 (d, *J* = 3.9 Hz, 1 H, H-1), 5.17 (d, *J* = 3.9 Hz, 1 H, H-1), 5.14 (d, *J* = 3.9 Hz, 1 H, H-1), 5.12 (d, *J* = 3.7 Hz, 1 H, H-1), 5.09 (d, *J* = 4.4 Hz, 1 H, H-1), 5.08 (d, *J* = 4.4 Hz, 1 H, H-1), 5.07 (d, *J* = 4.1 Hz, 1 H, H-1), 5.06 (d, *J* = 3.7 Hz, 1 H, H-1), 4.76−4.64 (m, 10 H, 8 × H-2, 2 × H-6), 4.55−3.60 (m, 32 H, 2 × H-1′, 8 × H-4, 8 × H-5, 14 × H-6), 2.14−1.97 (m, 69 H, 23 × CH_3) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 171.5 (C-2′), 171.0−169.3 (23 × C═O), 96.5 (C-1), 96.4−96.3 (5 × C-1), 96.2 (C-1), 95.6 (C-1), 77.2−69.4 (8 × C-2, 8 × C-3, 8 × C-4, 8 × C-5), 70.1 (C-6^I), 68.8 (C-1′), 62.7 (2 × C-6), 62.64 (C-6), 62.58 (C-6), 62.5 (C-6), 62.3 (2 × C-6), 20.9−20.7 (23 × CH_3) ppm. IR (drift KBr): ν = 1751, 1368, 1236, 1042 cm^{-1}. HRMS (MALDI): *m*/*z* calcd for $C_{96}H_{128}O_{65}Na$ $[M + Na]^+$ 2343.6608, found 2343.6603.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Copies of NMR spectra (including 2D NMR spectra) of all cyclodextrin derivatives. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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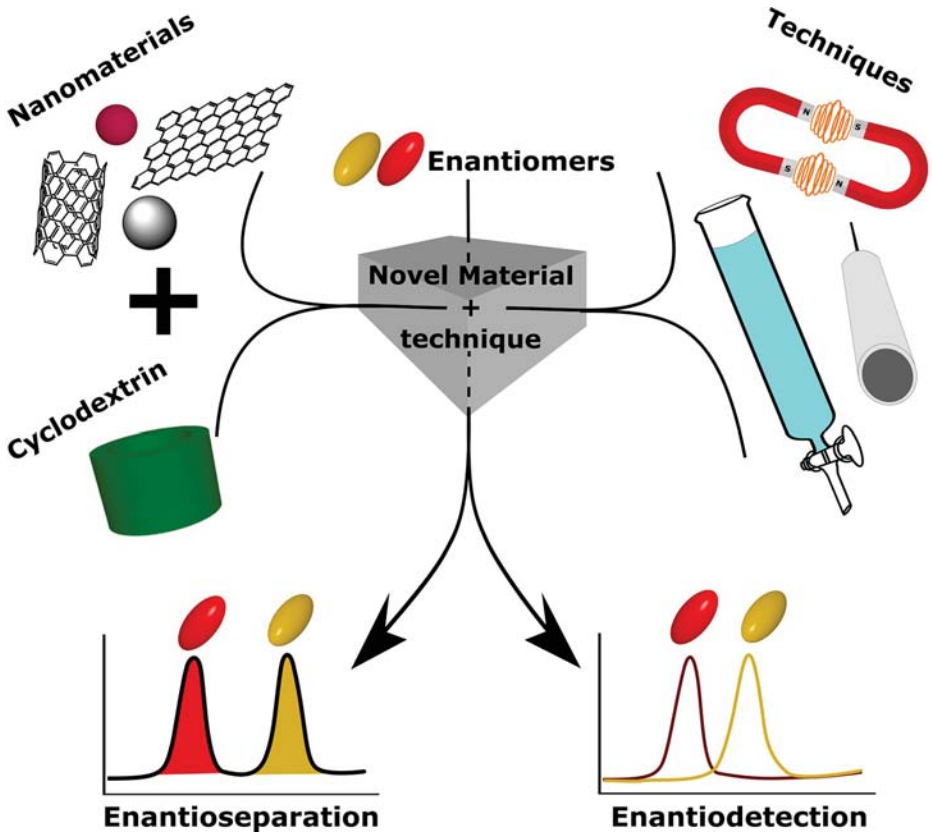
Special Collection

Cyclodextrin-Functionalised Nanomaterials for Enantiomeric Recognition

Christopher Hobbs,[a] Pavel Řezanka,[b] and Michal Řezanka*[a]



Cyclodextrins, which are glucose-based cyclic oligosaccharides, are materials that can act inherently as chiral selectors, with many reports of the application of cyclodextrins in enantioseparation. However, many studies have encountered the problem of insufficient enantioselective performance of the chiral selector. One of the main reasons is due to low surface concertation's, whereby interaction between the chiral selector and analyte usually occurs at a surface. Thus, scientists have been trying for the last two decades to overcome this problem, with the incorporation of nanomaterials being promising as they possess a large surface area which allows for the accommodation of a higher concentration of the chiral selectors. Herein, we outline nanomaterial-cyclodextrin conjugates that work in tandem to achieve or enhance enantioselectivity through various methods such as chromatography, adsorption, and removal using magnetic nanoparticles, or enantiorecognition using electrochemical techniques.

1. Introduction

Chirality, derived from the Greek χειρ (*kheir*, translated in English to "hand") is a vital attribute of asymmetric objects. For an object to be chiral, the object must not contain any improper axis of rotation (S_n). In the particular view-point of chemistry and biology, chiral molecules are crucial since many important processes occurring within the human body, beginning from an embryo, are chiral.[1] Therefore, recognition of chirality in studied molecules is critical and plays an increasingly important role in the current world.

Chiral recognition is not an easy task due to the fact that chiral molecules have identical chemical and physical properties in achiral environments. In addition, when a chiral selector is used for chiral recognition, a minimum three-point interaction should be present (Figure 1), first formulated by Ogston,[2] with at least one of these interactions being stereochemically dependent.

The importance of chiral recognition can be exemplified by the drug thalidomide,[3] where one of the enantiomers has a therapeutic effect, while the other enantiomer is teratogenic. Thus, in pharmacology, knowledge of the effects of individual enantiomers is crucial.[4] The enantioselective processes are of great significance also in biology[5] as well as chemistry, whether in asymmetric synthesis using chiral catalysts,[6] separation techniques[7] or electrochemistry.[8]

According to SciFinder Scholar,[9] articles dealing with chiral selectors in the last decade are utilising the following: cyclodextrins (36%), chiral ionic liquids (18%), polysaccharides (16%) and compounds used for chiral ligand-exchange (10%). Chiral materials can be used, as example, sensors (27%), stationary phases (25%), and catalysts (20%), whilst nanomaterials comprise 26% of them overall.

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1.1. Nanomaterials in chiral recognition

Nanomaterials are objects whereby at least one dimension lies within the nanometre range. Their biggest advantage in terms of chiral recognition is their high surface to volume ratio. Further, the surface is important for chiral recognition because it is the surface where the above-mentioned three-point interaction occurs. Therefore, the functional surface of a nanomaterial possess plenty of opportunities for applications in chiral recognition or discrimination.[10,11]

Chiral nanomaterials could be divided into two main types, either intrinsically or modified achiral nanomaterials. The difference between intrinsically chiral and modified achiral nanomaterials arises from where chirality originates from, whereby in the case of modified achiral nanomaterials, the chirality feature is introduced by incorporation of chiral structures (molecules). Intrinsically chiral nanomaterials could either be chiral by nature (e.g. carbon nanotubes)[12] or could be prepared by chiral template-driven process (e.g. chiral Au nanoclusters).[13] To achieve a desired chiral recognition, achiral nanomaterials are often functionalized with small chiral molecules like amino acids,[14] larger molecules (e.g. cyclodextrins[15]) or achiral nanoparticles (NPs) are interconnected to form chiral superstructures.[16] The functionalisation is usually performed on the surface, rather on the surface and inside of the nanomaterial.

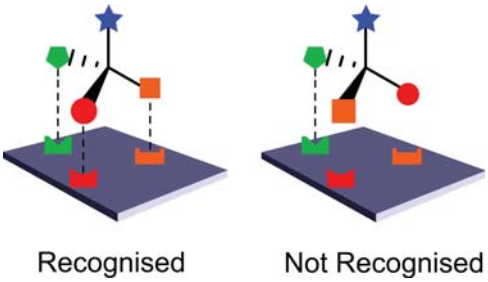

Figure 1. Three-point interaction model of chiral compounds.

1.2. Cyclodextrins in chiral recognition

Cyclodextrins (CDs) are naturally occurring glucose-based cyclic oligosaccharides. The most common CDs (α-, β- and γ-CD) differ in the number of α-D-glucopyranose units that are linked through an α(1→4) glycosidic bonds (Figure 2). CDs were firstly described by A. Villiers[17] in 1891, with CDs becoming used for chiral recognition[18] from 1977. A predominant feature, to which CDs are often exploited for in chiral recognition, is their chiral lipophilic cavity. This cavity allows for the association with various non-polar compounds.[19] Together with their other properties (e.g. ease of modification, nontoxicity, biodegradability, solubility in water and other polar solvents, etc.)[20] and their relatively low price, it makes them the one of the most widely used chiral selectors.

Many different classes of molecules are known to stereospecifically interact with CDs, including various drugs,[21,22] psychoactive substances,[23] amino acids,[24,25] alkaloids,[26,27] herbicides[28] and even chiral Au nanoclusters.[29] Most of these chemical compounds contain an aromatic ring in their molecule hence they can form stable complexes with β-CD due to its suitable cavity size.[19] This fact along with the cheap price (β-CD is several times cheaper than α- or γ-CD) are the main reasons for its prominence (70% of the publications focused on cyclodextrins according to SciFinder Scholar).[9] α-CD is used in 17% and γ-CD in 13% cases.

Apart from native (unmodified) CDs, their derivatives are also used in chiral recognition. Derivatization mostly occurs on the primary hydroxyl groups present on the narrower rim and the secondary hydroxyl groups present on the wider rim,[30] with the position of substitution often playing a prominent role in the chiral recognition process.[31] The most common groups which are present on CD derivatives are methyl, hydroxypropyl, and benzoyl (being neutral groups), as well as carboxylic acid, and sulfonic acid (acidic groups), and also amino (basic).[32] Amongst these derivatives, the sulphated CD, which was first utilized in separation techniques by Wu and Stalcup[33] in 1995, is capable to enantioseparate the largest number of different analytes.[34] Multiple forms or derivatives of CDs can also be used in a mixture to enhance chiral recognition,[35] or conversely, CDs can also be utilised in a mixture with other selectors for enhancement.[21] Somewhat surprisingly, even if the other selector used in conjunction with CD is non-chiral, such as ionic liquids, it still results in enhanced chiral recognition.[36]

Dr. Michal Řezanka finished his PhD focusing on organic chemistry at Charles University in Prague, Czech Republic in 2012, and in the same year joined as a postdoc at the Technical University of Liberec. He then carried out an internship in Paul D. Beer's group at the University of Oxford (2013/2014). Dr. Řezanka is interested in the synthesis of cyclodextrin derivatives and their uses in the wide range of chemistry-based applications, predominantly in the functionalisation of nanomaterials.

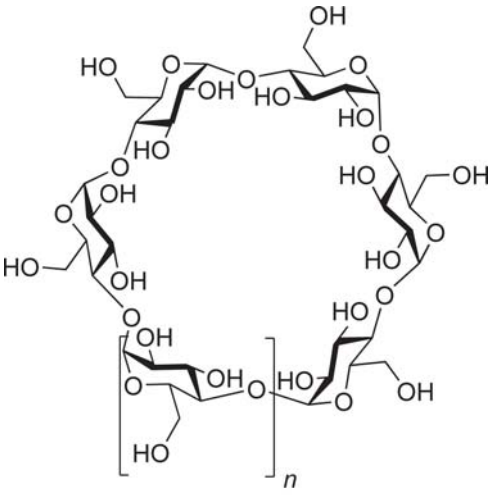

Figure 2. Structure of the most commonly used CDs.

CDs and their derivatives (mainly β-CD and its derivatives) are used in several chiral recognition techniques. For example in separation techniques as chiral selector in high performance liquid chromatography (HPLC; in mobile phase[37] or stationary phase[38]), gas chromatography (GC),[39] capillary electrophoresis (CE),[32] and supercritical fluid chromatography (SFC).[40]

The use of CDs in electrochemical methods is equally important.[41] They are exploited in differential pulse voltammetry,[42] square wave voltammetry,[43] cyclic voltammetry,[44] and linear sweep voltammetry.[45] Further, CDs are for example used in asymmetric synthesis,[46] as a part of enantioselective sensors[47] and also in nuclear magnetic resonance (NMR) as chiral nuclear magnetic resonance shift reagents.[48]

CDs can provide multiple types of interactions to molecules, such as hydrogen bonds, hydrophobic interactions, van der Waals interactions, and charge-transfer interactions[49] (and additional forms in cases with CD derivatives). A common approach for understanding the principle of chiral recognition involves the combination of CE and NMR techniques,[50] often supported by *in silico* quantum calculation using density functional theory[51] and with the aid of molecular dynamics.[52] Kano and Nishiyabu proposed that CDs adopt asymmetrically twisted structures in aqueous solution which results in specific chiral recognition.[53] They found that native and negatively

charged CDs are selective for *R* enantiomers of α-amino acids whereas positively charged CDs exhibit selectivity for *S* enantiomers and further, that CDs are not only able to recognize chirality based on the chiral centre, but also recognition based on axial chirality (binaphthyl derivatives) and helicity. The above-mentioned distortion of CDs was confirmed by Bikádi et al. by molecular dynamics calculation,[52] by measuring distances between the opposite glycosidic oxygen atoms of α-CD in the presence of selected analytes. On the other hand, some analytes do not allow for large scale distortion of the CD ring. This phenomenon was explained by the different orientation of various analytes within the cavity of CD. Analytes preferably form hydrogen bonds with the –OH groups of CDs if it is possible, with the example of tyrosine interacting with the –OH groups of CD and not interacting with the inner cavity. In comparison to tyrosine, phenylalanine interacts with the inner cavity of CD, forming an inner complex with α-CD.

2. Cyclodextrin functionalised nanomaterials in chiral recognition

2.1. Chromatography for enantioseparation

Enantiomeric separation using a racemic approach has been ever increasingly the focus of much research. This is largely due to the importance of the well-known differences incurred from chirality to certain actions or outcomes. As such, various techniques have been exploited and developed over the past few decades to achieve enantio-separation: gas chromatography, liquid chromatography (LC), reversed phase liquid chromatography (RP-LC), HPLC, capillary electrochromatography (CEC), thin layer chromatography (TLC), micellar chromatography, SFC, etc..[14] Each of these techniques exploits various materials with unique properties and mechanisms to achieve the desired enantioseparation. Research has progressed to linking the enantioselective properties of CDs to the particularly high surface area of NPs to be used within chromatography, as depicted in Figure 3, with the procedure resulting in enhanced enantioseparation (Table 1). Furthermore, the utilised NPs not only allow for greater surface area to achieve the desired enhancements, but the surface can also be readily modified which allows for unique functionality and conjugation into various systems.

Highlighted in Table 1, various NPs have been used in tandem with β-CD to form unique nanomaterials which were used in chromatography for the effective enantioseparation of numerous analytes. β-CD, as the chiral selector, interacts with one enantiomer preferentially via a combination of hydrophobic interactions (often through the phenyl moiety of the analyte), hydrogen bonding, and Van der Waals forces, which in combination with the employed chromatography technique and NPs, translates to the effective enantioseparation of a racemic solution.

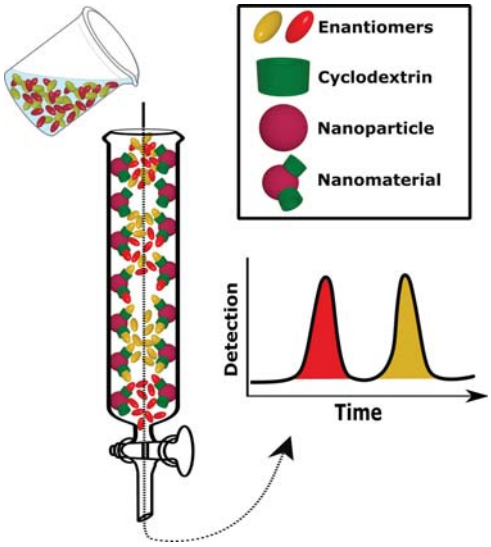

Figure 3. Schematic representation of a typical column chromatography set-up utilising high surface-to-volume nanoparticles linked to cyclodextrin for enhanced separation of enantiomers (not to scale).

Comparative experiments in CD-promoted chiral separation carried out using nanomaterials revealed that the chosen nanomaterial plays a key role in improving the resulting resolution.[54,55] For such a direct comparison, Zhou et al.[54] used open-tubular capillary electrochromatography (OT-CEC) with a stationary phase comprised of β-CD conjugated to polydopamine Au NPs. The resolution (R) for the tested amino acid and drug enantiomers increased when Au NPs were employed, with the drug Fexofenadine showing the greatest improvement for R, increasing from 0.35 to 1.43. The amino acids Phenylalanine, Tryptophan, and Histidine also displayed a significant increase in R with the utilization of Au NPs, from 1.87, 1.27, and 1.06 to 2.76, 2.20, and 2.19 respectively, hence portraying the worth of incorporation of NPs for chiral separation. Further, a comparative study of the performance in HPLC using graphene quantum dots (QDs) functionalized β-CD and unmodified β-CD provides further evidence for the positive influence NPs can provide. The study revealed that the incorporation of graphene QDs resulted in effective enantioseparation, whereby the same system without graphene QDs resulted in no enantioseparation.[55] The authors analysed 7 different chiral compounds, with R increasing in each case when the QDs were incorporated. Most notably was for the compounds benzoin and benzoin methyl ether, increasing from 0.00 and 0.10 to 0.50 and 0.89 respectively. Moreover, the authors suggest that, with the aid of molecular simulations, graphene QDs provides additional non-covalent interactions for when β-CD interact with analytes. This translates into a better performance of the nanomaterial used within the chiral stationary phase.

Table 1. Nanoparticle-cyclodextrin nanomaterials used for enantioseparation through chromatography. All listed nanomaterials incorporate β-CD as the enantioselective entity.

Nanomaterial	Analyte(s)	Technique	Reference
Au NPs	Tramadol hydrochloride, zopiclone, and two tetralone derivatives	OT-CEC	[61]
Au NPs	Brompheniramine, chlorphenamine maleate, pheniramine, and zopiclone	OT-CEC	[67]
Au NPs	Bifonazole, fexofenadine, lansoprazole, and omeprazole	OT-CEC	[62]
Au NPs	Zopiclone, chlorphenamine maleate, brompheniramine maleate, dioxopromethazine hydrochloride, carvediol, homatropine hydrobromide, homatropine methylbromide, venlafaxine, sibutramine hydrochloride, and tebutaline sulfate	OT-CEC	[63]
Au NPs	Chlorpheniramine, tropicamide, and zopiclone	OT-CEC	[58]
Multi-layered Au NPs	Meptazinol and its intermediates	OT-CEC	[56]
Co-ordinated Cu^{2+} and metal organic framework	Propranolol, esmolol, metopropolol, amilodipine, and sotalol	OT-CEC	[65]
Magnetite NPs with an ionic liquid	DNS-modifed amino acids, including; alanine, isoleucine, leucine, glutamic acid, methionine, and valine	OT-CEC	[68]
Poly(glycidyl methacrylate) NPs	Amlodipine, metoprolol, and propranolol	OT-CEC	[60]
Polydopamine–Au NPs conjugate	Fexofenadine, histidine, phenylalanine, promethazine, terbutaline, tropicamide, and tryptophan	OT-CEC	[54]
Periodic mesoporous organosilica	Leucine and proline	OT-CEC	[69]
Spherical mesoporous silica	4′-, or 7-methoxyflavanone, 2′-,4′-, or 6-hydroxyflavanone, hesperatin, hesperidin, naringenin, and naringin	OT-CEC	[64]
Polydopamine composites	Epinephrine, isoprenaline, norepinephrine, verpamil, terbutaline, carvedilol, and tryptophan	OT-CEC	[57]
Graphene oxide (GO) sheets with magnetite NPs	Tryptophan	OT-CEC	[70]
Au NPs	Chlorpheniramine, tropicamide, and zopiclone	CEC	[71]
Au NPs	Dinitrophenyl-labeled amino acids; Valine, leucine, glutamic acid, aspartic acid. Also carvedilol, chlorpheniramine, and zopiclone	CEC	[66]
Silica NPs with different generation dendrimer spacers (polyamidoamine)	Chlorpheniramine, nefopam, and verapamil	CEC	[72]
Polystyrene NPs	Propranolol	CEC	[73]
Graphene QDs	Benzoin, benzoin methyl ether, benzoin ethyl ester, 6,6′-dibromo-1,1′bi-2-napthol, *trans*-stilbene oxide, flavanone, and 6-hydroxyflavanone	HPLC	[55]
Au NPs on porous silica spheres	Fenthion, chlorpyrifos, chlorpyrifos–methyl, diazinon, fenchlorpyos, flavanone, propranolol, and equol, 6-hydroxyflavanone	RP-LC	[59]

There have been many strategies employed for enantioseparation utilising column chromatography, with the separation resolutions varying greatly across all published work relating to CD-based chromatography, outlined in Table 1. This wide variation is primarily due to the numerous chiral analytes used across all published work, with the unique properties of each analyte interacting with the column functionalised with CD in different manners. Nonetheless, although it is highly difficult to compare directly the separation resolution between different analytes and columns, the effectiveness of each strategy is evident whereby in optimised conditions (i.e. pH, buffer concentrations, applied voltages, selected analyte, etc.) the separation resolutions are often well above 2.0,[54,56–62] sometimes reaching above 4.0.[55,63–66] Furthermore, within the vast majority of listed published articles, the authors frequently analyse the stability of prepared columns and techniques extensively, along with adjustments to the methods, including changes to the polarity and pH of the mobile phase, for more in-depth analysis of interactions and enantioseparation. In this regard, the methods employed are often optimised for a wide variety of analytes, with proof of continual stability and inter-column repeatability also explored. It is also noteworthy that, as is apparent in Table 1, Au NPs or functionalised Au NPs are the most commonly used nanomaterial, due to ease of production, functionalisation, layering, and stability, accounting for half of all present published work. As such, future research should focus on previously unexplored areas rather than repeating the common template presented in previous work. Such work should explore and analyse more fundamental principles in utilising NPs with CDs, such as if the size or potentially the nature of the NP used influences separation. It would be expected that changes in the surface to volume ratio translates to differences in enantioseparation, however, this has not been explored as of yet. Further to this, the effect of the NP, although not directly involved in enantioselectivity, could influence CD-analyte interaction, a process which has been shown to be possible.[36] It would hence be appropriate to explore the use of various NPs of equal size but with different properties, such as Au, silica, QDs, carbon based, for imparted variation in conjugated CD enantioselectivity. In converse, although many analytes have been explored, a systematic study of changing the characteristics, such as size and polarity, of the chiral analyte should be explored to give better understanding on the mechanisms involved and the groups or classes of compounds which will be effectively separated.

2.2. Magnetic materials for enantioseparation

Integration of NPs with chiral selectors can provide vital additional functionality to the system for enantiomeric discrimination. This is in contrast to when NPs are exploited for (predominantly) enhancement rather than providing a crucial function, such as in techniques utilising chromatography which

is shown in the previous section. When CDs are linked with specially designed NPs, the functionality of the NP can be exploited to achieve enantioseparation via working in harmony with CDs. Specially designed NPs can provide many functions based on the intrinsic properties, with one such property capable of being exploited is NPs magnetic properties. When magnetic NPs are linked with enantioselective CD, the novel nanomaterial can be used for enantioseparation of a racemic solution, whereby a physical separation of complexed enantiomer with nanomaterial proceeds (Figure 4).

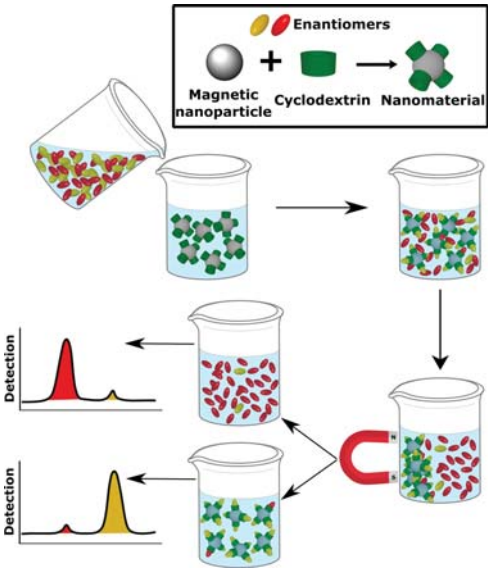

Figure 4. Schematic representation of a typical procedure utilising magnetic nanoparticles linked to cyclodextrin for separation of enantiomers (not to scale).

Numerous magnetic NPs linked with β-CD have been developed to achieve efficient enantioseparation (Table 2). Generally, enantioseparation is achieved with the novel nanomaterial whereby the magnetic NPs are conjugated with the enantioselective β-CD which is dispersed in a racemic solution of the chiral analyte. Exploiting the differences in the propensity for β-CD to selectively bind with the enantiomer, an external magnetic field can then be applied and physical separation of the nanomaterial, which contains bound enantiomer, and the solution containing the unbound enantiomer can ensue. The resulting separated samples are generally checked for purity via HPLC, or similar analysis techniques, to verify effectiveness of the enantioseparation. As apparent from Table 2, the most common chiral analytes used for enantioseparation via CD-linked magnetic NPs are the aromatic amino acids Phenylalanine, Tyrosine, or Tryptophan with β-CD. These analytes were likely chosen by the authors due to the strong chiral complexation which occurs with β-CD.[74,75]

The magnetic nanoparticles employed for enantiomeric separation has drastically changed over time, with nanomaterials comprised of the relatively simpler Fe_3O_4 NPs conjugated to cyclodextrin being the first material developed for this purpose.[76–81] However, these nanomaterials, although displaying chiral discrimination, were very limited in their capabilities for effective enantioseparation, with enantiomeric excess (e.e.) ranging from 12.3 to 73% in optimized conditions, with only one example of Tryptophan reaching 94% e.e.[78] This low effectiveness for complete separation lead to more complex nanomaterials being developed, which implemented additional functional properties and resulting in a more effective separation of chiral compounds. One such developed material was with the implementation of thermally responsive polymer branches attached to the magnetic NP and conjugation to CD.[82–84] The resulting nanomaterial allows for the adsorption of a chiral compound, physical separation, and subsequent desorption following thermal treatment, whilst the novel nanomaterial also has high repeatability and recycling for further enantioseparation. Utilising these more complex systems, and recycled repeatability, the effectiveness of separation drastically

Table 2. Magnetic nanoparticle-cyclodextrin nanomaterials used for enantioseparation through magnetic-based techniques. All listed nanomaterials incorporateβ-CD as the enantioselective entity.

Nanomaterial	Analyte(s)	Reference
Fe_3O_4 NPs	1-(1-napthyl)ethylamine	[80]
Fe_3O_4 NPs	Tryptophan	[79]
Fe_3O_4 NPs	*N*-(3,5-dinitrobenzoyl)phenylglycine, and *N*-(1-phenylethyl)phthalamic acid)	[76]
Fe_3O_4 NPs	Phenylalanine, tyrosine, and tryptophan	[78]
Fe_3O_4 NPs	Tryptophan	[77]
Fe_3O_4 NPs	Dansyl valine, dansyl phenylalanine, and dansyl leucine	[81]
Poly(*N*-isopropyl acrylamide-co-glycidyl methacrylate) polymer chains with Fe_3O_4 NPs loaded onto carbon nanotubes (CNTs)	Tryptophan	[82]
Conjugated Fe_3O_4-polydopamine-poly(*N*-isopropylacrylamide-*co*-glycidyl methacrylate) NPs	Tryptophan	[86]
Fe_3O_4–SiO_2–Au microspheres	Mandelic acid	[87]
Core/shell/shell structure comprised of Fe_3O_4/SiO_2/polymer	Tryptophan	[84]
Fe_3O_4 NPs with thermo-responsive polymer 'brushes'	Phenylalanine and tryptophan	[83]
Core-shell (Fe_3O_4/silica) NPs	Phenylalanine, tyrosine, and tryptophan	[88]
Poly(*N*-isopropylacrylamide) grafted to GO with Fe_3O_4 NPs	Tryptophan	[85]

increased, with e.e. reaching 100% under optimal conditions.[82,85,86] This is achieved through the formation of microenvironments within the thermally responsive polymer branches, in conjunction with the possibility to repeat the separation steps multiple times. However, this effective separation was achieved with only tryptophan, in all cases, as the chiral compound, which was noted previously to have shown high separation effectiveness with cyclodextrin in simpler systems (ie. 94% e.e. without thermally responsive brushes). Therefore, these systems should be further analysed with other chiral aromatic amino acids to further show the usefulness of the newly developed polymer branches.

Furthermore, the vast majority of published articles report enantioseparation of Tryptophan or Phenylalanine, both containing a hydrophobic region in the form of a benzene ring and amino groups, both of which have been shown to interact and influence complexation with cyclodextrin.[88] In this regard, further research should be focused on improving enantioseparation of molecules other than aromatic amino acids, such as commonly used drugs, which have a larger need and impact in common practice due to the large physiological difference in consumed enantiomers. Additionally, the potential application for using magnetic based nanomaterials for enantiomeric separation needs to be further revolutionised for up-scaled applications. To date, the application of such systems is highly limited for versatility and effectiveness in addition to having multiple steps. In this regard, future developments should include practicality elements for reasonable up-scaled applications in industry, whereby the need for effective enantioseparation is most needed.

2.3. Electrochemistry for enantiorecognition

In contrast to separation of chiral compounds, just as importantly is the enantioselective detection of enantiomers. Enantioselective recognition of chiral compounds is increasingly gaining attention, due to the understanding that enantiomers can elicit vastly different responses in a given system. For example, the role of many amino acid enantiomers within human physiology has been constantly analysed over the past century, with the presence of some enantiomers linked with various diseases such as neurological disorders as well as some forms of cancer.[89,90] Hence, the detection of enantiomers is a significant topic, with various electrochemical techniques giving the possibility to provide quick and sensitive detection of enantiomers. As depicted in Figure 5, there have been recent developments of using electrochemical measurements for detection of enantiomers through the use of a modified working electrode with immobilized nanomaterial and CD. This electrode is used to measure current/voltage responses of the enantiomers in solution, with the propensity of complexation between enantiomer and CD producing different responses.

Development of many novel electrochemical systems utilising the enantioselective properties of CDs with the high surface area to volume of NPs for enantiorecognition has been produced (Table 3). This combination between the properties of

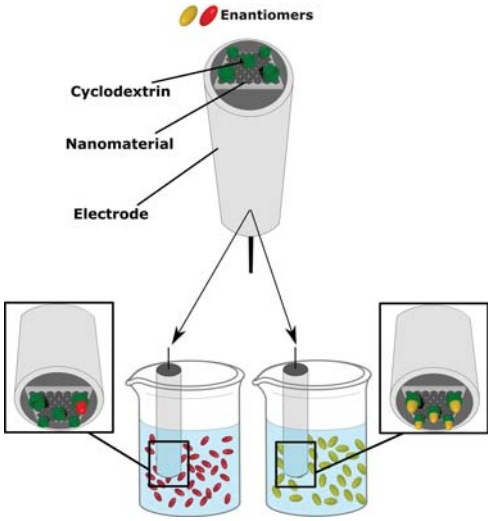

Figure 5. Schematic representation of a nanomaterial-cyclodextrin modified electrode used in current/voltage responses for the detection of enantiomers based on strength of complexation (not to scale).

CDs and NPs for use in electrochemistry often leads to highly sensitive detection of a wide range of chiral analytes. As is shown in Table 3, various NPs have been modified with β-CD (unless otherwise stated), with the CD employed as the enantioselective component, resulting in a novel nanomaterial which is capable of differentiating between chiral compounds via electrochemical techniques. Generally, the developed nanomaterial was immobilized onto a glassy carbon electrode (GCE), with the modified electrode used to probe a racemic solution for enantiorecognition.

As displayed in Table 3, the vast majority of applied electrochemical techniques utilize differential pulse voltammetry (DPV) for enantioselective detection due to the relative ease for determination of peak current and peak potential position differences. The principle mechanism involved for enantiorecognition in all articles presented in Table 3 (with exception of the final four articles utilising current trace techniques across a novel membrane) can be divided into two modes, being 'direct' or 'indirect' electrochemical responses. The direct response originates directly from the enantiomers, whereby generally a large current difference and small shifts in peak potential positions is observed due to the preferential binding of the L-form of the enantiomer.[44,91–104] The difference in current responses arising from the preferential incorporation of the L-form rather than D-form of the analyte within the CD cavity, is a linear response verse concentration which can then be used to interpolate unknown concentrations within enantiomeric solutions. The difference in current responses originates from the oxidation of the chiral compounds due to the propensity for interactions with the immobilized CD. For example, Niu et al.

Table 3. Nanoparticle-cyclodextrin nanomaterials immobilised onto a GCE (unless otherwise stated) for enantioselective sensing of chiral compounds using electrochemical based techniques. All listed nanomaterials incorporate β-CD as the enantioselective entity, unless otherwise stated.

Nanomaterial	Analyte(s)	Technique	Reference
carbon nanotubes (CNTs)-GO nanohybrids	Phenylalanine	Differential pulse voltammetry (DPV)	[109]
3D GO	Tryptophan	DPV	[94]
Reduced GO (rGO) and ferrocene composite	Phenylalanine	DPV	[108]
Cu^{2+} with carboxymethyl cellulose on rGO	Tryptophan	DPV	[97]
Nanocomposite of Pt/Pd nanowire and rGO	Ascorbic acid and isoascorbic acid	DPV	[95]
rGO	Phenylalanine	DPV	[104]
rGO	Cystine	DPV	[110]
rGO	Tryptophan	DPV	[111][a]
Graphene QDs	Tyrosine	DPV	[92]
Graphene QDs	Tryptophan	DPV	[112]
Graphene QDs	Tryptophan	DPV	[113]
Graphene QDs	Tryptophan	DPV	[114]
Au NPs with microporous metal–organic framework immobilized onto a gold electrode	Phenylalanine	DPV	[101][b]
Hollow carbon microspheres with Au NPs	Ascorbic acid and isoascorbic acid	DPV	[102]
Cu^{2+} and ammonia–ethanol co-treated chitosan	Tryptophan	DPV	[99]
Multi-walled CNTS (MWCNTs)/poly L-arginine with copper NPs	Tryptophan	DPV	[93]
MWCNTs with ferrocene	Tryptophan	DPV	[107]
MWCNT composites with polyethyleneimine and Au–Pt core-shell microspheres	Tryptophan	DPV	[98]
MWCNTs composite with ionic liquid	3,4-dihydroxyphenylalanine (DOPA)	DPV	[91]
Graphene nanosheets (GNS) incorporation into a carbon paste electrode	Moxifloxacin	DPV	[100][b]
GNS hybridised with Pt NPs	Tryptophan	DPV	[103]
MWCNTs	Atorvastatin calcium	Adsorptive stripping DPV	[115]
Fullerene composite linked to Au–Pd core-shell nanostructure	Tyrosine	Square wave voltammetry (SWV)	[43]
Hybridized rGO sheets	DOPA	SWV	[116][a]
rGO	Tartaric acid	Cyclic voltammetry (CV)	[44]
Au–cobalt–ferrite NPs coupled with a magneto nanocomposite graphene paste electrode	Tryptophan	CV	[96][b]
CNTs	Tartaric acid	CV	[117]
Graphene nanosheets	Tryptophan	CV	[106]
rGO with methylene blue	Mandelic acid, and phenyllactic acid	CV	[118]
Au NPs	Phenylalanine	CV	[105]
Nanochannel fabricated in a polyimide membrane	Tryptophan	Current traces	[119][c]
Utilizing the lumen of a protein pore (α-hymolysin) with copper (II)	Phenylalanine, tryptophan, and tyrosine	Current traces	[120][c]
Nanopore in pipet terminal	Catechin and ibuprofen	Current traces	[121][c]
Nanochannel fabricated in a polyethylene terephthalate membrane	Histidine	Current traces	[122][c]

[a] γ-CD was utilized instead of β-CD. [b] A GCE was not utilized, rather, the stated modified electrode was used instead. [c] Current across a membrane was measured rather than utilizing a modified GCE.

used a modified electrode consisting of CD incorporated within carboxymethyl cellulose and rGO for enantiorecognition of Tryptophan, whereby the peak potential shifted lightly by 34 mV and a large change in peak current observed, with L-Tryptophan producing 4.72 times the peak current compared to D-Tryptophan.[97] Similarly, Xu et al. reported current changes for the oxidation of Tryptophan enantiomers using a modified electrode consisting of CD, Pt NPs and graphene nanosheets, with L-Tryptophan resulting in a 1.30 times larger peak current compared to D-Tryptophan (with no change in peak potential observed).[103] In both these examples of 'direct responses', the unmodified electrode resulted in identical peak potentials and peak currents for the Tryptophan enantiomers.

Conversely to 'direct responses', 'indirect response' mechanisms for enantiorecognition exploit the difference in binding strength between enantiomers with CD for distinct changes in electrochemical measurements. One such method involves the electrochemical response of ferrocene being probed, which produces a high current response with a low onset potential. Therefore, it is the preferential binding of the L-form of an enantiomer within the cavity of CD which 'blocks' the electrode surface for the electrochemical response from ferrocene, resulting in a sharp decrease in the measured current.[105–108] Furthering this idea, a novel dual-signal method has been developed, whereby rhodamine B is bound within the CD cavity prior to electrochemical measurements.

Therefore, the electrode produces a distinct peak potential and current response from the incorporated rhodamine B, however, L-Phenylalanine can displace the bound rhodamine B from the CD cavity, whereby D-Phenylalanine does not. This results in the peak current response from rhodamine B dropping significantly whilst a second peak current at a

different potential position is observed, arising from now bound L-Phenylalanine.[109] Therefore, unlike the 'direct responses' mentioned previously, this competitive binding method produces distinct peak potential changes, with a change of 260 mV observed with L-Phenylalanine due to difference in oxidation peaks of Phenylalanine and rhodamine B. All of these techniques described above prob predominantly the distinct change in current response due to the preferential binding of one chiral form (generally the L-form) to the cavity of the incorporated CD. However, although there are sometimes small peak potential position changes between the chiral forms (ascribed to the change in immediate environment of the aromatic ring and formation of hydrogen bonds[92,95–97]), somewhat surprisingly, Zor et al. reports small current changes with large peak potential position differences between chiral forms of an enantiomer.[110,111] Through measurements of 'direct responses' from Tryptophan enantiomers, a modified electrode with CD and rGO was used to produce a 210 mV difference in peak potentials,[111] whilst a similar modified electrode produced a difference in oxidation peak potentials of 100 mV for cysteine enantiomers.[110] This is somewhat surprising as larger peak potential position changes suggests significant structural changes or environmental changes to the enantiomers, which should be further analysed and characterized. It should be noted that Xia et al. and Ma et al. reported the highest current response from the D-form compared to the L-form of the selected enantiomer, which they attributed to the favourable binding of the D-form to CD.[43,112] However, this larger current response for the D-form is likely due to some sort of blocking effect which the strongly bound L-form elicits (based on all previously explored papers, discussed previously). Nonetheless, all papers presented in Table 3 include the relevant controls, whereby the technique and method for electrochemical enantiorecognition is not possible without the inclusion of CD (ie. the modified electrode with no CD produces identical measurements from L- and D-forms of the enantiomer), thereby validating the need for the CD entity and displaying the enhancement effects of the nanomaterial for electrochemical responses.

In contrast to the methods and techniques explored above, nanochannels have been developed in combination with electrochemical measurements for the enantiorecognition of various analytes.[119–122] Gao et al. produced a nanopore using glass, which subsequently trapped a single CD molecule for enantiodiscrimination of catechin and ibuprofen.[121] The electrochemical mechanism used for detection was the changes in discrete current blocks from the sequential binding-release of individual molecules within the entrapped CD molecule. Generally, when the CD molecule within the nanopore binds to an analyte, a small drop in current (10 to 20 pA) is observed across the nanochannel, with the duration (1 to 20 ms) depending on the favourable binding due to the enantioselectivity of the incorporated CD molecule. Han et al. furthered this pioneering work, able to covalently attach multiple CD molecules within a single nanochannel.[122] This allowed for the enantiodiscrimination of Histidine, whereby current-voltage curves across the membrane displayed significant current changes (in the 100's of pA) with L-Histidine compared to D-Histidine due to incorporation with the bound CD within the nanochannel. Similarly, this concept was further explored by Xie et al. whereby current changes (in the 100's of pA) across a nanochannel containing covalently linked CD molecules was measured due to the favourable incorporation of L-Trypotphan.[119] The use of current changes across a membrane to detect chiral analytes was also exploited via the use of a non-covalently lodged CD molecule within a nanopore of an α-hemolysin mutant for the enantiodiscrimination of various aromatic amino acids.[120] Changes in current for this approach was observed in pA due to a single CD molecule being present within the nanopore, compared to the multiple CD molecules incorporated within the nanochannels discussed previously.

2.4. Other enantioselective techniques

Apart from employing chromatography techniques or magnetism for enantioseparation (Section 2.1 and 2.2, respectively), as well as enantiorecognition using electrochemistry (Section 2.3), a number of other unique techniques have been utilised with β-CD conjugated NPs for enantioselectivity (Table 4). Many different QDs have unique optoelectronic properties, which when conjugated with CDs, can be exploited for enantiorecognition of chiral compounds. For instance, changes in photoluminescence response of the QDs due to the formation of complexes between analyte and the conjugated CD can be used for sensing enantiomers.[123,124] Zhou et al. observed photoluminescence changes arising from complexation between the conjugated CD-QD material and enantiomer. Similarly, Wei et al. observed photoluminescence changes arising from complexation, however, a distinct wavelength shift was also characterized after complexing with one chiral form due to a selective hydrolysis step. Further to photoluminescence, others have used spectroscopic techniques based on the changes in fluorescence from the CD-QD conjugates for enantiorecognition.[125–127] The fluorescence intensity was observed to increase for the complexation with the L-form of the chosen chiral aromatic amino acids, however, interestingly, Zhou et al. reported that the photoluminescence intensity increased more for D-Tryptophan compared to L-Tryptophan, being the opposite result reported by Wei et al., although slightly different methods were used. A variation of observing changes in luminescence or fluorescence is detecting changes in the electrochemiluminescence, working in a similar principle, whereby the measured intensity of signal changes due to discriminative chiral complexation within the conjugated CD.[128,129] In contrast to the measurable emission spectra changes, absorbance profiles of the developed nanomaterial can be observed after complexation with integrated CD.[130] The change in absorbance can be noticed with the human eye via distinct change in colour, but more importantly, can be quantified with a spectrophotometer to determine enantiomeric excesses.

Apart from the above-mentioned enantiomeric detection strategies, various other conjugated CD-NPs can be used for

Table 4. Nanoparticle-cyclodextrin nanomaterials for enantioselective recognition or separation of chiral compounds using various techniques.

Nanomaterial	Analyte(s)	Technique	Reference
CdTe QDs	Histidine, phenylalanine, tryptophan, and tyrosine	Photoluminescence	[123]
Mn doped ZnS QDs	Tryptophan	Photoluminescence	[124]
Au/Pt NPs on MWCNT/silica coaxial nanocables with Ru dye on a GCE	Ascorbic acid and isoascorbic acid	Electrochemiluminescence	[129]
Ru dye conjugated Au NPs with rGO on a GCE	Proline	Electrochemiluminescence	[128]
CdSe QDs	Tyrosine	Fluorescence spectroscopy	[125]
CdSe/ZnS QDs	Tyrosine	Fluorescence spectroscopy	[127]
CdSe/ZnS QDs	Penicillamine	Fluorescence quenching or enhancements	[126]
Nanoporous GO	Asparagine	Adsorption in solution and separation	[133]
Assembly of Mg/Al layered double hydroxide nanosheets	Propranolol	Adsorption in solution and separation	[134]
Silica NPs	Amlodipine, chlorpheniramine, ephedrine, propranolol	CE	[135]
MWCNTs, polystyrene, TiO_2, or Al_2O_3	Clenbuterol	CE	[136]
MWCNTs	Metoprolol	Filtration using nanomaterial-based adsorbent	[131]
Silica nanochannels	Tryptophan	Physical separation using prepared membranes	[132]
Ag NPs	Phenylalanine, tryptophan, and tyrosine	Colorimetric assay from inclusion complexes	[130]
Ag NPs coated microspheres	Hydrobenzoin	Novel surface enhanced Raman spectroscopy platform	[137]
MWCNTs	Clenbuterol	TLC	[138]

enantioseparation, whereby the properties of the NP are exploited to aid in the separation process after formation of complexes with chiral analytes (Table 4). One such method utilises the novel nanomaterial to interact and complex with one of the chiral forms of the analyte, allowing for a second step to physically separate complexed material and solution resulting in enantioseparation of analyte.[131–134] The novel nanomaterial can also be employed within capillary electrophoresis for enantioseparation, whereby the CD bound material can be used as either a pseudostationary phase or within the running buffer,[135,136] both methods allowing for effective separation of various chiral analytes. However, both these processes require further separation of analyte and nanomaterial for real world applications, albeit being a relatively easier process than enantioseparation. Regardless, it is apparent that there are two emerging fields regarding NPs with CD for enantioselectivity, one being the use of QDs for fluorescence/luminescence detections, and the other being enantioseparation based on a capture-release process. The working principles and proof of concepts have been shown, however, compared to the three previous groups (chromatography techniques, magnetism techniques, and electrochemical techniques), it is apparent that these newer groups are in their infancy. It is likely that the use of QDs with CDs will continue to be explored across a wide range of analytes due to their increasing prominence in science along with their unique optoelectronic properties which holds promise for wide-spread applications in enantioselectivity.

3. Summary and outlook

Herein, we have summarized the use of cyclodextrin-modified nanomaterials for uses in chiral discrimination. From the published research in this particular area, it is apparent that there are three main categories to which enantioselectivity employing CD-NP nanomaterials can be placed, being enantioseparation using chromatography, enantioseparation using magnetism, or enantiorecognition using electrochemistry. Within the category of enantioseparation of chiral compounds using chromatography, it is evident that the vast majority use open-tubular capillary electrochromatography for effective chiral discrimination. Furthermore, the use of β-CD functionalized Au NPs prevails as the most commonly used NP. This is due to open-tubular capillary electrochromatography being an effective technique to produce high resolution of separation, whilst Au NPs are relatively easy to produce, functionalise, and also contain the desired high surface area which culminates in effective enantioseparation. CD-modified magnetite NPs dominate in physical separation of enantiomers within the magnetism category. These NPs can also be further be functionalised, such as with thermally responsive polymers to produce novel functional nanomaterials with dual actions. Within electrochemical based techniques for enantiorecognition, differential pulse voltammetry using the nanomaterial immobilize onto the working glassy carbon electrode is the most common method for sensing the chiral compounds. This is predominantly due to differential pulse voltammetry allowing for the current responses to the analyte along with any potential (voltage) shifts to be readily discerned with high sensitivity. Each of these techniques utilize the NPs in the desired technique to increase the effectiveness of enantiodiscrimination generally by their large surface area resulting in increasing the quantity of available CD units.

Current published research has shown that a large variety of chiral compounds can be differentiated through the use of nanomaterials comprised of CDs linked to a range of NPs.

Nonetheless, the overwhelming majority of these papers focus on comparative experimental procedures, often demonstrating not only the effectiveness of the novel nanomaterial used but also the necessity of each of the components utilised. Based on the outcomes, the author's give possible explanations as to how and why their combinations of procedure/nanomaterial/technique results in enantio-discrimination. However, there has been no in-depth study delving into the mechanisms as to the processes which the incorporation of NPs elicits chiral discrimination when linked the CD. In comparison, the mechanisms pertaining to CD being a chiral selector in solution is well studied.[50–53] This mode of chiral discrimination could potentially be altered through binding of larger nanoparticles or nanomaterials to the CD molecule. This is somewhat evident whereby incorporation of a non-chiral element improves the chiral selectivity of the CD.[36] Furthermore, another study revealed that the incorporation of non-chiral graphene QDs can elicit enantioseparation with CD, whereby the absence of the QDs displays minimal to no selectivity for various analytes.[55] Therefore, linking CD to a material can induce changes in the enantioselectivity of CD, including enhancement or diminishment of selectivity, which should be further explored and analysed. This work could potentially pave the way for exploiting previously unexplored nanomaterials with CDs. With the majority of the work presented here, the nanomaterials used were metallic nanoparticles, quantum dots, carbon nanotubes, or graphene oxide layers, all of which are expensive, especially compared to CD. Therefore, if novel cheap nanomaterials can be discovered and exploited for conjunction with CD, the successful application could lead to a new class of systems using CD for enantioselectivity. This has the potential to be utilised in a wide variety of areas, from research to industry, whereby the enantioselectivity of CD, as has been presented in this review, can be developed for a wide range of analytes to provide not only cheap, but also quick and sensitive separation and/or detection. Furthermore, one emerging area to be explored is exploiting cyclodextrin polymers as the base nanomaterial, electively used for direct enantioselectivity and hence bypassing the need for the expensive nanoparticle components often implemented. With the ever-increasing need for sensitivity and speed, using CDs for a development of a cheap enantioselective system could become wide-spread and common practice for the indispensable need of enantiomeric selectivity.

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Conflict of Interest

The authors declare no conflict of interest.

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Cyclodextrin-Polypyrrole Coatings of Scaffolds for Tissue Engineering

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Abstract: Polypyrrole is one of the most investigated conductive polymers used for tissue engineering applications because of its advantageous properties and the ability to promote different cell types' adhesion and proliferation. Together with β-cyclodextrin, which is capable of accommodating helpful biomolecules in its cavity, it would make a perfect couple for use as a scaffold for tissue engineering. Such scaffolds were prepared by the polymerisation of 6-(pyrrol-3-yl)hexanoic acid on polycaprolactone microfibres with subsequent attachment of β-cyclodextrin on the polypyrrole layer. The materials were deeply characterised by several physical and spectroscopic techniques. Testing of the cyclodextrin enriched composite scaffold revealed its better performance in in vitro experiments compared with pristine polycaprolactone or polypyrrole covered polycaprolactone scaffolds.

Keywords: cyclodextrin; pyrrole; polypyrrole; polycaprolactone; functionalisation; microfibres; tissue engineering; scaffold

1. Introduction

Tissue engineering [1,2] is a very complex science discipline that aims to develop methods for tissue renewal and, thus, tries to imitate or improve the processes that take place everywhere in nature. One promising material for fabricating scaffolds, which are used for achieving this goal, is polypyrrole (PPy) [3]. Pyrrole—a five-membered heterocycle occurring in natural compounds—is used for PPy preparation by oxidative polymerisation. PPy is one of the most investigated among conductive polymers used for tissue engineering applications because of its high stability, biocompatibility, simple preparation, structural tunability, and its ability to promote the adhesion and proliferation of different cell types [3–5]. Moreover, tests of PPy powder extracts for acute and subacute toxicity, haemolysis, and cell viability showed no negative results [6].

Cyclodextrins (CDs) are naturally occurring glucose-based cyclic oligosaccharides. Among them, β-CD (possessing seven glucose units in the cycle) has a prominent position because of its easy availability. Cyclodextrins are best known for their ability to accommodate lipophilic guests in their

cavity in an aquatic environment. This feature also plays a key role in their use in tissue engineering scaffolds, where CDs have proven to be beneficial [7–9]. The β-CD functionalised polymer could be used for the fabrication of supramolecular 3D scaffolds [10]. It has also been shown that β-cyclodextrin (β-CD) functionalised scaffolds promote performance in tissue engineering [11] by sequestration of growth factors [12], by an increase in oxygen concentrations in tissue engineered constructs [13], and by the regulation of collagen self-assembly [14]. Moreover, β-CD functionalised scaffolds are able to release drugs during cell culturing [15–17].

Based on the abovementioned findings, CD-PPy covered scaffolds would, thus, open the way for a versatile material, which can bind biomolecules on its surface (without the need of covalent bond attachment), thereby, enhancing cell adhesion, proliferation, or the possession of other desired features. Such a material aspires to have many advantages, including better attachment of biomolecules, easy modification of surfaces, the stability of the included biomolecules, and suitability as a scaffold for nerve tissue engineering. To the best of our knowledge, there are no known CD modified polypyrrole scaffolds used for tissue engineering.

Herein, we wish to report the preparation of microfibrous polycaprolactone (PCL) scaffolds functionalised with poly [6-(pyrrol-3-yl)hexanoic acid] (PPyHA) layer bearing β-CD units. The testing of a cyclodextrin enriched composite scaffold revealed its better performance in in vitro experiments compared to pristine PCL or PPyHA covered PCL scaffolds.

2. Materials and Methods

2.1. Synthesis of Pyrrole Monomer

Nuclear magnetic resonance (NMR) spectra were recorded using JEOL JNM-ECZR at 500 MHz for ^{1}H and at 126 MHz for ^{13}C. High-resolution mass spectrometry (HRMS) was measured using LTQ-Orbitrap Velos (APCI^{+}). Silica gel 60 (Merck, Darmstadt, Germany) was used for column chromatography.

An oven-dried flask was charged with 6-[(1-triisopropylsilyl)pyrrol-3-yl)]hexanoic acid (2 g, 5.92 mmol) [18], and evacuated and refilled with argon three times. Dry tetrahydrofurane (THF, 29 mL) was added using a cannula, and the reaction mixture was cooled to 0 °C. The reaction mixture was then treated with 1.05 eq. (6.22 mL, 1 M in THF) of tetrabutylammonium fluoride (TBAF) at 0 °C and stirred for 1 h. All volatiles were then evaporated under reduced pressure, and a semi-solid residue was purified by column chromatography on silica gel with a gradient mobile phase ($CHCl_3$:MeOH 40:1, 20:1, 5:1). 6-(pyrrol-3-yl)hexanoic acid (934 mg) was isolated as a grey solid in 87% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.24 (br s, 1H), 6.36 (ddd, J = 2.6, 2.0, 0.2 Hz, 1H), 6.23 (ddd, J = 2.5, 2.0, 1.6, 0.8 Hz, 1H), 5.70 (ddd, J = 2.7, 1.6, 0.5 Hz, 1H), 2.17 (t, J = 7.6 Hz, 2H), 1.97 (t, J = 7.6 Hz, 2H), 1.34 (quint, J = 7.3 Hz, 2H), 1.29 (quint, J = 7.4 Hz, 2H), 1.10 (quint, J = 7.7 Hz, 2H) (for more details see Figure S1); ^{13}C NMR (126 MHz, $CDCl_3$/MeOD) δ 175.6, 122.9, 116.8, 114.2, 107.2, 33.6, 30.4, 28.4, 26.2, 24.2 (for more details see Figure S2); HRMS (APCI^{+}): calcd for $C_{10}H_{15}NO_2$ 182.11756 [(M + H)$^{+}$)]; found m/z 182.11757 [(M + H)$^{+}$)].

2.2. Preparation of Scaffolds and Their Characterisation

2.2.1. Preparation of Electrospun PCL Fibres

Polycaprolactone (PCL) with a molecular weight of 80,000 (Merck) was electrospun. The spinning was carried out from a 10% (w/w) polymer solution in a solvent system composed of chloroform, ethanol and acetic acid (8/1/1, $v/v/v$;). The planar fibrous layer was prepared using a needleless electrospinning technology and carried out by Nanospider^{TM} 1WS500U (Elmarco, Liberec, Czech Republic). The 0.2 mm string and 0.5 mm slots were used. The forming fibres were collected on a spun bond layer placed 16 cm above the string and rolled by a speed of 10 mm/min. The applied voltages of −10 kV and +35 kV were used in the collector and the string, respectively. The temperature was

20 °C and relative humidity was 36–41%. For a morphology evaluation, samples were observed by scanning electron microscope (SEM) using a Tescan Vega3 SB Easy Probe. The samples were sputter coated with gold (7 nm) before analysis. The morphological analysis of the images from the SEM was performed by NIS Elements software (Nikon, Tokyo, Japan). The fibre diameter (1.13 ± 0.36 µm) was evaluated from 100 measurements.

Specific surface analysis (BET) was performed on the Autosorb iQ, Quantachrome instrument in a standard mode. The fibrous sample (291.8 mg) was put into a 12 mm glass BET cell and degassed for 24 h at 40 °C before measurement. Krypton was used for the analysis, and the data were processed using ASiQwin software (Figure S3). The resulting specific surface area of the analysed sample was 2.747 m^2/g.

2.2.2. Deposition of PPyHA Layer onto PCL

A glass vial containing 6-(pyrrole-3-yl)hexanoic acid (18 mg, 0.1 mmol) was charged with 3.4 mL of methanol. The PCL sheet (area weigh 5 mg/cm^2) was cut into 35 circles (diameter 0.9 cm) and the samples were immersed in the monomer solution for 60 min. 2.4 Eq. of $FeCl_3.6H_2O$ (65 mg, 0.24 mmol) in 0.6 mL of methanol was then added in one portion to reach the pyrrole monomer concentration of 2.5 mM. The reaction was shaken (150 rpm) at room temperature for 12 h while the samples became black. This indicated the formation of insoluble polypyrrole. All of the samples were rinsed thoroughly with methanol and sonicated five times in another methanol for 5 min. Finally, the samples were air and vacuum dried before being stored in a desiccator.

X-ray photoelectron spectroscopy (XPS) measurements of the prepared PCL-PPyHA sample were performed in an ultra-high vacuum chamber with a base pressure below 10^{-7} Pa. The spectra were taken at a normal emission angle using a Specs XR50 x-ray source with Al and Mg anodes ($h\upsilon$ = 1486.6 eV for Al K_α and $h\upsilon$ = 1253.6 eV for Mg K_α radiation, respectively) and a VSW HA100 hemispherical analyser with multi-channel detection. The samples were measured in an "as received" state without any cleaning of the surface. The chemical state of the samples was investigated by fitting C 1 s, O 1 s and N 1 s core level spectra in the KolXPD software. Spectral lines were represented by pseudo-Voigt functions, and a Shirley-type background was subtracted from the spectra.

Moreover, a Nicolet™ iZ™10 FT-IR Spectrometer (Thermo Scientific™, Waltham, MA, USA) and a Q500 thermogravimetric analyser (TA Instruments, New Castle, DE, USA) were used for further characterisation of the PCL-PPyHA scaffolds. The sample for thermogravimetric analysis (TGA) was placed on a platinum pan, and the thermal properties were analysed using a nitrogen atmosphere with a flow rate of 60 mL/min. The analysis ran from room temperature to 650 °C with a gradient of 10 °C/min. Temperature and weight loss were not correlated, and TA Universal Analysis software was used for data processing.

The electrical sheet resistance of the prepared scaffolds was examined by a standard two-point technique using the KEITHLEY 487 picoampermeter [19]. The contacts were prepared by the cathode sputtering of Au at a current of 40 mA and a sputtering time of 200 s. Under the conditions described, their thickness was approximately 75 nm. The measurements were carried out under reduced pressure (10 Pa) to eliminate the influence of air humidity. The electrical sheet resistance of the PCL-PPyHA scaffold was determined to be 2.22 ± 0.02 TΩ.

2.2.3. Immobilisation of CD onto PPyHA Modified PCL

PCL-PPyHA samples from the previous experiment were sonicated in a 2-(*N*-morpholino) ethanesulfonic acid (MES) buffer (10 mM, pH 6) until they were completely soaked with the solution. The samples were then transferred to 12 mL of fresh MES buffer. *N*-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide (EDC, 200 mg, 1.04 mmol) was dissolved together with *N*-hydroxysuccinimide (NHS, 300 mg, 2.6 mmol) in 8 mL of MES buffer and the solution was added in one portion to the reaction mixture. The reaction mixture was gently shaken at 150 rpm for 3 h at room temperature. After activation of carboxy groups, the solution was removed, and 20 mL of a fresh MES buffer with

dissolved 6^A-deoxy-6^A-amino-β-CD (47.2 mg, 41.6 μmol) was added. The functionalisation proceeded for 12 h at room temperature before the buffer solution was removed. The samples were rinsed with distilled water, sonicated 6 times for 5 min in water and then allowed to air dry. Finally, all of the samples were dried under a vacuum and stored in a desiccator.

A series of rhodamine B solutions in methanol was prepared as standard, and the fluorescence was measured in a quartz cuvette at a wavelength of 545 nm using a TECAN Spark spectrometer. PCL-PPyHA and PCL-PPyHA-CD samples (52 mg) were soaked in 4 mL of MeOH with rhodamine B (10^{-7} M) for 30 min, and the fluorescence was measured.

2.3. Cell Culturing

Samples of planar fibrous materials were tested in vitro using normal human dermal fibroblasts (NHDF). The cell line was obtained from ATCC (LGC Standards, Lomianki, Poland). The cells were cultured in a Fibroblast Growth Medium-2 BulletKit™ (FGM-2, Lonza, Basel, Switzerland) at 37 °C and 5% CO_2. The materials were cut into round samples (d = 10 mm), sterilised by 70% ethanol for 30 min and washed several times in phosphate-buffered saline (PBS, pH 7.4) before cell seeding. NHDF cells in passage 8 were seeded on the surface of the material in a concentration of 2000 cells/well. Evaluation of the material–cell interaction was performed after 1, 3, 7 and 14 days of incubation. Cell viability and proliferation were measured by the cell counting test (cck-8, Merck). 250 μL of 10% cck-8 solution (in FGM-2 medium) were added to each sample and incubated for 3 h at 37 °C and 5% CO_2. The absorbance of the solution after incubation was measured at 450 nm with the reference wavelength at 650 nm.

Visualisation of cells was carried out by fluorescence staining of fibroblasts. The samples were washed two times in PBS (pH 7.4), then fixed with 2.5% glutaraldehyde in PBS (30 min at 4 °C). After fixation, the samples were rinsed in PBS, then stained with 4′,6-diamidino-2-phenylindole (DAPI, Merck) together with fluorescein isothiocyanate labelled phalloidin (phalloidin-FITC) (Merck). The cells were treated in 0.1% Triton (Merck) for permeabilization, and the samples were washed with PBS and incubated with phalloidin-FITC (1 mg/mL stock solution diluted 1:1000 in PBS) for 30 min at room temperature (RT). Then the samples were washed with PBS and incubated with DAPI (5 min, RT). Finally, the samples were rinsed with PBS and analysed by a fluorescent microscope (NICON Eclipse Ti-e).

Multiple comparison pairs were analysed using a one-way ANOVA with Tukey post-hoc test. Significance was defined at a level of $p < 0.05$. For all conditions, $n = 4$ was used for analysis. All values are reported as mean +/− standard deviation.

2.4. Analysis of Protein Adsorption to Scaffolds

Interactions of bovine serum albumin (BSA) protein (Merck) with the prepared materials were tested with a BSA solution (50 mg/mL, in PBS with pH 7.4). Round samples (diameter of 1.6 cm, 10 mg) were incubated in 1 mL of BSA solution for 1 h (37 °C, gentle agitation). The samples were then washed twice in PBS followed by desorption of proteins. Desorption was carried out in 1 mL of PBS solution containing 1% sodium dodecyl sulphate (SDS) (Merck), and the samples were gently agitated for 1 h at RT. The protein solutions after desorption were analysed by electrophoresis (SDS-PAGE, Bio Rad) and by liquid chromatography. The experiment was carried out in quadruplicate. For SDS-PAGE, 10 μL of each sample were diluted (1:1) in a loading buffer (0.15 M Tris.HCl, pH 6.8, 30% glycerol (v/v)), 15% β-mercaptoethanol (v/v), 1% SDS (w/v), 0,01% bromophenol blue (w/v)). Twenty microlitres of the prepared samples were run using 10% gel (2 h, 110 V) with a wide-range molecular weight marker (Protein wide range MW Marker, Amresco, Solon, OH, USA). Subsequently, gels were stained with Coomassie blue solution (2.5% Coomassie brilliant blue R-250 (w/v) in 45% methanol (v/v) and 10% acetic acid (v/v)) and were evaluated after being washed several times in a destaining solution (45% methanol (v/v) and 10% acetic acid (v/v)). For the chromatography analysis, 200 μL of the samples were diluted in 1 mL of a solution of 100 mM NaH_2PO_4 (pH 6.8), 100 mM NaCl and 0.03% NaN_3.

The same solution was used as the mobile phase during the analysis. The Dionex Ultimate 3000 system with a single Phenomenex Yarra SEC-3000 column (3 µm particle diameter, 300 mm length and 4.6 mm inner diameter) and UV detection (DAD 3000 detector) at 279 nm were used.

3. Results and Discussion

3.1. Preparation of Scaffolds and Their Characterisation

The general route A (Figure 1) was followed for preparation of the PPyHA-CD modified scaffold with an electrospun PCL microfibrous platform as a starting material. First, *N*-(6^{A}-deoxy-β-cyclodextrin-6^{A}-yl)-6-(1*H*-pyrrol-3-yl)hexanamide (Figure 1) was synthesised [18] and used for polymerisation with ferric chloride as an oxidation agent [20]. Surprisingly, the polymerisation did not proceed either under the given reaction conditions or under other ones (using elevated temperature, different oxidizing agents or higher concentration of reagents) published elsewhere [21,22]. Such low reactivity is probably caused by the steric demands of bulky β-CD macrocycle, which blocks α-positions of the pyrrole ring. Thus, an alternative functionalisation sequence (route B, Figure 1) was proposed. 6-(pyrrol-3-yl)hexanoic acid was synthesised starting from its triisopropyl protected analogue [18] by simple cleavage of the protecting group using TBAF in THF at RT. The target pyrrole monomer was purified using column chromatography and isolated in 87% yield.

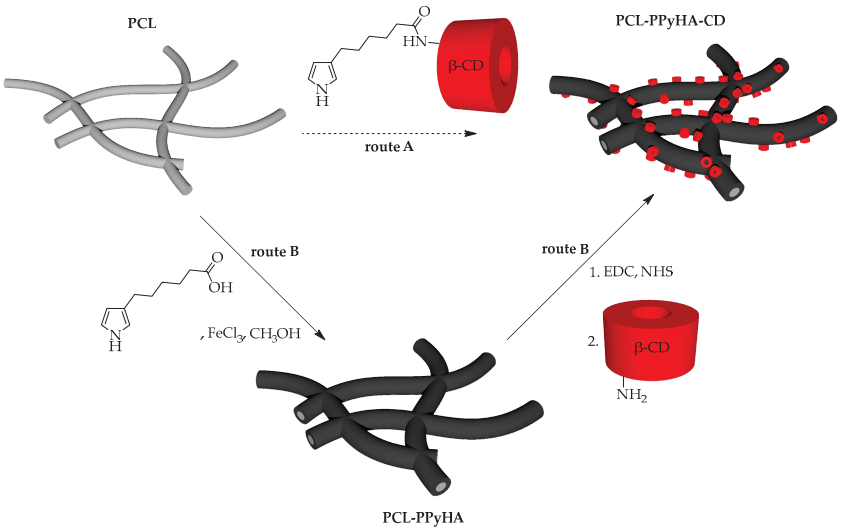

Figure 1. General routes for the preparation of poly [6-(pyrrol-3-yl)hexanoic acid]-cyclodextrins (PPyHA-CD) modified polycaprolactone (PCL) scaffolds. (**Route A**) direct polymerisation of pyrrole-CD conjugate onto PCL fibres. (**Route B**) stepwise functionalisation—polymerisation of 6-(pyrrol-3-yl)hexanoic acid and subsequent coupling with amino derivative of β-cyclodextrin (β-CD). Image not to scale.

Based on our experience with polypyrrole chemistry the choice of solvent was considered as one of the most critical parameters for obtaining a smooth polymer layer. The native pyrrole polymerises rapidly in water but slowly in alcoholic solvents due to deactivation of $FeCl_3$ as an oxidizing agent [23]. However, the hydrophobic character of the PCL scaffold does not allow the use of pure water as a solvent. Thus, 60% methanol in water (*v*/*v*) was used instead to ensure proper wetting of the PCL fibres and also to keep the polymerisation rate sufficient. As can be seen in Figure 2b, the resulting

PPyHA coating on the PCL fibrous matrix had several microscopic defects compared to pristine PCL (Figure 2a). Therefore, subsequent experiments were performed using a different methanol/water ratio (Figure 2c,d). Surprisingly, the polymerisation rate of the pyrrole derivative with the carboxylic group was sufficient even in pure methanol at room temperature, whereas pyrrole alone polymerises very slowly under these conditions [23]. Thanks to the unexpected reactivity and superior wettability of the PCL scaffold in methanol, a smooth layer of PPyHA without any structural defects was achieved (Figure 2d). Moreover, the pending side chains with terminal carboxylic groups represent a binding place for the final β-CD connection.

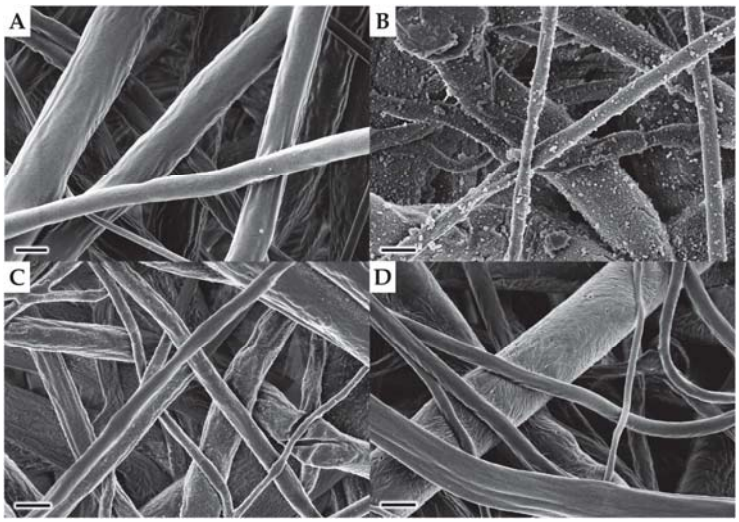

Figure 2. Scanning electron microscope (SEM) images of (**a**) PCL; and polycaprolactone-poly [6-(pyrrol-3-yl)hexanoic acid] (PCL-PPyHA) scaffolds prepared using (**b**) 60% methanol in water (*v*/*v*); (**c**) 95% methanol in water (*v*/*v*); and (**d**) 100% methanol. Scale bar 1 μm.

A number of analytical methods were used for confirmation of the successful deposition of the PPyHA film. Although the characterisation of thin layers on a flat metal surface can be easily studied by XPS [24], the analysis is far more complicated on fibrous scaffolds. PCL has a low melting point (60 °C) and, therefore, cannot be purged by energy-rich argon ions. Moreover, the penetration depth of the radiation is higher in organic materials, and, thus, the overall signal is a combination of the PCL matrix and the thin PPyHA layer on the surface. The spectra of oxygen and carbon were accompanied by surface impurities, and the elemental composition could not be quantified. However, successful coverage of the fibre surface was confirmed by the nitrogen spectrum (Figure 3). The nitrogen signal of the PCL-PPyHA sample is a combination of two out of four possibilities of how the nitrogen atom could be bonded in PPy [25,26]. The other two forms are not visible due to their low intensities.

Next, infrared (IR) spectroscopy was used to confirm the presence of functional groups in the PCL-PPyHA substrate. However, due to the very thin layer of PPyHA, it was not possible to measure it directly. Therefore, the PCL was washed out by $CHCl_3$, and the structure of the resulting PPyHA powder was analysed (Figure 4). A broad valence vibration of carboxylic O–H group between 3500 and 3050 cm^{-1} and the characteristic strong symmetrical stretching vibration of C=O group at 1714 cm^{-1} confirmed the presence of carboxylic acid on the polymer structure. Furthermore, the symmetric, as well as asymmetric vibrations of the corresponding alkyl $–CH_2–$ linker at 2932 and 2859 cm^{-1}, can be found as well.

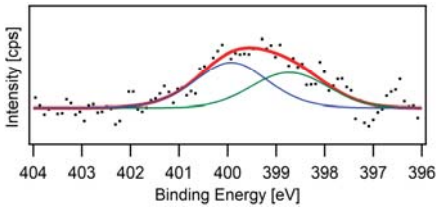

Figure 3. The X-ray photoelectron spectroscopy (XPS) spectrum of N 1 s core level (Mg K_α radiation) of the PCL-PPyHA sample (red). Blue and green lines represent two binding modes of the nitrogen atom.

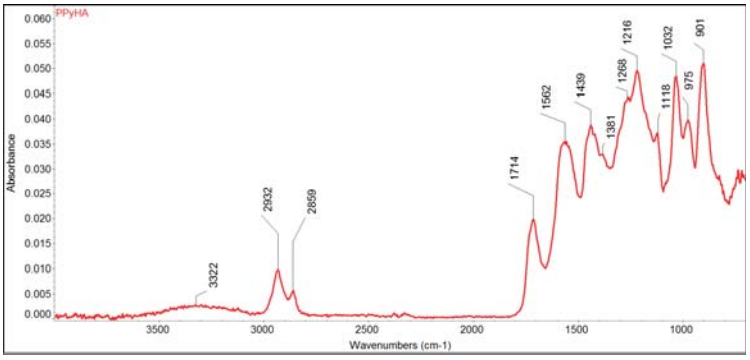

Figure 4. Infrared (IR) spectrum of the resulting PPyHA powder after washing off the PCL.

TGA was used for further characterisation of the deposited PPyHA layer (Figure 5). The decomposition profile was similar for both PCL and PCL-PPyHA because the PPyHA layer is very thin and does not influence the thermal properties of the matrix. Nevertheless, valuable information about the quantity of deposited polypyrrole could be deducted from the residual weight. While pristine PCL was decomposed nearly entirely (98.3%), the residual weight of powdered PPyHA was 38.2%. It is, therefore, possible to calculate the percentage of PPyHA on the PCL surface. The percentage of deposited PPyHA was 5.2% (w/w), which is in good agreement with our previously published results [27]. Moreover, such an analysis represents a fast and robust method for confirmation and also quantification of deposited polypyrrole between similar experiments.

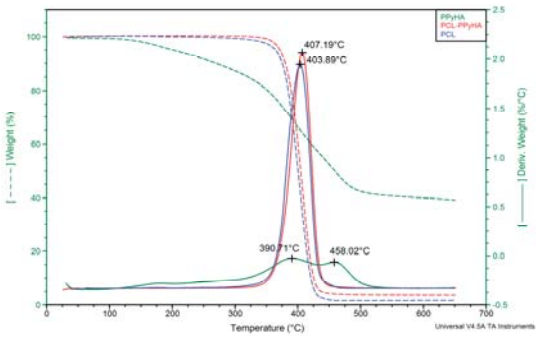

Figure 5. TGA curves and their derivatives (dashed) of PCL (blue), PCL-PPyHA (red) and PPyHA powder after washing off the PCL (green).

To prepare the final PCL-PPyHA-CD scaffold, commonly used 6^A-deoxy-6^A-amino-β-cyclodextrin [28,29] was conjugated onto a PCL-PPyHA scaffold with the aid of carbodiimide chemistry. First, carboxylic groups on the surface were activated with EDC/NHS in an MES buffer (pH 6). Then, the solution was replaced with a fresh buffer containing a dissolved β-CD amino derivative, and the scaffold was allowed to incubate overnight.

The maximum theoretical amount of CDs attached to the fibrous surface was estimated to be 0.25% (*w*/*w*). This calculation was based on the specific surface area of the fibres, the outer diameter of CDs, and the hexagonal packing arrangement with the highest possible density of molecules. Due to such a low relative number of CDs on the surface, the abovementioned methods used for characterisation of the PCL-PPyHA scaffold gave the same results for PCL-PPyHA-CD. Therefore, the amount of immobilised CD was measured indirectly by fluorescence labelling. The interaction of CDs with rhodamine B was exploited using the known ability of CDs to form inclusion complexes with various molecules. The PCL-PPyHA material was incubated with rhodamine B, and the results were used as a blank to exclude an interaction with the PPyHA layer. However, due to a low overall number of CDs on the surface of the PCL-PPyHA-CD sample, the difference was negligible. The experimental error could, therefore, be very high, and the amount of deposited CDs could not be determined exactly, even though, the results from cell culturing, protein adsorption (see below) and change in the electrical sheet resistance to 13.81 ± 0.31 TΩ suggests successful functionalisation of the PCL-PPyHA scaffold with CDs.

3.2. Cell Culturing and Protein Adsorption to the Scaffolds

All three prepared scaffolds (PCL, PCL-PPyHA and PCL-PPyHA-CD, Figure 1) were subjected to an in vitro experiment with NHDF cells. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cell viability assay together with fluorescence analysis was conducted on days 1, 3, 7 and 14. As shown in Figure 6, the performance of both functionalised materials was slightly worse than pristine PCL on testing days 1 and 3. However, there was a significant increase in cell activity for both samples compared to PCL. The cell viability was significantly higher compared to PCL on day 14 (PCL vs. PCL-PPyHA and PCL vs. PCL-PPyHA-CD; $p < 0.05$). Cells were well spread across the fibrous scaffold with no visible defects (Figures S4 and S5). The introduction of the thin carboxylic acid modified PPyHA layer onto the PCL surface helped cell proliferation and growth, which is in accordance with the abovementioned findings [3–5]. Moreover, the immobilised CDs work synergistically with PPyHA and dramatically increase cell proliferation.

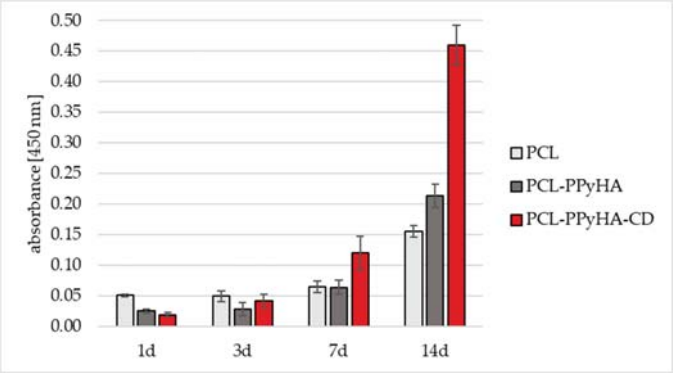

Figure 6. Cell viability analysis. Proliferation of normal human dermal fibroblasts (NHDF) on prepared scaffolds (PCL, PCL-PPyHA, PCL-PPyHA-CD) after 1, 3, 7 and 14 days.

In general, the adsorption of proteins significantly influences the properties of the materials giving them biological identity [30,31]. Therefore, the protein adsorption was measured to find out if the CD decorated scaffold is capable of accommodating helpful biomolecules. Scaffold samples (10 mg) were used for the adsorption experiment with a model BSA protein, whose concentration was quantified by SDS-PAGE and liquid chromatography after desorption. SDS-PAGE results revealed that the presence of CDs on the surface of the fibrous material leads to a greater degree of protein adsorption compared to the PCL-PPyHA scaffold (Figure 7). However, the pristine PCL scaffold adsorbs far more BSA than the two others. These results were confirmed by liquid chromatography, which showed that the amount of BSA adsorbed onto the materials was 67 ± 9 µg/mg of the PCL sample, 8 ± 3 µg/mg of the PCL-PPyHA sample and 21 ± 8 µg/mg of the PCL-PPyHA-CD sample. In the case of the PCL scaffolds, the best results for protein adsorption are in direct contradiction to the worst results from the cell viability analysis. It is generally known [32] that if the material is too hydrophobic, the proteins could be adsorbed in high amounts, but in a denatured state [33], causing the surface to be less attractive for the cells. The results suggest that BSA is well adsorbed to PCL, but in distorted conformation [34] unsuitable for proper interaction with membrane receptors. However, PCL-PPyHA and PCL-PPyHA-CD scaffolds are less covered, but with native proteins.

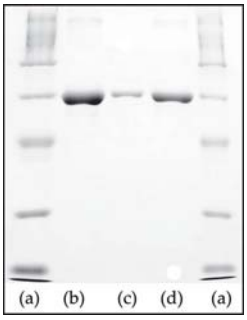

Figure 7. Sodium dodecyl sulphate electrophoresis (SDS-PAGE) results of adsorbed bovine serum albumin (BSA) onto the scaffolds. (**a**) marker; (**b**) PCL; (**c**) PCL-PPyHA; (**d**) PCL-PPyHA-CD.

4. Conclusions

A general platform for functionalisation of non-woven polymers for tissue engineering has been developed. An unexpected high polymerisation rate of a newly synthesised pyrrole monomer—6-(pyrrol-3-yl)hexanoic acid—in methanol was used as an advantage for the preparation of a smooth layer on the PCL fibrous matrix. Deposited polypyrrole bearing hexanoic acid was characterised by physical and spectroscopic techniques to verify its material structure. The surface pending carboxylic groups was subsequently used for conjugation of β-CD via an amide bond. Finally, the biological in vitro experiment revealed that our composite PCL-PPyHA-CD material is not only biocompatible but also greatly improves cell-material interaction. These results will, thus, serve as a cornerstone for future research focused on the design of bioinspired conductive polymers for neural tissue regeneration.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4360/11/3/459/s1, Figure S1: ^{1}H NMR spectrum ($CDCl_3$/MeOD, 298 K, 500 MHz), Figure S2: ^{13}C NMR spectrum ($CDCl_3$/MeOD, 298 K, 126 MHz), Figure S3: Multi-point BET plot, Figure S4: Fluorescent microscopy of cells seeded on PCL, PCL-PPyHA and PCL-PPyHA-CD scaffolds on days 1, 3, 7 and 14 after cell seeding, and Figure S5: SEM images of the PCL, PCL-PPyHA and PCL-PPyHA-CD scaffolds on days 1 and 14 after cell seeding.

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Conflicts of Interest: The authors declare no conflict of interest.

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ORIGINAL ARTICLE

Synthesis of cyclodextrin–pyrrole conjugates possessing tuneable carbon linkers

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Abstract
Cyclodextrins are naturally occurring cyclic oligosaccharides consisting of glucose units. The main feature of cyclodextrins is the ability to accommodate various lipophilic compounds in their interior, which determines them to be popular helpers to the mankind. However, there is still a demand for new derivatives for advanced applications. Herein, we report the synthesis of β-cyclodextrin–pyrrole conjugates. Their preparation is based on an amide bond formation or copper(I)-catalysed azide-alkyne cycloaddition between β-cyclodextrin and pyrrole derivatives. The main advantage of the synthetic approach lies in the possibility to attach the substituent in β-position, because polypyrroles possessing a substituent in this position are generally more conductive than the *N*-substituted ones. Moreover, the presented synthetic route is general and allows tuning the properties (various types of connections and lengths) of a linker. The presented cyclodextrin–pyrrole derivatives thus open the door for new applications in the field of sensors or tissue engineering.

Keywords β-Substitution · Amide · Click chemistry · Cyclodextrin · Pyrrole

Introduction

Pyrrole [1] is a crucial component of an enormous number of bioactive and naturally occurring compounds [2]. Pyrrole and its derivatives (with the exception of α-substituted derivatives) undergo oxidative polymerisation to produce inherently conductive polypyrrole (PPy). Generally, conjugated polymers are insulators in their undoped state, but higher conductivity can be achieved by doping. The properties of doped conjugated polymers can be simply tailored by the type of dopant and a method of preparation. Unlike the first discovered air sensitive conductive polyacetylene, PPy represents a step forward to new types of sensors, supercapacitors or molecular scaffold for tissue engineering [3–6]. Although the pristine PPy is a brittle material it proved useful in supporting cell adhesion, proliferation and growth in the form of composite material [7]. Surprisingly, Mao et al. [8] recently published an article dealing with preparation of PPy membrane with good mechanical properties even in liquid nitrogen. Instead of commonly used electrochemical method they synthesized PPy by template assisted chemical oxidation on the water/chloroform interface.

Cyclodextrins (CDs) are naturally occurring cyclic oligosaccharides consisting of glucose units. They have the shape of a hollow truncated cone and their main feature is the ability to form complexes with lipophilic compounds in water environment. Host–guest interactions between CDs and extracellular matrix proteins could enhance integrin binding and trigger signalling pathways leading to the better cell-material adhesion. Moreover, pyrrole-β-CD conjugates could also find their application as electrochemical sensors due to the CD complexation ability and polypyrrole conductivity, as it was described before [9–12].

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CDs are usually modified to allow a selective attachment to another molecule. The best known CD derivatives suitable for this purpose are 6^A-deoxy-6^A-azido and amino CDs [13, 14]. Although there are known pyrrole-CD conjugates [11, 15], CDs connected to pyrrole via nitrogen atom possess generally lower conductivity when compared to polymerized β-substituted pyrroles [16]. There are known [17–21] several β-substituted pyrrole derivatives suitable for well-established amide bond formation or for copper(I)-catalysed azide-alkyne cycloaddition [22–24]. However, no general synthetic route for these derivatives with different lengths of carbon chain between pyrrole and the functional group is available.

Herein, we would like to report the synthesis of new pyrrole-β-CD conjugates with tuneable carbon linkers and different types of connection (amide bond or triazole).

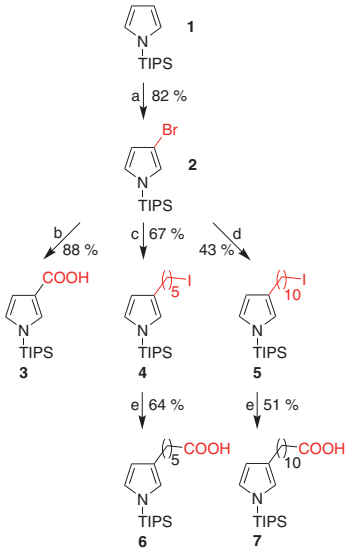
Results and discussion

We had to establish a general synthetic route for the above-mentioned derivatives. At the outset, commercially available TIPS-pyrrole **1** was brominated at β-position to furnish bromo derivative **2**, which was transformed into carboxylic acid **3** according to published literature [25] (Scheme 1). Next, new β-substituted iodoalkyl pyrroles with a different carbon chain length **4** and **5** were prepared by alkylation of lithio species generated in situ from compound **2**. Target molecules **6** and **7** were subsequently synthesised in good yields by metal-halogen exchange reaction quenched by CO_2 before acidic workup.

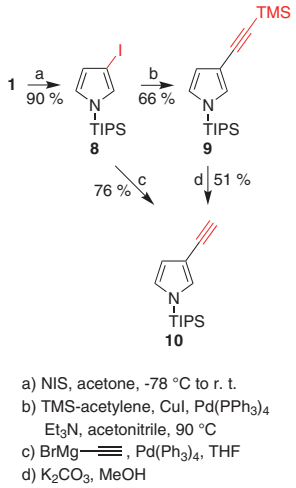
Compound **8** was prepared by iodination of TIPS-pyrrole **1** followed by Sonogashira cross-coupling reaction [26] providing **9** according to the published literature [27] (Scheme 2). However, only a moderate yield was achieved in the TMS-deprotection step due to competitive TIPS cleavage. Therefore, the target molecule **10** was prepared in a new, shorter way using Pd-catalysed Kumada [28, 29] reaction starting from iodopyrrole **8**.

Introduction of a triple bond to aromatic ring is normally achieved by Sonogashira reaction but the synthesis of aliphatic alkynes is far more challenging. There are several publications dealing with the synthesis of "pincer" catalyst which can supress competitive β-elimination and provide coupling products. Despite the great progress, there is no general method for the synthesis of aliphatic triple bonds from alkyl halogenides yet [30–34].

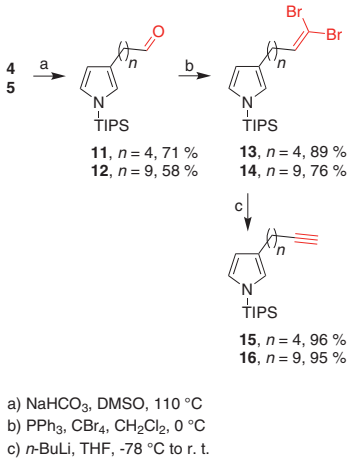
The direct alkylations of **2** (e.g. with TMS-protected 5-iodopent-1-yn) gave no product and therefore iodo derivatives **4** and **5** were used as starting materials instead. However, nucleophilic substitution with ethynylmagnesium bromide, sodium acetylene, TMS-protected lithium acetylene etc. did not lead to the desired products. Hence, we decided



Scheme 1 Synthesis of TIPS-protected pyrrolecarboxylic acids



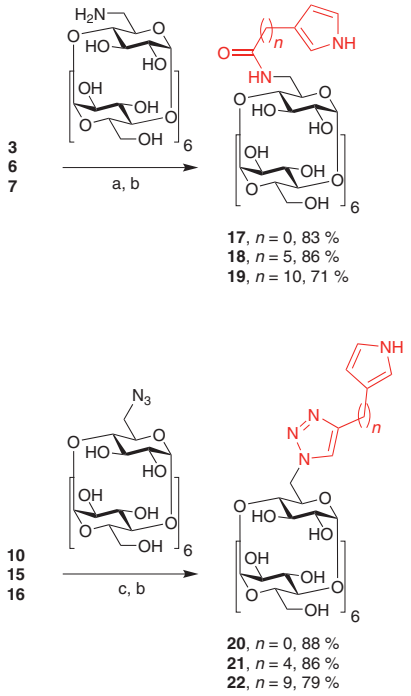
Scheme 2 Synthesis of TIPS-protected β-acetylenepyrrole



Scheme 3 Synthesis of triple bond substituted pyrroles

to use a traditional multistep procedure including the synthesis of the corresponding aldehydes and Corey-Fuchs [35] reaction as a final step (Scheme 3). The reduction of acids **6** and **7** or metalation of iodo derivatives **4** and **5** followed by addition of DMF or *N*-formylpiperidine furnished only in complex reaction mixtures with low yields of the products. On the contrary to the above described unsuccessful reactions, Kornblum oxidation [36, 37] of iodo derivatives **4** and **5** proceeded smoothly to yield aldehydes **11** and **12**, respectively. Corey-Fuchs reaction was used to transform the aldehydes to geminal dibromo alkenes **13** and **14**. The final elimination reaction with *n*-BuLi afforded target molecules **15** and **16** in excellent yields.

Target pyrrole–cyclodextrin conjugates **17–19** were prepared by the coupling of compounds **3, 6** and **7** with 6^A-deoxy-6^A-amino-β-cyclodextrin [38] (Scheme 4). First, the carboxyl group was activated with (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) followed by treatment with *N,N*-diisopropylethylamine (DIPEA) in DMF at room temperature. Silyl protecting group was easily cleaved by HF/Et_3N system to afford compounds **17–19** in 71–86% yields. Recently, Chmurski et al. [39]. reported an improved procedure for connection of 6^A-deoxy-6^A-azido-β-cyclodextrin [38] on various substrates by copper(I)-mediated azide-alkyne cycloaddition. Starting from pyrrole derivatives **10, 15** and **16** we adopted their protocol with minor changes. Briefly, Cu(I) was generated in situ by reducing $CuSO_4$ with ascorbic acid. Further, the catalytic system was stabilised with 0.2 Eq. of tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA) and the reaction proceeded in DMSO/H_2O at 60 °C under argon



Scheme 4 Synthesis of pyrrole-β-CD conjugates

atmosphere. Triazoles **20–22** were formed in 79–88% yields using the above mentioned HF/Et_3N mixture for the final deprotecting step.

Conclusion

In conclusion, we have synthesised pyrrole derivatives substituted in β-position with carboxyl group or triple bond, which is present on carbon chain with different lengths. Such derivatives are useful precursors for introduction of a wide range of molecules and for making modified PPy layers. Applicability of the pyrrole precursors was demonstrated by preparation of pyrrole-CD conjugates connected via amide bond or triazole ring. These final products are now under investigation in tissue engineering experiments.

Experimental

General information

All the reagents were obtained from common commercial sources and the solvents were used as obtained unless otherwise noted. NMR spectra were recorded using Agilent 400-MR DDR2 at 399.94 MHz for ^{1}H and at 100.58 MHz for ^{13}C. Samples were measured as solutions in deuterated solvents and referenced to a residual solvent peak. Chemical shifts are given in δ-scale and coupling constants *J* are given in Hz. HRMS spectra were recorded using GC-MS Autospec Ultima (EI) or LC-MS LTQ-Orbitrap Velos (ESI^+, ESI^- or $APCI^+$). Silica gel 60 and silica gel 60 F254-coated aluminum sheets (both Merck, Germany) were used for column and thin layer chromatography, respectively. Spots were detected under UV or by spraying with 50% aqueous H_2SO_4 solution and carbonization with a heat-gun.

Synthesis of pyrrole derivatives

Pyrrole derivatives [25, 27] **2, 3, 8, 9** and 6^A-deoxy-6^A-azido and amino β-CDs [38] were prepared according to the published literature.

General procedure for the synthesis of compound (4) and (5)

A dry argon-flushed flask was charged with **2** (302 mg, 1 mmol) and anhydrous THF (18 ml). The solution was cooled to 0 °C and *n*-BuLi (1.18 ml, 2 mmol, 1.7 M solution in THF) was added dropwise over 10 min and the reaction mixture was stirred for 1 h. In a second flask 1,5-diiodopentane or 1,10-diiododecane (6 mmol) was dissolved in 3 ml of dry THF and added to the solution of lithiated species via a cannula. The reaction mixture was allowed to reach room temperature and was stirred for 3 h before quenched with a saturated solution of NH_4Cl (30 ml). The aqueous layer was extracted with hexane (3 × 25 ml). The combined organic layers were washed with brine (1 × 50 ml), water (1 × 50 ml), dried over $MgSO_4$ and filtered. Solvents were evaporated under reduced pressure and the crude reaction mixture was purified by column chromatography on silica gel using hexane:EtOAc as the mobile phase with a gradient elution mixture (20:1, 10:1, 5:1).

3-(5-iodopent-1-yl)-1-(triisopropylsilyl)pyrrole (4)

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.70 (t, $J = 2.4$ Hz, 1H), 6.52 (s, 1H), 6.14 (dd, $J = 2.5$, 1.4 Hz, 1H), 3.19 (t, $J = 7.1$ Hz, 2H), 2.50 (t, $J = 7.4$ Hz, 2H), 1.86 (quint, $J = 7.4$ Hz, 2H), 1.60 (quint, $J = 7.3$ Hz, 2H), 1.49–1.37 (m, 5H), 1.09 (d, $J = 7.4$ Hz, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 126.0, 124.2, 121.2, 110.7, 33.7, 30.4, 30.0, 26.9, 18.0, 11.8, 7.5; HRMS ($APCI^+$): calcd for $C_{18}H_{34}INSi$ 420.15780 $[(M+H)^+]$; found *m/z* 420.15796 $[(M+H)^+]$; Yield 281 mg (67%).

3-(10-iododec-1-yl)-1-(triisopropylsilyl)pyrrole (5)

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.69 (t, $J = 2.4$ Hz, 1H), 6.51 (s, 1H), 6.14 (dd, $J = 2.4$, 1.3 Hz, 1H), 3.19 (t, $J = 7.1$ Hz, 2H), 2.47 (t, $J = 7.5$ Hz, 2H), 1.82 (quint, $J = 7.4$ Hz, 2H), 1.50–1.18 (m, 17H), 1.09 (d, $J = 7.5$ Hz, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 126.5, 124.0, 121.1, 110.7, 33.7, 31.2, 30.7, 29.7, 29.61, 29.57, 29.56, 28.7, 27.1, 18.0, 11.9, 7.5; HRMS ($APCI^+$): calcd for $C_{23}H_{44}INSi$ 490.23605 $[(M+H)^+]$; found *m/z* 490.23645 $[(M+H)^+]$; Yield 211 mg (43%).

General procedure for the synthesis of compound (6) and (7)

An oven-dried flask was charged with **4** or **5** (1 mmol) and anhydrous THF (5 ml) under argon atmosphere. The solution was cooled to − 78 °C and *t*-BuLi (1.09 ml, 1.85 mmol, 1.7 M solution in THF) was added dropwise over 10 min. The reaction mixture was stirred for 1 h at low temperature before bubbled with CO_2 for 30 min. The reaction was quenched with a saturated NH_4Cl solution (30 ml) and acidified with 1M HCl to neutral pH. The aqueous layer was extracted with $CHCl_3$ (3 × 30 ml), the combined organic layers were washed with water (1 × 50 ml), dried over $MgSO_4$ and filtered. Solvents were evaporated under reduced pressure and the crude reaction mixture was purified by column chromatography on silica gel using $CHCl_3$:MeOH with a gradient elution mixture (40:1, 20:1, 5:1).

6-[(1-triisopropylsilyl)pyrrol-3-yl]hexanoic acid (6)

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.68 (t, $J = 2.4$ Hz, 1H), 6.50 (m, 1H), 6.13 (dd, $J = 2.5$, 1.4 Hz, 1H), 2.49 (t, $J = 7.4$ Hz, 2H), 2.34 (t, 7.4 Hz, 2H), 1.66 (quint, $J = 7.42$ Hz, 2H), 1.59 (quint, $J = 7.42$ Hz, 2H), 1.50–1.33 (m, 5H), 1.08 (d, $J = 7.5$, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.1, 126.1, 124.1, 121.2, 110.7, 34.2, 30.8, 29.0, 26.9, 24.7, 18.0, 11.8; HRMS (ESI^-): calcd for $C_{19}H_{35}NO_2Si$ 336.2364 $[(M–H)^-]$; found *m/z* 336.2365 $[(M–H)^-]$; Yield 216 mg (64%).

11-[(1-triisopropylsilyl)pyrrol-3-yl]undecanoic acid (7)

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.68 (t, $J = 2.3$ Hz, 1H), 6.51 (m, 1H), 6.14 (dd, $J = 2.3$, 1.2 Hz, 1H), 2.47 (t,

$J = 7.5$ Hz, 2H), 2.34 (t, $J = 7.5$ Hz, 2H), 1.63 (quint, $J = 7.4$ Hz, 2H), 1.56 (quint, $J = 7.4$ Hz, 2H), 1.41 (m, $J = 7.5$ Hz, 3H), 1.35–1.24 (m, 6H), 1.09 (d, $J = 7.5$ Hz, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.7, 126.5, 124.0, 121.2, 110.7, 34.1, 31.2, 29.8, 29.63, 29.59, 29.58, 29.4, 29.2, 27.2, 24.8, 18.0, 11.9; HRMS (ESI^-): calcd for $C_{24}H_{45}NO_2Si$ 406.3147 [$(M–H)^-$]; found *m/z* 406.3147 [$(M–H)^-$]; Yield 208 mg (51%).

3-ethynyl-1-(triisopropylsilyl)pyrrole (10)

An oven dried flask was charged with the starting compound **8** (166 mg, 0.475 mmol), $Pd(PPh_3)_4$ (28 mg, 23.8 umol) and 2.5 ml of anhydrous THF under argon atmosphere. Ethynylmagnesium bromide (1.4 ml, 0.713 mmol, 0.5 M solution in THF) was added dropwise over 5 min and the stirring was continued for additional 30 min at room temperature. The reaction mixture was quenched with 10 ml of distilled water and the aqueous phase was extracted with Et_2O (3×10 ml). The combined organic layers were dried over $MgSO_4$ and filtered. All volatiles were removed under reduced pressure and the residual yellowish slurry was purified by column chromatography on silica gel using hexane:EtOAc (10:1) to afford 82 mg of compound **10** as a colourless oil in 76% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.03 (dd, $J = 1.8$, 1.5 Hz, 1H), 6.66 (dd, $J = 2.6$, 2.2 Hz, 1H), 6.41 (dd, $J = 2.7$, 1.4 Hz, 1H), 2.97 (s, 1H), 1.43 (m, $J = 7.5$ Hz, 3H), 1.09 (d, $J = 7.5$ Hz, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 129.4, 124.2, 114.1, 105.2, 79.9. 75.9, 17.8, 11.7; GC-HRMS (EI^+): calcd for $C_{15}H_{25}NSi$ 247.17563 (M^+); found *m/z* 247.17605 (M^+).

General procedure for the synthesis of compound (11) and (12)

Iodoalkylpyrrole **4** or **5** (2 mmol) was dissolved in 10 ml of DMSO and heated to 110 °C. Sodium bicarbonate (336 mg, 4 mmol) was then added in one portion. The reaction mixture was stirred at this temperature for 90 min and monitored by TLC. The excess of DMSO was evaporated under reduced pressure and the residue was dissolved in Et_2O before washed with H_2O. The aqueous phase was extracted three times with Et_2O (3×30 ml), the combined organic phases were extracted with brine (1×50 ml), dried over $MgSO_4$ and filtered. All volatiles were removed under reduced pressure and the crude product was purified by column chromatography on silica gel using hexane:$CHCl_3$ with a gradient elution mixture (1:1, 1:2, 1:4).

5-[(1-triisopropylsilyl)pyrrol-3-yl]-pentan-1-al (11)

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.74 (t, $J = 1.9$ Hz, 1H), 6.69 (t, $J = 2.3$ Hz, 1H), 6.51 (m, 1H), 6.12 (dd, $J = 2.3$, 1.5 Hz, 1H), 2.51 (t, $J = 7.3$ Hz, 2H), 2.43 (dt, $J = 7.1$, 1.9 Hz, 2H), 1.74–1.55 (m, 4H), 1.41 (m, $J = 7.5$ Hz, 3H), 1.08 (d, $J = 7.5$, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.2, 125.6, 124.5, 121.3, 110.6, 44.0, 30.6, 26.8, 22.0, 18.0, 11.8; HRMS ($APCI^+$): calcd for $C_{18}H_{33}NOSi$ 308.24042 [$(M+H)^+$]; found *m/z* 308.24104 [$(M+H)^+$]; Yield 437 mg (71%).

10-[(1-triisopropylsilyl)pyrrol-3-yl]-decan-1-al (12)

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.76 (t, $J = 1.9$ Hz, 1H), 6.68 (t, $J = 2.3$ Hz, 1H), 6.51 (s, 1H), 6.14 (dd, $J = 2.3$ Hz, 1.4 Hz, 1H), 2.47 (t, $J = 7.5$ Hz, 2H), 2.41 (dt, $J = 7.4$, 1.9 Hz, 2H), 1.62 (quint, $J = 7.4$ Hz, 2H), 1.56 (quint, $J = 7.5$ Hz, 2H), 1.42 (m, $J = 7.5$ Hz, 3H), 1.35–1.23 (m, 10H), 1.09 (d, $J = 7.5$ Hz, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.2, 126.5, 124.0, 121.2, 110.7, 44.1, 31.2, 29.58, 29.56, 29.5, 29.3 overlap, 27.1, 22.3, 18.04. 11.86; HRMS ($APCI^+$): calcd for $C_{23}H_{43}NOSi$ 378.31867 [$(M+H)^+$]; found *m/z* 378.31776 [$(M+H)^+$]; Yield 438 mg (58%).

General procedure for the synthesis of compound (13) and (14)

Tetrabromomethane (665 mg, 2 mmol) was dissolved in 6 ml of dry CH_2Cl_2 and the solution was cooled to 0 °C using an ice-water bath. Triphenylphosphine (1.05 g, 4 mmol) was added under stirring which resulted in a change of colour from colourless to orange-yellow. After 10 min, the aldehyde **11** or **12** (1 mmol) was added as a solution in dry CH_2Cl_2 (2 ml). The reaction mixture was stirred for additional 30 min and then filtered through the plug of silica gel.

1-(triisopropylsilyl)-3-(6,6-dibromohex-5-en-1-yl) pyrrole (13)

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.69 (t, $J = 2.3$ Hz, 1H), 6.52 (s, 1H), 6.38 (t, $J = 7.3$ Hz, 1H), 6.14 (dd, $J = 2.4$, 1.5 Hz, 1H), 2.50 (t, $J = 7.3$ Hz, 2H), 2.12 (q, $J = 7.4$ Hz, 2H), 1.60 (quint, $J = 7.5$ Hz, 2H), 1.45 (quint, $J = 7.5$ Hz, 2H), 1.43 (m, $J = 7.5$ Hz, 3H), 1.09 (d, $J = 7.5$ Hz, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.1, 125.9, 124.2, 121.3, 110.8, 88.5, 33.1, 30.5, 27.6, 26.8, 18.05, 11.9; GC-HRMS (EI^+): calcd for $C_{19}H_{33}Br_2NSi$ 463.07294 (M^+); found *m/z* 463.07294 (M^+); Yield 412 mg (89%).

1-(triisopropylsilyl)-3-(11,11-dibromoundec-10-en-1-yl)pyrrole (14)

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.68 (t, J = 2.4 Hz, 1H), 6.50 (s, 1H), 6.38 (t, J = 7.3 Hz, 1H), 6.14 (dd, J = 2.4 Hz, 1.4 Hz, 1H), 2.47 (t, J = 7.5 Hz, 2H), 2.08 (q, J = 7.3 Hz, 1H), 1.55 (quint, J = 7.3 Hz, 2H), 1.48–1.36 (m, 5H), 1.35–1.23 (m, 10H), 1.09 (d, J = 7.5 Hz, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.1, 126.5, 124.0, 121.2, 110.7, 88.5, 33.2, 31.2, 29.7, 29.61, 29.57, 29.5, 29.2, 28.0, 27.2, 18.0, 11.9; HRMS (APCI$^+$): calcd for $C_{24}H_{43}Br_2NSi$ 534.15838 [(M+H)$^+$]; found m/z 534.15894 [(M+H)$^+$]; Yield 405 mg (76%).

General procedure for the synthesis of compound (15) and (16)

A solution of dibromoolefine **13** or **14** (1 mmol) in dry THF (4 ml) was treated with *n*-BuLi (1.18 ml, 2 mmol, 1.7 M solution in hexane) at − 78 °C under argon atmosphere. After being stirred for 1 h at − 78 °C, the reaction mixture was allowed to reach room temperature and the stirring was continued for an additional hour. The reaction was quenched with a saturated solution of NH_4Cl (30 ml) followed by extraction with $CHCl_3$ (3 × 25 ml), combined organic phases were washed with distilled water (1 × 50 ml), dried over $MgSO_4$, filtered and concentrated. The product was purified by column chromatography on silica gel using hexane:$CHCl_3$ (5:1) as the elution mixture.

1-(triisopropylsilyl)-3-(hex-5-yn-1-yl)pyrrole (15)

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.69 (t, J = 2.4, 1H), 6.52 (m, 1H), 6.14 (dd, J = 2.5, 1.4, 1H), 2.51 (t, J = 7.3 Hz, 2H), 2.20 (dt, J = 7.1, 2.6 Hz, 2H), 1.93 (t, J = 2.6 Hz, 1H), 1.75–1.51 (m, 4H), 1.42 (m, J = 7.5 Hz, 3H), 1.09 (d, J = 7.5 Hz, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 125.9, 124.2, 121.2, 110.7, 85.0, 68.1, 30.2, 28.4, 26.6, 18.4, 18.0, 11.9; HRMS (APCI$^+$): calcd for $C_{19}H_{33}NSi$ 304.24550 [(M+H)$^+$]; found m/z 304.24601 [(M+H)$^+$]; Yield 291 mg (96%).

1-(triisopropylsilyl)-3-(undec-5-yn-1-yl)pyrrole (16)

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.69 (t, J = 2.4 Hz, 1H), 6.51 (m, 1H), 6.13 (dd, J = 2.4, 1.4 Hz, 1H), 2.47 (t, J = 7.5 Hz, 2H), 2.17 (dt, J = 7.1, 2.6 Hz, 2H), 1.93 (t, J = 2.6 Hz, 1H), 1.60–1.48 (m, 2H), 1.46–1.36 (m, 5H), 1.34–1.24 (m, 10H), 1.09 (d, J = 7.5 Hz, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 126.5, 124.0, 121.2, 110.7, 85.0, 68.16, 31.6, 29.66, 29.61, 29.58, 29.3, 28.9, 28.7, 27.1, 18.6, 18.0, 11.9; HRMS (APCI$^+$): calcd for $C_{24}H_{43}NSi$ 374.32375 [(M+H)$^+$]; found m/z 374.32419 [(M+H)$^+$]; Yield 354 mg (95%).

General procedure for the synthesis of compound (17–19)

Acids **3, 6** or **7** (0.662 mmol), 6^A-deoxy-6^A-amino-β-CD (500 mg, 0.441 mmol) and PyBOP (275 mg, 0.529 mmol) were dissolved in 11 ml of dry DMF. Then DIPEA (154 µl, 0.882 mmol) was added and the reaction mixture was stirred for 16 h at room temperature under argon atmosphere. Triethylamine (614 µl, 4.41 mmol) together with HF (44 µl, 0.882 mmol, 35% solution in H_2O) were added and the reaction mixture was stirred for an additional hour. All volatiles were evaporated under reduced pressure and the oily residue precipitated with acetone (100 ml). The white solid was collected by filtration on sintered glass funnel, washed with acetone, dichloromethane and ethyl acetate.

N-(6^A-Deoxy-β-cyclodextrin-6^A-yl)-1*H*-pyrrole-3-carboxamide (17)

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.05 (s, 1H), 7.44 (t, J = 5.4 Hz, 1H), 7.28 (m, 1H), 6.71 (dd, J = 4.6, 2.2 Hz, 1H), 6.42 (m, 1H), 6.01–5.39 (m, 14H), 4.95–4.65 (m, 7 H), 4.61–4.28 (m, 6H), 4.14–3.02 (m, 42H, overlap H_2O); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.3, 120.4, 119.4, 118.3, 107.2, 102.4–101.7, 101.2, 84.3, 81.9–81.3, 80.6, 73.5, 73.3–71.6, 70.3, 60.3–59.3; HRMS (ESI$^+$): calcd for $C_{47}H_{74}N_2O_{35}$ 1249.3964 [(M+Na)$^+$]; found m/z 1249.3961 [(M+Na)$^+$]; Yield 449 mg (83%).

N-(6^A-Deoxy-β-cyclodextrin-6^A-yl)-6-(1*H*-pyrrol-3-yl)hexanamide (18)

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.34 (s, 1H), 7.57 (t, J = 5.2 Hz, 1H), 6.60 (dd, J = 4.5, 2.3 Hz,), 6.49 (m, 1H), 6.11–5.25 (m, 15H), 5.04–4.71 (m, 7H), 4.62–4.32 (m, 6H), 3.94–3.02 (m, 42H, overlap H_2O), 2.36 (t, J = 7.5 Hz, 2H), 2.08 (m, 2H), 1.57–1.39 (m, 4H), 1.26 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.5, 122.5, 117.2, 114.5, 107.4, 102.3–101.6, 81.7–81.2, 73.2–71.8, 72.0, 60.2–59.5, 35.2, 30.7, 28.6, 26.6, 25.2; HRMS (ESI$^+$): calcd for $C_{52}H_{84}N_2O_{35}$ 1319.4747 [(M+Na)$^+$]; found m/z 1319.4743 [(M+Na)$^+$]; Yield 492 mg (86%).

N-(6^A-Deoxy-β-cyclodextrin-6^A-yl)-11-(1*H*-pyrrol-3-yl)undecanamide (19)

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.33 (s, 1H), 7.56 (t, J = 5.2 Hz, 1H), 6.59 (dd, J = 4.7, 2.4 Hz, 1H), 6.48 (m, 1H), 6.03–5.43 (m, 15H), 4.95–4.71 (m, 7H), 4.56–4.32 (m, 6H), 3.91–3.00 (m, 42H, overlap H_2O), 2.36 (t, J = 7.4 Hz, 2H),

2.08 (m, 2H), 1.56–1.36 (m, 4H), 1.35–1.04 (m, 12H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.5, 122.5, 117.2, 114.5, 107.4, 102.3–101.6, 83.6, 81.7–81.2, 73.1–72.9, 60.1–59.7, 35.1, 31.0, 29.05, 29.00, 28.96, 28.92, 28.78, 28.71, 26.6, 25.2; HRMS (ESI$^+$): calcd for $C_{52}H_{84}N_2O_{35}$ 1389.5529 [(M+Na)$^+$]; found *m/z* 1389.5522 [(M+Na)$^+$]; Yield 428 mg (71%).

General procedure for the synthesis of compound (20–22)

A flask was charged with 6^A-deoxy-6^A-amino-β-CD (500 mg, 0.431 mmol), pyrrole **10, 15** or **16** (0.647 mmol) and ascorbic acid (11 mg, 64.7 µmol as a solution in 0.5 ml of H_2O). Subsequently, a mixture of DMSO/H_2O (4:1, 38 ml) was added and argon was bubbled through the solution for 20 min. Next, TBTA (46 mg, 86.2 µmol) was added and the reaction mixture was heated to 60 °C under argon atmosphere. Finally, $CuSO_4$ (3.5 mg, 21.6 µmol as a solution in 500 µl of H_2O) was added via a cannula over 5 min and the stirring was continued for 16 h. The solvents were evaporated under reduced pressure and the solid residue was precipitated with acetone (100 ml), filtered and vacuum-dried. The TIPS-protected intermediate was dissolved without further purification in DMF (10 ml) and triethylamine (600 µl, 4.31 mmol) together with HF (43 µl, 0.862 mmol, 35% solution in H_2O) were added. The reaction mixture was stirred for 1 h before poured into acetone (150 ml). The white precipitate was collected by filtration on sintered glass funnel, washed with acetone, dichloromethane, ethyl acetate and dried.

6^A-Deoxy-6^A-[4-(1*H*-pyrrol-3-yl)-1,2,3-triazol-1-yl]-β-cyclodextrin (20)

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.85 (s, 1H), 8.06 (s, 1H), 7.11 (m, 1H), 6.76 (m, 1H), 6.34 (m, 1H), 5.85–5.47 (m, 14H), 5.05 (d, J = 3.4 Hz, 1H), 4.90–4.73 (m, 6H), 4.64–4.39 (m, 6H), 4.23 (m, 1H), 4.10 (m, 1H), 3.96–2.90(m, 40H); ^{13}C NMR (100 MHz,, DMSO-d_6) δ 143.7, 128.7, 119.8, 118.4, 114.9, 114.0, 105.8, 102.3-101.7, 101.0, 83.6, 82.2, 81.5, 81.4, 81.2, 80.5, 73.3–71.7, 69.8, 60.4–60.2, 60.0-59.4, 58.4; HRMS (ESI$^+$): calcd for $C_{48}H_{74}N_4O_{34}$ 1273.4077 [(M+Na)$^+$]; found *m/z* 1273.4075 [(M+Na)$^+$]; Yield 475 mg (88%).

6^A-Deoxy-6^A-{4-[4-(1*H*-pyrrol-3-yl)-but-1-yl]-1,2,3-triazol-1-yl}-β-cyclodextrin (21)

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.35 (s, 1H), 7.74 (s, 1H), 6.60 (dd, J = 4.6, 2.3 Hz, 1H), 6.50 (m, 1H), 5.90–5.55 (m, 15H), 5.03 (d, J = 3.3 Hz, 1H), 4.89–4.72 (m, 6H), 4.63–4.38 (m, 6H), 4.29 (m, 1H), 4.06–2.79 (m, 41H), 2.59 (t, J = 7.4 Hz, 2H), 2.41 (t, J = 7.3 Hz, 2H), 1.68–1.47 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 146.8, 122.7, 122.4, 117.2, 114.6, 107.5, 102.3–101.8, 101.1, 83.5, 82.0, 81.6–81.3, 80.7, 73.3–72.8, 72.6–71.7, 70.0, 62.5, 60.2–59.7, 58.8, 30.7, 28.8, 26.4, 24.9; HRMS (ESI$^+$): calcd for $C_{52}H_{82}N_4O_{34}$ 1329.4703 [(M+Na)$^+$]; found *m/z* 1329.4681 [(M+Na)$^+$]; Yield 485 mg (86%).

6^A-Deoxy-6^A-{4-[9-(1*H*-pyrrol-3-yl)non-1-yl]-1,2,3-triazol-1-yl}-β-cyclodextrin (22)

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.33 (s, 1H), 7.75 (s, 1H), 6.59 (dd, J = 4.7, 2.4 Hz, 1H), 6.48 (m, 1H), 6.03–5.37 (m, 15H), 5.03 (d, J = 3.5 Hz, 1H), 4.92–4.72, (m, 6H), 4.61–4.35 (m, 6H), 4.28 (t, J = 5.8 Hz, 1H), 4.13–2.82 (m, 41H), 2.56 (t, J = 7.6 Hz, 2H), 2.36 (t, J = 7.6 Hz, 2H), 1.58 (m, 2H), 1.48 (m, 2H), 1.43–1.03 (m, 10H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 146.8, 122.7, 122.5, 117.2, 114.5, 107.4, 102.2-101.7, 101.1, 83.5, 82.2–81.9, 81.6–81.3, 80.7–80.6, 73.3–71.7, 70.0, 60.2–59.7, 58.9–58.8, 31.0, 29.08, 28.95, 28.94, 28.93, 28.81, 28.78, 26.6, 25.0; HRMS (ESI$^+$): calcd for $C_{57}H_{92}N_4O_{34}$ 1399.5485 [(M+Na)$^+$]; found *m/z* 1399.5489 [(M+Na)$^+$]; Yield 469 mg (79%).

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Short Communication

The study of enantioselectivity of all regioisomers of mono-carboxymethyl-β-cyclodextrin used as chiral selectors in CE

This work documents the influence of the position of single carboxymethyl group on the β-cyclodextrin skeleton on the enantioselectivity. These synthesized monosubstituted carboxymethyl cyclodextrin (CD) derivatives, native β-cyclodextrin, and commercially available carboxymethyl-β-cyclodextrin with degree of substitution approximately 3 were used as additives into the BGE consisting of phosphate buffer at 20 mmol/L concentration, pH 2.5, and several biologically significant low-molecular-mass chiral compounds were enantioseparated by CE. The results indicate that different substituent location on β-cyclodextrin skeleton has a significant influence on the enantioseparation of the investigated enantiomers. The enantioselectivity of 2^1-*O*-regioisomer was better than with native β-cyclodextrin. Comparable results to native β-cyclodextrin were obtained for 6^1-*O*- regioisomer and the enantioselectivity of 3^1-*O*-regioisomer was even worse than with native β-cyclodextrin. Commercially available derivative of CD provides better resolutions than the monosubstituted carboxymethyl CD derivatives for most of the investigated analytes.

Keywords: CE / Chiral separation / Enantioselectivity / Monosubstituted carboxymethyl-β-cyclodextrin / Regioisomer
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1 Introduction

Cyclodextrins (CDs) and their derivatives are one of the most used chiral selectors in CE due to their ability to complex many substances in their cavity, stability, solubility, and low-UV absorption [1–3]. The most common CDs are α-, β-, and γ-CDs formed by 6, 7, or 8 α-1,4-linked D-glucopyranose units.

Specifically, β-CD is the most used chiral selector in CE [4–7], however, even this relatively universally applicable CD has its own limitations. For this reason, various charged CDs, cationic and anionic CD derivatives, have been synthesized and utilized as chiral additives in CE [8]. Cationic CDs are mostly amino derivatives and common anionic CDs are sulfated, sulfoalkylated, and carboxymethylated derivatives [2, 9–11].

Carboxymethylated β-CDs were used in CE for the first time in 1985 [12]. Nowadays mainly randomly substituted carboxymethylated β-CDs have been applied as chiral selectors for chiral separations of cathinone derivatives [13], quantification of zopiclone enantiomers and its impurities [14], enantioseparation of pramipexole and its *R*-enantiomer [15], lignans in plants [16], and several antimalarial drugs (primaquine, tafenoquine, mefloquine, chloroquine, and quinacrine) [17].

Although randomly substituted CDs are the most frequently used derivatives, because of their facile commercial availability, their application often leads to problems related to the limited reproducibility of the obtained chiral resolution for analytes, as a consequence of limited batch-to-batch reproducibility in their synthesis [18]. Moreover, we have shown previously [19] that different mono-carboxymethyl regioisomers of α-CD had different enantioseparation abilities. On the other hand, it is known that multichiral selector systems can be more selective than the single-chiral selector systems in some cases as the other authors have demonstrated [20–22].

In this work we used all three individual regioisomers of monosubstituted β-CD, specifically, 2^1-*O*-, 3^1-*O*-, and 6^1-*O*-carboxymethyl-β-CDs, then a mixture of these three isomers in molar ratio 1:1:1, and a randomly substituted commercial carboxymethyl-β-cyclodextrin with degree of substitution approximately 3 (RCMBCD), for our chiral CE experiments. The goal of the work was to critically evaluate the influence of the substituent position on chiral recognition of selected compounds.

Various biologically relevant racemic mixtures of chiral compounds (Fig. 1) were used for the investigation of the

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Abbreviations: CD, cyclodextrin; **CMACD**, carboxymethyl-α-cyclodextrin; **CMBCD**, carboxymethyl-β-cyclodextrin; **2CMBCD**, 2^1-*O*-carboxymethyl-β-cyclodextrin; **3CMBCD**, 3^1-*O*-carboxymethyl-β-cyclodextrin; **6CMBCD**, 6^1-*O*-carboxymethyl-β-cyclodextrin; **RCMBCD**, carboxymethyl-β-cyclodextrin with average degree of substitution ~3; **TB**, Tröger's base

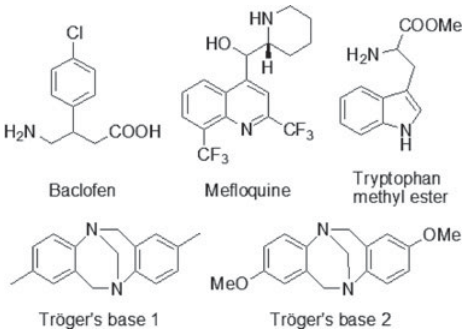

Figure 1. Structures of investigated low-molecular-mass chiral compounds.

enantioselectivity changes related to the utilization of individual three regioisomers of mono-carboxymethyl-β-CD as additives to the BGE in CE. All the analyzed chiral compounds had an appropriate chromophore allowing for the UV-VIS detection and basic group(s) allowing electrostatic interaction between the analyte and the chiral anionic selector (carboxylic group of the CMBCD). Specifically, baclofen (analog of γ-aminobutyric acid extensively used as a stereoselective agonist for gamma-aminobutyric acid B receptor), Tröger's bases (TB1 and TB2; chiral heterocyclic amines applicable for the recognition of bioactive compounds), mefloquine (antimalarial drug), and tryptophan methyl ester (amino acid derivative) were chosen for the experiments.

Racemic mixtures of baclofen [23], mefloquine [17], TB1, and TB2 have been previously separated by native β-CD, and tryptophan methyl ester enantiomers has been resolved by heptakis(2,6-di-*O*-methyl)-β-cyclodextrin [24] in CE mode.

2 Materials and methods

2.1 Chemicals and materials

Hydrochloric acid (30%) (Suprapur, Merck, Germany), 1 mol/L sodium hydroxide (Tripur, Merck, Germany), ACN (99.8%, LiChrosolv, Merck, Germany), orthophosphoric acid (50%), (5*S*,11*S*)-(+)-2,8-dimethyl-6*H*,12*H*-5,11-methano-dibenzo[b,f][1,5]diazocin (Tröger's base 1, TB1, 99.5%), baclofen (98%), tryptophan methyl ester hydrochloride (98%), mefloquine hydrochloride (98%), dimethyl sulfoxide (DMSO, 99.7%), β-cyclodextrin (βCD, 97%), carboxymethyl-β-cyclodextrin sodium salt (RCMBCD, average degree of substitution∼3), (all Sigma-Aldrich, Czech Republic), and ultrapure water (Milli-Q grade, Millipore, France) were used. 2,8-Dimethoxy-6*H*,12*H*-5,11-methanodibenzo[b,f][1,5]diazocin (Tröger's base 2, TB2) was prepared in the group of professor Kral. 2^1-*O*-, 3^1-*O*-, and 6^1-*O*-carboxymethyl-β-CDs (2CMBCD, 3CMBCD, and 6CMBCD) were synthesized by following reaction steps: monoalkylation (allylation or cinnamylation), peracetylation, oxidation of double bond, and Zemplene deacetylation [25–28]. The purity of CMBCDs was determined by NMR and for all derivatives was >95%.

2.2 Equipment

CE separations were performed with an Agilent CE instrument (Agilent 3D HPCE, Germany) equipped with UV-Vis diode-array detector. Bare fused-silica capillaries of 375/75 μm od/id and 58.5/50 cm total/effective length obtained from Polymicro Technologies (AZ, USA) were used.

2.3 CE conditions

Tryptophan methyl ester and baclofen were dissolved in water, mefloquine was dissolved in ACN, Tröger's bases were dissolved in DMSO, each at 10 mmol/L concentration. For the CE experiments, the analyte solutions were further diluted with water to the final concentration 1 mmol/L with the exception of TB2 that was diluted to the final concentration 0.2 mmol/L. The BGE was composed of 20 mmol/L sodium phosphate buffer, pH 2.5, and 10 mmol/L β-CD, RCMBCD, the individual monosubstituted CMBCDs (2CMBCD, 3CMBCD, and 6CMBCD), respectively, or the mixture of monosubstituted CMBCDs in molar ratio 1:1:1 at the total concentration 10 mmol/L (each at concentration 3.33 mmol/L). A new fused-silica capillary was first rinsed with 0.1 mol/L NaOH for 30 min, then with H_2O for 30 min. Between runs the capillary was rinsed for 2 min with 0.1 mol/L NaOH, for another 2 min with H_2O and finally 2 min with running buffer from a vial different from the one used for a subsequent analysis. The analytes were injected hydrodynamically by pressure of 1.5 kPa for 5 s. All the separations were performed at 20 kV (anode at the injection capillary end) with a voltage ramp time of 12 s. Detection was carried out at 207 nm and the capillary was thermostated at 25°C during the analyses.

3 Results and discussion

In our work, all the enantioseparations of the investigated compounds were carried out at pH 2.5. Such environment was intentionally chosen as at this pH, we obtained the best enantioseparation for similar analytes using monosubstituted carboxymethyl-α-CDs (CMACDs) [19]. At such low pH, the analytes are positively charged, on the other hand, CMBCDs are only partially dissociated. Thus, it looks like that electrostatic interaction does not play a significant role in the enantiorecognition. This seems to be a valid deduction also as it has been shown previously that at higher pH, when CMBCDs are dissociated completely, no separation of enatiomers has been obtained [19]. The application of 2CMBCD

Table 1. Migration times[a] (t_m), resolutions[b] (R), and numbers of theoretical plates[c] (N) measured for racemic mixtures of the investigated compounds using native β-CD, 2^I-*O*-CMBCD, 3^I-*O*-CMBCD, 6^I-*O*-CMBCD, RCMBCD, and the mixture of monosubstituted CMBCDs in ratio 1:1:1 at pH 2.5

Analyte		Selector					
		β-CD	2^I-*O*-CMBCD	3^I-*O*-CMBCD	6^I-*O*-CMBCD	RCMBCD	Mixture of CMBCDs
Baclofen	t_{m1} (min)	9.19	9.59	9.77	9.47	11.49	12.73
	t_{m2} (min)	9.19	9.63	9.79	9.47	11.59	12.73
	R	0	<0.5	<0.5	0	<0.5	0
	N_1 (m^{-1})	d)	d)	d)	d)	d)	d)
	N_2 (m^{-1})	d)	d)	d)	d)	d)	d)
Mefloquine	t_{m1} (min)	9.76	10.97	10.95	10.19	17.49	10.30
	t_{m2} (min)	9.79	10.98	11.21	10.19	21.79	10.43
	R	<0.5	0.5	1.0	0	6.9	<0.5
	N_1 (m^{-1})	d)	d)	94 000	d)	45 000	d)
	N_2 (m^{-1})	d)	d)	70 000	d)	25 000	d)
Tröger's base 1	t_{m1} (min)	17.63	16.61	16.77	19.73	28.70	15.08
	t_{m2} (min)	18.20	17.35	17.00	20.41	30.32	15.60
	R	1.9	2.8	0.7	2.1	3.7	1.8
	N_1 (m^{-1})	162 000	126 000	d)	139 000	172 000	109 000
	N_2 (m^{-1})	127 000	138 000	d)	139 000	173 000	100 000
Tröger's base 2	t_{m1} (min)	12.87	17.16	18.09	18.69	3.59	13.71
	t_{m2} (min)	13.25	17.81	18.42	19.34	3.59	14.15
	R	1.9	2.5	1.3	2.0	0	1.9
	N_1 (m^{-1})	143 000	159 000	169 000	97 000	d)	156 000
	N_2 (m^{-1})	121 000	128 000	142 000	116 000	d)	136 000
Tryptophan methyl ester	t_{m1} (min)	8.69	9.16	9.19	9.00	7.59	8.49
	t_{m2} (min)	8.71	9.25	9.21	9.06	7.77	8.50
	R	<0.5	<0.5	<0.5	<0.5	0.9	<0.5
	N_1 (m^{-1})	d)	d)	d)	d)	d)	d)
	N_2 (m^{-1})	d)	d)	d)	d)	d)	d)

a) t_{m1} corresponds to the first migrating peak, t_{m2} corresponds to the second migrating peak.
b) Resolution was calculated as $R = (t_{m2} - t_{m1})/(0.85 \times (W_1 + W_2))$, where W_1 and W_2 are peak widths at their half heights for the first and second migrating peak.
c) N_1 corresponds to the first migrating peak, N_2 corresponds to the second migrating peak; number of theoretical plates was calculated as $N = 5.54 \times (t_m/W)^2$.
d) Due to insufficient resolution of the peaks, the calculation of theoretical plates was impossible.

and 3CMBCD as additives to the BGE allowed for at least partial enantioseparations of all five investigated compounds (Table 1, Fig. 2). The 2CMBCD provided two baseline separations ($R \geq 1.5$) of Tröger's bases and three partial separations ($R < 1.5$) for the other three analytes. Five partial separations were achieved with 3CMBCD and in comparison to 2CMBCD lower resolutions were found with the exceptions for baclofen and mefloquine. The utilization of 6CMBCD as a chiral selector led to the baseline enantioseparation for two racemates only (Tröger's bases) and a slight indication of separation for tryptophan methyl ester was achieved. Two other compounds showed no enantioseparation. Values of resolution for TBs and tryptophan methyl ester in the presence of 6CMBCD ranged between values of resolution achieved with 2CMBCD and 3CMBCD (Table 1). Comparing the resolutions reached for TBs with the assistance of 2CMBCD, it can be clearly seen that the enantioseparation improved with respect to the application of the native β-CD as the chiral BGE additive. The resolutions of the analytes in the presence of the native β-CD and 6CMBCD were comparable. The commercial RCMBCD provided better resolutions than the single-isomer CMBCDs probably due to its higher degree of substitution (~3) with carboxymethyl groups. On the other hand for TB2, no separation was attained. Moreover, as already mentioned in the introduction, the significant drawback of the application of the commercial RCMBCD consists of a limited reproducibility of its synthesis leading to significant variability of the composition of the resulting product and this may result in fluctuation of migration times and resolution values obtained for the chiral analytes with different batches of RCMBCD [18]. Finally, the application of a mixture of three regioisomers of CMBCDs (in molar ratio 1:1:1) provided expected results as the obtained resolutions were close to the values obtained by averaging resolutions for the individual single-isomer CMBCDs. Thus, no improvement in separation selectivity as a consequence of a synergic effect of the three regioisomers in the mixture discussed by other authors [20–22] was observed, probably because the differences in the corresponding limit

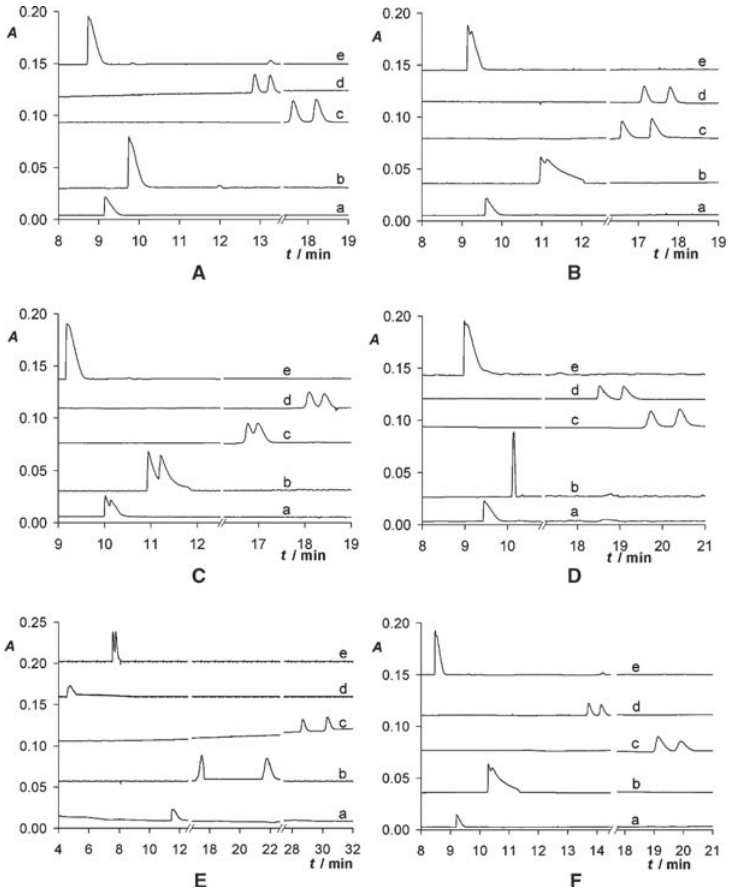

Figure 2. Electropherograms of the analyzed racemic mixtures of the studied compounds; Separated analytes: (A) baclofen; (B) mefloquine, (C) Tröger's base 1; (D) Tröger's base 2; (E) tryptophan methyl ester; used chiral selectors: A native β-CD; B 2^{I}-*O*-CMBCD; C 3^{I}-*O*-CMBCD; D 6^{I}-*O*-CMBCD; E RCMBCD; F 1:1:1 molar mixture of the individual monosubstituted CMBCDs; BGE: 20 mmol/L sodium phosphate, pH 2.5, 10 mmol/L chiral selector; fused-silica capillary: od/id = 375/75 μm, total/effective length 58.5/50.0 cm; voltage 20 kV; temperature 25°C.

mobilities of the individual regioisomers were too small to have any significant impact on the enantioseparation.

The above-mentioned observations correspond to our results obtained with 2^{I}-*O*-, 3^{I}-*O*-, and 6^{I}-*O*- CMACDs [19], which means that the substituent position in CMACD has a significant influence on its resulting enantioselectivity. Further, as expected, the separation abilities of α- and β-CD derivatives differ due to different size of their cavities. For instance for most of the investigated compounds, the best resolutions were achieved with the use of 2^{I}-*O*- derivative for CMBCD. On the other hand, the best resolutions were achieved with the application of 3^{I}-*O*- derivative for CMACD [19]. The use of 6^{I}-*O*- derivatives gave similar results comparable to native CDs indicating that for both CMACD and CMBCD interactions with the investigated analytes probably occur at secondary (wider) rim of CDs bearing hydroxyl groups at C2 and C3. Thus, the substitution at 6^{I}-*O*- position has no effect on enantiorecognition ability of the resulting CD derivative.

4 Concluding remarks

The influence of the carboxymethyl substituent location in mono-carboxymethyl-β-cyclodextrin on the enantioselectivity of biologically important compounds separated by chiral CE was studied. The results of the analyses, carried out in 20 mmol/L phosphate buffer at pH 2.5, demonstrate a significant influence of the carboxymethyl group position on the enantioseparations. Chiral separation of the analytes separated with 2^{I}-*O*-CMBCD added into the BGE provided better resolutions than with the native β-CD. On the other hand for 6^{I}-*O*-CMBCD comparable and for 3^{I}-*O*-CMBCD worse results to the native β-CD were obtained.

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Microreview

Substituted Cyclodextrins

Monosubstituted Cyclodextrins as Precursors for Further Use

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Abstract: Cyclodextrin derivatives find use in a broad field of applications (e.g., nanomaterials, biomedical applications, catalysis, separation techniques, sensors etc.), with monosubstitution allowing cyclodextrins to be attached to various types of surfaces or for a new feature to be introduced onto a cyclodextrin skeleton (recognition, catalysis, ...). Although an enormous number of monosubstituted cyclodextrin derivatives have been synthesised, this review focuses only on the synthesis of several interesting monosubstituted derivatives (allyl, cinnamyl, propargyl, formylmethyl, carboxymethyl, azido and amino) suitable for further modifications. These derivatives allow the synthesis of an unlimited number of desired cyclodextrin derivatives. Using a cyclodextrin derivative already monosubstituted with a suitable functional group is much easier than the optimisation of monosubstitution for every new cyclodextrin derivative desired.

1. Introduction

Cyclodextrins (CDs) have been known for 125 years. During that time CDs have become part of our lives due to their main feature – the ability to form supramolecular complexes with a great number of compounds.

The first mention of the substance that was later identified as cyclodextrin was published by Villiers[1] in 1891. The digestion of starch by *Bacillus amylobacter* (apparently the culture was not pure and also contained *Bacillus macerans*) yielded about 3 g of the crystals when starting from 1 kg of starch. The structure of this new compound was determined as $(C_6H_{10}O_5)_2 \cdot 3\,H_2O$. Villiers named the compound "celulosine", due to its resistance to acid hydrolysis – similar to that of cellulose – and because it also showed no reducing ability.

Twelve years later Schardinger studied starch digestion by microorganisms and isolated small quantities of two crystalline products.[2] These substances appeared identical to "celulosines" isolated by Villiers. Schardinger's next research laid the foundations of cyclodextrin chemistry. An interesting review on the history of CDs can be found in the recent publication by Crini.[3]

Over the years, the interest in CDs continued to grow. In 1981 the first international conference on CDs was held and CDs are currently widely used in our daily life. They are used mainly as food additives[4–6] (β-CD is approved in the European Union as food additive E 459), in pharmaceutics[7] or in cosmetics.[8]

1.1. Structures, Properties and Production of Cyclodextrins

CDs are cyclic oligosaccharides composed of α-D-glucopyranose units, which are linked together through α(1→4) glycosidic bonds. The most widely used CDs are referred to as α-, β- or γ-CD, with six, seven or eight glucopyranose units, respectively, in the macrocycle (Figure 1). However, there are many other known types of CDs. A CD with five glucose units in the cycle is called pre-α-CD and it was prepared by a synthetic route.[9] CDs can also contain more than eight glucose units. Although there are known CDs with dozens (or even several hundreds) of glucose units,[10–12] their use is scarce.

Cyclodextrins have the shape of a hollow truncated cone with primary hydroxy groups on the narrow (primary) rim (usually depicted as the bottom) and with secondary hydroxy groups on the wider (secondary, upper) rim (Figure 2). C–H bonds and glycosidic oxygen atom bridges are located inside

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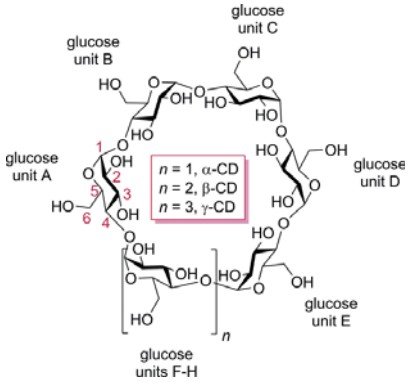


Figure 1. Structures and numbering of the most widely used CDs.

the cavity. This nonpolar environment is the cause of the significant lipophilicity of the cavity, whereas the high numbers of hydroxy groups on both rims result in good solubility of cyclodextrins in water (except in the case of β-CD).

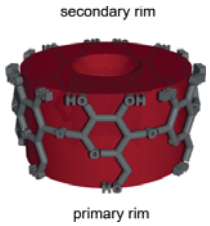


Figure 2. Shape of CDs.

CDs are fairly stable under standard experimental conditions. They are stable in alkaline solutions, and also, to a certain extent, resistant to acid hydrolysis (which occurs at a pH lower than 3.5 and temperatures higher than 60 °C).[13]

CDs are regarded as nontoxic,[14] as well as nonirritant to skin, eyes and mucosa upon inhalation.[15] When orally administered, only negligible amounts (<1 %) of CDs are absorbed, due to their nature and size. Absorbed CDs are almost exclusively excreted in urine without being significantly metabolised.[16]

Cyclodextrins are produced by the enzyme cyclomaltodextrin glucanotransferase (EC 2.4.1.19), synthesised by various microorganisms such as *B. macerans*, *Klebsiella oxytoca* and many others.[17] In nature, the synthesis of CDs by these microorganisms provides them with a monopoly on starch, because competing microorganisms are unable to utilise CDs.[18]

Demand for cyclodextrins is constantly increasing, so manufacturers have been devoting great efforts to improving the production process, either by modifying the properties of cyclomaltodextrin glucanotransferases or by finding better complexing agents to separate the desired CDs from their mixtures.[4,19,20]

1.2. Inclusion Complexes of Cyclodextrins

An important property of CDs is their ability to form inclusion complexes (host–guest complexes) with a wide variety of solid, liquid and gaseous compounds. These compounds (guests) should have suitable structural properties to allow them to occupy the cavities of CDs or at least to interact with the outer rims. The strength of the interaction depends on the steric conformity between the guest and the interior of the cavity and interactions between the atoms of the CD and the guest. It was believed that in aqueous solutions the nonpolar CD cavity is filled with polar molecules of water, to which this location is energetically disadvantageous. The entry of a nonpolar guest of a size and shape suitable for the CD cavity would thus reduce the energy of the system.[21] However, Liu and Guo[22] concluded that van der Waals and hydrophobic interactions are the major driving forces for the complexation process, whereas electrostatic interaction and hydrogen bonding can significantly affect the formation of the inclusion complex.

The cavity of a CD is a suitable host for a wide variety of organic compounds (predominantly of lipophilic character), and their complexation constants with CDs can be found in the review by Rekharsky and Inoue.[23] Although CD complexes usually have a CD/guest ratio of 1:1, many other CD/guest ratios are known,[24] and CDs can even form a wide variety of other supramolecular structures including catenanes and rotaxanes.[25,26]

2. General Methods for Synthesis and Characterisation of Monosubstituted Derivatives

CDs are modified for many reasons – for increased solubility or complexation properties, for attachment to various types of surfaces, or for introduction of a new feature onto a CD skeleton.

Strategies for modification depend on the purpose of the product. For example, if a more soluble CD is required by the pharmaceutical industry, then random conversion of hydroxy groups into, for example, sulfate (or other hydrophilic) groups is performed. However, the products are not single isomers and will differ in the detailed relative proportions of individual isomers from batch to batch.[27]

When a single-isomer CD derivative is desired, the easiest way is through the preparation of a persubstituted derivative. The direct synthesis of other single-isomer derivatives is more difficult, due to the number of theoretically possible isomers.[28] For a given CD, there are three monosubstituted, dozens of disubstituted, and more than one hundred trisubstituted isomers possible. However, scientists have been devoting great efforts to add more than one functional group to a CD skeleton. The efforts spent on this work have paid off, and many selectively multiply substituted CDs have been prepared.[29–34]

Besides the substituted CDs described above, there are also other single-isomer derivatives – CDs persubstituted at the 2-, 3- or 6-positions. They are often used in separation techniques,[35] as building blocks[36] or in nanotechnology.[37]

Of all these CD derivatives, the monosubstituted variants have achieved a prominent position because of their versatility. Monosubstituted derivatives are used in catalysis,[38–41] separation techniques,[35,42–44] biomedical applications,[45–47] nanotechnologies[37,48] and sensors,[49] or as components of supramolecular assemblies.[50–54]

The number of known monosubstituted CD derivatives is enormous and their listing would exceed the scope of this review. Thus, the aim of this paper is to provide a comprehensive view on the synthesis of monosubstituted derivatives suitable for further modifications. The reason for this approach is obvious – the modification of a CD already monosubstituted with a suitable functional group is much easier than the optimisation of monosubstitution for every new CD derivative desired.

This review therefore focuses only on α-, β- or γ-CDs monosubstituted with allyl, cinnamyl, propargyl, formylmethyl, carboxymethyl, azido or amino groups.

Allyl derivatives of CDs have been successfully applied in cross-metathesis[55–57] and, together with cinnamyl derivatives, could be oxidised to formylmethyl or carboxymethyl functional groups (see below). These derivatives could be then used for the Wittig reaction (so far applied only for formyl derivatives),[58–60] imine formation,[61,62] reductive amination[63] or the formation of amide bonds.[64,65] Amino-CD derivatives are used for amide formation as well.[66–69] Propargyl- and azido-CD derivatives are frequently used for copper(I)-catalysed azide–alkyne (Huisgen) cycloaddition.[36,70,71]

For other monosubstituted CD derivatives not covered by this review please refer to the previous ones.[38,72–76]

For the purposes of this review, the term "monosubstituted CDs" also refers to monosubstituted CDs with all the remaining hydroxy groups protected – methylated, acetylated, benzylated etc.

2.1. Strategies for Synthesis of Monosubstituted CD Derivatives

Monosubstitution of CDs is a great challenge for chemists. The hydroxy groups at positions 2, 3 and 6 compete against each other during substitution, thus making the process rather difficult. The hydroxy groups at position 6 are the most basic (least acidic and most accessible), those at position 2 are the most acidic, and those at position 3 are the least accessible. Moreover, the CD cavity tends to interfere with the substitution agent, making the functionalisation more complicated.[72]

When all of the hydroxy groups are deprotonated with an excess of a base, an electrophilic reagent will preferentially attack position 6, because it is most accessible. More reactive agents will attack the hydroxy groups less selectively, and therefore will react not only with the hydroxy groups at position 6, but also with the hydroxy groups on the secondary rim. Less reactive reagents will react selectively at position 6.

The hydroxy groups at position 2 are deprotonated first, because they are the most acidic (pK_a = 12.2).[74] The oxyanion formed is more nucleophilic than other non-deprotonated hydroxy groups. The use of a controlled amount of a strong base thus often predominantly leads to substitution at position 2.

Solvents play another important role during the substitution reaction. They can affect the nucleophilicity of oxyanions, as well as the strength and orientation of the complex of the substitution agent with the CD. If the complex is strong, the predominant product will be dictated by the orientation of the substitution agent in the cavity of the CD. In view of these factors, commonly achieved yields of monosubstituted derivatives are usually in the order of percent or up to a few dozen percent.

The methods for monosubstitution of cyclodextrins can be divided (according to the approach used) into two categories – direct and indirect:

a1) a "direct" method – in which a non-regiospecific substitution is performed and the CD derivatives obtained are purified by separation (chromatographic) methods,

a2) a "smart-direct" method – in which unique physicochemical features both of a CD and of a substitution agent are taken advantage of for a one-step and high-yielding substitution reaction,

a3) a "bio-direct" method – in which a monosubstituted CD is synthesised through an enzymatic reaction,

b1) an "indirect" method – in which several protection/deprotection steps allow the key monosubstitution step to be high-yielding,

b2) a "smart-indirect" method – in which selective high-yielding deprotection of persubstituted CDs is performed, and

b3) a "cyclisation-indirect" method – in which a monosubstituted CD is synthesised by cyclisation of an oligosaccharide.

The "direct" method was used, for example, for the synthesis of 2^A-*O*- and 3^A-*O*-cinnamyl-α-CD.[77] Treatment of α-CD with cinnamyl bromide gave these derivatives after purification by reversed-phase chromatography.

The most favoured of the monosubstitution reactions are the syntheses of 6^A-tosyl-β-CD (see Section 3.3). These methods could be regarded as "smart-direct" methods. Another example of this approach is the synthesis of 3^A-*O*-cinnamyl-β-CD.[78] This reaction takes advantage of the formation of a complex between the CD and cinnamyl bromide, which thus forms the monosubstituted product in a high yield. However, the number of "smart-direct" methods is limited and they cannot therefore be used for every CD derivative desired.

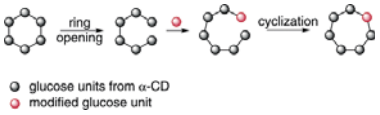
The use of the "bio-direct" methods has not been investigated thoroughly yet. CDs produced in this way are usually monosubstituted only with monosaccharides or esters.[79]

The "indirect" method often requires several reaction steps. It includes, for example, the synthesis of 2^A-*O*-carboxymethyl-β-CD.[80] The first step is the *tert*-butyldimethylsilylation of all hydroxy groups at positions 6. Subsequent alkylation with ethyl iodoacetate and deprotection steps yields 2^A-*O*-carboxymethyl-β-CD.

There are now several convenient methods available for the selective high-yielding deprotection of persubstituted CDs (see the beginning of Section 3). These methods allow one free hydroxy group to be obtained (while the others are still pro-

tected) for a subsequent modification. These methods are suitable for simple syntheses of almost any desired CD derivative and thus can be referred to as "smart-indirect" methods.

The "cyclisation-indirect" method is the most challenging variant because the synthesis is very difficult. Usually, the α-CD ring is opened, a new monosubstituted glucose unit is attached to the hexasaccharide, and the ring is closed again (Scheme 1).



Scheme 1. Synthesis of monosubstituted CDs by the "cyclisation" method.

2.2. NMR Characterisation of Monosubstituted Derivatives

There are currently two main methods used for determination of the position at which a substituent is attached to the CD skeleton: X-ray crystallography and NMR spectroscopy. However, NMR spectroscopy is used far more frequently than X-ray crystallography.

When determining whether a substituent is attached at position 2, 3 or 6, 2D NMR techniques (such as ^{1}H-^{1}H COSY, HSQC, HMBC) in combination with ^{1}H and ^{13}C (or APT or DEPT) are used. To find the substituent position in cases of *O*-substituted derivatives, HMBC cross-peaks are necessary to overcome the etheric oxygen. Once the atoms in the glucose unit bearing the substituent are determined, it is possible to determine the position of the substituent by using COSY and/or HMBC and HSQC (Figure 3).

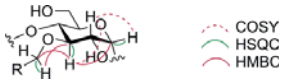


Figure 3. Determining substituent positions in mono-*O*-alkylated CD derivatives.

Although there have been attempts to determine the position of a substituent (a 4-sulfobutyl group) on the cyclodextrin skeleton solely from 1D spectra, it was only found that the signal of the C-6^A atom is shifted out of the usual region, unlike in the cases of the other two (2^A-*O* and 3^A-*O*) isomers.[81]

However, Řezanka and Jindřich[82] suggested a quick method for determining the position of a substituent on a CD skeleton by use only of ^{1}H NMR spectra measured in $CDCl_3$. This method is designed for peracetylated mono-*O*-substituted CD derivatives (Figure 4).

It was found that the signal of the hydrogen atom at position 2 on the glucose unit bearing the substituent (H-2^A) is shifted upfield to approximately 3.3 ppm. The characteristic signal – dd (approx. J = 10 and 3 Hz) – could thus be observed outside of the region usual for acetylated CDs (Figure 5, a).

When a CD is monosubstituted at position 3, the H-3^A signal is shifted upfield (approx. about 1.5 ppm). Unfortunately, this signal is thus overlapped with other peracetylated CD signals. However, this shift causes a decrease in the integral intensity of the H-3 signals by one (Figure 5, b).

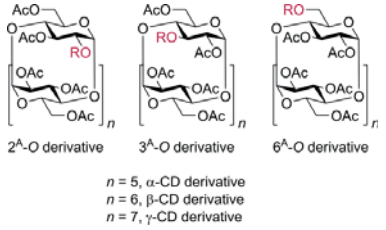


Figure 4. Peracetylated mono-*O*-substituted CD derivatives.

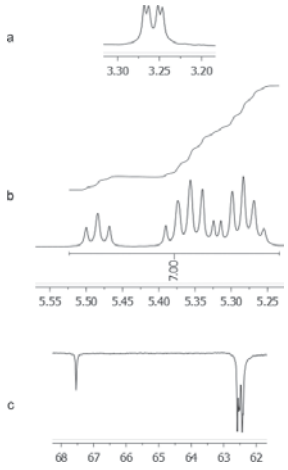


Figure 5. Characteristic NMR signals (a, b: ^{1}H NMR; c: DEPT) for peracetylated (a) 2^A-*O*-substituted, (b) 3^A-*O*-substituted, and (c) 6^A-*O*-substituted γ-CD derivatives.

Table 1. ^{1}H NMR ($CDCl_3$) shifts of H-2^A signals in otherwise peracetylated 2^A-*O*-substituted CD derivatives.

Entry	CD	Substituent	H-2^A signal (ppm)	Ref.
1	α-CD	allyl	3.32	[82]
2	α-CD	cinnamyl	3.38	[82]
3	α-CD	formylmethyl	3.34	[82]
4	α-CD	carboxymethyl	3.43	[82]
5	β-CD	allyl	3.27	[83]
6	β-CD	allyl	3.28	[55]
7	β-CD	propargyl	3.55	[84]
8	β-CD	carboxymethyl	3.38	[83]
9	β-CD	3,3-bis(chlorodimethylstannyl)propanyl	3.59	[84]
10	β-CD	4-(1,2-dicarbadodecaboran-1-yl)but-2-en-1-yl	3.34	[56]
11	β-CD	4-{8,8′-μ-(disulfido)-[3,3′-commocobalt(III)-bis(1,2-dicarbaundecaborate)]-8-yl}but-2-en-1-yl	3.34	[56]
12	β-CD	4-{8,8′-μ-(sulfido)-[3,3′-commocobalt(III)-bis(1,2-dicarbaundecaborate)]-8-yl}but-2-en-1-yl	3.24	[56]
13	β-CD	5,5,6,6,7,7,8,8,9,9,10,10,10-tridecafluorodec-2-en-1-yl	3.30	[55]
14	γ-CD	allyl	3.28	[85]
15	γ-CD	propargyl	3.48	[85]
16	γ-CD	formylmethyl	3.30	[85]
17	γ-CD	carboxymethyl	3.43	[85]

If none of the changes described above is observed, then the isomer is substituted in position 6. This could be confirmed by the measurement of APT or DEPT spectra. The C-6^A methylene signal is shifted downfield (to approx. 68 ppm) and could thus be found outside the usual region for C-6 signals (Figure 5, c).

A literature survey (of cases in which sufficient NMR spectroscopic data are given) confirmed that this method is usable with all known peracetylated mono-*O*-substituted α-, β- and γ-CD derivatives (Table 1, Table 2, and Table 3). H-2^A signals are shifted to the 3.2–3.6 ppm region, the integral intensity for H-3 signals is decreased by one H atom, and C-6^A is shifted to the 66–71 ppm region.

Table 2. Peracetylated 3^A-*O*-substituted CD derivatives, for which decreases in the ^{1}H NMR ($CDCl_3$) integral intensities of H-3 signals corresponding to one H atom were observed.

Entry	CD	Substituent	Ref.
1	α-CD	allyl	[82]
2	α-CD	cinnamyl	[82]
3	α-CD	formylmethyl	[82]
4	α-CD	carboxymethyl	[82]
5	α-CD	2-bromoprop-2-en-1-yl	[82]
6	β-CD	allyl	[83]
7	β-CD	allyl	[56]
8	β-CD	cinnamyl	[78]
9	β-CD	propargyl	[84]
10	β-CD	carboxymethyl	[78]
11	β-CD	3,3-bis(chlorodimethylstannyl)propanyl	[84]
12	β-CD	4-(1,2-dicarbadodecaboran-1-yl)but-2-en-1-yl	[56]
13	γ-CD	allyl	[85]
14	γ-CD	propargyl	[85]
15	γ-CD	formylmethyl	[85]
16	γ-CD	carboxymethyl	[85]

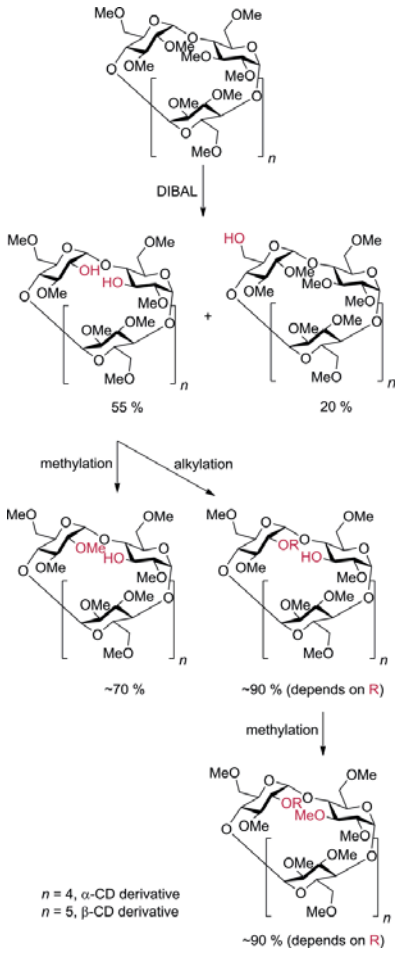
Table 3. ^{13}C NMR ($CDCl_3$) shifts of C-6^A signals in otherwise peracetylated 6^A-*O*-substituted CD derivatives.

Entry	CD	Substituent	C-6^A signal (ppm)	Ref.
1	α-CD	allyl	68.26	[55]
2	α-CD	cinnamyl	68.05	[82]
3	α-CD	formylmethyl	70.20	[82]
4	α-CD	5,5,6,6,7,7,7-heptafluorohept-2-en-1-yl	68.33	[55]
5	α-CD	5,6,6,6-tetrafluoro-5-(trifluoromethyl)hex-2-en-1-yl	68.32	[55]
6	α-CD	5,5,6,6,7,7,8,8,9,9,10,10,10-tridecafluorodec-2-en-1-yl	68.34	[55]
7	β-CD	allyl	67.6	[78]
8	β-CD	propargyl	67.67	[84]
9	β-CD	carboxymethyl	70.5	[78]
10	β-CD	3,3-bis(chlorodimethylstannyl)propanyl	69.07	[84]
11	β-CD	4-(1,2-dicarbadodecaboran-1-yl)but-2-en-1-yl	68.12	[56]
12	β-CD	4-{8,8′-μ-(disulfido)-[3,3′-commocobalt(III)-bis(1,2-dicarbaundecaborate)]-8-yl}but-2-en-1-yl	68.37	[56]
13	β-CD	α-D-glucopyranosyl	65.9	[86]
14	β-CD	5,5,6,6,7,7,7-heptafluorohept-2-en-1-yl	67.77	[55]
15	β-CD	4-{8,8′-μ-(sulfido)-[3,3′-commocobalt(III)-bis(1,2-dicarbaundecaborate)]-8-yl}but-2-en-1-yl	68.28	[56]
16	β-CD	5,6,6,6-tetrafluoro-5-(trifluoromethyl)hex-2-en-1-yl	67.78	[55]
17	β-CD	5,5,6,6,7,7,8,8,9,9,10,10,10-tridecafluorodec-2-en-1-yl	67.82	[55]
18	γ-CD	allyl	67.58	[55]
19	γ-CD	propargyl	67.5	[85]
20	γ-CD	formylmethyl	69.7	[85]
21	γ-CD	carboxymethyl	70.1	[85]
22	γ-CD	5,5,6,6,7,7,7-heptafluorohept-2-en-1-yl	67.79	[55]
23	γ-CD	5,6,6,6-tetrafluoro-5-(trifluoromethyl)hex-2-en-1-yl	67.81	[55]
24	γ-CD	5,5,6,6,7,7,8,8,9,9,10,10,10-tridecafluorodec-2-en-1-yl	67.81	[55]

3. Synthesis of Monosubstituted CD Derivatives

There are currently two "smart-indirect" methods available for the preparation of monosubstituted derivatives. These methods allow selective high-yielding deprotection of persubstituted CDs. The free hydroxy groups obtained can be modified by standard reactions to almost any desired monosubstituted CD derivative. Both these methods take advantage of selective deprotection by diisobutylaluminium hydride (DIBAL).

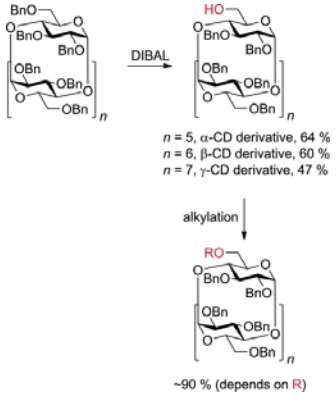
The first method uses DIBAL for selective de-*O*-methylation of permethylated α- or β-CD (Scheme 2).[87] The deprotection



Scheme 2. Selective de-*O*-methylation of CDs.

affords permethylated 2^A,3^B-dihydroxy α- or β-CD and permethylated 6^A-hydroxy α- or β-CD in 55 % and 20 % yields respectively. Permethylated 2^A,3^B-dihydroxy derivatives can either be methylated at position 2^A (forming the permethylated 3-hydroxy derivative)[83,88] or first alkylated at position 2^A and then methylated at position 3^B (forming an permethylated 2^A-alkyl derivative).[89,90] Both options take advantage of the higher reactivity of the hydroxy group at position 2, as mentioned above. These three options allow the synthesis of permethylated 2^A-*O*, 3^A-*O* or 6^A-*O* derivatives regardless of the alkylation agent used.

The second method uses DIBAL as well, but for selective de-*O*-benzylation of perbenzylated α-, β- or γ-CD.[91] The deprotection affords perbenzylated 6^A-hydroxy α-, β- or γ-CD in 64 %, 60 % and 47 % yields, respectively (Scheme 3). These derivatives can be used for subsequent alkylation, followed by removal of all remaining benzyl groups. However, the alkylation agent has to be chosen wisely to avoid unwanted reduction



Scheme 3. Selective de-*O*-benzylation of CDs at position 6.

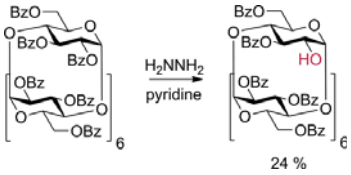
Table 4. Reaction conditions and yields for known monosubstituted allyl, cinnamyl and propargyl CD derivatives.

Entry	CD	Alkylation agent	Reaction conditions	Yields [%] at positions			References
				2	3	6	
1	α-CD	allyl bromide	LiH, LiI, DMSO	24	–	6[a]	[94]
2	α-CD	allyl bromide	LiH, LiI, DMSO	32	–	–	[95]
3	α-CD	allyl bromide	LiH, LiI, DMSO	27[b]	8[b]	–	[82]
4	α-CD	allyl bromide	2 equiv. NaOH, H_2O/CH_3CN	17[b]	10[b]	–	[82]
5	α-CD	allyl bromide	2 equiv. NaOH, DMF	6[b]	2[b]	–	[82]
6	α-CD	allyl bromide	5 equiv. NaOH, H_2O	8[b]	3[b]	7[b]	[82]
7	α-CD	allyl bromide	25 equiv. NaOH, $CuSO_4$, H_2O	9[b]	–	–	[82]
8	α-CD	allyl bromide	excess NaOH, H_2O[c]	–	–	13[b]	[82]
9	α-CD	allyl bromide	excess NaOH, H_2O[c]	–	–	14	[55]
10	β-CD	allyl bromide	LiH, LiI, DMSO	40	–	–	[96]
11	β-CD	allyl bromide	LiH, LiI, DMSO	29	–	–	[97]
12	β-CD	allyl bromide	LiH, LiI, DMSO	27	–	–	[94]
13	β-CD	allyl bromide	NaH, DMF	4	–	–	[98]
14	β-CD	allyl bromide	EtONa, DMSO	25	–	–	[99]
15	β-CD	allyl bromide	2 equiv. NaOH, H_2O	10	–	–	[100]
16	β-CD	allyl bromide	2 equiv. NaOH, H_2O	9	–	–	[55]
17	β-CD	allyl bromide	11 equiv. NaOH, H_2O	26[b]	10[b]	12[b]	[83]
18	β-CD	allyl bromide	11 equiv. NaOH, H_2O	–	–	6[b,d]	[78]
19	β-CD	allyl bromide	25 equiv. NaOH, $CuSO_4$, H_2O	27	–	–	[99]
20	β-CD	allyl bromide	excess NaOH, H_2O[c]	–	–	17	[55]
21	γ-CD	allyl bromide	LiH, LiI, DMSO	6[b]	1[b]	–	[85]
22	γ-CD	allyl bromide	2 equiv. NaOH, H_2O/CH_3CN	19[b]	11[b]	–	[85]
23	γ-CD	allyl bromide	excess NaOH, H_2O[c]	–	–	18	[55]
24	α-CD	cinnamyl bromide	LiH, LiI, DMSO	9	21	–	[82]
25	α-CD	cinnamyl bromide	NaH, DMSO	9	21	–	[77]
26	α-CD	cinnamyl bromide	2 equiv. NaOH, H_2O/CH_3CN	< 1[b]	3[b]	–	[82]
27	α-CD	cinnamyl bromide	2 equiv. NaOH, DMF	5[b]	3[b]	–	[82]
28	α-CD	cinnamyl bromide	5 equiv. NaOH, H_2O	5[b]	7[b]	–	[82]
29	α-CD	cinnamyl bromide	excess NaOH, H_2O[c]	–	–	6	[82]
30	β-CD	cinnamyl bromide	2 equiv. NaOH, H_2O/CH_3CN	–	32	–	[78]
31	α-CD	propargyl bromide	LiH, LiI, DMSO	30	–	–	[71]
32	β-CD	propargyl bromide	LiH, LiI, DMSO	40	–	–	[71]
33	β-CD	propargyl bromide	LiH, LiI, DMSO	39	–	–	[101]
34	β-CD	propargyl bromide	LiH, LiI, DMSO	39	–	–	[102]
35	β-CD	propargyl bromide	LiH, LiI, DMSO	29	–	–	[103]
36	β-CD	propargyl bromide	LiH, LiI, DMSO	29	–	–	[104]
37	β-CD	propargyl bromide	11 equiv. NaOH, H_2O	25[b]	11[b]	13[b]	[84]
38	γ-CD	propargyl bromide	LiH, LiI, DMSO	4[b]	–	–	[85]
39	γ-CD	propargyl bromide	2 equiv. NaOH, H_2O/CH_3CN	13[b]	8[b]	–	[85]
40	γ-CD	propargyl bromide	excess NaOH, H_2O[c]	–	–	13	[85]

[a] No characterisation was given and it was shown that the 6^A-*O* isomer was in fact the 3^A-*O* isomer.[82] [b] Yields determined after separation as peracetylated isomers. [c] An excess of NaOH was used (typically 40–50 equiv.). [d] Other monosubstituted isomers were reported, but not isolated.

during the deprotection step, usually done by H_2 with Pd catalyst.[59] Alternatively, the deprotection step on an perbenzylated CD substituted with a group labile to H_2 reduction (e.g., propargyl) should be carried out after using this group for CD functionalisation.[70]

There is also another method similar to selective de-*O*-benzylation. Selective de-*O*-benzoylation of β-CD is carried out with hydrazine and yields a deprotected hydroxy group at position 2^A (Scheme 4).[92]



Scheme 4. Selective de-*O*-benzoylation of β-CD at position 2.

Other methods for CD monofunctionalisation depend on the substituent agent used and are discussed in the following Sections. When browsing literature looking for suitable reaction conditions for the preparation of a monosubstituted CD derivative, please read the purification and characterisation part carefully. Precipitation of a reaction mixture after alkylation as the only purification step is not sufficient. Without verification given at least in the form of copies of NMR spectra one could expect unreacted CD and multiply substituted CDs present in the product (for examples see ref.[65,93]).

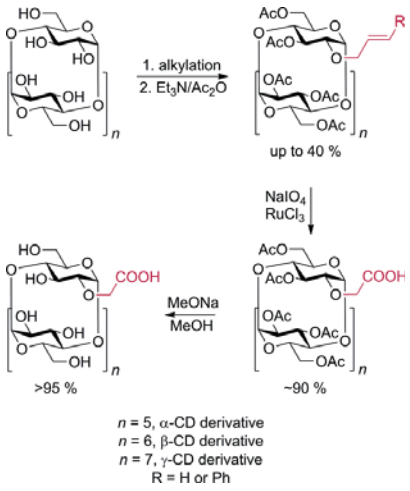
The direct monosubstitution of CDs with alkylating agents is used very often. The ratios of 2^A-*O*, 3^A-*O* or 6^A-*O* derivatives vary depending on the solvent, base and the alkylation agent used. Table 4 summarises yields and reaction conditions for known monosubstituted allyl, cinnamyl and propargyl CD derivatives. These single derivatives are usually separated from the unreacted CD and multiply substituted CDs by chromatography on silica gel (with MeOH/acetonitrile, *n*PrOH/H_2O/aq. NH_3 or $CHCl_3$/MeOH/H_2O elution mixtures) or reversed-phase silica gel (with MeOH/H_2O elution mixtures). Alternatively, a mixture of monosubstituted CDs may be separated first. The mixture is then acetylated (usually with Et_3N/Ac_2O), and monosubstituted isomers are separated as peracetates on silica gel with use of $CHCl_3$/MeOH elution mixtures. The following Sections focus on the synthesis of CD derivatives monosubstituted variously at position 2, 3 or 6.

3.1. Synthesis of Derivatives Substituted at Position 2

As mentioned above, permethylated α- or β-CD derivatives can be selectively de-*O*-methylated at position 2^A in high yields. Selective de-*O*-benzoylation is not as important as the de-*O*-methylation due to its relatively low yields.

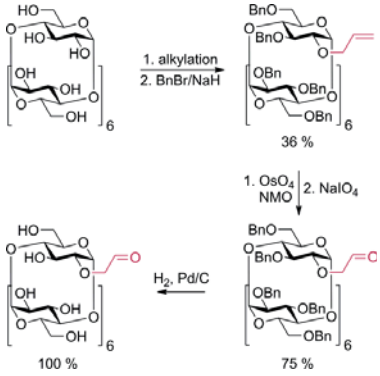
The direct methods take advantage of the fact that the hydroxy group at position 2 is the most acidic one. Therefore, the use of a controlled amount of a strong base often leads to substitution predominantly at this position. This feature can also be clearly seen in Table 4. The best results for alkylation are achieved with LiH in DMSO together with a catalytic amount of LiI. This method for CD mono-*O*-alkylation was first used by Hanessian,[94] and the success of this method was confirmed by other scientists in the synthesis of 2^A-*O*-allyl- or propargyl-α- and -β-CDs in up to 40 % yields (Table 4, Entry 10). However, this method fails when applied for the synthesis of cinnamyl-α-CD derivatives. γ-CDs exhibit generally lower yields for this and also for other methods. 2^A-*O*-Allyl-β-CD can be further permethylated[97,105] or perbenzylated.[96]

Direct introduction of the carboxymethyl group (e.g., by use of a haloacetate) to produce 2^A-*O*-carboxymethyl-CDs is rather difficult, because many isomers are formed.[106,107] 2^A-*O*-Carboxymethyl-α-, -β- or -γ-CDs thus have to be prepared by oxidation of the corresponding peracetylated allyl or cinnamyl derivatives followed by Zemplén deacetylation (Scheme 5).[78,82,83,85,108–110] Per-*O*-methyl-2^A-*O*-carboxymethyl-β-CD was prepared from per-*O*-methyl-2^A-*O*-allyl-β-CD by oxidation with $NaIO_4/RuCl_3$[83] or from per-*O*-methyl-2^A-*O*-formylmethyl-β-CD with $NaClO_2/NaH_2PO_4$[111] and also by treatment of permethylated 2^A-*O*-hydroxy-β-CD with iodoacetate.[112]



Scheme 5. Synthesis of 2^A-*O*-carboxymethyl-CDs.

2^A-*O*-Formylmethyl-α- and -β-CDs were prepared by reductive ozonolysis (O_3/Me_2S) of the corresponding allyl derivatives.[61,62,95] Alternatively, 2^A-*O*-formylmethyl-β-CD could be synthesised by oxidation of per-*O*-benzyl-2^A-*O*-allyl-β-CD followed by removal of benzyl groups (Scheme 6).[96] Per-*O*-methyl-2^A-*O*-formylmethyl-β-CD was prepared from per-*O*-methyl-2^A-*O*-allyl-β-CD by oxidation either with O_3/PPh_3[105,113] or with OsO_4/*N*-methylmorpholine-*N*-oxide (NMO) followed by $NaIO_4$.[114] Per-*O*-acetyl-2^A-*O*-formylmethyl-α- and -γ-CD were synthesised from the corresponding peracetylated allyl derivatives by reductive ozonolysis (O_3/Me_2S).[82,85]



Scheme 6. Synthesis of 2^A-*O*-formylmethyl-β-CD.

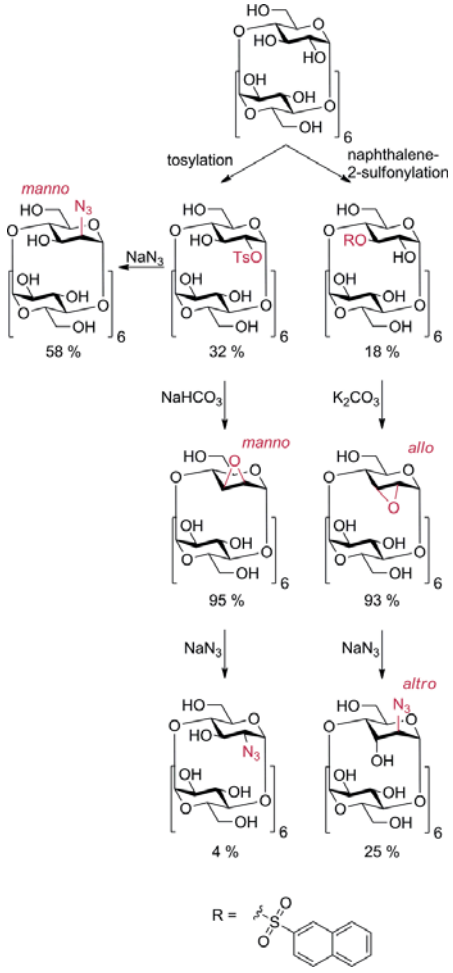
Syntheses of 2^A-azido-2^A-deoxy-CDs are used very rarely (in relation to the far more favoured 6^A-derivatives) due to complications with the inversion of configuration at the 2^A carbon atom during the substitution step. There are two key intermediates in the synthesis of azido derivatives of β-CD substituted at position 2^A: 2^A-*O*-tosyl-β-CD[115,116] and 3^A-*O*-(naphthalene-2-sulfonyl)-β-CD.[117,118] Both can be synthesised regioselectively. 2^A-*O*-Tosyl-β-CD can be directly substituted to give 2^A-azido-2^A-deoxy-*manno*-β-CD[119] or can be transformed into 2^A,3^A-*manno*-epoxy-β-CD. 3^A-*O*-(Naphthalene-2-sulfonyl)-β-CD gives rise to 2^A,3^A-*allo*-epoxy-β-CD under basic conditions (Scheme 7).[117,120,121]

Opening of 2^A,3^A-*allo*-epoxy-β-CD with NaN_3 afforded 2^A-azido-2^A-deoxy-*altro*-β-CD, whereas opening of 2^A,3^A-*manno*-epoxy-β-CD afforded the "real" 2^A-azido-2^A-deoxy-β-CD, but in a very low yield of 3.6 % (Scheme 7).[120] However, the latter method affords the inaccessible "undistorted" 2^A derivative.

Although authors often do not differentiate between 2^A-azido-2^A-deoxy-*manno*-β-CD, 2^A-azido-2^A-deoxy-*altro*-β-CD and 2^A-azido-2^A-deoxy-β-CD,[118,122,123] the cyclodextrin with one mannosidic or altrosidic subunit could have the cavity slightly distorted and could be less acid-resistant than the native β-CD.[120] Moreover, Martina et al.[124] found the 2^A-azido-2^A-deoxy-*manno*-β-CD synthesised by Poon et al.[123] to be the 3^A derivative rather than the 2^A derivative.

2^A-Azido-2^A-deoxy-*manno*-β-CD and 2^A-azido-2^A-deoxy-*altro*-β-CD could also be permethylated on all remaining hydroxy groups.[122,125,126]

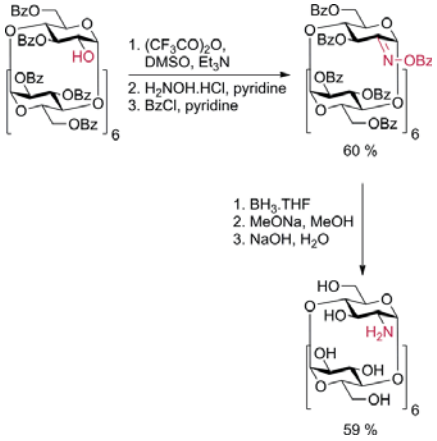
2^A-Amino-2^A-deoxy-β-CD was prepared by the insertion of a new substituted glucose unit into the α-CD ring (Scheme 1). However, this methodology is very inconvenient and requires advanced understanding of oligosaccharide synthesis. Those interested in this approach can find more details in the work by Sakairi et al.[127,128] 2^A-Amino-2^A-deoxy-β-CD was also synthesised by the de-*O*-benzoylation methodology (Scheme 4), in which the free hydroxy group was converted into an amine via an oxime (Scheme 8).[92]



Scheme 7. Synthesis of 2^A-azido-2^A-deoxy derivatives of β-CD.

There are also other 2^A-amino-2^A-deoxy stereoisomers of glucose: 2^A-amino-2^A-deoxy-*manno*-β-CD and 2^A-amino-2^A-deoxy-*altro*-β-CD. Together with 2^A-amino-2^A-deoxy-β-CD, they were prepared from the corresponding azido derivatives by reduction with PPh_3.[120,122] Their permethylated derivatives were prepared similarly.[122,125]

Microsummary: The most general approach for the synthesis of CDs monosubstituted at position 2 is the selective de-*O*-methylation methodology. However, for the synthesis of non-methylated *O*-alkylated derivatives, LiH (with a catalytic amount of LiI) in DMSO should be used.



Scheme 8. Synthesis of 2^A-amino-2^A-deoxy-β-CD.

3.2. Synthesis of Derivatives Substituted at Position 3

Permethylated 3^A-hydroxy α- or β-CD derivatives could be conveniently obtained in high yields through the selective de-*O*-methylation methodology described above. The free hydroxy group could then be converted into a desired functional group by means of standard reactions.

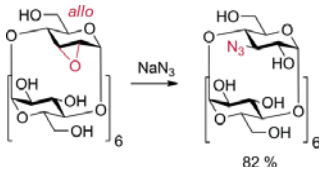
Because of the relative lack of reactivity of hydroxy groups at position 3, described above, the preparation of these derivatives is rather difficult. However, there is one exception: the direct substitution of β-CD with cinnamyl bromide (Table 4, Entry 30) in H_2O/CH_3CN mixture with 2 equiv. NaOH as a base.[78] 3^A-*O*-Cinnamyl-β-CD is obtained in quite a high yield – 32 % – thanks to the formation of a complex of cinnamyl bromide and β-CD during the reaction. Application of the same conditions to α-CD (Table 4, Entry 26)[82] led only to poor yields of 2^A-*O*-cinnamyl-α-CD and 3^A-*O*-cinnamyl-α-CD (< 1 % and 3 %, respectively), which confirms the complex formation only in the case of β-CD. Surprisingly, the highest yield (21 %) of 3^A-*O*-cinnamyl-α-CD is achieved when using a strong base (NaH or LiH) in DMSO (Table 4, Entries 24 and 25).[77,82] Unfortunately, the reason for this selectivity over the 2^A-*O* derivative is unknown.

Generally the best results for the syntheses of 3^A-*O*-allyl- or -propargyl-α-, -β- or -γ-CD were achieved either under the same conditions as for the synthesis of 3^A-*O*-cinnamyl-β-CD mentioned above (2 equiv. NaOH, H_2O/CH_3CN),[82,85] or under conditions in which 11 equiv. of NaOH in H_2O were used.[83,84] Yields around 10 % were obtained under these conditions. Moreover, the latter set of conditions can be used when all three monosubstituted CDs are required for a study.

3^A-*O*-Carboxymethyl-α-, -β- or -γ-CD and their peracetylated or permethylated derivatives were prepared in a similar way as the 2^A-*O* derivatives (see above).[78,82,83,85,108–110]

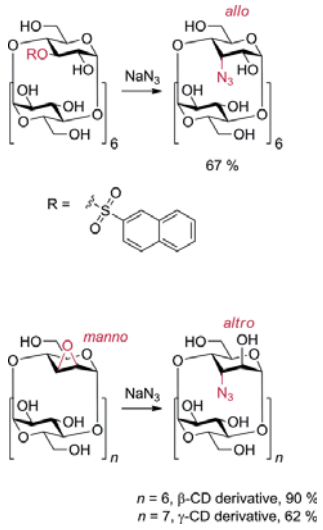
3^A-*O*-Formylmethyl-β-CD was synthesised from 3^A-*O*-cinnamyl-β-CD by reductive ozonolysis (O_3/Me_2S).[63] Per-*O*-acetyl-3^A-*O*-formylmethyl-α- and -γ-CD were synthesised from the corresponding peracetylated allyl or cinnamyl derivatives by reductive ozonolysis as well.[82,85]

The synthesis of 3^A-azido-3^A-deoxy-CDs also suffers from the inversion of configuration at the 3^A carbon atom during the substitution step. However, the situation is much better in this case than in that of the 2^A derivatives, because opening of $2^A,3^A$-*allo*-epoxy-β-CD affords 3^A-azido-3^A-deoxy-β-CD in reasonable yields – up to 82 % (Scheme 9).[120,129]



Scheme 9. Synthesis of 3^A-azido-3^A-deoxy-β-CD.

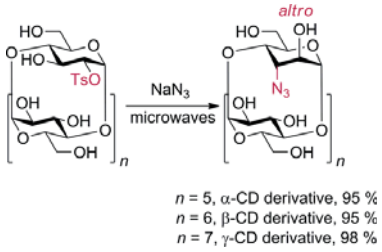
There are also other 3^A-azido-3^A-deoxy stereoisomers of glucose available. 3^A-Azido-3^A-deoxy-*allo*-β-CD was synthesised through a the substitution reaction from 3^A-*O*-(naphthyl-2-sulfonyl)-β-CD,[130] whereas 3^A-azido-3^A-deoxy-*altro*-β- or -γ-CD were synthesised by opening of the corresponding $2^A,3^A$-*manno*-epoxy CD derivatives (Scheme 10).[120,131]



Scheme 10. Synthesis of 3^A-azido-3^A-deoxy CD derivatives.

3^A-Azido-3^A-deoxy-*altro*-α-, -β- or -γ-CDs were synthesised by direct treatment of the corresponding 2^A-*O*-tosyl derivatives of the CDs with NaN_3 (Scheme 11).[124] These reactions proceed through the $2^A,3^A$-*manno*-epoxy CD derivatives.

3^A-Amino-3^A-deoxy-*altro*-α-, -β- or -γ-CDs are nowadays commercially available from common suppliers and so their syntheses are described only briefly. They could be synthesised



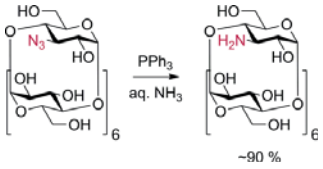
Scheme 11. Synthesis of 3^A-azido-3^A-deoxy-*altro*-α-, -β- and -γ-CD.

from the corresponding tosyl (α-CD,[132,133] β-CD,[115,116,134] and γ-CD[135]) derivatives by treatment with 28 % aq. ammonia[136] and were characterised by NMR in detail.[137]

An alternative synthesis proceeds through opening of 2^A,3^A-*manno*-epoxy-α-[132,138], -β-[139] or -γ-CD[140,141] with aq. ammonia or by the reduction of the above azide derivatives with PPh_3.[120,131]

As in the case of 2^A-azido derivatives, some authors are confused by 3^A-amino-3^A-deoxy-*altro* and "normal" (*gluco*) diastereoisomers. Miyauchi et al.[142,143] claimed the synthesis of 3^A-amino-3^A-deoxy-α-CD even though they themselves described the preparation according to Ikeda et al. (who synthesised the *altro* derivative; see above)[132] Hopefully it is just a mistake in the structure and the authors forgot to mention that the prepared derivative is in the *altro* form, as it seems to be in the case of an article by Miyawaki et al.[144]

Unlike its diastereoisomer, 3^A-amino-3^A-deoxy-β-CD is not commercially available and has to be synthesised from the corresponding azide (see above) by reduction with PPh_3 (Scheme 12).[120,129,145,146]



Scheme 12. Synthesis of 3^A-amino-3^A-deoxy-β-CD.

Microsummary: As in the case of CDs monosubstituted at position 2, the most general approach for substitution at position 3 is the selective de-*O*-methylation methodology. 3^A-*O*-Cinnamyl-β-CD (regioselectively synthesised in relatively high yield) could be used for the synthesis of other non-methylated CD derivatives. Moreover, 3^A-amino-3^A-deoxy-*altro*-CDs are commercially available.

3.3. Synthesis of Derivatives Substituted at Position 6

Derivatives substituted at position 6 benefit from the presence of a primary hydroxy group at this position. This hydroxy group is the most accessible one and due to its nature there is no problem with inversion of configuration during substitution of, for example, a tosyl group for some other moiety. Moreover, the attachment of a CD to a surface through its primary rim allows the wider secondary rim to be accessible for guest molecules. All of these factors make CD derivatives substituted at position 6 the most highly favoured ones.

CD derivatives monosubstituted at position 6 could be conveniently obtained in high yields through the selective de-*O*-benzylation methodology described above. Selective de-*O*-methylation is not as important as the de-*O*-benzylation due to its relatively low yield.

The syntheses of 6^A-*O*-allyl, -cinnamyl and -propargyl CD derivatives proceed with the best yields when an excess of NaOH (relative to the number of hydroxy groups in the CD) in H_2O is used (Table 4).[55,82,85] The yields are usually above 10 % and no other monosubstituted derivatives are formed, which facilitates the separation process. This confirms the above comment that the deprotonation of all the hydroxy groups with an excess of base results in an electrophilic attack at position 6, because it is the most accessible one.

6^A-*O*-Carboxymethyl-α-, -β- or -γ-CDs and their peracetylated or permethylated derivatives were prepared in a similar way as the 2^A- and 3^A-*O* derivatives (see above).[78,82,83,85,108–110,112]

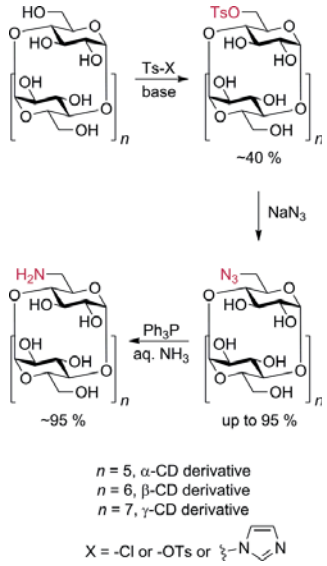
6^A-*O*-Formylmethyl CD derivatives were prepared only as peracetates. Per-*O*-acetyl-6^A-*O*-formylmethyl-α- and γ-CDs were synthesised from the corresponding peracetylated allyl derivatives by reductive ozonolysis (O_3/Me_2S).[82,85]

Most 6^A-amino- or 6^A-azido-α-, -β- or -γ-CD derivatives are commercially available. However, they are still synthesised by researchers for reasons of cost. Usually, α-, β- or γ-CD is first tosylated and then nucleophilic substitution with NaN_3 is performed. When an amino derivative is needed, the reduction of an azido derivative with PPh_3 is carried out (Scheme 13).

Usually, TsCl,[147–154] Ts_2O[155] or 1-tosylimidazole[156–159] is used for the introduction of the tosyl group onto a cyclodextrin skeleton. Although some authors do not purify the product, it is recommended the crude product be treated by chromatography[148,151,159] or recrystallised from water[149,152,153,157] or 50 % MeOH in H_2O.[160,161] Subsequent substitution of the tosyl CD derivative with sodium azide is carried out in water[148–150,159] or DMF.[147,152,157] The final step, when the amine is formed, involves reduction with triphenylphosphine in the presence of aqueous ammonia[148,152,159] or water.[149]

Permethylated,[122,162–165] peracetylated[164–166] or perbenzylated[167,168] 6^A-amino- or 6^A-azido-α-, β- or γ-CD derivatives are also available. They are usually obtained by persubstitution of azido derivatives (followed by reduction in the case of amino derivatives).

Microsummary: The most widely used 6^A-amino- or 6^A-azido-CDs can either be synthesised in high yields or most of them can be purchased from the suppliers. The selective de-*O*-benzylation methodology is often used for other 6^A-substituted CD derivatives.



Scheme 13. Synthesis of 6^A-amino- and 6^A-azido-CD derivatives.

4. Conclusion

This review summarises the syntheses of several basic CD derivatives that can subsequently be used to prepare specific derivatives monosubstituted at positions 2, 3 or 6. The choice of the right position is crucial because, as has been shown, not only the size of the cavity, but also the position of the attached substituent has a great influence on a CD derivative's properties.[169]

There are several available general methods for the synthesis of monosubstituted CD derivatives, which use selective deprotection of permethylated, perbenzylated or perbenzoylated CDs. These methods allow the synthesis of desired monosubstituted CD derivatives regardless of the alkylation or other substitution agent used.

Direct monosubstitution of CDs with allyl, cinnamyl or propargyl bromides can be targeted to some positions selectively. Position 2 is attacked when LiH in DMSO with a catalytic amount of LiI is used. The selective substitution of β-CD with cinnamyl bromide results in the formation of 3^A-*O*-cinnamyl-β-CD in quite a high yield due to complex formation between cinnamyl bromide and β-CD during the reaction. The best method for the other 3^A-*O* derivatives uses 2 equiv. of NaOH in H_2O/acetonitrile or 11 equiv. of NaOH in H_2O. An excess of NaOH in H_2O yields 6^A-O derivatives selectively.

Monosubstituted carboxymethyl and formylmethyl-CD derivatives are synthesised by the oxidation of the corresponding allyl or cinnamyl derivatives.

The synthesis of 6^A-azido- and 6^A-amino-CD derivatives is the most popular, whereas the introduction of these groups at positions 2^A or 3^A frequently suffers from the inversion of configuration at the carbon atom caused by epoxy ring opening or by substitution of arylsulfonyl derivatives.

Moreover, it has been shown that the previously suggested quick method for determining a substituent position on a CD skeleton solely from ^{1}H NMR spectra is applicable to all peracetylated mono-*O*-substituted α-, β- and γ-CD derivatives.

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Keywords: Synthetic methods · Regioselectivity · Cyclodextrins

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REVIEW

Synthesis of substituted cyclodextrins

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Abstract
Cyclodextrins are naturally occurring, non-toxic and biodegradable cyclic oligosaccharides. The main feature of cyclodextrins is the ability to encapsulate lipophilic compounds, which has led to many applications. Although many cyclodextrin derivatives have become available on the market, their price is in the range of fine chemicals, and thus, they are still often synthesised in laboratories. The actual number of cyclodextrin derivatives exceeds 11,000, but new cyclodextrin derivatives are still needed for more advanced applications. Therefore, many beginners in cyclodextrin chemistry struggle with a reliable choice of a synthetic route. This review focuses on cyclodextrin derivatives that can be subsequently modified. Indeed, the modification of an already substituted cyclodextrin with a suitable functional group is much easier than the optimisation of the substitution for every new cyclodextrin derivative desired. This review describes the synthesis of different types of cyclodextrin derivatives: persubstituted, randomly substituted, persubstituted at selected positions, selectively substituted and monosubstituted cyclodextrins.

Keywords Cyclodextrin · Derivative · Synthesis · Substitution · Selectivity · Random polymers

Introduction

The history of cyclodextrin derivatives goes back to the beginning of the twentieth century when cyclodextrin properties were examined and the first derivatives were prepared (Crini 2014). Since then, cyclodextrin derivatives have come a long way, and they can currently find their use in many kinds of human activities: in pharmaceutical and biomedical applications (di Cagno 2017; Liao et al. 2017; Adeoye and Cabral-Marques 2017; Ioele et al. 2017; Qiu et al. 2017; Alvarez-Lorenzo et al. 2017; Muankaew and Loftsson 2018); nanotherapeutics (Swaminathan et al. 2016; Antoniuk and Amiel 2016; Mejia-Ariza et al. 2017; Venuti et al. 2017; Arima et al. 2017; Peng et al. 2017); cosmetics, toiletries and personal care (Sharma and Baldi 2016); nutrition industry (Astray et al. 2009; Fenyvesi et al. 2016; Sharma and Baldi 2016); textile and packing industry (Radu et al. 2016; Sharma and Baldi 2016); separation techniques (Řezanka et al. 2014; Zhou et al. 2015; Adly et al. 2016; Saz and Marina 2016; Zhu and Scriba 2016); and as artificial enzymes or catalysts (Kryjewski et al. 2015; Macaev and Boldescu 2015; Aghahosseini and Ramazani 2016; Letort et al. 2016; Bai et al. 2017).

Inclusion complexes of cyclodextrin derivatives

The key property of cyclodextrin derivatives lies in their ability to complex compounds in their cavity. This could be illustrated by the most known application of a cyclodextrin derivative in daily life: the use of octakis[6-(2-carboxyethylthio)-6-deoxy]-γ-cyclodextrin sodium salt (sugammadex) in Bridion® (Donati 2008). It is used after a surgery to bind the neuromuscular blocking agent in its cavity instead of relying on rocuronium pharmacokinetic properties or on the inhibition of acetylcholine breakdown with a reversal agent.

The shape and size of guests forming complexes with cyclodextrin or its derivatives are variable, and therefore, the strength of a complex depends on their interactions (Liu and Guo 2002) as well as on the cavity size (Szente and Fenyvesi 2017). In the case of cyclodextrin derivatives, the substituents present on a cyclodextrin skeleton should not be overlooked—as they could significantly affect the

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formation of the inclusion complex. For example, an association constant of the abovementioned sugammadex with rocuronium bromide is 10^7 M^{-1} (Bom et al. 2002), while native γ-cyclodextrin has the constant only in the order of 10^4 M^{-1} (Cameron et al. 2002). The association constants for complexes of cyclodextrin derivatives with a wide variety of guests can be found in the review by Rekharsky and Inoue (1998).

Cyclodextrin derivatives properties

Nomenclature usually used by researchers for the description of cyclodextrin derivatives is depicted in Fig. 1. A glucose unit possesses substitution sites at positions 2, 3 and 6. Glucose units are named A, B, C etc., respectively, in superscript. Simplicity also often wins over precision. For example, per-*O*-methyl-2^{A}-*O*-allyl-β-cyclodextrin refers to β-cyclodextrin, where allyl substituent is at position 2 at one glucose unit and all 20 the other hydroxyl groups are protected with methyl groups.

As it was already shown in the previous section, substituents present on a cyclodextrin skeleton play a key role in its properties. For example, cyclodextrins with hydrophobic chains could form micelles or vesicles in water environment (Sallas and Darcy 2008), other cyclodextrins are capable of catalysing decomposition of organophosphorus compounds

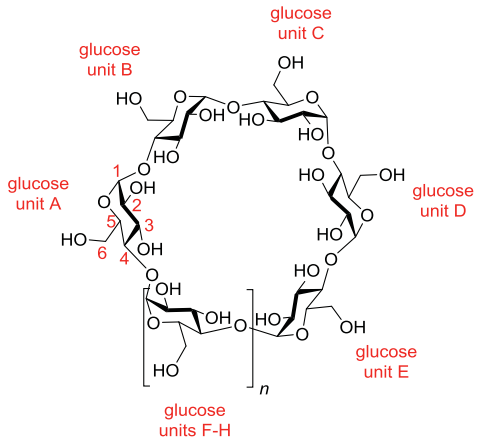

Fig. 1 Structure and numbering of α-, β-, and γ-cyclodextrin. Glucose units are named A, B, C etc., respectively, and carbon atoms in each unit are numbered as usual

(Letort et al. 2016), and positively charged cyclodextrin derivatives are used in chiral separations in capillary electrophoresis as they are able to interact with carboxylic acids (Tang and Ng 2008).

The dependence of solubility on substituents could be demonstrated in the following example. Only 1.9 g of β-cyclodextrin is soluble in 100 ml water at room temperature (Szejtli 1998), while almost 24 g of per-*O*-methyl-β-cyclodextrin could be dissolved in the same amount of water (Szente and Szejtli 1999). Moreover, when heated, the solubility of the β-cyclodextrin increases, while in the case of the latter its solubility decreases. Permethylated cyclodextrins are also well soluble in most organic solvents.

Cyclodextrin derivatives

The number of known cyclodextrin derivatives is huge. A search in SciFinder® for any substituted cyclodextrin skeleton revealed there are more than 2000 derivatives for α-cyclodextrin, almost 8000 for β-cyclodextrin, and more than 1000 for γ-cyclodextrin. Listing of more than 11,000 α-, β-, and γ-cyclodextrin derivatives would exceed the possibilities of this review. Thus, the aim of this review is to provide a comprehensive view on the synthesis of favourite or interesting cyclodextrin derivatives, especially on those which are suitable for further modifications. The reason for this approach is obvious—the modification of a cyclodextrin already substituted with one or more suitable functional groups is much easier than the optimisation of substitution for every new cyclodextrin derivative desired. The review is divided into several sections, each focused on one type of cyclodextrin derivatives: persubstituted cyclodextrins, randomly substituted cyclodextrins, cyclodextrins persubstituted at selected positions, selectively substituted cyclodextrins, and monosubstituted cyclodextrins (Fig. 2). A mini summary could be found at the end of these sections as a help for busy readers.

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Substituted cyclodextrins

When native cyclodextrins are not suitable for a given application, their derivatives come into play. When a single isomer cyclodextrin derivative is desired, the most straightforward way is to synthesise a persubstituted derivative. The direct synthesis of other single isomer derivatives is more challenging due to the number of theoretically possible isomers (Wenz 1994). The number of possible isomers is three

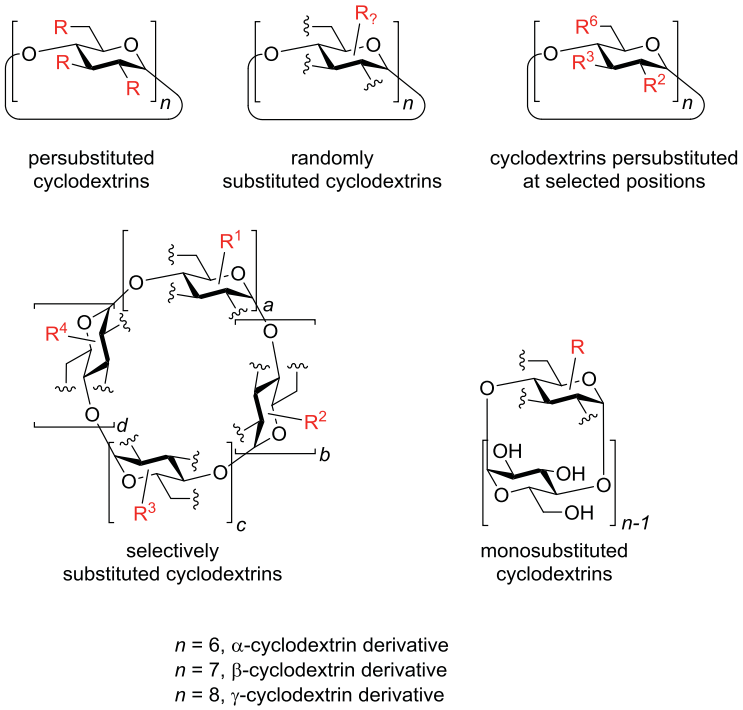


Fig. 2 Schematic representation of the cyclodextrin derivatives types

for monosubstituted, dozens for disubstituted, and more than one hundred for trisubstituted derivatives.

For the purpose of this review, the term monosubstituted cyclodextrins or selectively substituted cyclodextrins also refers to corresponding cyclodextrin derivatives with all the remaining hydroxyl groups protected—methylated, acetylated, benzylated, etc.

Reactivity of cyclodextrins

The modification reactions on cyclodextrins take place at the hydroxyl groups. As the hydroxyl groups are nucleophiles, the reaction proceeds via an electrophilic attack. However, a selective substitution of cyclodextrins is a great challenge for chemists as there are three types of hydroxyl groups present in one glucose unit (at position 2, 3 or 6). Moreover, several glucose units of which cyclodextrins are composed make the process rather difficult. Hydroxyl groups at positions 2, 3 and 6 compete against each other during the reaction. Fortunately, there are at least some differences among them. Hydroxyl groups at positions 6 are primary and at positions 2 and 3 are secondary (Khan et al. 1998).

Bases first deprotonate the hydroxyl groups at position 2, because they are the most acidic, having $pK_a = 12.2$ (Sallas and Darcy 2008). The oxyanion formed is more nucleophilic than other non-deprotonated hydroxyl groups. When all the hydroxyl groups are deprotonated with an excess of a base, an electrophilic reagent reacts at position 6, because it is the most accessible.

Substitution at position 3 is the most difficult one. Fortunately, some reagents interfere with the cavity of cyclodextrins, making this process much easier. On the other hand, such interference of a reagent with the cavity could be a complication in other modifications of cyclodextrins and should be always taken into account.

Solvents play another important role during the modification of a cyclodextrin. They can affect both nucleophilicity of oxyanions, as well as the strength of a complex with a substitution agent. If the complex is strong, the predominant product will be driven by the orientation of the substitution agent in the interior of cyclodextrin. Considering the

abovementioned facts, the commonly achieved yields of substituted cyclodextrin derivatives are very low. Exceptions to this rule will be of interest in the next sections.

Persubstituted cyclodextrin derivatives

A great variety of persubstituted cyclodextrins, i.e. cyclodextrin derivatives, where every hydroxyl group is substituted by the same functional group, are available from common commercial sources. However, they are still synthesised by researches because of their simple synthesis and lower overall cost when compared to the commercial ones. The modification usually aims to increase the solubility of cyclodextrins—either in organic solvents or water—or to use them in deprotection reactions—see below.

As it was stated above, the persubstitutions proceed smoothly and they are usually carried out by the reaction of a cyclodextrin with an excess of the reagent, e.g. alkyl halogenide, in the presence of a base. Per-*O*-methylated cyclodextrins are obtained by the reaction of a corresponding cyclodextrin with NaH and methyl iodide in *N*,*N*′-dimethylformamide (Stefanache et al. 2014) or dimethyl sulfoxide (Szejtli et al. 1980). Similarly, per-*O*-benzylated cyclodextrins are prepared by the reaction of a cyclodextrin with benzyl halogenide (Bjerre et al. 2007). Per-*O*-acetylated cyclodextrins are usually prepared by the reaction of a cyclodextrin with acetanhydride. The reaction is promoted by acids (Jicsinszky et al. 2015) or bases (Lian et al. 2014).

Mini summary Persubstituted cyclodextrin derivatives are prepared by the reaction of native cyclodextrin with an excess of the reagent.

Randomly substituted cyclodextrin derivatives

Randomly substituted cyclodextrin derivatives are modified at various positions, and they are usually characterised by degree of substitution. The exact structure and ratio of single derivatives forming the mixture of randomly substituted cyclodextrins is unknown. As well as in the case of persubstituted derivatives, randomly substituted cyclodextrin derivatives are largely available from common commercial sources.

The purchase of randomly substituted cyclodextrin derivatives conceals many pitfalls. It was shown there could be a significant difference in the relative abundances of the isomers with the same degree of substitution between two batches (Estrada and Vigh 2012). Moreover, the authors also found the information about degree of substitution from the supplier may be affected by an error.

Researches should be careful when preparing randomly substituted cyclodextrin derivatives as they do not know the exact composition of the single isomers in the product. As it has been shown before (Řezanka et al. 2016), diverse single cyclodextrin isomers have different properties and their ratio could therefore affect the properties of randomly substituted cyclodextrin derivatives. They could be synthesised using conditions similar to preparation of persubstituted derivatives. The only need is to add a lesser amount of a reagent that is needed for a fully substituted derivative.

Random cyclodextrin polymers

The randomly substituted cyclodextrin derivatives also include a group of random cyclodextrin polymers, where cyclodextrins are interconnected to the other ones in a random way. Such polymers could be synthesised via three approaches: (1) cyclodextrins or their derivatives are directly cross-linked by a suitable agent; (2) cyclodextrins are first randomly modified with reactive groups, which are subsequently used for the attachment onto a polymer backbone; and (3) cyclodextrin is substituted with a functional group that is available for polymerisation, e.g. double bond. The first approach often gives branched polymers, whereas the second and the third result in linear polymers substituted with cyclodextrins.

The best-known example of the first method is the direct reaction of epichlorohydrin—2-(chloromethyl)oxirane—with a cyclodextrin (van de Manakker et al. 2009; Morin-Crini and Crini 2013; Concheiro and Alvarez-Lorenzo 2013; Gidwani and Vyas 2014; Morin-Crini et al. 2018). If the degree of cross-linking is sufficiently high, the resulting polymer becomes insoluble in water. Epichlorohydrin could be used for cross-linking of randomly substituted cyclodextrin derivatives as well (Zhang et al. 2012). Other commonly used cross-linking agents for the synthesis of random cyclodextrin polymers are, e.g. epoxides, acid chlorides or isocyanates (Mocanu et al. 2001; Concheiro and Alvarez-Lorenzo 2013; Karoyo and Wilson 2015; Taka et al. 2017).

An example of the second approach, where cyclodextrins are first randomly modified by reactive groups and subsequently attached onto a polymer backbone, is the use of randomly carboxymethylated β-cyclodextrin for the attachment onto chitosan (Krauland and Alonso 2007; Prabaharan and Gong 2008).

The third approach requires the introduction of functional groups with the ability to be polymerised. This requirement is usually fulfilled by the synthesis of cyclodextrin ester of acrylic acid—e.g. by the reaction of cyclodextrin with *m*-nitrophenyl acrylate. Polymerisation of randomly acryloylated cyclodextrins is then initialised by potassium persulfate (Mocanu et al. 2001; Zhang et al. 2009).

Mini summary Randomly substituted cyclodextrin derivatives are prepared by the reaction of native cyclodextrins with fewer equivalents of the reagent than the number of hydroxyl groups is. Random cyclodextrin polymers are most often synthesised by cross-linking with epichlorohydrin.

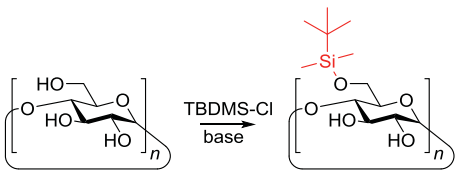
Cyclodextrins persubstituted at selected positions

Cyclodextrins persubstituted at selected positions include cyclodextrins persubstituted either at 2 or 3 or 6 positions or any combination of thereof (Fig. 2). Synthesis of these derivatives is based on the different reactivity of hydroxyl groups described above and often employs the protection/deprotection methodology to achieve a desired derivative. The most favourite reactions are those carried out at position 6, because it possesses primary hydroxyl groups. Cyclodextrins could be easily substituted at this position with *tert*-butyldimethylsilyl or halogens. Such derivatives are useful precursors for amphiphilic cyclodextrin derivatives (Sallas and Darcy 2008).

Syntheses based on per-6-*O*-(*tert*-butyldimethylsilyl) cyclodextrins

Synthesis of per-6-*O*-(*tert*-butyldimethylsilyl)-α-, β- and γ-cyclodextrins is carried out by the reaction of native cyclodextrin with *tert*-butyldimethylsilyl chloride and BaO (Takeo et al. 1988, 1989), pyridine (Fugedi 1989; Ashton et al. 1996) or imidazole (Vincent et al. 1997; Maynard and Vigh 2000) (Scheme 1). Among the bases, pyridine gave the best yields (Ashton et al. 1996).

Hexakis(6-*O*-*tert*-butyldimethylsilyl)-α-cyclodextrin could be protected at positions 2 and 3 by acetyl, methyl or benzyl groups. Subsequent deprotection of silyl groups by BF_3 with tetrahydrofurane, sodium methanolate or tetrabutylammonium fluoride results in useful derivatives, where hydroxyl groups at positions 6 are ready for any modification desired (Scheme 2) (Takeo et al. 1988). The reactions proceed similarly with β- (Jullien et al. 1994; Ashton et al. 1996; Vincent et al. 1997; Kirschner and Green 2005) or γ-cyclodextrin derivatives (Jullien et al. 1994). These derivatives were also used for syntheses of other useful precursors (Uccello-Barretta et al. 2005).



up to 95 %

n = 6, α-cyclodextrin derivative
n = 7, β-cyclodextrin derivative
n = 8, γ-cyclodextrin derivative
TBDMS-Cl = *tert*-butyldimethylsilyl chloride

Scheme 1 Synthesis of per-6-*O*-(*tert*-butyldimethylsilyl)-α-, β- and γ-cyclodextrins

Syntheses based on per-6-halogeno-per-6-deoxy cyclodextrins

Another group of favourite starting materials for synthesis of cyclodextrins persubstituted at selected positions are cyclodextrins perhalogenated at position 6. They are synthesised from native cyclodextrins by the reaction with triphenylphosphine and bromine (Takeo et al. 1974) or iodine (Gadelle and Defaye 1991; Fernandez et al. 1995; Ashton et al. 1996; Benkhaled et al. 2008) in *N*,*N*′-dimethylformamide (Scheme 3). However, the synthesis of bromo derivatives has been declining due to more convenient handling of iodine. Alternatively, *N*-halosuccinimides may be used as agents for a more convenient procedure (Chmurski and Defaye 2000).

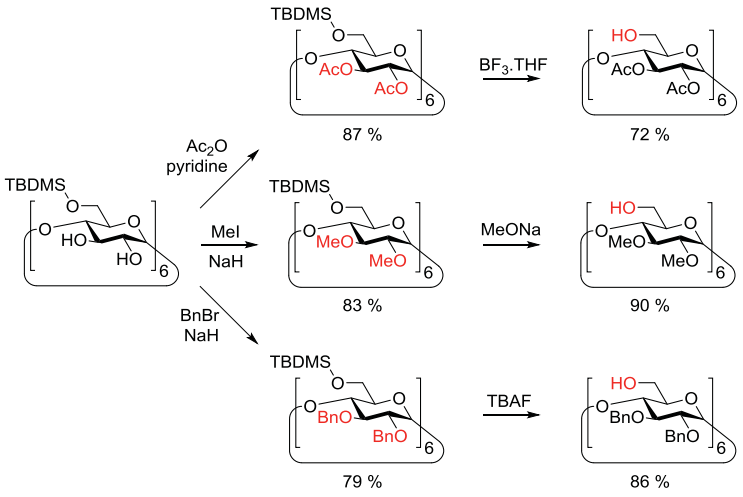
These halogen derivatives are very useful precursors and could be easily transformed for example into azides and amines (Ashton et al. 1996; Gorin et al. 1996) or thiols (Rojas et al. 1995; Gorin et al. 1996) by standard procedures (Scheme 4). The remaining hydroxyl groups of these derivatives could be peracetylated (Boger et al. 1978; Baer et al. 1992), methylated (Boger et al. 1978) or benzylated (Jullien et al. 1994) by the same methods described above.

Jicsinszky et al. have recently described the use of per-6-iodo-per-6-deoxy-β- and γ-cyclodextrins for the synthesis of azido or thio derivatives in a planetary ball mill under solvent-free conditions. The authors found out the mechanochemical synthesis not only simplified the isolation and purification processes, but also allowed easy scale-up (Jicsinszky et al. 2016). Per-6-azido-per-6-deoxy cyclodextrins are the perfect starting materials for nowadays favourite copper-catalysed azide-alkyne cycloaddition reactions (Faugeras et al. 2012; Letort et al. 2016).

Other syntheses

$BaO/Ba(OH)_2$ was successfully applied for the synthesis of per-2,6-di-*O*-alkyl cyclodextrin derivatives (Bergeron et al. 1976). Moreover, when the position 6 is protected, the reaction proceeds to per-2-*O*-alkyl cyclodextrin derivatives (Kraus et al. 2002).

The selective deprotection strategy was also used for acetolysis of perbenzylated α-cyclodextrin (Angibeaud and Utille 1991). The reaction yielded hexakis(2,3-*O*-dibenzyl-6-*O*-acetyl)-α-cyclodextrin, in which either acetyl or benzyl groups could be selectively deprotected (Scheme 6). Moreover, perbenzylated α-cyclodextrin was also used for deprotection by triethylsilane and iodine (Guitet et al. 2012). The method was originally developed for debenzylation of multiple-*O*-benzylated mono- and disaccharides (Pastore et al.



Scheme 2 Synthesis of α-cyclodextrins protected at positions 2 and 3

Scheme 3 Synthesis of per-6-halogeno-per-6-deoxy-α-, β- and γ-cyclodextrins

2011) and in the case of α-cyclodextrin proceeds at position 3, which is normally the least accessible (Scheme 5).

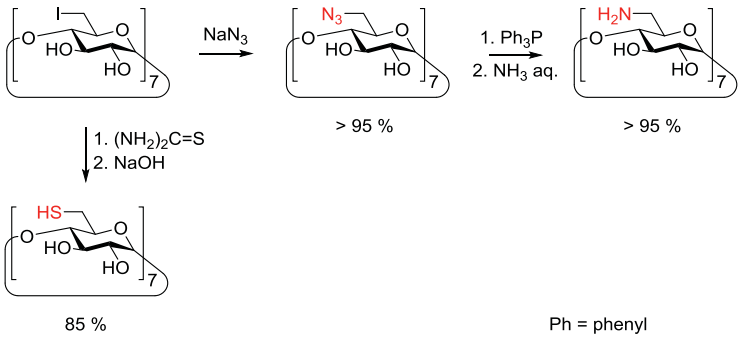
Mini summary Synthesis of cyclodextrins persubstituted at selected positions utilises the different reactivity of hydroxyl groups and employs protection/deprotection methodology to achieve a desired derivative. Most of the syntheses begin with the substitution at position 6—either by *tert*-butyldimethylsilyl or a halogen. BaO/$Ba(OH)_2$ direct the substitution to positions 2 and 6. When position 6 is blocked, the substitution proceeds selectively at position 2. The selective substitution at otherwise the least accessible position 3 could be achieved by deprotection of perbenzylated cyclodextrin by triethylsilane with iodine.

Selectively substituted cyclodextrins

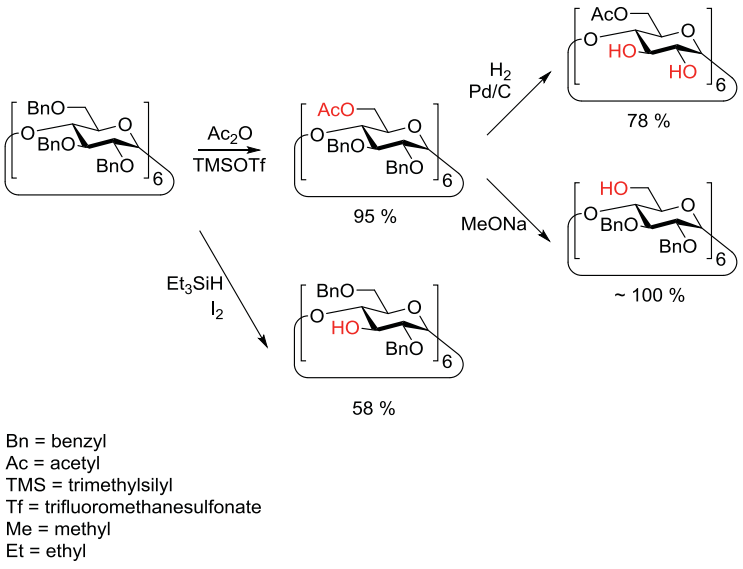
Selectively substituted cyclodextrins fill the gap between all the above-discussed cyclodextrin derivatives and monosubstituted cyclodextrins. That is, they are single isomer compounds with a known structure, where two or more substituents are attached to the cyclodextrin skeleton. Their synthesis is the most challenging among all cyclodextrin derivatives, as the number of possible isomers starts at dozens and ends at millions for different substituents at different positions.

Syntheses based on direct substitution

One of the first attempts to synthesise selectively substituted cyclodextrins was the use of disulfonates for the selective modification of selected glucose units at position 6 (Scheme 6). $6^A,6^B$ derivative is formed when β-cyclodextrin is reacted with 4,6-dimethoxybenzene-1,3-disulfonyl



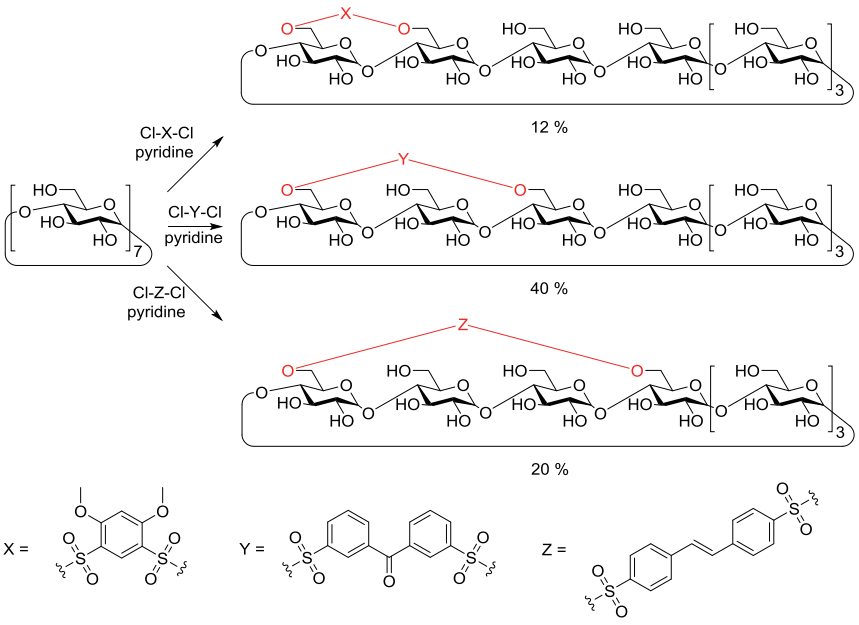
Scheme 4 Synthesis of azido, amino and thio derivatives of β-cyclodextrin



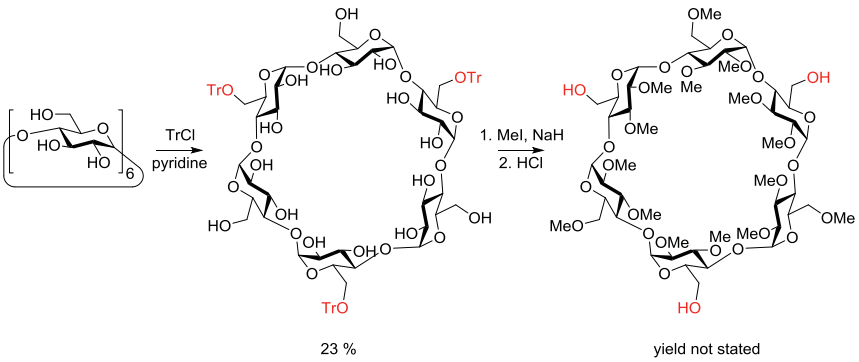
Scheme 5 Selective deprotection of perbenzylated α-cyclodextrin

chloride (Breslow et al. 1990). The use of benzophenone-3,3′-disulfonyl chloride led to $6^A,6^C$ derivative, while *trans*-stilbene-4,4′-disulfonyl chloride led to $6^A,6^D$ derivative (Tabushi et al. 1981). The disulfonates could be transformed into diiodo (Breslow et al. 1990), diazido, diamino (Tabushi et al. 1977; DiBlasio et al. 1996) or dithio derivatives (Tabushi et al. 1977) by common reactions.

When a trisubstituted cyclodextrin derivative is needed, the reaction of α-cyclodextrin with triphenylmethyl chloride, so-called "trityl chloride", comes into play. The reaction yields symmetrically trisubstituted cyclodextrins due to sterical hindrance (Scheme 7). Protection of all the remaining hydroxyl groups by methyl iodide and hydrolysis of trityl groups proceeded to a useful trisubstituted precursor—per-*O*-methyl-$6^A,6^C,6^E$-trihydroxy-α-cyclodextrin (Boger et al. 1979). The quantitative analysis of α-cyclodextrin tritylation was studied later by ultra-fast liquid chromatography (Yoshikiyo et al. 2015).



Scheme 6 Regioselective synthesis of disulfonate cyclodextrin derivatives



Tr = triphenylmethyl
Me = methyl

Scheme 7 Synthesis of symmetrically trisubstituted α-cyclodextrin

Syntheses based on selective debenzylation

Syntheses based on selective deprotection of persubstituted cyclodextrins have became very popular as they are high-yielding and proceed very smoothly. The sterical properties of a reagent together with cyclodextrin reactivity play the key role in the deprotection reaction. Selective deprotection of perbenzylated cyclodextrins by diisobutylaluminium

hydride represents the most favourite method for synthesis of selectively substituted cyclodextrins, as well as monosubstituted cyclodextrins—see below. It was first described by Pearce and Sinaÿ (2000). The method involves a selective deprotection of two opposite benzyl groups in high yields and it is applicable to α-, β-, and γ-cyclodextrin (Scheme 8). Benzyl groups are removed from glucose units A and D at position 6, but in the case of γ-cyclodextrin also from glucose units A and E. It has been proposed the reaction involves at least two molecules of diisobutylaluminium hydride and the mechanism occurs by a stepwise process (Sollogoub 2013). Free hydroxyl groups could be subsequently used for organic chemistry transformations leading to the desired cyclodextrin derivative (Petrillo et al. 2009; Volkov et al. 2015).

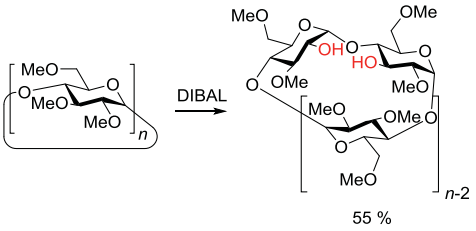
The step-by-step debenzylation and substitution reactions could lead to introduction of two different functional groups (Guieu and Sollogoub 2008a) or even to far more complicated structures—cyclodextrins trisubstituted (Guieu and Sollogoub 2008b; Rawal et al. 2010), tetrasubstituted (Rawal et al. 2010; Sollogoub 2013), pentasubstituted (Guieu and Sollogoub 2008b) or hexasubstituted at position 6, even with all the substituents different from each other (Wang et al. 2014). Moreover, together with Et_3SiH/I_2 hexakis-*O*-debenzylation at position 3 (Guitet et al. 2012) (see above), they allow a simultaneous selective deprotection on both the primary and secondary rim.

Syntheses based on other selective deprotections

Shortly after the selective bis-*O*-debenzylation was discovered, another selective double diisobutylaluminium hydride deprotection from Sinaÿ saw the light of day (du Roizel et al. 2002). It was found out both per-*O*-methyl α- and β-cyclodextrin were able to undergo a regioselective bis-*O*-demethylation, but on the secondary rim (Scheme 9). This opened the way for a direct access to $2^A,3^B$ derivatives (Letort et al. 2015).

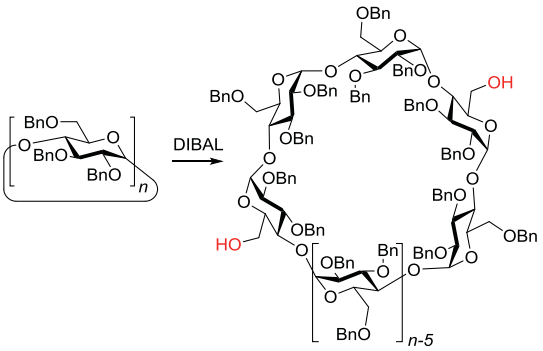
As it was shown by Xiao et al. (2013), the reaction requires 2^A methoxy group and an oxygen atom present at position 3^B to proceed. The authors also showed it was possible to carry out two or even three bis-*O*-demethylations on permethylated α- or β-cyclodextrins. However, in contrast to the multiple bis-*O*-debenzylations described above, these bis-*O*-demethylations are carried out in one step.

Mini summary The synthesis of selectively substituted cyclodextrins is quite a challenge due to the high number of theoretically possible isomers. However, selective bis-*O*-debenzylation (forming $6^A,6^D$ derivatives) and bis-*O*-demethylation (forming $2^A,3^B$ derivatives) make this



n = 6, α-cyclodextrin derivative
n = 7, β-cyclodextrin derivative
Me = benzyl
DIBAL = diisobutylaluminium hydride

Scheme 9 Selective bis-*O*-demethylation



n = 6, α-cyclodextrin derivative, 82 %
n = 7, β-cyclodextrin derivative, 83 %
n = 8, γ-cyclodextrin derivative, 59 %
(together with $6^A,6^E$ derivative)

Bn = benzyl
DIBAL = diisobutylaluminium hydride

Scheme 8 Selective bis-*O*-debenzylation

process much easier. The deprotections could be used multiple times or combined with the other methods. Moreover, the use of selective 6^A,6^B-, 6^A,6^C-, 6^A,6^D-sulfonations, or 6^A,6^C,6^E-tritylation is also possible.

Monosubstituted cyclodextrins

Monosubstituted cyclodextrins could be either substituted at positions 2, 3 or 6. Nowadays, there are two main methods for their synthesis: direct and indirect (Řezanka 2016). When using the first method, a cyclodextrin is directly reacted with a substitution agent. The second indirect method is based on a high-yielding deprotection step of a persubstituted cyclodextrin, such as permethylated, perbenzylated etc. This method is more universal than the first one, as the substitution of the free hydroxyl group usually results in high yields regardless the nature of the substitution agent used.

The syntheses of monosubstituted allyl, cinnamyl, propargyl, formylmethyl, carboxymethyl, azido and amino cyclodextrin derivatives have recently been studied in detail (Řezanka 2016). The following sections thus summarise the main findings and add information about other derivatives, which could be used as precursors for further synthesis.

Monosubstitution at position 2

The direct method takes advantage of the above-mentioned fact the hydroxyl group at position 2 is the most acidic. The use of a strong base thus mostly leads to substitution predominantly at this position and the yields reach up to 40%. However, sometimes the desired 2^A-*O* substituted derivative is hard to separate from its isomer and the purification step is done after peracetylation (Řezanka and Jindřich 2011; Bláhová et al. 2013). Peracetylation has also other advantages such as easy distinguishing between 2, 3 and 6 isomers directly from ^{1}H nuclear magnetic resonance spectrum (Řezanka 2016) and protecting the rest of hydroxyl groups against side reactions, for example, when oxidation of double bond is needed. Peracetylated derivatives could be easily deprotected by Zemplén deacetylation (Řezanka et al. 2010).

It is also possible to use the indirect method for the synthesis of cyclodextrin monosubstituted at position 2. As it has been mentioned above, regioselective bis-*O*-demethylation yields permethylated 2^A,3^B-dihydroxy derivatives (Scheme 9). The diol could be selectively alkylated at position 2 and subsequently methylated on the remaining hydroxyl group yielding permethylated 2-*O* derivative (Guan et al. 2009; Řezanka et al. 2015). A great variety of permethylated α-cyclodextrin derivatives was prepared using this methodology (Xiao et al. 2013).

Monosubstitution at position 3

As it has been mentioned above, position 3 is the least accessible, which results in difficulties during the synthesis of such derivatives. However, there are several methods allowing substitution at position 3. Jindřich and Tišlerová (2005) found the alkylation of β-cyclodextrin with cinnamyl bromide result selectively in 3^A-*O*-cinnamyl-β-cyclodextrin in a good yield. This behaviour is caused by the inclusion of cinnamyl bromide in the cavity. The resulting complex has the reactive centre of the alkylation agent oriented towards position 3.

The abovementioned regioselective bis-*O*-demethylation (Scheme 9) is also useful for the synthesis of permethylated cyclodextrin derivatives monosubstituted at position 3. The 2^A,3^B-diol could be selectively methylated at position 2 and the free hydroxyl group at position 3 serves as the reaction centre for further modifications (Xiao et al. 2013).

Monosubstitution at position 6

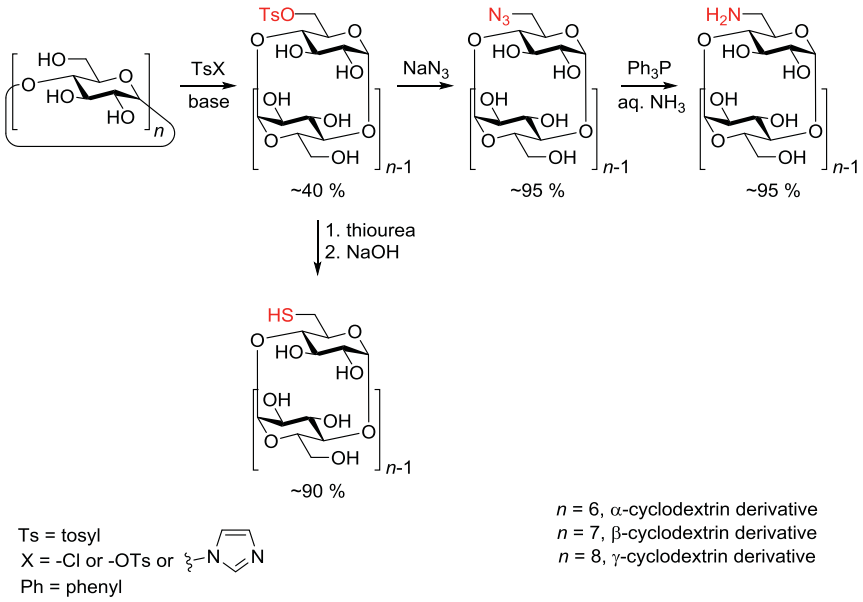
Cyclodextrins monosubstituted at position 6 represent unique precursors for the attachment to another molecule or to a surface. Connection through position 6 leaves the wider rim of a cyclodextrin open for interactions of guests with the cavity. Moreover, cyclodextrins monosubstituted at position 6 are easy to synthesise compared to the other derivatives. The reason has already been mentioned above—the hydroxyl group at position 6 is primary and the least acidic. The deprotonation of all hydroxyl groups thus predominantly leads to the substitution at this position, as there is the lowest steric hindrance. Although a lot of cyclodextrins monosubstituted at position 6 are commercially available, they are still synthesised by researchers due to their high price.

The most favourite derivative is 6^A-deoxy-6^A-tosylcyclodextrin is used for the synthesis of other useful precursors: azide, amino and thio derivatives (Scheme 10) (Martinelli et al. 2014; Řezanka 2016).

The best-known indirect method for the synthesis of cyclodextrin derivatives monosubstituted at position 6 is selective debenzylation of perbenzylated α-, β-, and γ-cyclodextrins (Pearce and Sinaÿ 2000). The reactions proceed in very good yields, unusual for monosubstituted cyclodextrins. The free hydroxyl group could be subsequently modified and benzyl groups removed by H_2 on Pd (Lindbäck et al. 2012).

The second indirect method is the abovementioned regioselective bis-*O*-demethylation. It provides per-*O*-methyl-6^A-hydroxy-α- or β-cyclodextrin as by-products in 20% yield (du Roizel et al. 2002).

Mini summary The direct syntheses of monosubstituted cyclodextrin derivatives benefit from the different reactivity of hydroxyl groups. 2^A-*O* substituted derivatives are



Scheme 10 Synthesis of cyclodextrins monosubstituted at position 6

obtained using a strong base. 3^A-*O* substituted derivatives are synthesised using selective introduction of cinnamyl group. Cyclodextrins monosubstituted at position 6 are the most favourite ones and 6^A-deoxy-6^A-tosyl-cyclodextrins overshadow all the other derivatives, as it is the most used precursor for further synthesis. Deprotection of methyl groups from permethylated cyclodextrins could lead selectively to either 2, 3, or 6 monosubstituted derivatives and debenzylation of perbenzylated cyclodextrins furnishes per-*O*-benzyl-6^A-hydroxy-cyclodextrins selectively in high yields.

Conclusion

A lot of cyclodextrin derivatives have become available on the market over the years. However, their price is in the range of fine chemicals, and thus, they are still often synthesised in laboratories. Randomly substituted cyclodextrin derivatives are the only exception. Synthesis of persubstituted cyclodextrin derivatives remains more or less the same and the methods for cyclodextrins persubstituted at selected positions are now very well examined.

The synthesis of selectively substituted and monosubstituted cyclodextrin derivatives has changed much over the years. Originally used direct methods subside, and the indirect methods are now on the rise. The only exceptions are 6^A-deoxy-6^A-tosyl-α-, β- and γ-cyclodextrins. They are the most favourite precursors for further syntheses.

The synthesis of tosyl derivatives is quick, high yielding, using cheap chemicals and the tosyl group is suitable for further reactions. Moreover, the synthesis requires only an easy purification process, i.e. recrystallisation, which is the key step. Cyclodextrin derivatives aspiring to be similarly successful should fulfil these conditions.

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Anion recognition in water by a rotaxane containing a secondary rim functionalised cyclodextrin stoppered axle†

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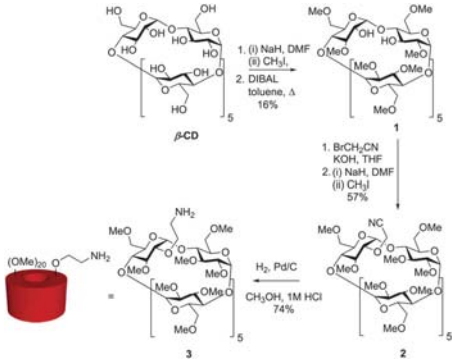
The synthesis of a water soluble [2]rotaxane is reported using hydrophilic secondary rim functionalised permethylated β-cyclodextrin derivatives as the axle stopper groups. The rotaxane recognises halide anions in pure water with impressive selectivity over sulfate.

Anion binding proteins found in nature achieve a high degree of selectivity and affinity through encapsulation of the target guest anion within a three-dimensional array of hydrogen bond donors, in a binding domain buried deep within the protein and shielded from the surrounding aqueous solvent.[1,2] Given the need to recognise, sense and extract anions in aqueous biological, medical, environmental and industrial contexts,[3,4] achieving this level of recognition with synthetic anion receptors remains a significant challenge for anion supramolecular chemistry. Despite the significant expansion and development of the field of anion recognition, the binding of anions in pure water – a necessity if the application of such receptors in aqueous environments is to be achieved – remains underdeveloped. Whilst there are countless anion hosts reported to date able to function in organic solvents, the majority of such receptors undergo a dramatic drop in anion binding affinity in organic–aqueous solutions, and binding is often completely negated in water. Indeed, examples of receptors able to function in pure water are rare, usually employing high positive charge or Lewis-acidic metals, with the former typically operating over a restricted pH range and involving multiple protonation equilibria.[5–10] Taking inspiration from the encapsulation of anions observed in anion binding proteins, we have synthesised a wide range of mechanically interlocked anion receptors in recent years, such as rotaxanes and catenanes, which bind the guest species in a three-dimensional binding cavity formed between the interlocked components.[11] Such mechanically bonded hosts have been demonstrated to exhibit a high level of selectivity and affinity for the templating anion in competitive aqueous–organic solvent mixtures.[12–17] Recently, as part of an investigation into halogen bonding interactions in water, we prepared a series of halogen bonding and hydrogen bonding rotaxanes capable of recognising halide anions in pure water.[18] Aqueous solubility was achieved by employing permethylated β-cyclodextrin derivatives as the rotaxanes' axle stopper motifs, which had been mono-functionalised on the narrow, primary rim of the cyclodextrin's truncated cone-like structure.

Herein we report the first example of a rotaxane featuring secondary rim mono-functionalised permethylated β-cyclodextrin derivatives as the axle stopper groups, and demonstrate the ability of the hydrogen bonding interlocked host to recognise halide anions in pure water with noteworthy selectivity over sulfate which is not bound.

In order to prepare the secondary-rim functionalised permethylated β-cyclodextrin (β-CD) stopper derivatives, a selective permethylated β-CD de-*O*-methylation procedure was employed (Scheme 1).[19]



Scheme 1 Synthesis of secondary-rim functionalised permethylated β-cyclodextrin **3**.

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† Electronic supplementary information (ESI) available: Experimental procedures, titration protocol and copies of NMR spectra. See DOI: 10.1039/c5cc00171d

Permethylated β-CD was prepared from the native β-CD by a deprotonation/methylation procedure, before selective de-methylation using diisobutylaluminium hydride (DIBAL) afforded $2^A,3^B$ diol **1** in 16% yield. This was subsequently alkylated selectively on the 2^A-position with bromoacetonitrile, and following methylation of the remaining hydroxyl group, nitrile derivative **2** was isolated in 57% yield.[20] Subsequent reduction of **2** afforded the desired 2^A-*O*-aminoethyl-per-*O*-methyl-β-cyclodextrin[20] **3** in 74% yield. The position of the amino-ethyl substituent on the 2^A-position of the cyclodextrin skeleton was confirmed by 2D NMR spectroscopy techniques (see ESI†).

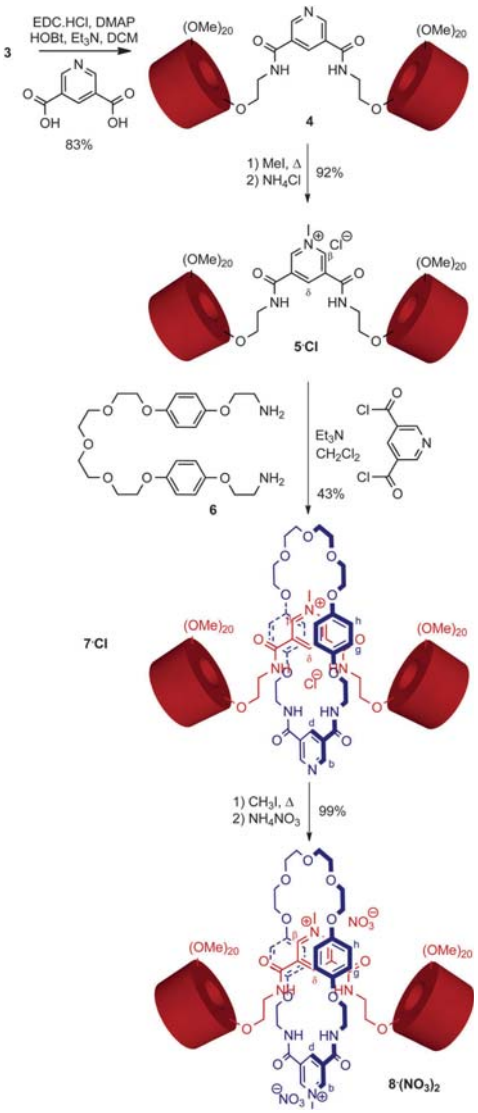
EDC-mediated coupling of two equivalents of 2^A-*O*-aminoethyl-per-*O*-methyl-β-cyclodextrin **3** with 3,5-pyridine dicarboxylic acid afforded 3,5-bis-amide pyridine compound **4**, which was subsequently methylated using methyl iodide to afford axle $\mathbf{5}^+$ as the iodide salt. This was exchanged to the chloride salt **5·Cl** using an anion-exchange resin, in preparation for chloride-templated rotaxane synthesis (Scheme 2).

Chloride anion templation methodology[11] was used to prepare rotaxane **7·Cl**. Axle **5·Cl** was stirred with bis-amine **6**[21] (10 eq.), 3,5-bis-chlorocarbonyl pyridine (10 eq.) and NEt_3 in dry CH_2Cl_2 (Scheme 2). Purification by size exclusion chromatography and preparatory silica gel chromatography afforded rotaxane **7·Cl** in 43% yield, which was characterised using ^1H and ^{13}C NMR spectroscopy, and high-resolution electrospray mass spectrometry (HRMS). Protons b and d are shifted downfield in the rotaxane product, whilst the hydroquinone protons h and g are significantly split and perturbed upfield (Fig. 1). This is characteristic of aromatic donor–acceptor interactions between the electron-rich hydroquinones in the macrocycle and the electron deficient pyridinium motif of the axle, and is indicative of the interlocked nature of the two components in the rotaxane. This was further confirmed by through-space interactions between the macrocycle and axle components observed in the ^1H–^1H ROESY NMR spectrum (see ESI,† Fig. S3).

Methylation of the pyridine moiety of the macrocycle component using CH_3I afforded the dicationic rotaxane $\mathbf{8}^{2+}$ as the mixed chloride–iodide salt in 99% yield (Scheme 2). To probe the anion binding properties of the rotaxane in water, it was necessary to exchange the halide counter ions to the corresponding water soluble nitrate salt, which was achieved by passing a solution of the rotaxane through a nitrate-loaded anion exchange resin. The hydroquinone proton signals of rotaxane **8·(NO$_3$)$_2$** remain upfield shifted and split following methylation and anion exchange (Fig. 2), and through-space interactions in the ^1H–^1H ROESY NMR spectrum between the two components are observed (see ESI,† Fig. S4), which taken together confirm that the interlocked nature of the rotaxane is preserved.

The anion recognition properties of rotaxane **8·(NO$_3$)$_2$** were investigated *via* ^1H NMR titration experiments, by adding increasing amounts of the sodium salts of Cl^-, Br^-, I^- and SO_4^{2-} in D_2O. It proved possible to monitor the shifts in the pyridinium protons as a function of anion concentration. Upon addition of halide anions to rotaxane **8·(NO$_3$)$_2$**, perturbations of the internal cavity protons d and δ were observed, with negligible movement of the external pyridinium protons b and β (Fig. 3), indicating that the halide anion is bound within the rotaxane's interlocked hydrogen bonding cavity (Fig. 4).

WinEQNMR[22] analysis of the titration data (Fig. 5), monitoring the perturbation of the macrocycle cavity pyridinium proton d,



Scheme 2 Synthesis of rotaxane **8** $(NO_3)_2$.

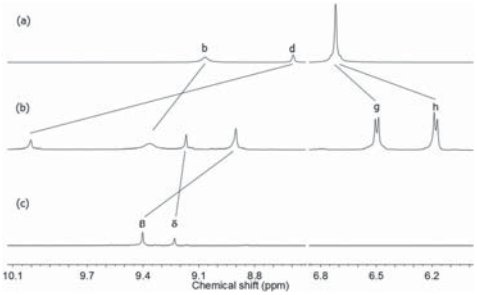


Fig. 1 Comparison of truncated ^{1}H NMR spectra of (a) macrocycle, (b) rotaxane **7·Cl** and (c) axle **5·Cl** (1 : 1 $CDCl_3/CD_3OD$, 500 MHz, 298 K). For atom labels see Scheme 2.

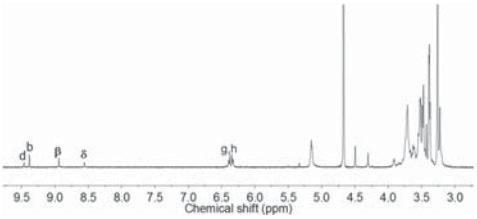


Fig. 2 ^{1}H NMR in D_2O of dicationic rotaxane **8·(NO$_3$)$_2$**.

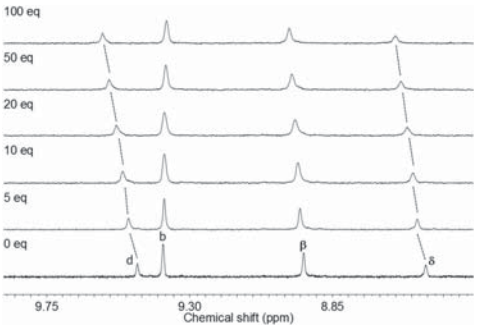


Fig. 3 Changes in the ^{1}H NMR spectrum of rotaxane **8·(NO$_3$)$_2$** upon addition of NaI (D_2O, 500 MHz).

determined 1 : 1 stoichiometric anion association constants shown in Table 1.

The halides bind with modest association constant values in the order $I^- > Br^- > Cl^-$, which presumably results from a combination of size and shape host–guest complementarity between the larger halides and the rotaxane's interlocked binding cavity,[12] and the Hofmeister series[23] bias towards the binding of the less solvated, larger halide anions. Importantly in contrast, addition of sulfate resulted in negligible perturbation of the internal cavity protons d and δ (<0.03 ppm after

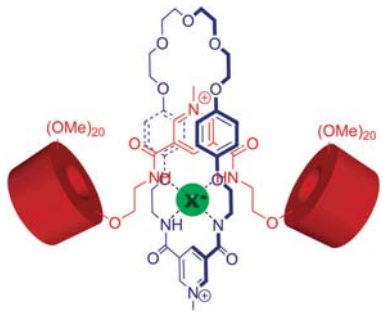


Fig. 4 Representation of halide binding within the interlocked binding domain of rotaxane **8·(NO$_3$)$_2$** *via* four convergent hydrogen bonds (X^- = Cl^-, Br^- or I^-).

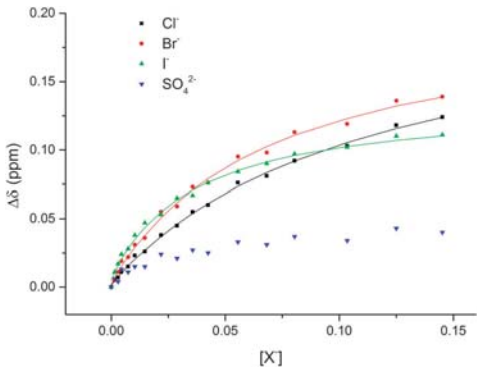


Fig. 5 Plots of chemical shift of binding cavity protons d against anion concentration in D_2O. Actual data represented by symbols, calculated 1 : 1 binding isotherms represented by solid lines.

Table 1 Anion association constants of rotaxane **8·(NO$_3$)$_2$** in D_2O and complexation induced chemical shift changes of proton d

Anion[a]	K_a[b] [M^{-1}]	Δδ(d)[d]
Cl^-	10	0.12
Br^-	15	0.14
I^-	35	0.11
SO_4^{2-}	—[c]	0.03

T = 298 K. [a] Anions added as the sodium salt. [b] Calculated using chemical shift data of proton d. Errors estimated to be <10%. [c] Binding too weak to be quantified. [d] Chemical shift change (ppm) of proton d after addition of 120 equiv. of anion.

120 equivalents), thus demonstrating that the di-anionic sulfate anion is not bound by the rotaxane, despite the receptor possessing a double positive charge.

It has previously been shown that dihydrogen phosphate and acetate oxoanions are unable to penetrate such geometrically restrained interlocked rotaxane binding domains and associate

weakly on the periphery. In this case, the lack of perturbation of the external pyridinium protons b and β, or internal cavity protons d and δ, indicate that there is no quantifiable sulfate association with the rotaxane in water. This level of halide selectivity over oxoanions, particularly those bearing multiple units of negative charge such as sulfate, is particularly rare. Indeed, the few examples reported in the literature that compare halide and oxoanion binding in aqueous media are found to be selective for the di-anionic sulfate anion over the mono-charged halides.[24–28]

The halide association constants for rotaxane **8·(NO$_3$)$_2$** are of comparable magnitude with those reported previously for the analogous rotaxane host in which the cyclodextrin stoppers are functionalised on the primary rim (see ESI,† Fig. S5, K_a = 15, 35 and 50 M^{-1} for Cl$^-$, Br$^-$ and I$^-$ respectively),[18] as opposed to the wider, secondary rim in this case. This indicates that the structure of the stopper motif plays a minor role in influencing the anion binding affinity, which suggests the ability of the bulky cyclodextrin derivatives to exclude solvent from the rotaxane's binding cavity is somewhat limited. Indeed, the use of an ethylene spacer group between the pyridinium bis-amide motif and the cyclodextrin groups in rotaxane **8·(NO$_3$)$_2$** significantly increases both the conformational flexibility of the system and the accessibility of the solvent to the binding cavity, yet causes only a modest reduction in halide binding affinity. Importantly, this indicates that a wider range of water solubilising derivatives, such as α- and γ- cyclodextrin, or dendrimer-type motifs may be usefully employed for water soluble rotaxane host synthesis, without a detrimental effect on the strength of anion association.

In conclusion, we have prepared the first example of a rotaxane exploiting secondary rim functionalised permethylated β-cyclodextrin derivatives as the axle stopper groups. Such cyclodextrin derivatives impart excellent aqueous solubility on the rotaxane host structure, which recognises halide anions in water with impressive selectivity over sulfate.

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Enantiomeric recognition of amino acid ester salts by β-cyclodextrin derivatives: an experimental and computational study

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Abstract

β-cyclodextrin derivatives bearing benzoyl (β-CD-**1**) and naphthoyl (β-CD-**2**) moieties have been synthesized from salicylic acid and 3-hydroxy-2-naphthoic acid by a convenient method in 60% and 58% yields, respectively, and were tested for enantiomeric recognition of amino acid ester derivatives. Their ability to discriminate between various L-/D- amino acid methyl ester hydrochloride salts was examined using the ^{1}H NMR titration method in DMSO-d_6 at 25 ^{o}C. β-CD-**2** produced a fairly good discrimination of tryptophan ester salts with a binding constant of 4041 M^{-1} for L-salt compared with 2864 M^{-1} for D-salt, which corresponds to a difference of -0.21 kcal mol^{-1} in binding free energies. The binding free energies obtained from molecular dynamic calculation by MM/PB(GB)SA are consistent with those obtained from the experimental results.

Keywords: Beta-cyclodextrin derivatives, enantiomeric discrimination, ^{1}H NMR titration, molecular dynamic calculations, MM-PB(GB)SA

Introduction

Cyclodextrins (CDs) are cyclic polysaccharides usually made up of six to eight D-glucose units (α, β, γ-CD) linked at the C_1 and C_4 carbon atoms by α-1,4-glycosidic bonds. Because of their hydrophilic exteriors and hydrophobic cavities, they can form inclusion complexes with a wide range of guest molecules with a suitable shape and size in water like the hydrophobic pockets in enzymes, and catalyse or promote organic reactions through weak interactions between CDs and substrate molecules[1,2]. From the various types of cyclodextrins, β-cyclodextrin is widely used

since it is readily available and can accommodate a wide range of guest molecules because of its suitable cavity.

Molecular recognition of substrates by cyclodextrins occurs by noncovalent interactions in the hydrophobic cavity of the water-soluble, cyclic sugar oligomers. Some modified cyclodextrins have successfully been employed for enantiomer separations in several areas of science and technology[3,4]. The inclusion complex of host-guest systems serves our understanding of the cooperation of several weak forces working between a receptor and substrate, which include dipole-dipole, electrostatic, van der Waals, hydrogen bonding, and hydrophobic interactions[5].

Enantiomeric recognition of chiral amino acids by synthetic and natural host compounds is a good example of one of the most challenging subjects in modern host-guest chemistry[6], because host-guest chiral recognition plays an important role in biological processes, and asymmetric catalytic reactions[7]. A number of synthetic model compounds have been designed and synthesized as a chiral host molecule that help chemists understand the basis of the mechanism of host-guest complexations and their chiral recognition. Although significant advances in modelling techniques and the comparative simplicity of the host-guest system have been achieved, we still need to understand the molecular recognition at an atomic level and interpret experimental data by using computational tools and to ultimately help the design of new hosts for targeted molecular guests. Molecular dynamic simulations (MD) have been employed as current methods for understanding molecular recognition processes occurring in organisms at an atomic level, and consequently free energy calculations, initially developed for biological systems, have become a powerful tool in estimating quantitatively molecular interactions in host-guest chemistry. Nevertheless, they have also been applied to supramolecular systems of organic structures[8,9], even in predicting chiral discrimination[10,11].

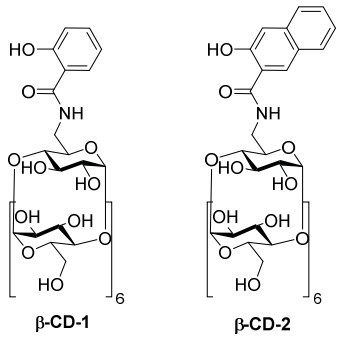
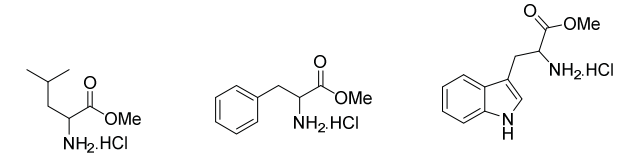


Figure 1. Structure of CD derivatives β-CD-**1** and β-CD-**2**.

The molecular mechanic-Poisson-Boltzmann (Generalized Born) surface area [MM/PB(GB)SA] is one of the most valuable methods,[12] which has successfully been applied to estimate the binding free energies of different biological systems.[13]

The present study reports the synthesis of two β-cyclodextrin derivatives bearing benzo (β-CD-**1**) and naphtho (β-CD-**2**) moieties from salicylic acid and 3-hydroxy-2-naphthoic acid, respectively (Figure 1), and investigates their inclusion complexation behaviour with L/D-amino acid ester salt derivatives (LeuOMe.HCl, PheOMe.HCl, TrpOMe.HCl) (Figure 2) by the ^{1}H NMR titration method in DMSO-d_6 at 25 ^{o}C. Molecular dynamic calculations were also applied to estimate binding free energies for the complexes using MM-PB(GB)SA.



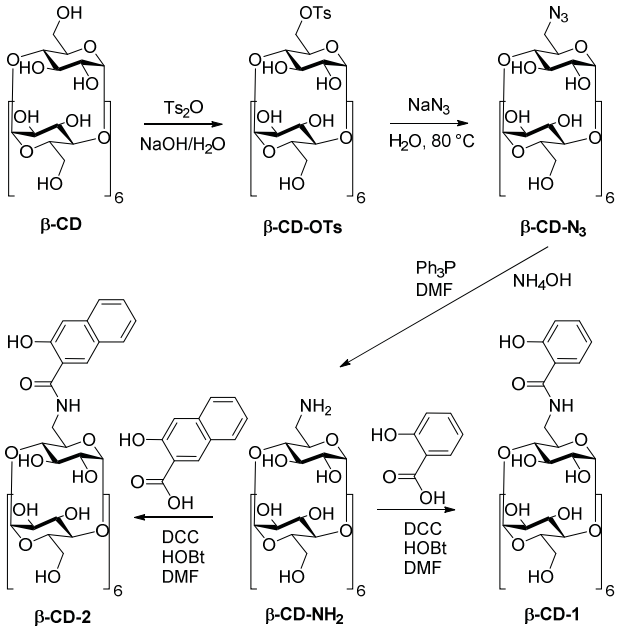
L- or D-LeuOMe.HCl L- or D-PheOMe.HCl L- or D-TrpOMe.HCl

Figure 2. Amino acid ester salts used as the guest.

Result and Discussion

Synthesis

At the outset 6^A-deoxy-6^A-amino- β-cyclodextrin was prepared *via* 6^A-tosyl[16] and 6^A-deoxy-6^A-azido derivatives according to the previously described procedures.[17] 6^A-deoxy-6^A-amino- β-cyclodextrin was used to prepare novel β-CD-**1** and previously synthesized β-CD-**2**.[15] As expected, the reactions proceeded uneventfully to furnish the desired products in yields of 60% and 58%, respectively (Scheme 1).



Scheme 1. Synthesis of β-CD-**1** and β-CD-**2**.

Enantiomeric recognition

Amino acid ester salts are widely used in enantiomeric recognition studies for three reasons: (i) ammonium salts form stronger hydrogen bonds than amines, which increases enantioselectivity, (ii) ammonium salts containing an aromatic moiety enable cation-π interactions, (iii) the ester moiety in amino acid ester salts contributes to the strength of hydrogen bonding due to its acceptor property and if a proton exists in the macrocycle it also contributes to enantiomeric recognition by forming a hydrogen bond with it.

NMR has become a routinely used tool for the study of host-guest supramolecular chemistry, and there are now hundreds of reports on studies where NMR titration was used to measure intermolecular association.[31,32] When macrocycles absorb different frequencies in free and complexed states, the differences in chemical shifts in the NMR spectra may suffice for an estimation of the thermodynamics of enantiomeric recognition. In NMR titration experiments, the addition of varying concentrations of guest molecules results in a gradual shifting of several chemical signals upfield or downfield.

Typical ^{1}H NMR spectral changes upon the addition of amino acid ester salts to a modified cyclodextrin solution are shown in Figure 3.

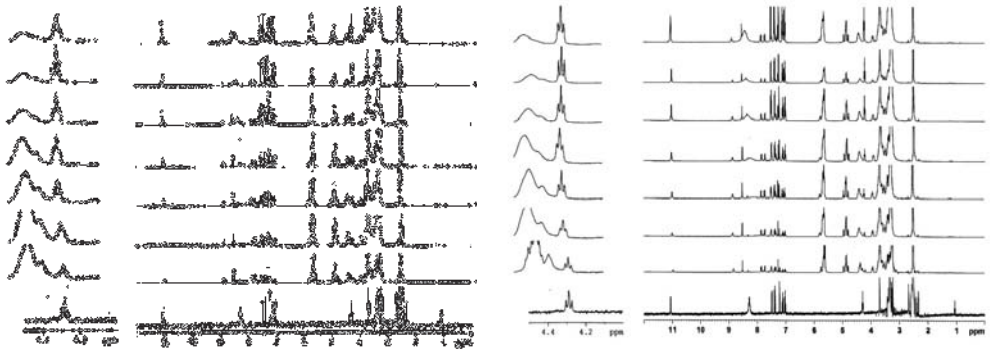


Figure 3. ^{1}H NMR spectral changes in chiral β-CD-**2** (4.40 mM) upon the addition of D-TrpOMe.HCl (left) and L-TrpOMe.HCl (right) in DMSO-d_6 at 25 ^{o}C.

These results indicate the formation of an inclusion complex between modified cyclodextrins and amino acid ester salts with a 1:1 stoichiometry, which was confirmed by a Job plot (Figure 4).

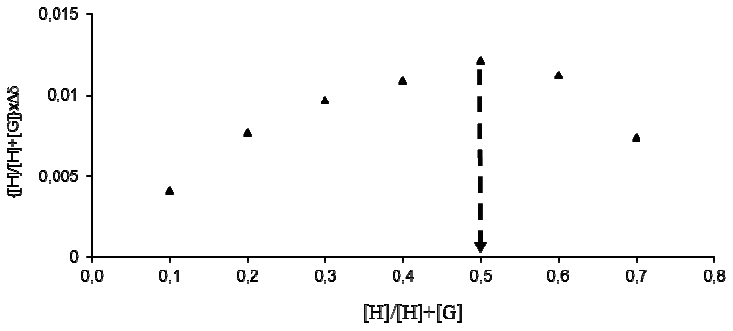


Figure 4. Job plot for the complex of L-PheAlaOMe.HCl with β-CD-**2**.

The determination of binding constants for chiral host-guest interaction provides information about the capability of hosts to recognize enantiomers of the chiral guest under given sets of

conditions. Correlation of the degree of recognition with the structural features of the CD-guest complexes is essential in understanding the origin of the chiral recognition. The ^{1}H NMR titration technique was employed to calculate the binding constants for each enantiomer of LeuOMe.HCl, PheOMe.HCl and TrpOMe.HCl methyl ester hydrochloride salts with β-CD-**1** and β-CD-**2** (Table 1). The chemical shift of the –C*H* (methine,δ= 4.3125 ppm) signal in the amino acid salts was monitored against their concentration. A typical plot for the complexation of β-CD-**2** with L-PheOMe.HCl is shown in Figure 5. The calculated 1/Δδ values are plotted against $1/\Delta\delta_{max}$ to give an excellent linear relationship (r^2= 0.9989) with a slope of 0.0183. Although the binding constants are relatively large, the discrimination of the enantiomers by the hosts is too small and the difference especially for leucine is within experimental errors.

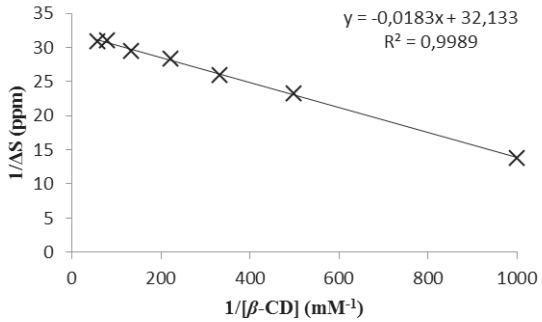


Figure 5. Typical plot of 1/Δδ versus 1/[β-CD] for the complex of β-CD-**2** with L-PheAlaOMe.HCl.

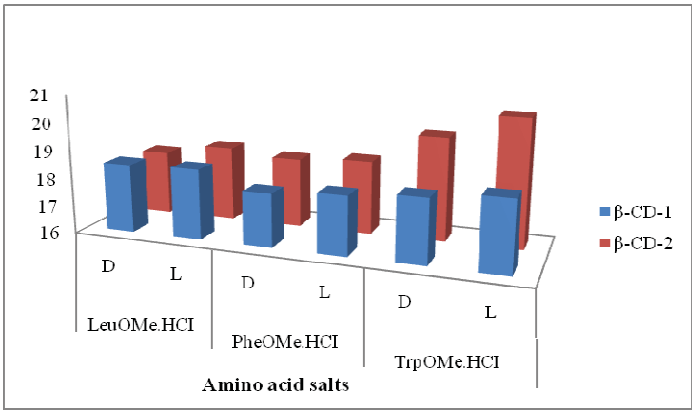


Figure 6. Gibbs free energy changes (-ΔG^{o}) as a function of amino acids for the inclusion complexes of modified β-CD-**1**and β-CD-**2.**

Table 1. Binding constants (K_a), Gibbs free energy changes ($-\Delta G^o$) and enantioselectivity K_L/K_D for the complexation of the L-/D-guest with β-CD-**1** and β-CD-**2** in DMSO-d_6

Host	Guests	Ka(M^{-1})	K_L/K_D	$-\Delta G^o$ (kcal mol^{-1})	$-\Delta\Delta G^o$(kcal mol^{-1})*
β-CD-**1**	D-LeuOMe.HCl	1737.17±0.95	1.02	4.41±0.95	-0.02
	L-LeuOMe.HCl	1764.85±0.15		4.43±0.87	
	D-PheOMe.HCl	1376.22±0.75	1.07	4.28±0.19	-0.05
	L-PheOMe.HCl	1483.78±0.79		4.33±0.53	
	D-TrpOMe.HCl	1609.18±0.30	1.09	4.37±0.97	-0.06
	L-TrpOMe.HCl	1767.78±0.85		4.43±0.28	
β-CD-**2**	D-LeuOMe.HCl	1635.02±0.79	1.16	4.38±0.92	-0.09
	L-LeuOMe.HCl	1906.10±0.29		4.47±0.96	
	D-PheOMe.HCl	1755.90±0.16	1.06	4.42±0.61	-0.03
	L-PheOMe.HCl	1855.56±0.18		4.45±0.30	
	D-TrpOMe.HCl	2863.79±0.31	1.41	4.71±0.91	-0.21
	L-TrpOMe.HCl	4040.75±0.23		4.92±0.29	

*$\Delta\Delta G^o$=-(ΔG^o_L-ΔG^o_D)

Extensive studies on molecular recognition by cyclodextrins have shown that an important characteristic of complexation is the simultaneous operation of several weak forces working between the guest and CDs, which determine how the size and shape of a guest molecule fit into the host cavity. Data indicate that both β-CDs form more stable complexes with L-enantiomers of all guests (Table 1). This may be attributed to the strict geometrical complementary relationship between the β-CD cavity and amino acids.[33] The results also demonstrate that the host β-CD-**2** forms stronger complexes with amino acid salts compared to those with β-CD-**1**, possibly because of the bulkier and more hydrophobic character of the naphtha group compared with the benzyl group. On the other hand, β-CD-**1** shows poor enantioselectivity for three amino acid ester salts. The highest enantioselectivity was observed for tryptophan (K_L/K_D= 1.09). However, β-CD-**2** shows better enantioselectivity for amino acids compared with β-CD-**1**, particularly for tryptophan (K_L/K_D= 1.41, $\Delta\Delta G^0$= -0.21 kcal mol^{-1}, Figure 6). The larger enantioselectivity found by β-CD-**2** may be attributed to the difference in the size of the site arms, naphtha and benzyl. It was found that both hosts bind and discriminate tryptophan, possibly due to stronger π-π interactions between CD (hosts) and Trp (guest) molecules in addition to weaker non-covalent interactions (hydrogen bonding, van der Waals, dipol-dipol etc.). The reason for the better selectivity presented by β-CD-**2** for this guest may be associated with the larger size of napthyl compared with benzyl in β-CD-**1**. The better selectivity found for tryptophan by both hosts may be attributed to the indole ring, better fitted to the cavity compared with phenyl and isopropyl in phenylalanine and leucine. It seems that the size and shape of the guest molecule and the structural change of the host molecule administrate the complexation phenomena to some extent. Hence, the induced fit and the geometrical complement between the host and the guest play a

crucial role in the chiral recognition of amino acid molecules. The stability of the inclusion complex with modified cyclodextrin should depend on the condition of strict size-fit between host and guest.

Computational modelling

Although significant information has been obtained regarding the stability and selectivity of the complexes of modified β-CDs with methyl esters of amino acids, one still cannot reach a conclusion about the mode of these complexes and the main driving forces behind the enantioselectivity imposed by these hosts and hence the truer of molecular recognition at an atomic level. Molecular dynamic simulations (MD) have been employed to understand the dynamic behaviour of these complexes and hence the binding energies for each complex were calculated from the dynamic trajectories of MM/PB(GB)SA. The molecular dynamic calculations were performed for both CDs from 0 K to 700 K. The reason for MD calculations at higher temperature is to avoid the possible traps of conformers in local minima. RMSD changes obtained for the CDs at 700 K compared to their starting coordinates as a function of time during the MD are presented in Figure 7. They show that the major conformational fluctuation occurs in the cyclodextrin cavities of β-CD-**1** and β-CD-**2**. The conformers with a higher population for each CD are presented in Figure 8. They show both β-hydroxyl phenyl and naphthyl groups are located perpendicular to the cavity of both hosts. The molecular dynamic calculations were also performed for the complexes of CDs with enantiomers of methyl esters of amino acid salts. RMSD changes obtained for each complexes compared to their starting coordinates as a function of time during the MD are presented in Figure 9. They show that the complexes of salts with both CDs does not experience large conformational changes, except that the complex of leucine salts with CDs have larger RMSD, more underlined with β-CD-**1**, possibly due to the large conformational fluctuations of the salts due to weaker binding of the site arm, isopropyl compared with phenyl and indole rings in other salts. It is evident that the receptors do not show large dynamic oscillation (Figure 9).

The lowest energy conformers for each complex of β-CD-**1** and β-CD-**2** with enantiomers of amino acid ester salts obtained from the cluster analyses are superimposed to see the mode of action behind the discrimination. They are displayed in Figures 10. They show that all of the site arms of salts are included in the cavity of β-CD-**1** and β-CD-**2** except for L-leucine where its methyl ester function tends to be located within the cavities of both hosts in an opposite direction to β-hydroxy phenyl and naphthyl groups. The MD calculations show that the ammonium ion interacts with the carbonyl groups for all the complexes of both hosts (Figure 11).

They also indicate that the indole ring of tryptophan better fits into the cavities of both hosts compared with the phenyl and isopropyl groups in phenylalanine and leucine salts, which is consistent with the experimental observations. Beside atom distance analyses (between methane hydrogen in the salts and C5 in CDs) demonstrate that the ligands are held within the cavity during MD simulations (Figure 11). Consistency with the experimental observations is also found for the discrimination of the enantiomers of these amino acid salts. The binding free

energy obtained for the D-phenylalanine salt by MM-PBSA (see Table 2) is somehow too large and stands out of the scale obtained for the rest of the enantiomers.

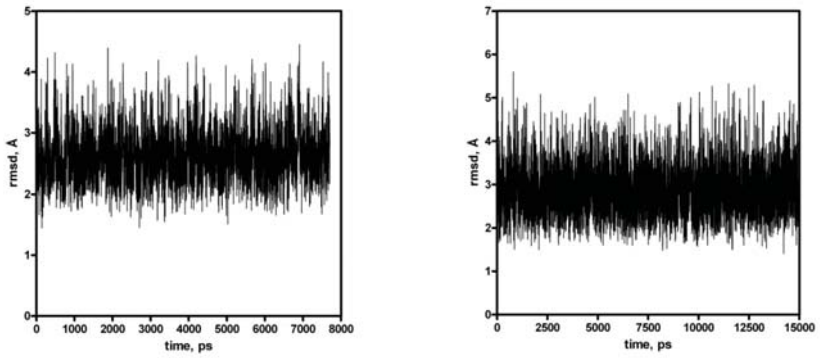


Figure 7. RMSD changes obtained for β-CD-**1** (left) and β-CD-**2** (right) compared to their starting coordinates as a function of time during MD performed at 700 K.

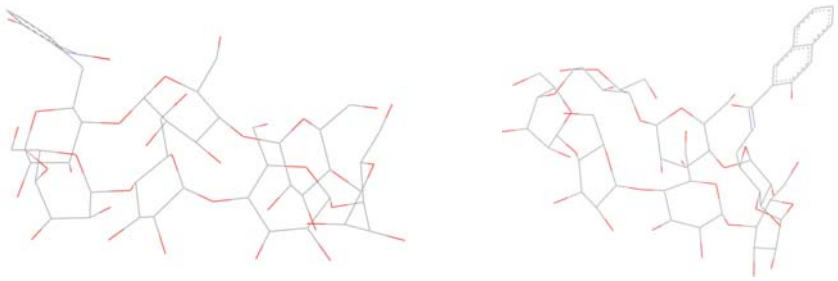


Figure 8. The lowest energy conformers of β-CD-**1** (left) and β-CD-**2** (right).

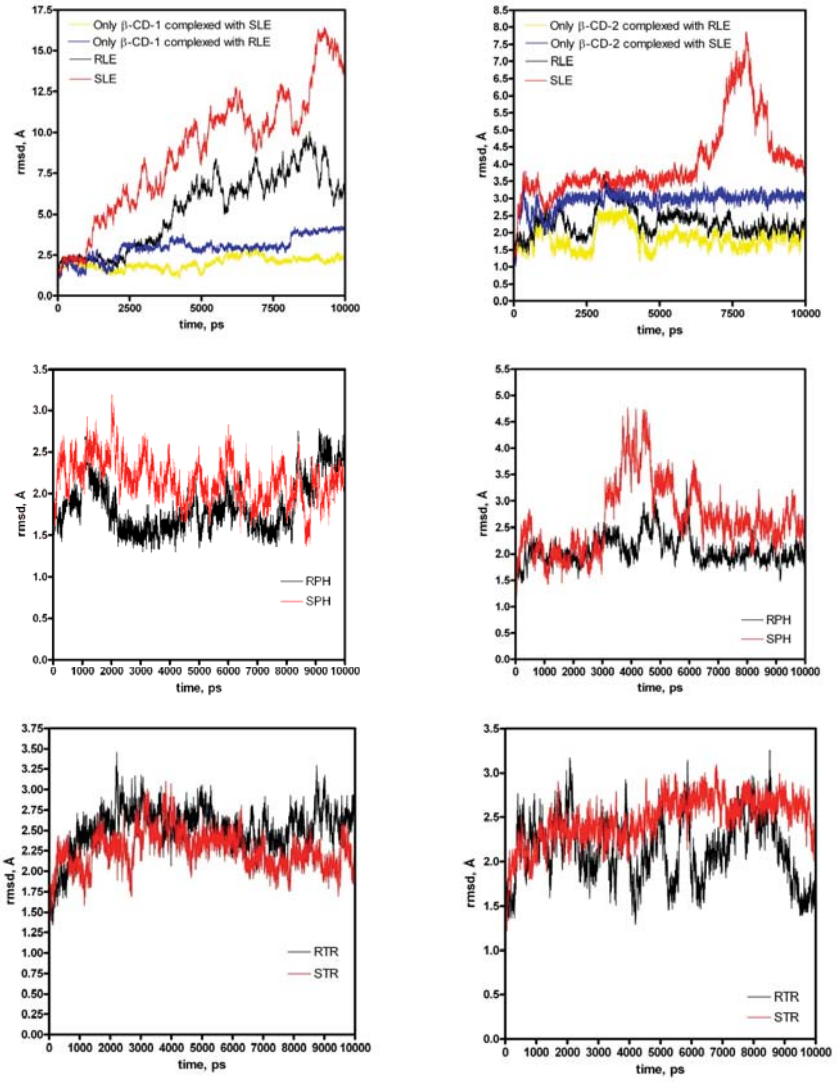


Figure 9. RMSD changes obtained for the complexes of β-CD-**1** (left column) and β-CD-**2** (right column) with amino acid ester salts compared to their starting coordinates as a function of time during MD performed at 298 K. (RLE: D-LeuOMe.HCl; SLE: L-LeuOMe.HCl; RPH: D-PheOMe.HCl; SPH: L-PheOMe.HCl; RTR: D-TrpOMe.HCl; STR: L-TrpOMe.HCl)

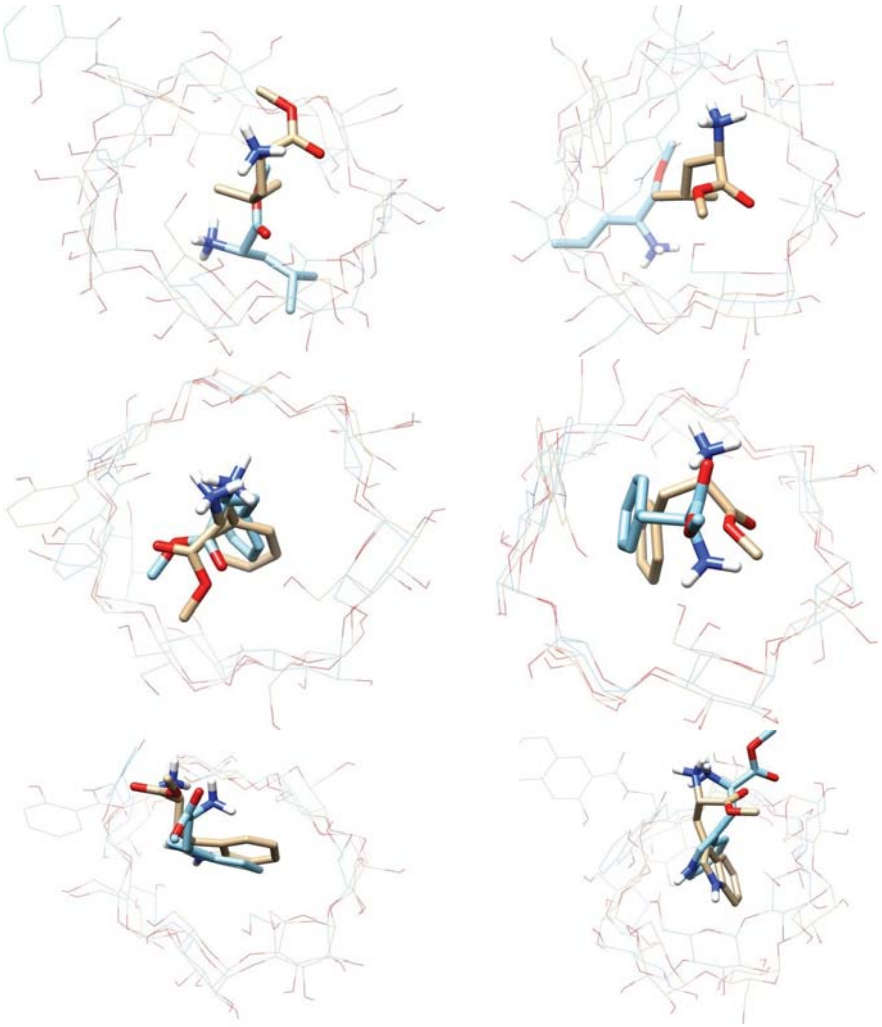


Figure 10. The superimposed lowest energy conformers of β-CD-**1** (right column) and β-CD-**2** (left column) with methyl esters of leucin (top row), phenylalanine (middle row) and tryptophan (bottom row).

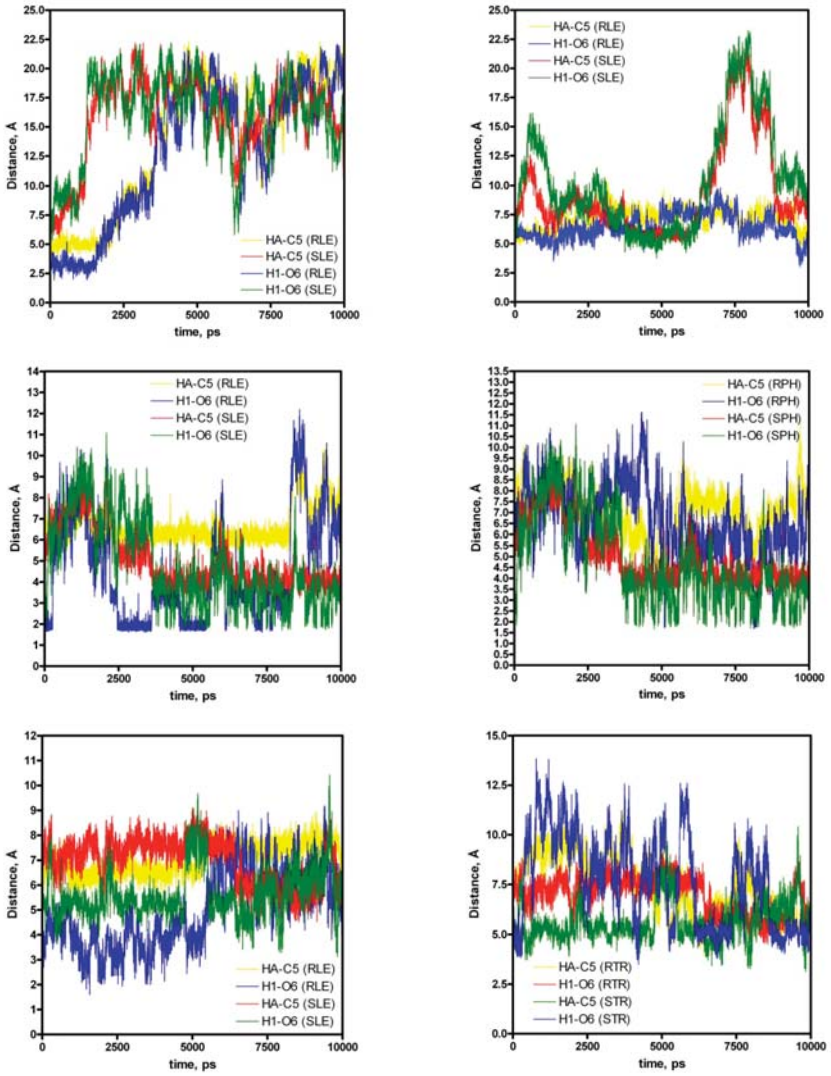


Figure 11. Distances between CH (HA) in the ligand and (C5) in the host and between one of ammonium hydrogens in the ligand and carbonyl oxygen in the receptor obtained from MD simulations for the complexes of β-CD-**1** (left column) and β-CD-**2** (right column) with amino acid ester salts at 298 K. (RLE: D-LeuOMe.HCl; SLE: L-LeuOMe.HCl; RPH: D-PheOMe.HCl; SPH: L-PheOMe.HCl; RTR: D-TrpOMe.HCl; STR: L-TrpOMe.HCl)

Table 2. Binding energies obtained from MD calculations by MM-PB(GB)SA for the complexes of β-CD-**1** and β-CD-**2**

Hosts	Ligands	ΔG_{GB} (kcal mol^{-1})	ΔG_{PB} (kcal mol^{-1})	$\Delta\Delta G_{GB}$ (kcal mol^{-1})	$\Delta\Delta G_{PB}$ (kcal mol^{-1})
β-CD-**1**	D-LeuOMe.HCl	0.268±0.051	0.982±0.018	-0.062	0.053
	L-LeuOMe.HCl	0.330±0.069	0.929±0.019		
	D-PheOMe.HCl	-6114±1605	-6114±1605	---	---
	L-PheOMe.HCl	-14.45±2.28	-11.88±3.06		
	D-TrpOMe.HCl	-17.51±2.44	-15.93±2.48	1.36	0.86
	L-TrpOMe.HCl	-18.87±1.98	-16.79±1.91		
β-CD-**2**	D-LeuOMe.HCl	-9.80±1.08	-7.60±1.28	-3.27	-2.29
	L-LeuOMe.HCl	-6.53±1.47	-5.31±2.14		
	D-PheOMe.HCl	-6.99±1.24	-4.70±1.39	-1.15	-0.26
	L-PheOMe.HCl	-5.84±0.95	-4.44±1.04		
	D-TrpOMe.HCl	-14.04±1.78	-12.01±2.17	3.16	6.22
	L-TrpOMe.HCl	-17.20±2.43	-18.22±2.52		

Conclusions

Two novel derivatives of β-cyclodextrin were prepared and their binding and enantioselective properties for methyl esters of some amino acid hydrochloride salts were investigated by the ^{1}H NMR titration technique. The result indicate that hosts have a significant ability to accommodate all amino acid salts and also that the host bearing the naphtho group has more binding and discrimination ability for the salts compared to the host bearing the benzo group. The molecular dynamic calculations are in agreement with those obtained by experimental observations and also give a detailed picture of complexes at an atomic level. Hence, these results may be useful in the understanding of the biochemical processes occurring in the cell.

Experimental Section

General. All of the chemicals were reagent grade unless otherwise specified. D- and L-amino acid methyl ester hydrochlorides and β-cyclodextrin (≥ 97%) were obtained from Aldrich Chemical Co. Silica gel 60 (Merck, 0.040-0.063 mm) and silica gel/TLC-sheets (F254) were used for flash column chromatography and TLC. Melting points were determined with a Gallenkamp Model apparatus with open capillaries. Infrared spectra were recorded on a Mattson 1000 FTIR model spectrometer. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded

on a Bruker AV400 high performance digital FT-NMR spectrometer. The chemical shifts (δ) and coupling constants (J) were expressed in parts per million and hertz, respectively.

NMR titration

A range of stock solutions of the ligands ($0–10^{-4}$ M) containing a constant amount of the CDs (10^{-3} M) in DMSO-d_6 was prepared and their ^{1}H NMR spectra (16 scans, sweep width of 20.7 ppm, digital resolution of 18, pulse angle of 30°, delay time of 1 sec) were collected at 298 K at ambient probe temperature and calibrated using tetramethylsilane as an internal reference. The changes in the chemical shift of –C*H* (methine) in amino acid methyl ester salts against their concentration were fitted to Equation 4, derived from Equations 1-3. The binding constants of the complexes of D- and L-amino acids ester salts with β-CD-**1** or β-CD-**2** were calculated by the Benesi-Hildebrand method[14].

$$CD + G \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{complex} \quad (\mathbf{1})$$

$$K_{diss} = k_1/k_{-1} = [CD][G]/[[\quad (\mathbf{2})$$

$$[G]_{tot} = [G]_{free} + [G]_{complex} \quad (\mathbf{3})$$

$$1/\Delta\delta = 1/(K_a.\Delta\delta_{max}.[G]_{tot}) + 1/\Delta\delta_{max} \quad (\mathbf{4})$$

where $\Delta\delta = (\delta_G - \delta_{obs})$, and $\Delta\delta_{max} = (\delta_G - \delta_{CD:.G})$, $\Delta\delta_{max}$ is the difference in chemical shift between the complexed and the free guest at saturation, $[G]_{tot}$ is the total concentration of guest and K_{diss} is the dissociation constant between CD and the guest.

Synthesis of β-CD-1

6^A-Deoxy-6^A-((2-hydroxybenzoyl)amino)- β-cyclodextrin. The synthesis was adapted from a previously published method.[15] Solutions of *N*,*N*′-dicyclohexylcarbodiimide (91 mg, 0.44 mmol) in DMF (4 mL) and hydroxybenzotriazole (68 mg, 0.44 mmol) in DMF (4 ml) were added to a solution of salicylic acid (61 mg, 0.44 mmol) in DMF (5 mL) cooled to 0 ^{o}C. The reaction mixture was stirred for 0.5 hours at 0 ^{o}C and a solution of 6^A-deoxy-6^A-amino- β-cyclodextrin[16,17] (500 mg, 0.44 mmol) in DMF (17 mL) was added dropwise. The reaction mixture was then stirred for another hour at 0 ^{o}C and 48 hours at room temperature. The reaction was quenched by pouring the mixture into acetone (300 mL). The precipitate was filtered out and purified by crystallization from water to yield 333 mg (60%) of the title compound as a white powder. Mp 228 ^{o}C (decomp.). IR (KBr): 3507, 3280, 3155, 3117, 3058, 1642, 1150, 1030 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 12.39 (s, 1 H, Ar-O*H*), 8.67 (s, 1 H, -N*H*-), 7.82 (d, *J* 7.6 Hz, 1 H, Ar-*H*), 7.38 (t, *J* 7.7 Hz, 1H, Ar-*H*), 6.94–6.82 (m, 2 H,Ar-*H*), 5.94–5.51 (m, 14 H, 7 x CD-O*H* (2), 7 x CD-O*H* (3)), 4.95–4.75 (m, 7 H, 7 x CD-*H* (1)), 4.56–4.33 (m, 6 H, 6 x CD-O*H* (6)), 3.92-3.10 (m, 42 H, 7 x CD-*H* (2), 7 x CD-*H* (3), 7 x CD-*H* (4), 7 x CD-*H* (5), 14 x CD-*H* (6)) ppm. ^{13}C NMR (DMSO-d_6): δ = 168.61 (-*C*ONH-), 159.65 (Ar-*C*-OH),133.52(Ar-*C*), 128.15(Ar-*C*),

118.51(Ar-*C*), 117.21(Ar-*C*), 115.56(Ar-*C*), 102.39–101.88 (7 x CD-*C* (1)), 84.16–59.91 (7xCD-*C* (2), 7 x CD-*C* (3), 7 x CD-*C* (4), 7 x CD-*C* (5), 7 x CD-*C* (6)) ppm.

Computational modelling

All of the molecular dynamic (MD) simulations were conducted using the Assisted Model Building with Energy Refinement (AMBER version 11.0)[18] suite of programmes on TR-Grid clusters based on Linux (TUBITAK). The structure of β-cyclodextrin was obtained from the X-ray structure (3CGT.pdb) found in the protein data bank (http://www.pdb.org).[19] The complexes were prepared by manually locating the site chain of each enantiomer into the cavity of the hosts using Discovery Studio Visualizer.[20] All of the atoms and charges for α-D-glucopyranoside units in the CDs were assigned as described in GLYCAM-06.[21] Atomic partial charges were calculated by AM1-Bcc (Austin model with bond and charge correction[22] using the antechamber module of AMBER (v11) for the modified residues by the introduction of benzyl and naphtyl amides and ff99SB[23] atom types were assigned for the benzyl and naphthyl parts. Xleap, as implemented in AMBER, was employed to prepare the parameter/topology and coordinate files and to solvate and neutralize the system for MD simulations. The complexes were solvated in a TIP3P[24] water box, which is a very popular solvation model for molecular dynamic simulations because of its simplicity and computational efficiency, with dimensions of 10 Å from the solute. Ptraj as implemented in AMBER was used for coordinate root-mean squared deviation (RMSD) and atom distance analyses. Three dimensional structures were displayed using Chimera (UCSF).[25]

To test the validity of the employed parameters and also to determine the dynamic behaviour of each host, they were heated from 0 to 700 K in a vacuum for a period of 15 ns. The complexes in explicit water were minimized in two stages; in the first, the hosts and guest were kept fixed and only the water molecules were allowed to move in 1000 steps (2500 steps with the steepest descent and 2500 steps with the conjugate gradient method, respectively) with a restraint of 500 kcal mol^{-1} $Å^{-2}$. In the second stage, all of the atoms were allowed to move in 2500 steps (1250 steps with the steepest descent and 1250 steps with the conjugate gradient method, respectively). Heating was performed in a canonical ensemble for 200 ps with a restraint of 10 kcal mol^{-1} $Å^{-2}$ on the complex. The final simulations, the production phase, were performed for 10 ns in the canonical ensemble at 300 K and 1 atm without any restraint. The step size for the entire simulation was 2 fs. A Langevin thermostat and barostat were used for coupling the temperature and pressure. A SHAKE algorithm was applied to constrain all of the bonds containing hydrogen atoms.[26] The no-bonded cut off was kept at 10 Å, and long range electrostatic interactions were treated by the particle mesh Ewald (PME)[27] method with a fast Fourier transform grid having a spacing of approximately 0.1 nm. Trajectory snapshots were taken every 0.2 ps, and were finally used for analysis. The Ptraj module of AMBER was used to obtain root mean square deviation (RMSD) changes during the molecular dynamic simulations, which are presented in GraphPad Prism 4. Cluster analyses were performed using Chimera.[25]

MM-PB(GB)SA

The Molecular Mechanics-Poisson-Boltzmann Surface Area (MM-PBSA)/Molecular Mechanics-Generalized Born Surface Area (MM-GBSA) module of AMBER (v11) was applied to compute the binding free energy (ΔG_{bind}) of each complex.[28,29] For each complex, a total number of 200 snapshots were extracted from the last 10 ns of the complex trajectories. In the MM-PB(GB)SA, the binding free energy of a ligand (L) to a receptor (R) to form the complex (RL) is described by Equation 5.

$$\Delta G = G(\mathrm{RL}) - G(\mathrm{L}) - G(\mathrm{R}) \quad (\mathbf{5})$$

The free energy of individual species in Equation 1 is given by Equation 6.

$$G(\mathrm{X}) = H(\mathrm{X}) - TS(\mathrm{X}), \text{ and hence } G(\mathrm{X}) = U(\mathrm{X}) + \mathrm{PV}(\mathrm{X}) - TS(\mathrm{X}) \quad (\mathbf{6})$$

The method is frequently described in the literature.[30]

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Review

Application of cyclodextrins in chiral capillary electrophoresis

CE represents a very powerful separation tool in the area of chiral separations. CD-mediated chiral CE is a continuously flourishing technique within the frame of the electromigration methods. In this review, a brief overview of the synthetic procedures leading to modified CDs is provided first. Next, selected aspects related to the utilization of CDs in chiral CE are discussed specifically in the view of recently published data. Advantages of CDs and basic principles of chiral CE are remained. The topic of the determination of binding constants is touched. Particular attention is paid to the effort aiming at better understanding of the molecular level of the enantiorecognition between CDs and the analyte in the solution. Powerful approaches extensively utilized in this field are NMR, molecular modeling, and computer simulations. Then, a summary of applications of CDs in the CE enantioseparations is given, covering years 2008–2013. Finally, the general trend of modified CDs use in separation science is statistically evaluated.

Keywords:
CD / CE / Chiral analysis / Enantioseparation / Synthesis
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1 Introduction

Chiral compounds relate to each other as an object and its mirror image. This outstanding property has created a lot of interest in various fields of human activities, including analytical chemistry and separation science [1]. The main and very practical reason consists in the well-known fact that enantiomers can have different pharmacologic, metabolic, and/or toxicologic activities in living organisms [1]. Consequently, there is a very strong motivation to develop separation methods capable of resolving individual enantiomers. Chiral separations represent the most intriguing and, by some measures, the most difficult separation problem, as the molecules to be resolved have the same molecular mass and physicochemical properties, except for the rotation of the polarized light. Currently, there are four preferred techniques applied for the separation of enantiomers, specifically, GC, HPLC, supercritical fluid chromatography, and CE. CDs are the most popular and widespread chiral selectors (CSs) used as an additive in electrodriven separation methods [1,2]. This review is focused specifically on the synthesis and the application of CD derivatives in chiral CE with an accent on the new development within this field in the last 6 years (2008–2013).

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Abbreviations: CS, chiral selector; **DIBAL**, diisobutylaluminium hydride; **EMD**, electromigration dispersion; **EMO**, enantiomer migration order; **ROESY**, rotating frame nuclear Overhauser effect spectroscopy

2 Synthesis of CD derivatives

CDs are composed of D(+)-glucopyranose units that are linked through an α(1,4)-glycosidic bonding. The molecule is shaped like a truncated cone. Depending on the number of glucopyranose units α-, β-, and γ-CDs, consisting of six, seven, and eight units, respectively, are distinguished (Fig. 1). These CDs are called the native CDs and some basic physicochemical data are summarized in Table 1.

There are also CDs with a higher number of glucopyranose units in the cycle, however, these are not much of a practical use, as they are rare and have worse enantioselectivity due to the lack of rigidity of their skeleton [5–8]. The interior cavity of a CD is hydrophobic, which allows hydrophobic interactions between CD and an analyte. The exterior is hydrophilic, with the wider rim of the molecule containing secondary hydroxyl groups in the 2- and 3-positions, and the bottom rim composed of primary hydroxyl groups in the 6-position. Derivatization of the hydroxyl groups leads to derivatized CDs, as is discussed in more detail hereafter.

2.1 Randomly substituted CDs

Randomly substituted CDs are substituted in various positions with various degree of substitution and exact structure and ratio of single derivatives forming a mixture of randomly substituted CDs is unknown. Although randomly substituted CDs are widely available from common commercial sources and their synthesis is claimed in patents [9,10], they are still prepared in laboratory from time to time. Randomly substituted CDs are usually synthesized using more than one

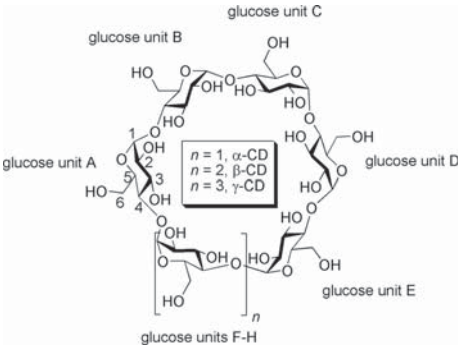


Figure 1. Numbering of glucose units and atoms in CD derivatives.

Table 1. Properties of native CDs [3, 4]

Property	α-CD	β-CD	γ-CD
Number of glucose units	6	7	8
Molecular mass (g/mol)	973	1135	1297
Solubility in water at 25°C (g/100 mL)	14.5	1.85	23.2
Cavity diameter (primary/secondary rim; nm)	0.47/0.52	0.60/0.64	0.75/0.83
Cavity length (nm)	0.8	0.8	0.8

equivalent of an alkylation (substitution) agent (with respect to a CD) in the presence of a base and the aim is to study the degree of substitution or distribution of substituents on a CD skeleton (Table 2).

2.2 Specifically substituted CDs

Specifically substituted CDs include persubstituted CDs with the same substituent in all positions, CDs persubstituted in 2-, 3-, or 6-positions with the same substituent in one type of positions (either 2-, 3-, or 6-positions), multiple substituted CDs with a specific substituent in specific positions, and monosubstituted CDs with only one substituent per the whole CD molecule.

2.2.1 Persubstituted CDs

Persubstituted CD derivatives are also commercially available. However, in contrast to randomly substituted derivatives, there is only one resulting isomer possible and there is no risk of variability among the prepared CDs' batches. The synthesis is carried out using an excess (with respect to the number of hydroxyl groups) of an alkylation (substitution) agent and a base (Table 2).

Table 2. Synthesis of randomly substituted and persubstituted CDs

Product	Alkylation (substitution) agent	Reference
Randomly substituted		
Methylated β-CD	Methyl chloride	[10]
Methylated β-CD	Methyl iodide	[9, 10]
Methylated β-CD	Dimethyl sulfate	[11]
Sulfated β-CD	Sulfur trioxide pyridine complex	[12]
Sulfoethylated β-CD	Sodium 2-bromo-ethanesulfonate	[13]
Sulfopropylated β-CD	1,3-Propanesultone	[14, 15]
Sulfobutylated β-CD	1,4-Butanesultone	[15]
Sulfobutylated γ-CD	1,4-Butanesultone	[16]
Carboxymethylated β-CD	Chloroacetic acid	[14, 17]
Carboxymethylated β-CD	Sodium chloroacetate	[18]
2-Hydroxypropylated β-CD	(*S*)-propylene oxide	[19]
Persubstituted		
Per-*O*-methyl-β-CD	Methyl iodide	[20, 21]
Per-*O*-hydroxyethyl-β-CD	Ethylene carbonate	[22]
Per-*O*-hydroxyethyl-γ-CD	Ethylene carbonate	[22]
Per-*O*-(2-hydroxypropyl)-β-CD	Propylene carbonate	[22]
Per-*O*-(2-hydroxypropyl)-γ-CD	Propylene carbonate	[22]
Per-*O*-carboxymethyl-β-CD	[a]	[23]

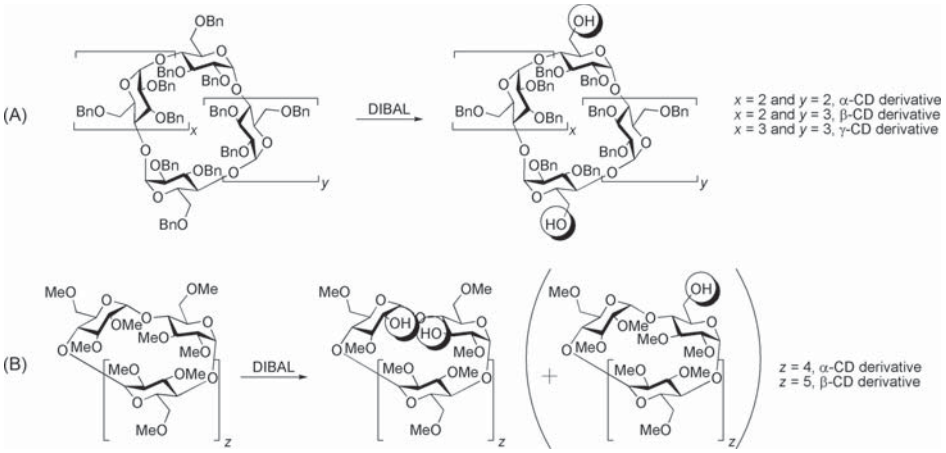
a) Prepared via per-*O*-allyl-β-CD.

2.2.2 CDs persubstituted in 2-, 3-, or 6-positions

Synthesis of CD derivatives persubstituted in 2-, 3-, or 6-positions takes advantage of the different reactivity of 2-, 3-, and 6-hydroxyl groups (for numbering of atoms see Fig. 1). 2-Hydroxyl groups are the most acidic and 6-hydroxyls are the most basic [24]. It is also common to use a protection-deprotection methodology to achieve a desired derivative.

Vigh and coworkers devoted a great effort in preparing single-isomer derivatives of α-, β-, and γ-CD persubstituted in 2-, 3-, or 6-position [25–37]. Their methodology for the synthesis of CD derivatives persubstituted in 2-, 3-, or 6-position is based on the selective protection of all 6-hydroxyl groups with *tert*-butyldimethylsilyl [38]. The protection (acetylation, methylation) of the remaining secondary hydroxyl groups allows a deprotection and subsequent sulfatization reaction in 6-position. The synthesis of CD derivatives carrying nonidentical substituents in all of the 2-, 3-, and 6-positions [34–36] moreover involves migration of the protection group from 2- to 3-position [39] or usage of protection-deprotection steps in 2-position with a benzyl group [37]. These methodologies could be used for the synthesis of new CD selectors tailored almost for any analyte.

Another approach to the synthesis of CD derivatives persubstituted in 6-position is the selective reaction of iodine and triphenylphosphine with β-CD. The resulting heptakis(6-deoxy-6-iodo)-β-CD can be converted via 6-azido derivative to heptakis(6-deoxy-6-amino)-β-CD [40]. A similar derivative—heptakis(6-deoxy-6-bromo)-β-CD—has been used as a precursor for the synthesis of multiple cationic compounds [41, 42].



Scheme 1. (A) Selective de-*O*-benzylation and (B) selective de-*O*-methylation.

2.2.3 Multiple-substituted CDs

Multiple-substituted CDs are single-isomer derivatives that fill the gap between CDs persubstituted in 2-, 3-, or 6-position and monosubstituted derivatives. Although the synthesis of multiple substituted CDs often requires sophisticated methods, there are a few procedures that allow an easy synthesis of such derivatives. Diisobutylaluminium hydride (DIBAL) allows regioselective de-*O*-benzylation of per-*O*-benzyl-α-, β-, or γ-CD to obtain perbenzylated 6^A,6^D-dihydroxy α-CD, β-CD, or 6^A,6^E-dihydroxy γ-CD [43] (Scheme 1A). A proposed mechanism for this de-*O*-benzylation includes coordination of two DIBAL molecules to the perbenzylated CD, hydride transfer from the complex at glucose unit D to the proximal benzyl group at glucose unit A, and a second hydride transfer back to the benzyl group at glucose unit D. DIBAL has been also used for de-*O*-methylation of per-*O*-methyl-α- or β-CD to obtain permethylated 2^A,3^B-dihydroxy α- or β-CD (and permethylated 6^A-hydroxy α- or β-CD as byproduct) [44] (Scheme 1B). These compounds are useful precursors for the synthesis of new CD selectors and they have been successfully used for the synthesis of multiple anionic CD derivatives [21, 45].

2.2.4 Monosubstituted CDs

Monosubstituted CD derivatives (as well as derivatives persubstituted in 2-, 3-, or 6-position) take advantage of the different reactivity of 2-, 3-, and 6-hydroxyl groups and their synthesis often requires a protection-deprotection methodology to achieve a desired product. The regioselective synthesis based on a substitution reaction of 6^A-*O*-tosylcyclodextrins is the most popular way leading to monosubstituted CD derivatives. 6^A-*O*-tosyl-α-, β- and γ-CDs are synthesized by the reaction of a corresponding CD with tosyl chloride [46]. 6^A-*O*-tosyl-β-CD is also synthesized by a reaction with tosylimidazole [47] or *p*-toluenesulfonic anhydride [48]. 6^A-*O*-tosylcyclodextrins are then predominantly used as precursors for the synthesis of cationic [49–54] and less often for negatively charged [55] 6^A-*O*-substituted CDs.

As the substitution in other than 6-position is more difficult, the number of such derivatives applied in CE is scarce. However, there are some methodologies providing all sets of 2-, 3-, and 6-monosubstituted CD derivatives. Luna et al. have synthesized a complete set of three monosubstituted sulfobutyl β-CD derivatives [56]. It has been also demonstrated that it is possible to prepare the complete sets of 2^A-*O*-, 3^A-*O*-, and 6^A-*O*-carboxymethyl-α-CDs [57–59]; β-CDs [21, 60, 61]; and γ-CDs [62]. The synthesis is based on regioselective monoallylation of CDs followed by acetylation of the remaining hydroxyl groups, oxidation of the double bond to carboxylic acid, and final deacetylation.

The synthesis of permethylated sets of 2-, 3-, and 6-monosubstituted β-CD derivatives can be carried out using the above-mentioned regioselective de-*O*-methylation of per-*O*-methyl-β-CD (Scheme 1B) [44]. Permethylated 2^A,3^B-dihydroxy β-CD could be transformed into either permethylated 2^A-hydroxy or 3^A-hydroxy β-CD [63]. Together with the already mentioned permethylated 6^A-hydroxy β-CD, they form a complete set of precursors ready for further transformations.

3 Selected aspects related to chiral CE based on CDs

Although CDs are known since 19th century [64], their use in electromigration methods is dated 100 years later. This

fact was caused by a slow development of the corresponding methods. CDs have been utilized as selectors of achiral substances first, and only a few years later as CSs [65].

3.1 Advantages of CDs

The advantage of using CDs in electromigration methods stems from the possibility of their derivatization. A required enantioseparation and selectivity can be achieved by introducing a suitable functional group into the molecule of a CD. Utilization of different functional groups at a varying number leads to an almost unlimited figure of potential CSs [66, 67]. The total number of analytes that can be successfully separated by CDs is countless also due to the fact that it is not necessary to have a functional group on the analyte and yet it can be separated as shown in the example of chiral-branched hydrocarbons that were separated using a CD-based chiral stationary phase in GC [2]. There are practically no restrictions concerning the structure of the analyte for a successful chiral separation with the aid of CDs [67]. For instance amino acid derivatives can be separated by CDs regardless the position of their amino group (α, β, γ, or δ) [2]. This is a great advantage compared to other CSs (e.g., proteins or antibiotics), which have the ability to separate only an amino acid with the amino group in position α. CDs can also separate enantiomers not only with central chirality, but also with planar or axial chirality. Polychlorinated biphenyls [68] and allenic acids [69] have been successfully enantioseparated based on these mentioned features. CDs are also able to separate enantiomers possessing chiral centers provided by heteroatoms, such as sulfur, silicon, phosphorus, and nitrogen [70, 71].

The ability of CDs to form chiral complexes with many different substances is mainly the consequence of a large number of chiral centers in CDs; for example, there are 35 chiral centers in β-CD [67]. Another important factor is the shape of a CD molecule and the accessibility to substitution that significantly increase the range of applications. The analyte–CD interaction itself takes place on the basis of induced adaptation, that is, the shape of CD is changed during the interaction, which also contributes to its broad chiral selectivity [67]. The disadvantage of this effect is usually a relatively low stability (binding) constant of the analyte–CD complex compared to other rigid selectors (e.g., bile acids and ligand-exchange complexes). The chiral recognition can be further extended by using ionizable CDs where pH can be adjusted to achieve the required ionization and thus the desired chiral separation properties [72–74].

Moreover, derivatization of CDs allows their application in all environments, that is, aqueous, polar organic, and nonpolar organic. The indisputable advantage is that even the nonderivatized CDs can also be used in all of these environments, but in some cases in significantly lower concentrations than with the use of CD derivatives. Depending on the environment, the type of analyte–CDs interaction changes, which results in a different selectivity [74–77].

The application of CDs in electromigration methods is very convenient. Due to their rather flexible structure, separation factors usually do not reach values higher than 2 [67, 73]. However, highly efficient electromigration methods are commonly able to separate analytes with separation factors even lower than 1.01. Therefore, CE methods (along with GC) are frequently used for such chiral separations [67, 73]. As the efficiency of separation systems is strongly dependent on the conductivity of a BGE, it is necessary to carefully select suitable electrolyte conditions especially when using charged CDs [34, 78, 79]. The necessity of a separation condition optimization for charged CDs is balanced by their wide range of applications.

CDs can separate enantiomers, as well as regioisomers. For example, chiral drugs can be separated together with their metabolites in one analysis [80–83], major and minor reaction products can be separated at the same time [84], and samples that contain less than 0.3% [85–89] or even less than 0.1% [90–98] of enantiomeric impurity can be reliably characterized.

Applicability of CDs and their derivatives in various environments also enables a selection of solvent mixtures where an analyte is well soluble. Therefore, it is not necessary to find a tradeoff between solubility of an analyte and CD [69, 99].

3.2 Principles of chiral CE

Chiral CE separations mediated by CDs are based on two mechanisms, one of them can be considered chromatographic and the other one electrophoretic [100]. The ability of a CD to stereoselectively recognize individual enantiomers of a specific analyte is a chromatographic principle irrespectively the fact that in CE CDs are not fixed in the separation system (as it is common in HPLC) but instead they are dissolved in BGE and thus create a pseudo phase. On the other hand, the analyte, in free or complexed form with CD, is driven in CE by an electrophoretic principle governed by the charge density of the moving species. As a consequence, undoubtedly the term EKC is, to be exact, more precise than plain CE to describe the real situation correctly.

Wren and Rowe [101] introduced a basic equation describing the separation of the individual enantiomers based on complexation equilibria:

$$\Delta\mu = \frac{\mu_1 + \mu_2 K_1[C]}{1 + K_1[C]} - \frac{\mu_1 + \mu_2 K_2[C]}{1 + K_2[C]}, \quad (1)$$

where $\Delta\mu$ is a difference in effective electrophoretic mobilities of the individual enantiomers of a given analyte, μ_1 is the effective electrophoretic mobility of the analyte in a free solution (where the mobilities of the free enantiomers in achiral media are the same), μ_2 is the effective electrophoretic mobility of the analyte–CS complex (here it is expected that the effective electrophoretic mobility of the analyte–CS complexes for both the enantiomers are the same), K_1 and K_2 are the complex equilibrium constants (stability or binding constants) of the first and second enantiomer of the analyte

with the CS, respectively, and [*C*] is the concentration of the CS. The Eq. (1) can be rearranged to:

$$\Delta\mu = \frac{[C](\mu_1 - \mu_2)(K_2 - K_1)}{1 + [C](K_1 + K_2) + K_1 K_2 [C]^2}. \quad (2)$$

From Eq. (2), it is evident that the effective mobility difference of the individual enantiomers will be zero if $K_1 = K_2$ or $\mu_1 = \mu_2$. In addition, $\Delta\mu$ will be zero also if $[C] = 0$ or [*C*] is very large. This model implies that in between those two extremes in concentration of the selector, some values of [*C*] give a maximum effective mobility difference and hence a maximum separation of the two enantiomers. This optimal concentration has also been derived by Wren and Rowe as follows:

$$[C]_{opt} = \frac{1}{\sqrt{K_1 K_2}}. \quad (3)$$

In the original Eq. (1), the same effective electrophoretic mobilities of the analyte–selector complex of both the enantiomers of a given analyte have been assumed. However, in general, these mobilities may differ and a more general version of Eq. (1) has been derived considering different analyte–selector complex mobilities for the individual enantiomers [102, 103]:

$$\Delta\mu = \frac{\mu_1 + \mu_{cR} K_1[C]}{1 + K_1[C]} - \frac{\mu_1 + \mu_{cS} K_2[C]}{1 + K_2[C]}, \quad (4)$$

where μ_{cR} and μ_{cS} are effective mobilities of the respective complexes of the CS with the *R*- and *S*-enantiomers of the analyte. Then, an enantioseparation can be reached as a result of a difference in the complex equilibrium constants ($K_1 \neq K_2$) or, alternatively, based on the difference in complex mobilities ($\mu_{cS} \neq \mu_{cR}$). The combination of both contributions is also possible. If the enantioselectivity is only due to differences of complexation constants, then Eq. (2) is valid. On the other hand, if only a difference in complex mobilities of the individual enantiomers contributes to the enantioseparation, the following equation has been derived [102, 104]:

$$\Delta\mu = \frac{K[C](\mu_{cR} - \mu_{cS})}{1 + K[C]}. \quad (5)$$

A very detailed discussion of many related fundamental aspects of chiral separations by CE has been provided in several elaborate reviews, for example, in [105, 106].

Recently, an interesting study focused specifically on the phenomenon of the CE enantioseparation in the case of equal binding constants of the enantiomers with a CS has been published [107]. The authors proved experimentally, combining CE and NMR data, that indeed a sole mobility difference between the temporary diastereomeric associates may be responsible for the successful separation of the enantiomers in chiral CE. The observed reversal of the migration order of the enantiomers of the probe compounds (ketokonazole and terconazole), depending on the concentration of the CS (HP-β-CD), were explained as the consequence of the dual separation mechanism. In particular, at lower concentrations of the selector, the difference between the binding constants determined the migration order of the enantiomers being the major contribution to the separation, while at higher concentration range of HP-β-CD, the differences in complex mobilities played the main role in the separation process, which resulted in an enantiomer migration order (EMO) reversal.

An extraordinarily high enantioselective separation capability of some commercially available randomly substituted CD mixtures has been discussed and explained by Dubsky et al. recently [108]. The approach is based on their multi-CS enantioseparation model [109]. The model shows that multi-CS systems consisting of a CDs' mixture of various degrees of substitution are significantly more complex than those with only one CD. First of all, the binding constant is a weighed sum of all the individual binding constants. However, what is even more important, the overall mobility of an enantiomer–CS complex in a mixed system (referred to as a limit mobility in the paper) is no longer an independent physicochemical constant. Instead, it depends on the distribution of this enantiomer between the chiral and the achiral environments (i.e., the binding constants of the enantiomer with the individual constituents of the mixed CS system). Thus, the overall limit mobilities of the two respective enantiomer–CS complexes mix both the thermodynamic and electrophoretic enantioselective mechanisms together. The model shows that in single-CS systems, the two enantiomers of a chiral compound are separated usually predominantly on the thermodynamic basis (the consequence of inequality of their distribution constants), while related limit complex mobilities are often almost the same for both enantiomers. On the other hand, in a multi-CS system, the thermodynamic and electrophoretic enantioselective mechanisms are mixed, which usually leads to different overall limit mobilities of the complexed enantiomers. It is evident that this mixed thermodynamic/electrophoretic enantioselective effect is inherent in multi-CS systems. Undoubtedly, in many cases it may lead to a significant improvement in resolution. However, it should also be mentioned clearly, specifically in the context of the study, that indeed it is reasonable to expect enhanced enantioseparation when CDs of the same chemistry differing only in the degree of substitution are mixed (as it is in the case described in the study). On the other hand, if two or more CSs of distant chemical structures are mixed, then it is not rational a priori to expect an improvement in chiral resolution with relation to the single CD systems. It is simply because the separation mechanisms responsible for a chiral separation in the case of individual single CDs may differ significantly and thermodynamic/electrokinetic effects can be directed against each other with an overall negative impact on enantioseparation.

3.3 Determination of binding constants

Interactions between an analyte and a complexation agent can be characterized by binding constants. CE belongs to the most frequently used experimental techniques applied to serve that purpose [110]. The reasons are various: high

resolving power of CE, experimental speed of the measurements, and low consumption of samples. The calculations of the binding constants are usually based on the following equation:

$$\mu = \frac{\mu_A + \mu_{AC} K[C]}{1 + K[C]}, \quad (6)$$

where μ is the effective mobility of the analyte A; μ_A and μ_{AC} are the mobilities of the free and complexed analyte, respectively; K is a binding constant; and $[C]$ concentration of the complexation agent C. It is a common practice to linearize Eq. (6) in effort to obtain K from experimental data. However, a nonlinear regression provides the best results of a high precision and accuracy [111, 112]. It should be also mentioned that there are many potential problems complicating the determination of K values in real situations. A viscosity increase, an ionic strength change, and, as a consequence, a temperature change of BGE can take place in the capillary when the complexation agent is added into the running buffer. The mentioned factors may have a significant influence on the determination of binding constants and they should not be ignored [110].

In this context, it is relevant to mention two recent papers focused specifically on a usually overlooked effect [113, 114]; the impact of a possible complexation of buffer constituents with complexation agents (for instance CDs) on common buffer properties. It has been shown that this phenomenon can significantly influence the ionic strength of BGE, pH, and conductivity. As a consequence, the application of interacting buffers can radically change the results of CE separation and it can also easily lead to misleading data on solute-CS binding constants determined in such environment. Thus, in general a possible interaction of buffer constituents with a complexation agent should be taken into account and its absence/presence should be always assessed before the measurement of binding constants takes place.

An interesting paper on CE methods suitable for the determination of all binding parameters in systems with simultaneous borate and CD complexation agents has been published by Svobodova et al. [115]. The proposed approach enables the determination of all binding parameters in those systems and even the binding constants of interaction of a neutral analyte with a neutral CD. Only one set of CE experiments is required to obtain a good quality results. The authors concluded that in their specific case the interaction of the analytes with CD governed the chiral recognition, while the complexation of the analytes with borate was responsible for electromigration.

3.4 NMR spectroscopy as a tool to study selector–selectand interactions

NMR spectroscopy represents a nonseparative analytical technique frequently applied to investigate noncovalent interactions. Currently, it is impossible in general to predict the result of a chiral CE recognition process a priori. NMR techniques and molecular modeling serve as complementary and supporting tools to direct chiral CE measurements. They may help to explain some specific effects observed in chiral CE as, for instance, the reason for the opposite migration order of the enantiomers when different CDs are used, or they may be of assistance in choosing the optimal conditions for an enantioseparation by CE. NMR techniques allow for the quantitation of the host–guest equilibria [116] in terms of stability constant with a dynamic range from 10 to 10^4 [117, 118]. It is also a reliable tool to determine complex stoichiometry [118, 119] and reveal the geometry of a diastereomeric complex [53, 116, 118, 120–122]. Several research groups are active in this field. Specifically, an interesting study recently presented has been focused on a comparison of chiral CE separations in fully aqueous environment versus nonaqueous BGE [123]. Despite CE in nonaqueous BGE is already an established technique, not much information is available on the mechanism of the chiral recognition in such an environment. Nonaqueous electrolytes enable to use CSs and analytes with a low solubility in water, to reduce adsorption onto the capillary wall, and to generate reduced electric current. Moreover, organic solvents with lower dielectric constants than water constitute, in principle, a more favorable environment for chiral discrimination due to their ability to promote intermolecular interactions [124]. It has been hypothesized that the separation of enantiomers involving their interaction with chiral buffer additives might be very different in aqueous versus nonaqueous BGE [125, 126]. However, direct proofs have been missing for long time. In the study [123] the enantiomers of propranolol were separated by chiral CE in aqueous and nonaqueous methanolic BGEs with single isomer well-characterized sulfated derivatives of β-CD. It was found that the EMO of the analyte was inverted when an aqueous buffer was replaced with a nonaqueous BGE in the case of one CD derivative, but remained the same in the case of the other applied CD selector. Rotating frame nuclear Overhauser effect spectroscopy (ROESY) was used to design a possible molecular mechanism responsible for this interesting behavior of the system. The results showed a significant difference in the mechanism of interactions in aqueous and nonaqueous environment. In aqueous BGE, propranolol created an inclusion complex, and in nonaqueous BGE, a complex of external type with heptakis(2,3-dimethyl-6-sulfo)-β-CD. The exact opposite behavior was found in the case of the heptakis(2,3-diacetyl-6-sulfo)-β-CD selector.

All of this shows that the current understanding of the molecular recognition mechanisms of the individual enantiomers in aqueous and nonaqueous BGEs is usually highly oversimplified. It is evident that multiple forces operate in the complex formation between chiral solutes and chiral CDs, which leads to a wide variety of complexes possessing different affinity patterns of enantiomers toward CSs. Still a question has remained open on how much the affinity pattern of an analyte to a selector is related to EMO in CE. In Professor Chankvetadze's group, they employ NMR techniques very frequently in their effort to obtain supporting and complementary data to chiral CE experiments [118]. They proposed that not only enantioselectivity of the selector for enantiomers

is responsible for EMO, but also other aspects might play a significant or even decisive role. They explicitly mentioned a possible influence of differences in electrophoretic mobilities of the complexes of the individual enantiomers with a given selector (vide supra) [107]. The mentioned phenomena have been further studied within the same scientific group using several different analytes and CDs [127–130]. The authors have concluded that (i) changes in the chemical structure of CDs can lead to a reversal of enantiomer affinity pattern in aqueous and nonaqueous electrolytes; (ii) changes of the geometry of analyte–CD complexes do not always result in a substantial change in the chiral recognition ability or chiral recognition pattern; (iii) the degree of chiral recognition, and the efficiency of enantiomer separations, which rely on the formation of external complexes between the analyte and the CD molecules, can be as good as the efficiency of separations where analyte/CD inclusion complexes are formed.

A combination of chiral CE with NMR experiments is frequently used also within Beni's group. They have studied various chiral compounds using native CDs and their derivatives as CSs [119, 122, 131]. For characterization of the complexes and the enantioseparation, the apparent averaged stability constants, resolution, and migration order were determined. The binding constants determined with CE were confirmed with NMR titrations. The resolution data correlated with the differences in the apparent stability constants of the enantiomers; the larger the difference between the *K* values of the enantiomers, the higher the resolution observed [122]. The average stochiometries of the complexes were found to be 1:1, independently of the applied CD and analyte. Information on the geometry of the complex and direction of inclusion into the CD cavity was obtained from 2D ROESY data. The effects of dual CD systems on separation were also studied. Interestingly, in the case of derivatized pregabalin, the application of dual CD systems did not provide a higher CE resolution than the most suitable single CD systems [119]. However, when they optimized a chiral CE method for antidiabetic drug sitagliptin, an unambiguous improvement of resolution was observed in most of the applied dual CD systems with respect to single CDs.

3.5 Molecular modeling

Computer-aided molecular modeling techniques have been proposed as supporting tools for obtaining information on 3D structures and the interaction taking place in inclusion complexes. Various studies have been performed to investigate CD inclusion complexes with the goal to comprehend the mechanism of the complex forming and to correlate the experimental data. Parametric model 3 (PM3) [132–136] and PM6 [137, 138] semiempirical methods, AutoDock molecular docking [139–141], molecular dynamic simulations [142–145], ONIOM (B3LYP/6–31G*:PM3) [136], and ChemBio 3D using the MM2 molecular mechanics algorithms [146] have been employed. Molecular mechanics calculations have been utilized also in works of Chai and coworkers [147–149]. At present, a precise calculation of small energy differences still remains a challenging task. Unfortunately, in some instances the CD model structures used for the calculations do not correspond exactly with the CDs utilized in the real chiral CE experiments (for instance the model works with persubstituted CD, however, real CE separation is carried out with a mixture of CDs possessing a broad degree of substitution). In some cases the results obtained by the theoretical calculations seem to contradict the experimental results. This might be due to the fact that these calculations have often been performed in the gas phase and the effect of the solvent is not considered. Nevertheless, the molecular modeling studies may significantly contribute to the understanding of the nature of intermolecular forces responsible for guest–CD interactions, especially when used in combination with other instrumental techniques as ROESY and NMR. Thus, frequently a combination of chiral CE with molecular modeling and NMR experiments has been found to be the most reliable approach [118, 124, 138, 142]. Moreover, besides NMR and molecular modeling, other supporting techniques are also used from time to time, specifically MS [138] and/or circular dichroism [121].

3.6 Computer simulations of chiral CE

Several mathematical models have been developed for the description of analyte mobility in enantioselective CE separation based on 1:1 complex formed between analytes and CSs. The above-mentioned mobility difference model (Wren) [150, 151] did not consider dissociation equilibria of the analytes as a function of the pH of the BGE. The improved model developed by Vigh and coworkers implemented dissociation equilibria and in addition to that the authors also included the effects of competing binding equilibria of the dissociated and nondissociated forms of the analytes [152–154]. The same research group developed a peak resolution model [155] and the charged resolving agent migration model [156, 157].

Recently, a comprehensive 1D dynamic simulator for electrophoretic separation, GENTRANS, has been extended for handling electrokinetic chiral separations with a neutral ligands by Thormann and coworkers [158]. The code is intended to study the 1:1 interaction of monovalent weak and strong acids and bases with a single monovalent weak and strong acid or base additive, including a neutral CD. The authors considered complexation constants and specific mobilities of the formed 1:1 analyte–selector complexes in solution. First, they experimentally obtained the appropriate constants of several chiral cationic model drugs in the presence of neutral CDs by CZE at low pH, and then compared simulated electropherograms and isotachopherograms to those reached experimentally. Finally, the resulting simulated data were compared to those obtained by a new version of SIMUL 5 Complex recently developed by Gas and coworkers [159–161] (vide infra). It was shown that the modified GENTRANS is a valuable tool to investigate the dynamics of chiral separations.

Several important papers on modeling of CE enantioseparations have been published by Professor Gas and coworkers. Very recently, the theory of equilibria, migration, and dynamics of interconversion of a chiral analyte in electromigration enantioseparation system involving a mixture of CSs for chiral recognition has been proposed and an appropriate mathematical model elaborated [109]. Then, the theoretical assumptions have been proved experimentally using commercially available mixture of highly sulfated β-CDs as CSs and lorazepam as a model analyte undergoing interconversion during the separation process [162]. SIMUL 5 Complex, a 1D dynamic simulation software designed for electrophoresis based on a numerical solution of the governing equations, has been updated, implementing a new mathematical model considering complex-formation equilibria [159]. The simulation supports any number of analytes complexed by one complex-forming ligand (typically CD) when the complexation stoichiometry is 1:1. In the second part of their work, the authors experimentally verified the validity of the new model [161]. Two testing systems containing a neutral CS were used with a fully charged analyte. In the third system, a positively charged CS was utilized and neutral enantiomers were injected as the analytes. It was claimed that SIMUL 5 Complex was a suitable tool to predict the results of enantiomer separations and to optimize the chiral CE separation conditions.

Moreover, by performing simulations the existence of unexpected electromigration dispersion (EMD) effects caused by the complexation process itself have been revealed [160]. EMD is often caused by conductivity or pH effects. However, the authors proved that also complexation can lead to a significant EMD. Thus, this effect was studied in more detail in two additional papers. As a result, a complete mathematical model of EMD in systems containing a neutral complex forming agent and a fully charged analyte was introduced [163]. The model has been implemented into the new version of PeakMaster 5.3. The improved software is now applicable for the optimization of the separation conditions providing minimal EMD effects and giving symmetrical and narrow peaks. Its practical feasibility has been verified experimentally [164].

The Vigh's model [154] has been employed by Hammitzsch-Wiedemann and Scriba in their detailed study of a pH-dependent and selector concentration dependent reversal of the EMO in CE [165]. They realized that in real experiments pH-dependent EMO reversal was observed for peptides using neutral and charged CDs [166–172] and similarly the reversal took place also as a function of the selector concentration change for various analytes [173–176]. In their effort to explain this phenomenon, they elaborated a separation selectivity model for CE enantioseparation of weak bases in the presence of uncharged CSs as a function of pH and chiral ligand concentration. Experimental proof of suitability of the developed mathematical model was provided by the migration behavior of the enantiomers of Ala-Tyr and Asp-PheOMe in the presence of heptakis(2,6-di-*O*-methyl)-β-CD as a CS [177].

Asensi-Bernardi et al. have used directly available structural data of 40 structurally unrelated drugs and pesticides to model their chiral CE separation mediated by highly sulfated β-CD [178]. Their simplified discriminant partial least squares-based quantitative structure–property relationship approach resulted in a consistent, predictive, and descriptive model. It has been further converted into an explicate equation capable of predicting the enantioresolution level of new compounds based on four structure properties available in an online open database.

3.7 Generic strategy for the development of a chiral CE method

CE practitioners are very interested in having generic methods for the development of chiral separations to speed up the tedious process of the method development. Indeed, several interesting papers have been published dealing with this topic [179–181]. More recently, an important paper has been published by Ates et al. [182]. As there are a lot of commercially available CSs, the experimental possibilities for the analysts responsible for the development of chiral CE methods are numberless. The practical development of an appropriate separation chiral CE method for a specific compound of interest is often not an easy task especially when no relevant data on that specific case can be found in the literature. Then generic method development strategies can represent a valuable supporting tool. The authors of the paper provided an overview of the strategies elaborated in this area not only for chiral electrophoretic techniques, but also for chiral HPLC. Moreover, the proposed procedures are fast and require only a limited number of experiments to achieve a good result. The generic strategy recommended for CE is based on the application of highly sulfated α-, β-, and γ-CDs in the first step. Then, if acceptable results are not reached, dual CD systems combining a negatively charged highly sulfated CD with a neutral CD are applied. The overall optimization procedure is described elaborately and all the decisions executed during the method optimization procedures are supported by a comprehensively commented decision diagram. This tool can be found very beneficial mainly within the community of CE practitioners frequently involved in a chiral method development.

4 CDs as additives in chiral CE

CDs have been used in CE since 1985 [183] and they are usually applied as additives into the BGE. Thus, CDs represent a pseudo phase as they are not fixed in the system. Besides the choice of an appropriate CD, it is also necessary to carefully select the BGE, that is, type of buffer, its pH, ionic strength, and (in some cases) a suitable additional organic solvent improving the solubility of analytes to reach a required chiral separation [2, 103]. In the case of water insoluble analytes, fully nonaqueous CE can be the

solution [94, 123, 124, 127, 184–193]. Native CDs are often used for the separation of compounds that have an aromatic moiety or a long hydrocarbon chain in their structure capable of entering the CD's hydrophobic cavity [2, 103]. For polar compounds (for instance some amino acids and their derivatives) suitable CD derivatives are used [130, 194–200].

CDs and their derivatives can be classified in several different ways (e.g., according to their charge or the type of the original CD). For the purpose of this paper the following sorting is used:

(i) Native (nonderivatized) CDs.
(ii) Randomly substituted CDs.
(iii) Specifically substituted CDs.
(iv) Mixtures of CDs (mostly dual systems consisting of CDs mentioned in i–iii).
(v) Miscellaneous systems containing CD(s) with other selector(s).

4.1 Native CDs

Wide applicability of CDs in electromigration methods is related to a large number of chiral centers in their molecules (every glucose unit has five stereogenic centers) and also to the shape of the molecules. As already mentioned, the interior of the cavity is hydrophobic as it is made of nonpolar bonds (C-H and glycosidic bridges). On the other hand, there are primary and secondary hydroxyl groups on the CDs' rim, which provide a good CD solubility in water (Table 1). The only exception in this sense is β-CD, whose significantly lower solubility is probably caused by a strong intramolecular hydrogen bonding among hydroxyl groups. A very important feature of all CDs is the diameter of the cavity (Table 1). Based on that parameter, it is possible to assume whether an analyte is able to form an inclusion complex with a given CD or not. The inclusion complexes are formed preferably in aqueous environment. In nonaqueous environment the interaction between an analyte and a CD frequently occurs on the outer surface of CD [201].

Native CDs are easily available at a very low price. On the other hand, the chiral selectivity is usually low and due to their zero charge only charged analytes can be separated by CE. This problem can be partially solved by the application of MEKC [130, 202]. Moreover, it is also appropriate to mention that a zero charge of native CDs brings one significant advantage as these CDs have only a slight influence on conductivity of the BGE. Recent applications of native CDs in chiral CE are summarized in Table 3.

Table 3. Recent applications of native CDs in chiral CE

Name of selector	Examples of applications
α-CD	Jasmonic acid [203]; norephedrine [128]; ANDA-CC-penicillamine [204]; trimipramine [205]; tryptophan [206]; vincamine, vinpocetine, and vincadifformine [122]; four basic compounds [58]; five N,S *exo*-heterodisubstituted *O*-carborane ligands [207]; Tic-hydantoin derivatives [208]; binaphthol and its mono derivatives [174, 209]; acyclic nucleoside phosphonate [210]
β-CD	Amisulpride [211]; DTAF-aspartic acid [212]; baclofen [138]; catechin, chlorpheniramine, and promethazine [213]; ephedrine [130]; epicatechin [214]; fluvastatin [215]; ANSA-fucose and FMOC-pipecolic acid [216]; metoprolol [217]; binaphthol and its mono derivatives [209]; 6,6′-dibromo-1,1′-binaphthyl-2,2′-diol [218]; norephedrine [128]; penicillamine [202]; ANDA-CC-penicillamine [204]; 1,4-bis(9-*O*-quininyl)phthalazine [219]; dioxopromethazine [220]; propranolol [221]; salsolinol and its derivatives [136]; NBD-F-serine [194]; Tröger's base derivatives [222, 223]; DNS-tyrosine and DNS-threonine [195]; vincamine, vinpocetine, and vincadifformine [122]; zopiclone [224]; β-agonists [225–227]; nine amino acids [228]; three amino acids and 13 DNS amino acids [196]; 12 carboxytetramethylrhodamine succinimidyl ester-amino acids [197]; FITC amino acids [198, 199]; model sample (labeled amino acids) [229]; four basic compounds [61]; drugs [230–232]; three dipeptides [233]; Tic-hydantoin derivatives [208]; six acyclic nucleoside phosphonates [210]
γ-CD	FITC-arginine; ephedrine [130]; FITC-methionine and FITC-leucine [200]; ornithine [234]; serine [235]; Tröger's base derivatives [222]; vincamine, vinpocetine, and vincadifformine [122]; two β-agonists [227]; 11 FITC amino acids [198]; two racemic tetrahydrobenzimidazoles [236]; five basic drugs [237]; Tic-hydantoin derivatives [208]; binaphthol and its mono derivatives [209]; modified pregabalins [119]

ANDA-CC, 7-amino-1,3-naphthalenedisulfonic acid monopotassium salt—cyanuric chloride; ANSA, 5-amino-2-naphthalene-sulfonic acid; DNS, dansyl chloride; DTAF, 5-(4,6-dichlorotriazinyl)aminofluorescein; FITC, fluorescein isothiocyanate; FMOC, fluorenylmethyloxycarbonyl chloride; NBD-F, 4-fluoro-7-nitro-2,1,3-benzoxadiazol; Tic, tetrahydroisoquinoline.

4.2 Randomly substituted CDs

Randomly substituted CDs often provide a compromise between native and persubstituted CDs and, in some cases, they are actually mixtures of native and substituted CDs, as it is obvious for instance with use of 2-hydroxypropylated CDs of the average degree of substitution below 1 [121, 158, 209, 218]. Randomly substituted CDs are frequently employed in electromigration methods. In these cases, the exact composition of the selectors in randomly substituted CDs is unknown and instead an average number of substituents per CD (in fact the lowest and the highest number of substituents) is used for the description of such mixed systems. This also implies that the number of substituents on each glucose unit is unknown. Nevertheless, these randomly substituted CDs can be very useful in enantioseparations because their separation ability

Table 4. Recent applications of randomly substituted CDs in chiral CE

Name of selector	Examples of applications
Neutral CDs	
Acetylated β-CD	Fluvastatin [215]
Ethylated β-CD	Four adrenergic β2-agonists [250]
Glucosylated β-CD	Naproxen and warfarin [247]
Hydroxyethylated β-CD	Four adrenergic β2-agonists [250]
2-Hydroxypropylated α-CD	6,6′-Dibromo-1,1′-binaphthyl-2,2′-diol [218]; binaphthol and its mono derivatives [174, 209]; ANDA-CC-penicillamine [204]; trimipramine [205]; tryptophan [121]; vincamine, vinpocetine, and vincadifformine [122]; Tic-hydantoin derivatives [208]; modified pregabalins [119]
2-Hydroxypropylated β-CD	(9-Anthryl)methoxyacetic acid and (9-anthryl)hydroxyacetic acid [253]; four triadimenols [149]; amlodipine [254–256]; naphthalene-2,3-dicarboxaldehyde-aspartic acid [257]; chlorpheniramine [258]; etodolac [87]; flinderoles and borreverine [259]; fluvastatin [215]; imazethapyr [260]; ketoconazole and terconazole [107]; four tetrahydrobenzimidazoles [236]; lamivudine [261]; methadone [158]; methamphetamine [262]; three naphthalene-2,3-dicarboxaldehyde amino acids [263]; binaphthol and its mono derivatives [174, 209]; 6,6′-dibromo-1,1′-binaphthyl-2,2′-diol [218]; naproxen, warfarin, and pranoprofen [247]; ofloxacin, omeprazole [88]; *threo*-methylphenidate [265]; pheniramine [266, 267]; propranolol and brompheniramine maleate [268]; vincamine, vinpocetine, and vincadifformine [122]; vinpocetine [269]; zolmitriptan [270]; five β-agonists [227]; four adrenergic β2-agonists [250]; four nonnatural carboxylic amino acid [271]; four nonnatural *N*-methylamino acids [272]; isochromene derivatives [273]; 20 basic drugs [274]; five basic drugs [230]; four drugs [231]; three drugs [232]; dipeptide β-alanyl-tyrosine and its derivatives [186]; Tic-hydantoin derivatives [208]; modified pregabalins [119]; aldo-bis(3′-indolyl) monosaccharides [275]; chiral oxazolidine derivative of tocainide [276]
2-Hydroxypropylated γ-CD	Dapoxetine [251]; flavan-3-ols [277]; flinderoles and borreverine [259]; eight tetrahydrobenzimidazoles [236]; iodiconazole and related triadimenol analogues [147, 278]; binaphthol and its mono derivatives [174, 209]; 6,6′-dibromo-1,1′-binaphthyl-2,2′-diol [218]; primaquine [279]; vincamine, vinpocetine, and vincadifformine [122]; isochromene derivatives [273]; Tic-hydantoin derivatives [208]; modified pregabalins [119]
(2,3-Dihydroxy)propylated β-CD	Twenty basic drugs [274]
(3-Hydroxy)propylated β-CD	Twenty basic drugs [274]
Methylated α-CD	Vincamine, vinpocetine, and vincadifformine [122]
Methylated β-CD	Aminoglutethimide [133], naproxen [246], naproxen and pranoprofen [247]; rotigotine and related chiral impurities [252]; sibutramine [248, 249]; vincamine, vinpocetine, and vincadifformine [122]; four adrenergic β2-agonists [250]; modified pregabalins [119]
Methylated γ-CD	Dapoxetine [251]; vincamine, vinpocetine, and vincadifformine [122]
Anionic CDs	
Carboxyethylated β-CD	Four drugs [325], modified pregabalins [119]
Carboxymethylated α-CD	Vincamine, vinpocetine, and vincadifformine [122]; nine dipeptides [240]; five antimalarial drugs [280]; two β-lactams [281]
Carboxymethylated β-CD	Twenty triadimenols [149]; anisodamine, atenolol, and metoprolol [282]; 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol [283]; fluvastatin [215]; matairesinol [284]; mirtazapine and its metabolites [81]; nateglinide [285]; ofloxacin [286]; trihexyphenidyl [287]; pramipexole [85]; propranolol and 4-hydroxypropranolol [288]; sibutramine [249]; vincamine, vinpocetine, and vincadifformine [122]; five β-agonists [227]; five β-adrenergic antagonists [289]; five β-blockers [290]; four basic compounds [61]; six antimalarial drugs [280]; five β-lactams [281]; 10 dipeptides and three tripeptides [240]; ruthenium(II) complexes [291]; stimulants [292]
Carboxymethylated γ-CD	Vincamine, vinpocetine, and vincadifformine [122]; four antimalarial drugs [280]; two β-lactams [281]; five dipeptides and one tripeptide [240]; ruthenium(II) complexes [291]
Phosphated β-CD	Modified pregabalins [119]
Phosphated γ-CD	Tolterodine and methoxytolterodine [89]
Succinylated β-CD	Catechin [326], four antimalarial drugs [280]
Sulfated α-CD	Amlodipine [241], borreverines [259], helquats [293], ornidazole [98], tolterodine and methoxytolterodine [89], verapamil [92], benzoxazolinonic aminoalcohols [294], two basic drugs [295], five basic drugs [296], seven pharmaceutical compounds [297], 7 2,3-naphthalenediamine-monosaccharides [298], saccharide-naphthimidazole derivatives [299], eight dipeptides and two tripeptides [240]

is different from single isomers [174, 204, 205, 229]. Sometimes, the migration order of enantiomers can be reversed as a consequence of the change of the degree of substitution [89,122,128,129,165,174,177,187,209,218,233,238–241].

The reproducibility of the preparation procedures leading to randomly substituted CDs might be uncertain [242] and even batch to batch reproducibility of the product of one supplier may be limited [12]. This often negatively affects the

Table 4. Continued

Name of selector	Examples of applications
Sulfated β-CD	Amlodipine [241], Betti bases [300], borreverine [259], bupivacaine [178], 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone [283], cetirizine [91, 301], citalopram [97], hydroxyflavanones [324], fluoxetine [302], helquats [293, 303], 4,5-disubstituted imidazoles [304], lorazepam [108, 162], 3-hydroxymexiletine [305], modafinil [134, 306], nicotine and its related compounds [307], nicotine butyric acid [90], ofloxacin and ornidazole [132], 3,4-dihydroxyphenylalanine [238, 308–310], phenylalanine [308], isoprenaline [92], rotigotine and related chiral impurities [252], thioridazine [311], tolterodine and methoxytolterodine [89], tryptophan [312], tyrosine [238, 308], benzoxazolinonic aminoalcohols [294], 13 new amphetamine-like designer drugs [313], 12 basic analytes [314], 19 cathinone derivatives [315], impurity of clopidogrel [316], four antimalarial drugs [280], four basic drugs [295], five basic drugs [296], six basic drugs [230], five pharmaceuticals [310], seven pharmaceutical compounds [297], Tic-hydantoin derivatives [208], four ketamines [196], tetrahydroisoquinoline-derived neurotoxins [317], 12 dipeptides and three tripeptides [240], five nucleoside phosphoramidate derivatives [318], ruthenium(II) complexes [291], metabolites of verapamil [80]
Sulfated γ-CD	Benzoxazolinonic aminoalcohols [294, 319], amlodipine [241], atenolol [92], helquats [293], ketamine and norketamine [320], methadone and its metabolite [83], methamphetamine and its derivatives [321], piperidinic benzoxazolinones [322], tryptophan [312], 16 β-carboline derivatives [323], four antimalarial drugs [280], four basic drugs [295, 296], seven pharmaceutical compounds [297], five nucleoside phosphoramidate derivatives [318], Tic-hydantoin derivatives [208], five dipeptides and one tripeptide [240], ruthenium(II) complexes [291], chiral oxazolidine derivative of tocainide [276]
Sulfobutylated α-CD	Five antimalarial drugs [280]
Sulfobutylated β-CD	Bupivacaine [327], baclofen [328], imperanene [329], isradipine [330], modafinil [331], *m*-nisoldipine [332], pantoprazole [93], sitagliptin [131], vincamine, vinpocetine, and vincadifformine [122], antiarrhythmic agent [333], four antimalarial drugs [280], 12 β-lactams [281], modified pregabalins [119]
Sulfobutylated γ-CD	Vincamine, vinpocetine, and vincadifformine [122]
Sulfo(2-hydroxy)propylated β-CD	Modified pregabalins [119]
Sulfo(2-hydroxy)propylated γ-CD	Modified pregabalins [119]
Sulfopropylated α-CD	Three dipeptides and two tripeptides [240], modified pregabalins [119], β-substituted tryptophan analogues [334]
Sulfopropylated β-CD	Eight dipeptides and two tripeptides [240], modified pregabalins [119], β-substituted tryptophan analogues [334]
Sulfopropylated γ-CD	Three dipeptides [240], modified pregabalins [119]
Cationic CDs	
Quaternary ammonium β-CD	Abscisic acid [200], three acyclic nucleoside phosphonates [210]

ANDA-CC, 7-amino-1,3-naphthalenedisulfonic acid monopotassium salt—cyanuric chloride; Tic, tetrahydroisoquinoline.

repeatability of CE separations. Moreover, it has been found that both the degree of substitution [243, 244] and the position of the substituents [245] on a CD skeleton have a significant influence on the separation. The application of mixtures of CDs instead of a well-defined single isomer therefore causes difficulties when the goal is to study the interaction processes, as the resulting system is too complex and insufficiently characterized.

Examples of commercially available randomly substituted CDs are neutral methylated CDs [119, 122, 133, 246–252] and 2-hydroxypropylated CDs [87, 88, 107, 119, 121, 122, 147, 149, 158, 174, 186, 204, 205, 208, 209, 215, 218, 227, 230–232, 236, 247, 250, 251, 253–279], and negatively charged carboxymethylated CDs [61, 81, 85, 122, 149, 215, 227, 240, 249, 280–292] and sulfated CDs [80, 83, 89–92, 97, 98, 108, 132, 134, 162, 178, 196, 208, 230, 238, 240, 241, 252, 259, 276, 280, 283, 291, 293–324]. The only commercially available positively charged randomly substituted CD is quaternary ammonium β-CD that has been used for the separation of negatively charged analytes [200, 210].

Besides using MEKC with native CDs as chiral additives, MEKC is also used with neutral randomly substituted CDs for the separation of neutral analytes [257, 269]. Neutral randomly substituted 2-hydroxypropylated β-CD has been immobilized on the inner capillary wall and then applied in the form of open-tubular CEC [264]. A summary on recent applications of randomly substituted CDs in the field of chiral CE is provided in Table 4.

4.3 Specifically substituted CDs

Wide range of CD derivatives with exactly known numbers and positions of substituents on CD glucose units is also used in electromigration methods. The most common derivatives have the same substituents on all the given carbon atoms of the glucose units (persubstituted derivatives at a given position or persubstituted derivatives where all the hydroxyl groups are replaced with the same functional group), for example, heptakis(2-*O*-sulfo-3-*O*-methyl-6-*O*-acetyl)-β-CD [37]

Table 5. Recent applications of specifically substituted CDs in chiral CE

Name of selector	Examples of applications
Neutral CDs (monosubstituted derivatives)	
6^A-Phenylcarbamoyl-β-CD	Halogen aryl alcohols, propranolol, and pindolol [336]
Neutral CDs (persubstituated derivatives)	
Heptakis(2,3-di-*O*-acetyl)-β-CD	Ephedrine [130]
Heptakis(2,6-di-*O*-methyl)-β-CD	Ephedrine [130], 4,5-disubstituted imidazoles [304], 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, codeine, and methadone [158], repaglinide [342], warfarin [200, 343], five β-agonists [227], cinchona alkaloids [344], glitazone compounds [345], four dipeptides [165, 177], modified pregabalins [119]
Per-*O*-methyl-α-CD	Ketoprofen [129], modified pregabalins [119]
Per-*O*-methyl-β-CD	Dapoxetine [251], ephedrine [130], ibuprofen [200], ketoprofen [129], lipoic acid [335], binaphthol and its mono derivatives [174, 209], 6,6′-dibromo-1,1′-binaphthyl-2,2′-diol [218], tulobuterol [227], modified pregabalins [119]
Per-*O*-methyl-γ-CD	Ketoprofen [129], amine derivaties [86], modified pregabalins [119]
Anionic CDs (monosubstituted derivatives)	
2^A-*O*-carboxymethyl-α-CD	Three basic compounds [58]
3^A-*O*-carboxymethyl-α-CD	Five basic compounds [58]
6^A-*O*-carboxymethyl-α-CD	Four basic compounds [58]
2^A-*O*-carboxymethyl-β-CD	Metoprolol [217], five basic compounds [61]
3^A-*O*-carboxymethyl-β-CD	Five basic compounds [61]
6^A-*O*-carboxymethyl-β-CD	Three basic compounds [61]
6^A-Succinyl-β-CD	Catechin [326]
6^A-Deoxy-6^A-sulfoethylthio-β-CD	Basic model compounds [55]
6^A-Deoxy-6^A-(6-sulfooxy-5,5-bis-sulfooxymethyl)hexylthio-β-CD	Basic model compounds [55]
Anionic CDs (persubstituated derivatives)	
Heptakis(2,3-di-*O*-acetyl-6-*O*-sulfo)-β-CD	Amphetamine and its related compounds [96], bupivacaine [124], ephedrine [130], norephedrine [128], propranolol [123, 124, 187], talinolol [127]
Heptakis(2-*O*-methyl-3-*O*-acetyl-6-*O*-sulfo)-β-CD	Carvedilol [193]
Heptakis(2,3-di-*O*-methyl-6-*O*-sulfo)-β-CD	Bupivacaine [124], carvedilol [193], norephedrine [128], propranolol [123, 124], 3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyranes [191], talinolol [127], ephedrine [130]
Heptakis(2-*O*-sulfo-3-*O*-methyl-6-*O*-acetyl)-β-CD	Twenty-seven weak base analytes [37]
Heptakis(6-*O*-sulfo)-β-CD	Ephedrine [130], lorazepam [108]
Heptakis(6-*O*-sulfobutyl)-β-CD	Glitazone compounds [345]
Cationic CDs (monosubstituted derivatives)	
6^A-Deoxy-6^A-amino-β-CD	Mandelic acid [92], modified pregabalins [119]
6^A-Deoxy-6^A-(3-hydroxypropyl)amino-β-CD	Flurbiprofen [192], five drugs [188], modified pregabalins [119]
6^A-Deoxy-6^A-(2-hydroxypropyl)amino-β-CD	Flurbiprofen [94]
6^A-Deoxy-6^A-(2-aminoethyl)amino-β-CD	Vicinal diols [337], FITC amino acids [338]
6^A-Deoxy-6^A-(2-hydroxyethyl)ammonium-β-CD	Eight DNS-amino acids, 14 hydroxy and carboxylic acids [53]
6^A-Deoxy-6^A-(2-methoxyethyl)ammonium)-β-CD chloride	Twenty-four DNS-amino acids, anionic and ampholytic analytes [52]
6^A-Deoxy-6^A-(3-methoxypropyl)ammonium-β-CD chloride	Twenty-two ampholytic and acidic racemates [120]
6^A-Deoxy-6^A-(2-hydroxpropyl)trimethylammonium-β-CD tetrafluoroborate	Eight drugs [213]
6^A-Deoxy-6^A-((2*S*,3*S*)-2,3-*O*-isopropylidene-1,4-tetramethylenediamine)-β-CD	Ten DNS-amino acids [51]
6^A-Deoxy-6^A-ammonium-6^C-deoxy-6^C-butylimidazolium-β-CD dichloride	Fourteen ampholytic and acidic racemates [339]
6^A-Deoxy-6^A-[4-(2-aminoethyl)imidazolyl]-β-CD	Tryptophan [340]
6^A-Deoxy-6^A-[*N*-(2-methylamino)pyridine]-β-CD	FITC amino acids [338]
6^A-Deoxy-6^A-pyrrolidine-β-CD chloride	Eighteen DNS-amino acids, anionic and ampholytic analytes [50]
6^A-Deoxy-6^A-(*N*-methyl-pyrrolidine)-β-CD chloride	Eight DNS-amino acids, anionic and ampholytic analytes [50]
6^A-Deoxy-6^A-(*N*-(2-hydroxyethyl)-pyrrolidine)-β-CD chloride	Eight DNS-amino acids, anionic and ampholytic analytes [50]
6^A-Deoxy-6^A-(2-hydroxymethyl-pyrrolidine)-β-CD chloride	Eight DNS-amino acids, anionic and ampholytic analytes [50]
6^A-Deoxy-6^A-[3-(4-ammoniobutyl)-imidazol-1-ium]-β-CD dichloride	Eight DNS-amino acids [54]
6^A-Deoxy-6^A-[(3*R*,4*R*)-dihydroxypyrrolidine]-β-CD chloride	Fifteen DNS-amino acids, anionic and ampholytic analytes [341]
Amphiphilic CDs (monosubstituted derivatives)	
6^A-*O*-(2-hydroxyl-3-betainylpropyl)-β-CD	Four drugs [232]

DNS, dansyl chloride; FITC, fluorescein isothiocyanate.

Table 6. Recent applications of CD mixtures in chiral CE

Name of selectors	Examples of applications
Carboxymethylated β-CD/α-CD	Camphorquinone [346, 347]
Carboxymethylated β-CD/β-CD	Seven β-lactams [281]
Carboxymethylated β-CD/2-hydroxypropylated β-CD	Six β-lactams [281]
Carboxymethylated β-CD/methylated β-CD	Seven β-lactams [281]
Carboxymethylated β-CD/heptakis(2,6-di-*O*-methyl)-β-CD	Stimulants [292]
Carboxymethylated β-CD/per-*O*-methyl-β-CD	Fourteen β-lactams [281]
Carboxymethylated β-CD/sulfated α-CD	Risperidone and its metabolites [82]
Carboxymethylated β-CD/sulfated β-CD	Nateglinide [285]
Sulfobutylated β-CD/β-CD	Twelve β-lactams [281]
Sulfobutylated β-CD/2-hydroxypropylated β-CD	Fifteen β-lactams [281]
Sulfobutylated β-CD/methylated β-CD	Twelve β-lactams [281], seven ergostane and lanostane derivatives [239]
Sulfobutylated β-CD/per-*O*-methyl-β-CD	Seventeen β-lactams [281]
Sulfobutylated γ-CD/6^A-deoxy-6^A-(3-hydroxy)propylamino-β-CD	Imperanene [329]
Sulfobutylated γ-CD/2-hydroxypropylated α-CD	Imperanene [329]
Sulfobutylated γ-CD/2-hydroxypropylated β-CD	Imperanene [329]
Sulfobutylated γ-CD/sulfobutylated α-CD	Imperanene [329]
Sulfobutylated γ-CD/sulfobutylated β-CD	Imperanene [329]
2-Hydroxypropylated β-CD/sulfated α-CD	9-Hydroxyrisperidone [351]
2-Hydroxypropylated β-CD/sulfated β-CD	Thioridazine [311]
2-Hydroxypropylated γ-CD/acetylated γ-CD	Polycyclic musks [348]
2-Hydroxypropylated γ-CD/γ-CD	Polycyclic musks [348]
Heptakis(2,6-di-*O*-methyl)-β-CD/sulfated β-CD	4,5-Disubstituted imidazoles [304]
Heptakis(2,6-di-*O*-methyl)-β-CD/heptakis(6-*O*-sulfobutyl)-β-CD	Glitazone compounds [345]
Heptakis(2,6-di-*O*-methyl)-β-CD/6^A-deoxy-6^A-(3-hydroxypropyl)amino-β-CD	Aspartic acid, glutamic acid [350]
Methylated β-CD/sulfated β-CD	Rotigotine and related chiral impurities [252]
β-CD/octakis(6-*O*-sulfo)-γ-CD	FMOC amino acids [352]
β-CD/per-*O*-sulfo-β-CD	Thioridazine [311]
β-CD/succinylated β-CD	Glycidyl tosylate [353]
β-CD/2-hydroxypropylated β-CD	Trimipramine [349]
β-CD/sulfated β-CD	Hydroxyflavanones [324]

FMOC, fluorenylmethyloxycarbonyl chloride.

Table 7. Recent applications of CDs in the mixture with other types of selectors in chiral CE

Name of selectors	Examples of applications
Ionic liquid/β-CD	FMOC-pipecolic acid [216], phenylalanine and tryptophan [354], β-agonists [225], 12 drugs [95]
Ionic liquid/glucosylated β-CD	Naproxen, warfarin, and pranoprofen [247]
Ionic liquid/methylated β-CD	Naproxen, warfarin, and pranoprofen [247]
Ionic liquid/2-hydroxypropylated β-CD	Naproxen, warfarin, and pranoprofen [247], propranolol [355], 10 drugs [356]
Ionic liquid/heptakis(2,3-di-*O*-methyl-6-*O*-sulfo)-β-CD	3,4-Dihydro-2,2-dimethyl-2*H*-1-benzopyranes [191]
18-Crown-6 ether/β-CD	Three primary amines [137]
18-Crown-6 ether/heptakis(2,6-di-*O*-methyl)-β-CD	Stimulants [292]
18-Crown-6 ether/sulfated β-CD	Betti bases [300]
Sodium taurodeoxycholate/β-CD	Five FITC-amino acids [357], nine FMOC-amino acids [358]

FITC, fluorescein isothiocyanate; FMOC, fluorenylmethyloxycarbonyl chloride.

and per-*O*-methyl-β-CD [119, 129, 130, 174, 200, 209, 218, 227, 251, 335].

Monosubstituted derivatives where only one functional group is attached to a CD in a known position are less common. For example 6^A-phenylcarbamoyl-β-CD has been used in packed CEC [336]. The reason for their less frequent application is related with their more difficult synthesis as described in Section 2.2.4.

The main purpose of the utilization of specifically substituted CDs is the enhancement of enantiomeric separation in some cases, but what is even more important, they are very beneficial for detailed studies of interaction with analytes in real systems. For instance sets of 2^A-*O*-, 3^A-*O*- and 6^A-*O*-carboxymethyl-α-CDs [57–59] and β-CDs [61] have been investigated and the influence of carboxymethyl group position on analyte–CS interaction has been studied in detail.

Similarly, the influence of the number of substituents has been evaluated for succinylated β-CD [326] and it has been shown that the best resolution for catechin enantiomers has been achieved with CD with only one succinyl group in the CD molecule.

Unlike randomly substituted CDs where the only positively charged derivative is commonly used, there are several positively charged specifically substituted CDs available. They are mainly 6^A-deoxy-6^A-alkylamino-β-CDs [52, 53, 92, 94, 119, 120, 188, 192, 213, 337, 338] or 6^A-substituted heterocyclic β-CD derivatives [50, 54, 338–341]. For example, Xiao et al. have used derivatives of 6^A-deoxy-6^A-pyrrolidine-β-CD chloride to study the interaction of carboxylic acid derivatives with these CDs and they have confirmed that electrostatic interaction plays very important role in enantioseparation [50, 341].

Moreover, an amphiphilic CD, 6^A-*O*-(2-hydroxyl-3-betainylpropyl)-β-CD, has been employed for enantioseparation of several drugs [232]. Table 5 gives a survey on applications of specifically substituted CDs.

4.4 Mixtures of CDs

It has been found that the application of CD mixtures frequently leads to enhancement of enantioseparation. When a sole CD is not able to separate the enantiomers then a second CD derivative can be added to the BGE and in some cases, the required separation might be obtained. Such mixtures are referred to as dual systems. One of these CDs is often negatively charged, for instance carboxymethylated β-CD [82, 281, 285, 292, 346, 347], sulfated β-CD [252, 285, 304, 311, 324], or sulfobutylated β-CD [239, 281, 329], and the other is frequently neutral. Dual systems consisting of two neutral CDs are relatively rare [348, 349], and to the best of our knowledge a system containing two positively charged CDs has never been used. The CDs added to the BGE are mostly randomly substituted or native CDs and dual systems with both specifically substituted CDs appear only scarcely [345, 350]. In most cases the resulting enantioseparation is better than a simple sum of the contributions of the individual CDs, that is, usually a significant synergistic effect occurs. On the other hand, it should be mentioned that this is not always the case in real instances. A summary on the recent use of CDs mixtures is provided in Table 6.

4.5 Miscellaneous mixtures containing CDs

Besides the systems mentioned above, a BGE can also consist of a CD with some other selector added (i.e., usually the resulting system consists of a chiral and achiral additive in a given ratio). The non-CD selectors can interact with CD itself and thus change the CD's binding properties. A typical example of this behavior are ionic liquids that have become popular in the last few years in many fields [95, 191, 216, 225, 247, 354–356]. Ionic liquids change both the EOF (due to their charge) and the interaction abilities of CDs with analytes. The other types

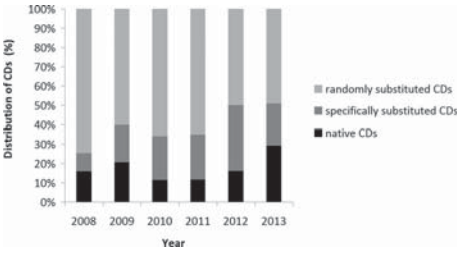


Figure 2. Relative representation of the CDs use in successful chiral CE separations in years 2008–2013.

of selectors used in combination with CDs are 18-crown-6 ether and its derivatives [137, 292, 300]. Unlike ionic liquids, crown ethers do not interact with CDs, but they interact with analytes and help to enhance enantioseparation by interacting with analytes via a mechanism that is different from that of CDs. The third type of selectors represent detergents that are typically used in the MEKC technique. Beside the commonly used SDS (already mentioned above in relation with MEKC), a chiral detergent, specifically sodium taurodeoxycholate, has been studied in combination with CDs [357, 358]. Recent applications of such mixed systems are listed in Table 7.

Based on our literature survey, the relative number of single isomer CDs (including native and specifically substituted CDs) successfully applied for chiral CE separations doubled (an increase from 25 to 50%) in the last six years (Fig. 2). This is predominantly caused by two factors. First, the number of newly synthesized CD derivatives is increasing and they are mostly specifically substituted and synthesized for specific purposes. Second, due to the intense effort to get an insight into the mechanism of interactions between CDs and analytes, well-defined CDs with exactly known structure are growingly used. These two factors lead to a certain relative decrease in the number of randomly substituted CDs although they are still irreplaceable for enantioseparation of many real samples.

5 Conclusions

It can be concluded unambiguously that chiral CE mediated by CDs is continuously flourishing. New CD derivatives have been prepared and utilized in electromigration methods. The potential of selected spectroscopy methods, molecular modeling, and simulation software has been realized within several scientific groups and significant contributions have been brought. In recent years it seems that there is a trend directed to the preferential use of CDs with well-defined structures mainly in studies aimed at the explanation of separation mechanisms. In the future, a continuous growth of the utilization of many supplementary techniques in the effort to gain a detailed knowledge on the equilibria occurring in the

solution and the recognition mechanism can be expected. Furthermore, it is highly probable that new CD derivatives will be introduced and their potential evaluated in chiral CE.

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Research Article

Influence of substituent position and cavity size of the regioisomers of monocarboxymethyl-α-, β-, and γ-cyclodextrins on the apparent stability constants of their complexes with both enantiomers of Tröger's base

Enantiomers of Tröger's base were separated by capillary electrophoresis using 2^{I}-*O*-, 3^{I}-*O*-, and 6^{I}-*O*-carboxymethyl-α-, β-, and γ-cyclodextrin and native α-, β-, and γ-cyclodextrin as chiral additives at 0–12 mmol/L for β-cyclodextrin and its derivatives and 0–50 mmol/L for α- and γ-cyclodextrins and their derivatives in a background electrolyte composed of sodium phosphate buffer at 20 mmol/L concentration and pH 2.5. Apparent stability constants of all cyclodextrin–Tröger's base complexes were calculated based on capillary electrophoresis data. The obtained results showed that the position of the carboxymethyl group as well as the cavity size of the individual cyclodextrin significantly influences the apparent stability constants of cyclodextrin–Tröger's base complexes.

Keywords: Capillary electrophoresis / Chiral separation / Cyclodextrin / Regioisomers / Stability constants
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1 Introduction

Although cyclodextrins (CDs) have been known since the end of 19th century [1], they were used in electromigration methods for the first time only nearly 100 years later. CDs were originally applied for the separation of achiral compounds [2]. Later, specifically in 1988, the first papers on the chiral separation utilizing CDs in CE format were published [3,4]. Since that time dozens of CD derivatives have been synthesized and employed as chiral selectors. However, still it is not an easy task to find a suitable CD derivative for the separation of a specific pair of enantiomers. Generally, the mechanism of the formation of CD–analyte complex is only partially known and predictions on the separability of the enantiomers solely based on the chemical structure of a given analyte and a chiral selector are practically impossible. Usually the inclusion type of complexation is expected but the structural details of the interactions are rarely known, for instance, in the case if they are obtained from independent investigation by NMR analysis of CD–analyte complexes in the liquid phase or from X-ray analysis of crystals of these complexes in the solid phase. The empirically gained data in real experiments are very useful in both the effort to clarify the core of the chiral recognition and the attempt to develop at least semiempirical models providing a reliable prediction of a given chiral separation problem. Due to the above-mentioned facts the experiments devoted to the study of the influence of position, type, and extent of CD modification with various substituents on the enantioseparation of specific groups of chiral compounds are important and desired [5–8]. Such a study dealing with the influence of the extent of the modification of the CD with a specific substituent on chiral separation has been carried out for instance by Maruszak et al. in 2001 [9]. Beside other CDs, they studied three different methylated β-CDs, namely, randomly methylated β-CD with a degree of substitution of 1.6–2.0, heptakis(2,6-di-*O*-methyl)-β-cyclodextrin, and heptakis(2,3,6-tri-*O*-methyl)-

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Abbreviations: **CD**, cyclodextrin; **HVL**, Haarhoff–van der Linde; **TB**, Tröger's base; **CM**, carboxymethyl; **2CMACD**, 2^{I}-*O*-carboxymethyl-α-cyclodextrin; **3CMACD**, 3^{I}-*O*-carboxymethyl-α-cyclodextrin; **6CMACD**, 6^{I}-*O*-carboxymethyl-α-cyclodextrin; **2CMBCD**, 2^{I}-*O*-carboxymethyl-β-cyclodextrin; **3CMBCD**, 3^{I}-*O*-carboxymethyl-β-cyclodextrin; **6CMBCD**, 6^{I}-*O*-carboxymethyl-β-cyclodextrin; **2CMGCD**, 2^{I}-*O*-carboxymethyl-γ-cyclodextrin; **3CMGCD**, 3^{I}-*O*-carboxymethyl-γ-cyclodextrin; **6CMGCD**, 6^{I}-*O*-carboxymethyl-γ-cyclodextrin

β-cyclodextrin. They have not observed any significant difference in the separation ability of these CDs derivatives for enantioseparation of four chiral neurotransmitters. Busby et al. synthesized and applied heptakis(2-*O*-methyl-6-*O*-sulfo)-β-cyclodextrin for chiral separations of a group of weak bases [10]. They concluded that previously studied heptakis(2-*O*-methyl-3-*O*-acetyl-6-*O*-sulfo)-β-cyclodextrin [11] proved to be a better resolving agent. Within the same research group also heptakis(2-*O*-sulfo-3-*O*-methyl-6-*O*-acetyl)-β-cyclodextrin have been prepared and analytically characterized [12]. The extensive evaluation and comparison of this compound with the other β-CD derivatives, specifically with heptakis(2-*O*-methyl-3-*O*-acetyl-6-*O*-sulfo)-β-cyclodextrin and heptakis(2-*O*-methyl-3,6-di-*O*-sulfo)-β-cyclodextrin led to the conclusion that enantioseparation selectivity was different for some of the analytes studied.

Beside the studies on persubstituted CD derivatives there are also papers focused on the enantioselectivity of monosubstituted CDs. In such case three isomers may exist substituted at the position C2, C3, or C6, respectively [13–15]. The results revealed that both the position of carboxymethyl group within the CD skeleton and the size of the CD cavity strongly influenced the enantioseparation ability of these selectors toward the studied analytes. Kim et al. studied the chiral separation of catechin enantiomers by CE using mono-, di-, and trisuccinyl-β-cyclodextrin substituted at the primary hydroxyl groups of CD as chiral selectors [16]. They have concluded that the optimal separation conditions have been reached utilizing monosubstituted CD. On the other hand, the chiral selectors with the higher degree of substitution had a broader pH range of (±)-catechin separation when compared with monosuccinyl-β-cyclodextrin.

The other aspect frequently studied with CDs utilized as the chiral additives to the BGE is the influence of a substituent type in a given CD on the stability constants with analytes. CE is a suitable method for the determination of apparent and/or thermodynamic stability constants of various complexes in solution [17–21]. Significant differences in the strength of the formed CD–analyte complexes have been demonstrated experimentally by Vincent et al. when they compared heptakis(2,3-di-*O*-acetyl-6-*O*-sulfo)-β-cyclodextrin with heptakis(6-*O*-sulfo)-β-cyclodextrin, the latter being much stronger complexing agent for all the analytes studied in low and high pH BGEs [22, 23]. Similarly, they have found that heptakis(2-*O*-methyl-3-*O*-acetyl-6-*O*-sulfo)-β-cyclodextrin interacted more strongly with the chiral solutes than heptakis(2,3-di-*O*-methyl-6-*O*-sulfo)-β-cyclodextrin but less strongly than heptakis(2,3-di-*O*-acetyl-6-*O*-sulfo)-β-cyclodextrin [11].

In this work, we investigated for the first time the influence of the substitution of all nine regioisomers of monosubstituted α-, β-, and γ-CDs, namely, 2^{I}-*O*-, 3^{I}-*O*-, and 6^{I}-*O*-carboxymethyl-α-, β-, and γ-cyclodextrins, on their apparent stability constants with the enantiomers of Tröger's base (TB). TB is a well-known and interesting compound exhibiting chirality not related to the presence of more common asymmetric carbon in its structure but instead of that two bridgehead stereogenic nitrogen atoms. This compound and its derivatives have many outstanding properties and potential applications [24–26]. We used it as the suitable chiral model compound. We believe that our study represents a valuable contribution potentially utilizable in the development of models aiming to describe chiral separation processes in real systems.

2 Materials and methods

2.1 Chemicals and materials

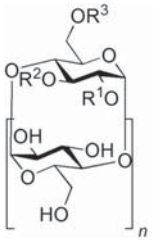
Hydrochloric acid (30%; Suprapur, Merck, Germany), 1 mol/L sodium hydroxide (Tripur, Merck), orthophosphoric acid (50%), DMSO (99.7%), (5*R*,11*R*)-(–)-2,8-dimethyl-6*H*,12*H*-5,11-methanodibenzo[b,f][1,5]diazocine ((–)-Tröger's base, (–)-TB, 99.5%), (5*S*,11*S*)-(+)-2,8-dimethyl-6*H*,12*H*-5,11-methanodibenzo[b,f][1,5]diazocine ((+)-Tröger's base, (+)-TB, 99.5%), α-cyclodextrin (98%), β-cyclodextrin (97%), γ-cyclodextrin (98%), all from Sigma–Aldrich, Czech Republic, and ultrapure water (Milli-Q grade, Millipore, France) were used. Syntheses of 2^{I}-*O*-, 3^{I}-*O*-, and 6^{I}-*O*-carboxymethyl-α-cyclodextrins (2CMACD, 3CMACD, 6CMACD), 2^{I}-*O*-, 3^{I}-*O*-, and 6^{I}-*O*-carboxymethyl-β-cyclodextrins (2CMBCD, 3CMBCD, 6CMBCD), and 2^{I}-*O*-, 3^{I}-*O*-, and 6^{I}-*O*-carboxymethyl-γ-cyclodextrins (2CMGCD, 3CMGCD, 6CMGCD) were described previously [27–29] (Fig. 1).

2.2 Equipment

CE separations were performed with an Agilent CE instrument (Agilent 3D HPCE, Germany) equipped with UV-Vis diode-array detector. Bare fused-silica capillary of 375/75 μm od/id and 58.5/50 cm total/effective length obtained from Polymicro Technologies (AZ, USA) was used.

2.3 CE conditions

Individual enantiomers of the TB were dissolved in DMSO at 10 mmol/L concentration. For the CE experiments, the analyte solutions were mixed together in the ratio 1:1 v/v to form a racemic solution, which was further diluted with water to the final concentration 1 mmol/L. For the determination of migration order of particular enantiomers the mixture of TB enantiomers at the ratio 1:2 v/v was used. The BGE consisted of 20 mmol/L sodium phosphate buffer, pH 2.5 (20 mmol/L orthophosphoric acid adjusted to appropriate pH with 1 mol/L NaOH), and various concentrations of the studied CDs (0–50 mmol/L for α-CD and its derivatives, 0–12 mmol/L for β-CD and its derivatives, and 0–50 mmol/L for γ-CD and its derivatives). A new fused-silica capillary was first rinsed with 1 mol/L NaOH for 30 min, then with H_2O for 30 min. Between the runs the capillary was rinsed at 99.4 kPa first with 0.1 mol/L NaOH for 2 min then with H_2O also for 2 min, and finally with running buffer again for 2 min (for the capillary washing a different buffer solution than for the subsequent analysis was used). The analytes



	n	R^1	R^2	R^3
2CMACD	5	-CH_2COOH	-H	-H
3CMACD	5	-H	-CH_2COOH	-H
6CMACD	5	-H	-H	-CH_2COOH
2CMBCD	6	-CH_2COOH	-H	-H
3CMBCD	6	-H	-CH_2COOH	-H
6CMBCD	6	-H	-H	-CH_2COOH
2CMGCD	7	-CH_2COOH	-H	-H
3CMGCD	7	-H	-CH_2COOH	-H
6CMGCD	7	-H	-H	-CH_2COOH

Figure 1. Structures of monocarboxylated CDs used in this study as chiral additives.

were injected hydrodynamically by pressure of 1.5 kPa for 5 s. All the separations were performed at 20 kV (anode at the injection capillary end) with a voltage ramp time of 12 s. Detection was carried out at 207 nm and the capillary was thermostatted at 25 °C during the analyses.

2.4 Calculation of the apparent stability constants

Due to the fact that peaks in real electropherograms are not perfectly symmetrical, we fitted experimentally acquired peaks of TB by Haarhoff–van der Linde (HVL) function Eq. (1) [30] using Origin software version 6.0 (OriginLab Corporation, USA) and we used a parameter a_1 as a marker of true peak position ($t_{R,corr}$) in a subsequent calculation of effective mobility instead of a plain migration time represented by the top of the peak.

$$\mathrm{HVL}_\delta(t; a_0, a_1, a_2, a_3) = \frac{\frac{a_0 \cdot a_2}{a_2 \cdot a_{3\delta}\sqrt{2\pi}} \cdot e^{-0.5 \cdot [(t-a_1)/a_2]^2}}{\frac{1}{e^{a_{3\delta}}-1} + 0.5 \cdot \left[1 + \mathrm{erf}\left(\frac{t-a_1}{\sqrt{2}\cdot a_2}\right)\right]} \quad (1)$$

The effective mobility (μ_{eff}) of the specific TB enantiomer was calculated according to Eq. (2):

$$\mu_{eff} = \frac{l \cdot L}{U \cdot t_{R,corr}} - \mu_{EOF} \quad (2)$$

where l is the effective length of the capillary, L is the total length of the capillary, U is the applied voltage, and μ_{EOF} is the mobility of the EOF, which can be calculated according to Eq. (3):

$$\mu_{EOF} = \frac{l \cdot L}{U \cdot t_{DMSO}} \quad (3)$$

where t_{DMSO} is the migration time of DMSO (a marker of the EOF).

In the next step, we implemented the correction on viscosity variation related to the addition of native CDs to the BGE. For that purpose, we used previously described procedure based on the measurement of the current in the CE system [31]. As a result, the viscosity-corrected effective mobility (μ_{corr}) was calculated according to Eq. (4):

$$\mu_{corr} = \mu_{eff} \frac{I_0}{I} \quad (4)$$

where I_0 is the current in the absence of CD and I is the current in the presence of a specific CD. For carboxymethyl-CDs the viscosity corrections obtained for the appropriate native CDs were applied (more detailed explanation is provided in Section 3).

Further the effective mobilities of the individual enantiomers of TB obtained in the presence of carboxymethyl-CDs corrected to viscosity were recalculated to 0.014116 mol/L ionic strength (the ionic strength of the 20 mmol/L sodium phosphate buffer, pH 2.5) according to Eqs. (5) and (6) modified for an univalent electrolyte and based on the equations postulated by Onsager and Fuoss [32] and further modified by Pitts [33]:

$$\mu_{corr} = \mu_{lim} - \left(\frac{e^3}{40.97\pi} \cdot \sqrt{\frac{N_{AV}}{(\varepsilon k T)^3}} \cdot \mu_{lim} + \frac{e^2}{6\pi\eta} \cdot \sqrt{\frac{N_{AV}}{\varepsilon k T}}\right) \cdot \frac{\sqrt{2I}}{1 + d\sqrt{\frac{2e^2 N_{AV} I}{\varepsilon k T}}} \quad (5)$$

$$\mu_{corr,IS} = \mu_{lim} - \left(\frac{e^3}{40.97\pi} \cdot \sqrt{\frac{N_{AV}}{(\varepsilon k T)^3}} \cdot \mu_{lim} + \frac{e^2}{6\pi\eta} \cdot \sqrt{\frac{N_{AV}}{\varepsilon k T}}\right) \cdot \frac{\sqrt{2 \cdot 0.014116}}{1 + d\sqrt{\frac{2e^2 N_{AV} \cdot 0.014116}{\varepsilon k T}}} \quad (6)$$

where ε is the permittivity of the solution (6.954×10^{-10} F/m), k is the Boltzmann constant, e is the elementary charge, N_{AV} is the Avogadro constant, T is the absolute temperature of the solution (298 K), η is the viscosity of the solution (8.937×10^{-4} Pa·s), d represents the mean distance of closest approach for the ion (1 nm), μ_{lim} is the limiting ionic mobility of the charged analyte at zero ionic strength, and I is the ionic strength calculated using well-known Eq. (7):

$$I = \frac{1}{2} \cdot \sum_{i=1}^{s} c_i z_i^2 \quad (7)$$

where s is the number of species, c_i is the concentration of the ion i and z_i is its charge number. The concentration of the charged fraction of the carboxymethyl-CD was calculated based on its known pK_a value (3.6) [34]. The values of ionic

strength of the BGE for all additions of carboxymethyl-CDs are available in Supporting Information.

The calculation of the apparent stability constants, K', and the ionic mobility of the complexed TB with CD, μ_{TB-CD}, was based on approach published elsewhere [35] utilizing Eq. (8); calculations were carried out utilizing Origin software and applying a nonlinear regression:

$$\mu_{corr,IS} = \frac{\mu_{TB} + \mu_{TB-CD} \cdot K' \cdot [CD]}{1 + K' \cdot [CD]} \quad (8)$$

μ_{TB} is the ionic mobility of the free TB at the absence of CD in the BGE and it is obtained experimentally as well as $\mu_{corr,IS}$ values, i.e. the effective mobility corrected to viscosity recalculated to 0.014116 mol/L ionic strength. [CD] is the equilibrium concentration of the CD in the system. However, as the correct migration time of the analyte (parameter a_1 in the HVL function) corresponds with the migration time of the analyte at its infinite dilution, the concentration of CD bound in the complex is limiting to zero, and consequently $[CD] = c_{CD,}$ where c_{CD} is the analytical (total) concentration of CD. The apparent stability constant is defined by Eq. (9):

$$K' = \frac{[TB - CD]}{[CD] \cdot [TB]} \quad (9)$$

where [TB–CD] is the equilibrium concentration of the complex of TB with CD and [TB] is the equilibrium concentration of the TB.

3 Results and discussion

In the present work, all the CE separations were carried out with 20 mmol/L sodium phosphate buffer, pH 2.5, as the BGE. This electrolyte was chosen because of our positive experience as we reached successful enanatioseparations of TB using all the monosubstituted CDs previously [13–15]. TB is positively charged at such pH (pK_a 4.75) [15] and degree of dissociation of the monosubstituted carboxymethyl-CDs (pK_a 3.6) is very limited [34].

Our previous results demonstrated a significant influence of the substituent position on a CD and the size of a CD cavity on the chiral separation. Here, we intended to determine apparent stability constants, K', of the complexes of TB with all the studied selectors. Before the calculations of the constants several factors potentially influencing the separation process and the calculations of the apparent stability constants were considered. As the calculations of K' values are based on the determination of migration times and subsequently effective mobilities at various concentrations of the added CD derivatives and such values might be influenced not only by the extend of the complexation of the TB with a given CD derivative but also by other factors, specifically, by the peak shape, the viscosity, and the ionic strength of the used electrolyte, we studied these three effects in more detail.

The obtained peaks in real electropherograms (Fig. 2) were not perfectly symmetrical. Thus, we fitted acquired peaks of TB in the presence of α-CD at different concentration in the BGE (Fig. 2) by the HVL function [36] using Origin software.

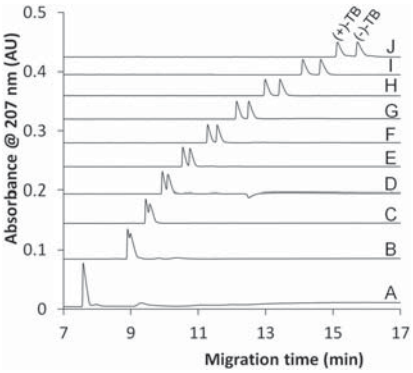


Figure 2. Electropherograms of the analyzed racemic mixture of TB. The separation of the enantiomers of TB was obtained at various concentration of α-CD in the BGE, specifically at: (A) 0, (B) 5, (C) 6.5, (D) 9, (E) 12, (F) 16, (G) 21, (H) 28, (I) 37, and (J) 50 mmol/L. The other conditions: detection at 207 nm, the concentration of the BGE 20 mmol/L sodium phosphate, pH 2.5; fused-silica capillary: od/id = 375/75 μm, total/effective length 58.5/50.0 cm; voltage 20 kV; temperature 25 °C.

The potential influence of viscosity change related to the addition of the CD derivative into the BGE on the effective mobility of the analyte was also evaluated. Previously described experimental setup was utilized to serve that purpose [31]. It was found out that the influence of viscosity change is relatively significant and increasing with the increasing CD cavity size (1.9, 2.7, and 4.2% for α-, β-, and γ-CD at 10 mmol/L, respectively). For carboxymethyl-CDs, the viscosity corrections obtained for the appropriate native CDs were applied due to the fact that the degree of dissociation of carboxymethyl-CDs was very low (only 4.7% at pH 2.5). Moreover, the viscosity of neutral and fully charged CDs is similar [18, 19].

Figure 3 shows the effective mobility corrected to viscosity of (+)-TB and (–)-TB in the presence of α-CD at various concentrations in the BGE as typical example of the obtained dependences. As can be seen the fit of the effective mobilities corrected to viscosity utilizing Eq. (8) is very good.

Finally, because the addition of the partially charged carboxymethyl-CDs into the BGE leads to a slight change of ionic strength of the electrolyte, the mobilities of the charged analytes were recalculated to constant (specifically to 0.014116 mol/L) ionic strength according to Eqs. (5) and (6). A comparison of $\mu_{corr,IS}$ with corresponding μ_{corr} values revealed only a very slight difference (about 0.1%).

As it was mentioned above the carboxymethyl-CDs are slightly dissociated at pH 2.5. Consequently, the enantiomers of TB can create complexes with both forms of the carboxymethyl-CDs, the neutral and the ionized one. Thus, the observed migration times represent weighted average

Table 1. Apparent stability constants (K'), and the effective mobilities corrected to viscosity recalculated to constant (0.014116 mol/L) ionic strength ($\mu_{corr,IS}$) for complexes of the studied (+)-TB/(–)-TB with CDs including SDs and selectivity

	$K'_{(+)\text{-TB–CD}}$ (L/mol)	$K'_{(-)\text{-TB–CD}}$ (L/mol)	$\mu_{corr,IS,(+)\text{-TB–CD}}$ (10^{-9} $m^2V^{-1}s^{-1}$)	$\mu_{corr,IS,(-)\text{-TB–CD}}$ (10^{-9} $m^2V^{-1}s^{-1}$)	Selectivity[a]
α-CD	49 ± 1	54 ± 1	6.25 ± 0.15	5.75 ± 0.12	1.09
2CMACD	33 ± 3	40 ± 3	4.20 ± 0.43	4.00 ± 0.32	1.05
3CMACD	85 ± 5	122 ± 7	8.10 ± 0.36	7.47 ± 0.45	1.08
6CMACD	44 ± 5	50 ± 6	5.68 ± 0.62	5.31 ± 0.51	1.07
β-CD	219 ± 8	303 ± 8	6.91 ± 0.13	6.49 ± 0.20	1.06
2CMBCD	271 ± 12	439 ± 15	4.37 ± 0.10	3.75 ± 0.18	1.16
3CMBCD	230 ± 11	276 ± 11	5.17 ± 0.22	4.56 ± 0.29	1.13
6CMBCD	269 ± 17	372 ± 20	4.53 ± 0.17	4.18 ± 0.26	1.08
γ-CD	22 ± 5	27 ± 5	4.69 ± 0.27	4.24 ± 0.22	1.11
2CMGCD	45 ± 6	53 ± 6	6.22 ± 0.82	5.76 ± 0.66	1.08
3CMGCD	928 ± 21	948 ± 33	5.68 ± 0.03	5.18 ± 0.05	1.10
6CMGCD	28 ± 3	36 ± 3	6.26 ± 0.69	5.80 ± 0.55	1.08

2CMACD, 2^1-*O*-carboxymethyl-α-cyclodextrin; 3CMACD, 3^1-*O*-carboxymethyl-α-cyclodextrin; 6CMACD, 6^1-*O*-carboxymethyl-α-cyclodextrin; 2CMBCD, 2^1-*O*-carboxymethyl-β-cyclodextrin; 3CMBCD, 3^1-*O*-carboxymethyl-β-cyclodextrin; 6CMBCD, 6^1-*O*-carboxymethyl-β-cyclodextrin; 2CMGCD, 2^1-*O*-carboxymethyl-γ-cyclodextrin; 6CMGCD, 6^1-*O*-carboxymethyl-γ-cyclodextrin.
a) Selectivity was calculated as the ratio of $\mu_{corr,IS}$ values of the complex (+)-TB–CD versus (–)-TB–CD.

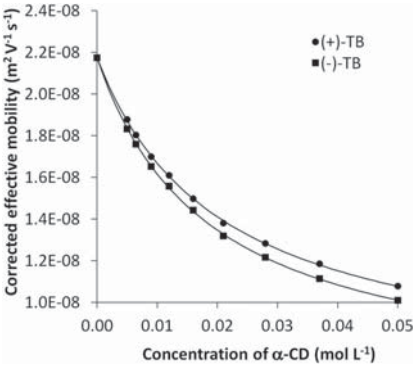


Figure 3. Dependence of the effective mobility corrected to viscosity of TB enantiomers on α-CD concentration and their fits according to Eq. (8).

values and the calculated apparent stability constants are, in strict sense, apparent weighted average stability constants. Values of K' and $\mu_{corr,IS}$ of the complexes TB–CD are summarized in Table 1. Concerning the native CDs, it is evident that β-CD forms much stronger complex with TB than the other two native CDs. Undoubtedly, the size of the cavity plays a significant role in the strength of the interaction. Similarly, the apparent stability constants for carboxymethyl-β-CD are in most cases higher than for carboxymethyl derivatives of α- and γ-CD. However, even within the same type of CD (i.e., carboxymethyl substituent at a given CD) the apparent stability constants differ up to about 30 times for 3CMGCD and 6CMGCD. Thus, the change even in one carboxymethyl group position on one single glucose unit within the entire γ-CD cycle leads to such significant change in the apparent stability constant. γ-CD and its derivatives are of interest also by the other reason. While the apparent stability constant for the native γ-CD is one of the lowest found, in contrast, by far the highest was the apparent stability constant for 3CMGCD. Such pronounced difference in the apparent stability constants is probably related to the more flexible structure of γ-CD, where the induced cavity shape change effect can occur. The more rigid structure of α- and β-CD does not allow for such a deformation of their skeleton.

The results obtained on the apparent stability constants are in a good agreement with the previously published conclusions (vide supra) demonstrating that not only cavity size but also the position of the substituent play very important role in the complex formation ability and, in many cases, it may be even more critical than the cavity size itself.

On the other hand, the selectivity values did not show such significant differences as the apparent stability constants (Table 1). The values of selectivity are comparable for both α-CD and γ-CD and their derivatives (≤1.11). The selectivity for two β-CD derivatives (2^1-*O*-carboxymethyl-β-cyclodextrin and 3^1-*O*-carboxymethyl-β-cyclodextrin) is slightly better (1.16 and 1.13) than for α- and γ-CD and their derivatives.

4 Concluding remarks

In this work, the racemic mixture of TB was used for the study of the influence of the CD cavity size and the position of carboxymethyl group in monocarboxymethyl-α-, β-, and γ-CD on the enantioseparation and their respective apparent stability constants. The results clearly demonstrate a significant influence of the substituent location in the monosubstituted CD as well as the size of the CD cavity on both the chiral

separation and the apparent stability constants. Considering the native CDs solely the highest apparent stability constants were achieved for β-CD (219 L/mol for (+)-TB and 303 L/mol for (–)-TB). Taking into account all of the studied CD derivatives, the highest apparent stability constants were obtained for 3^{I}-*O*-carboxymethyl-γ-CD (928 L/mol for (+)-TB and 948 L/mol for (–)-TB) and the highest selectivity was achieved for 2^{I}-*O*-carboxymethyl-β-CD.

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Short Communication

The influence of the substituent position in monocarboxymethyl-γ-cyclodextrins on enantioselectivity in capillary electrophoresis

Three newly synthesized chiral selectors, namely, 2^1-*O*-, 3^1-*O*-, and 6^1-*O*-carboxymethyl-γ-cyclodextrin, native γ-cyclodextrin, and commercially available carboxymethylated γ-cyclodextrin with degree of substitution of 3–6 were used as additives in a background electrolyte composed of phosphate buffer at 20 mmol/L concentration and pH 2.5. This system was used for the analysis of several biologically significant low-molecular-mass chiral compounds by capillary electrophoresis. The results confirmed that the position of carboxymethyl group influences the enantioseparation efficiency of all the studied analytes. The 2^1-*O*- and 3^1-*O*- regioisomers provide a significantly better resolution than native γ-cyclodextrin, while the 6^1-*O*-regioisomer gives only a slightly better enantioseparation than native γ-cyclodextrin. The application of γ-cyclodextrin possessing higher number of carboxymethyl groups led to the best resolution for the majority of the compounds analyzed.

Keywords: Capillary electrophoresis / Chiral separation / Enantioselectivity / Regioisomers / Substituted cyclodextrins
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Additional supporting information may be found in the online version of this article at the publisher's web-site

1 Introduction

Cyclodextrins (CDs) and their derivatives have been used as additives in CE since 1985 [1]. Initially, they were used for the separation of achiral compounds, often geometric isomers, later also for enantioseparations [2]. The main advantage of CDs stems from their facile derivatization resulting in the ability of fine tuning of separation selectivity, excellent solubility in various solvents including water (except for β-CD), high chemical stability, and low UV absorption [3–9]. The capacity of CDs to form chiral complexes with many compounds is firstly related to a large number of chiral centers in CDs and, secondly, the shape of the CD molecule that allows the formation of inclusion complexes with analytes where the CD serves as a pseudostationary phase [10].

There are three most common CDs, specifically, α-, β-, and γ-CD. The molecule of the last and the biggest one, γ-CD, is formed by eight α-1,4-linked D-glucopyranose units containing 40 chiral centers and with 8–10 Å internal diameter of its cavity. Thus, γ-CD can form complexes with guests as big as substituted pyrenes [5]. γ-CD has very good solubility in water and it can be used up to 180 mmol/L concentration in aqueous solutions. Both native and derivatized γ-CDs are used for separation purposes. The most frequent is the application of neutral γ-CD derivatives, namely, 2-hydroxypropylated and methylated γ-CD, and negatively charged γ-CD, usually sulfated and carboxymethylated γ-CD [11–13]. Carboxymethylated γ-CD was used for instance for the enantioseparation of citalopram [14], vincamine, vinpocetine, and vincadifformine [15], primaquine, quinocide, tafenoquine, and mefloquine [16], aromatic tricyclic β-lactams [17], dipeptides and tripeptides [18], and ruthenium(II) complexes [19].

Although randomly substituted CDs are the most frequently used derivatives, because of their facile commercial availability, their application often leads to difficulties related to the limited reproducibility of the obtained chiral resolution for analytes. This is a consequence of the limited batch-to-batch reproducibility in their synthesis [20]. Moreover, we have shown previously that the individual monocarboxymethyl regioisomers of α- and β-CD have different enantioseparation abilities [21, 22].

In this work, we have used all three individual regioisomers of monosubstituted γ-CD, specifically, 2^1-*O*-, 3^1-*O*-, and 6^1-*O*-carboxymethyl-γ-CDs (CMGCD), and a randomly substituted commercial carboxymethylated γ-CD with degree of substitution (DS) of 3–6 (RCMGCD), for chiral CE

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Abbreviations: CD, cyclodextrin; **CMGCD**, carboxymethyl-γ-CD; **DS**, degree of substitution; **RCMGCD**, carboxymethylated γ-CD with average DS ~3–6; **TB**, Tröger's base

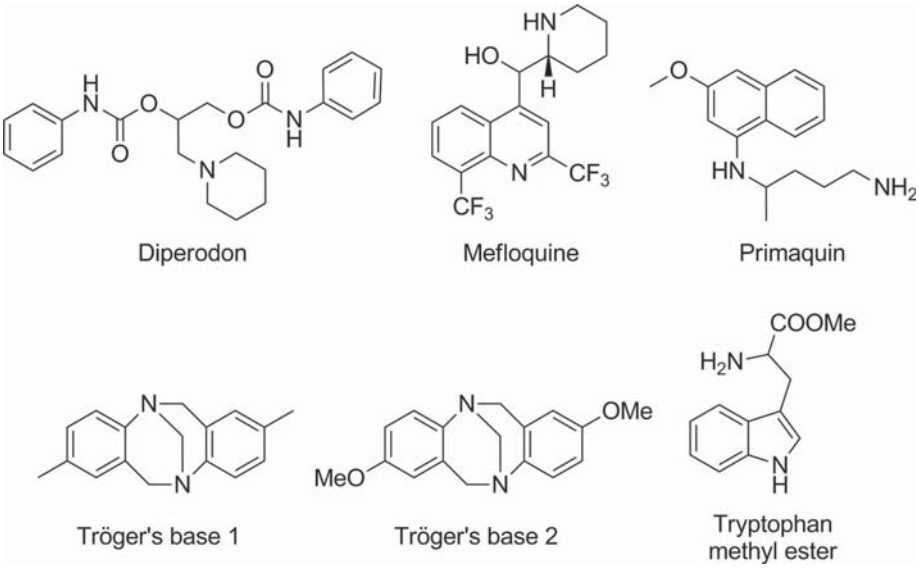

Figure 1. Structures of the investigated biologically significant chiral compounds.

experiments. The goal of the work was to critically evaluate the influence of the substituent position on chiral recognition of the selected biologically important compounds.

Racemic mixtures of several biologically important chiral compounds (Fig. 1) have been used for the investigation of the enantioselectivity changes related to the utilization of individual three regioisomers of CMGCD as additives to the BGE in CE. All the analyzed chiral compounds have an appropriate chromophore allowing for the UV-VIS detection and basic group(s) allowing electrostatic interaction between the analyte and chiral anionic selector. Specifically, diperodon (local anesthetic), Tröger's bases TB1 and TB2 (chiral heterocyclic amines applicable for the recognition of bioactive compounds), mefloquine and primaquine (antimalarial drugs), and tryptophan methyl ester (amino acid derivative) have been chosen for the CE experiments.

Some of these analytes, namely, primaquine [16, 22], TB1 [22] and TB2 [22] have been separated previously utilizing a mixture of carboxymethylated α-CDs, and TB1 employing a mixture of highly sulfated α-CDs [23]. Racemic mixtures of diperodon [24], mefloquine [16, 21, 25], primaquine [16], TB1 [21, 26–28], TB2 [21, 26, 27], and tryptophan methyl ester [21] have been successfully separated by native β-CD and carboxymethylated β-CDs. Tryptophan methyl ester enantiomers have also been resolved by heptakis(2,6-di-*O*-methyl)-β-CD [29] in CE mode. Native γ-CD has been previously used only for the enantioseparation of TB1 and TB2 [26] and carboxymethylated γ-CD only for the chiral separation of diperodon [24].

However, here, for the first time, we have utilized our synthesized well-defined CMGCD in chiral CE and studied the influence of the substituent position on enantioseparation.

2 Materials and methods

2.1 Chemicals and materials

Hydrochloric acid (30%; Suprapur, Merck, Germany), 1 mol/L sodium hydroxide (Tripur, Merck), acetonitrile (99.8%, LiChrosolv, Merck), orthophosphoric acid (50%), 2,8-dimethyl-6*H*,12*H*-5,11-methanodibenzo[b,f][1,5]diazocine (TB1, 99.5%), diperodon hydrochloride (98%), tryptophan methyl ester hydrochloride (98%), mefloquine hydrochloride (98%), primaquine (98%), DMSO (99.7%), γ-CD (97%), carboxymethylated γ-CD sodium salt (RCMGCD, average DS ~3–6) (all Sigma–Aldrich, Czech Republic), and ultrapure water (Milli-Q grade, Millipore, France) were used. 2,8-Dimethoxy-6*H*,12*H*-5,11-methanodibenzo[b,f][1,5]diazocine (TB2) was prepared by the group of Professor Kral. 2^1-*O*-, 3^1-*O*-, and 6^1-*O*-CMGCDs (2^1-*O*-CMGCD, 3^1-*O*-CMGCD, and 6^1-*O*-CMGCD) were synthesized by Zemplen deacetylation of previously described per-*O*-acetyl-2^1-*O*-, 3^1-*O*-, and 6^1-*O*-CMGCD [30], for more details see Supporting Information. The purity of CMGCDs was determined by NMR spectroscopy and for all derivatives was >95%.

Table 1. Migration time[a] (t_m), resolution[b] (R), and number of theoretical plate[c] (N) measured for racemic mixtures of the investigated compounds using native γ-CD, 2^I-*O*-CMGCD, 3^I-*O*-CMGCD, 6^I-*O*-CMGCD, and RCMGCD at pH 2.5

Analyte		Selector				
		γ-CD	2^I-*O*-CMGCD	3^I-*O*-CMGCD	6^I-*O*-CMGCD	RCMGCD
Diperodon	t_{m1} (min)	13.69	12.25	11.97	11.78	52.82
	t_{m2} (min)	13.78	12.43	11.97	11.89	53.97
	R	<0.5	<0.5	0	<0.5	1.1
	N_1 (mm^{-1})	d)	d)	d)	d)	104
	N_2 (mm^{-1})	d)	d)	d)	d)	92
Mefloquin	t_{m1} (min)	9.83	11.13	10.97	10.03	11.08
	t_{m2} (min)	9.83	11.23	10.97	10.03	11.29
	R	0	<0.5	0	0	1.1
	N_1 (mm^{-1})	d)	d)	d)	d)	164
	N_2 (mm^{-1})	d)	d)	d)	d)	118
Primaquine	t_{m1} (min)	5.57	7.51	7.78	6.82	20.43
	t_{m2} (min)	5.63	7.82	8.13	6.95	23.07
	R	<0.5	1.9	2.2	0.5	10.7
	N_1 (mm^{-1})	d)	75	100	388	330
	N_2 (mm^{-1})	d)	101	88	10	278
TB1	t_{m1} (min)	9.83	12.15	12.54	11.19	35.95
	t_{m2} (min)	10.00	12.53	12.86	11.53	37.11
	R	1.1	2.6	1.9	2.4	2.4
	N_1 (mm^{-1})	202	308	210	261	220
	N_2 (mm^{-1})	134	259	221	219	219
TB2	t_{m1} (min)	9.65	11.49	12.01	10.65	12.03
	t_{m2} (min)	9.77	11.75	12.30	10.86	12.17
	R	0.9	2.0	2.0	1.5	1.2
	N_1 (mm^{-1})	346	360	301	309	395
	N_2 (mm^{-1})	157	288	250	195	404
Trp-Me	t_{m1} (min)	5.45	8.99	8.96	8.26	9.39
	t_{m2} (min)	5.45	9.06	9.01	8.26	9.50
	R	0	<0.5	<0.5	0	0.9
	N_1 (mm^{-1})	d)	d)	d)	d)	328
	N_2 (mm^{-1})	d)	d)	d)	d)	188

a) t_{m1} corresponds to the first migrating peak, t_{m2} corresponds to the second migrating peak.
b) Resolution was calculated as $R = (t_{m2} - t_{m1}) / (0.85 * (W_1 + W_2))$, where W_1 and W_2 are peak widths at their half heights for the first and second migrating peak.
c) N_1 corresponds to the first migrating peak, N_2 corresponds to the second migrating peak; number of theoretical plates was calculated as $N = 5.54 * (t_m/W)^2$.
d) Due to insufficient resolution of the peaks, the calculation of theoretical plates was impossible.

2.2 Equipment

CE separations were performed with an Agilent CE instrument (Agilent 3D HPCE, Germany) equipped with UV-Vis diode-array detector. Bare fused-silica capillaries of 375/75 μm od/id and 58.5/50 cm total/effective length obtained from Polymicro Technologies (AZ, USA) were used.

2.3 CE conditions

Tryptophan methyl ester, diperodon, and primaquine were dissolved in water, mefloquine was dissolved in acetonitrile, TBs were dissolved in dimethyl sulfoxide, each at 10 mmol/L concentration. For the CE experiments, the analyte solutions were further diluted with water to the final concentration 1 mmol/L with the exception of TB2 that was diluted to the final concentration 0.2 mmol/L. The background electrolyte was composed of 20 mmol/L sodium phosphate buffer, pH 2.5 (20 mmol/L orthophosphoric acid adjusted to appropriate pH with 1 mol/L NaOH), and 10 mmol/L γ-CD, RCMGCD or the individual monosubstituted CMGCDs (2^I-*O*-CMGCD, 3^I-*O*-CMGCD, and 6^I-*O*-CMGCD). A new fused-silica capillary was first rinsed with 0.1 mol/L NaOH for 30 min, then with H_2O for 30 min. Between the runs, the capillary was rinsed at 99.4 kPa for 2 min with 0.1 mol/L NaOH, for another 2 min with H_2O, and finally 2 min with running buffer from a vial different from the one used for a subsequent analysis. The analytes were injected hydrodynamically by a pressure of 1.5 kPa for 5 s. All the separations were performed at 20 kV

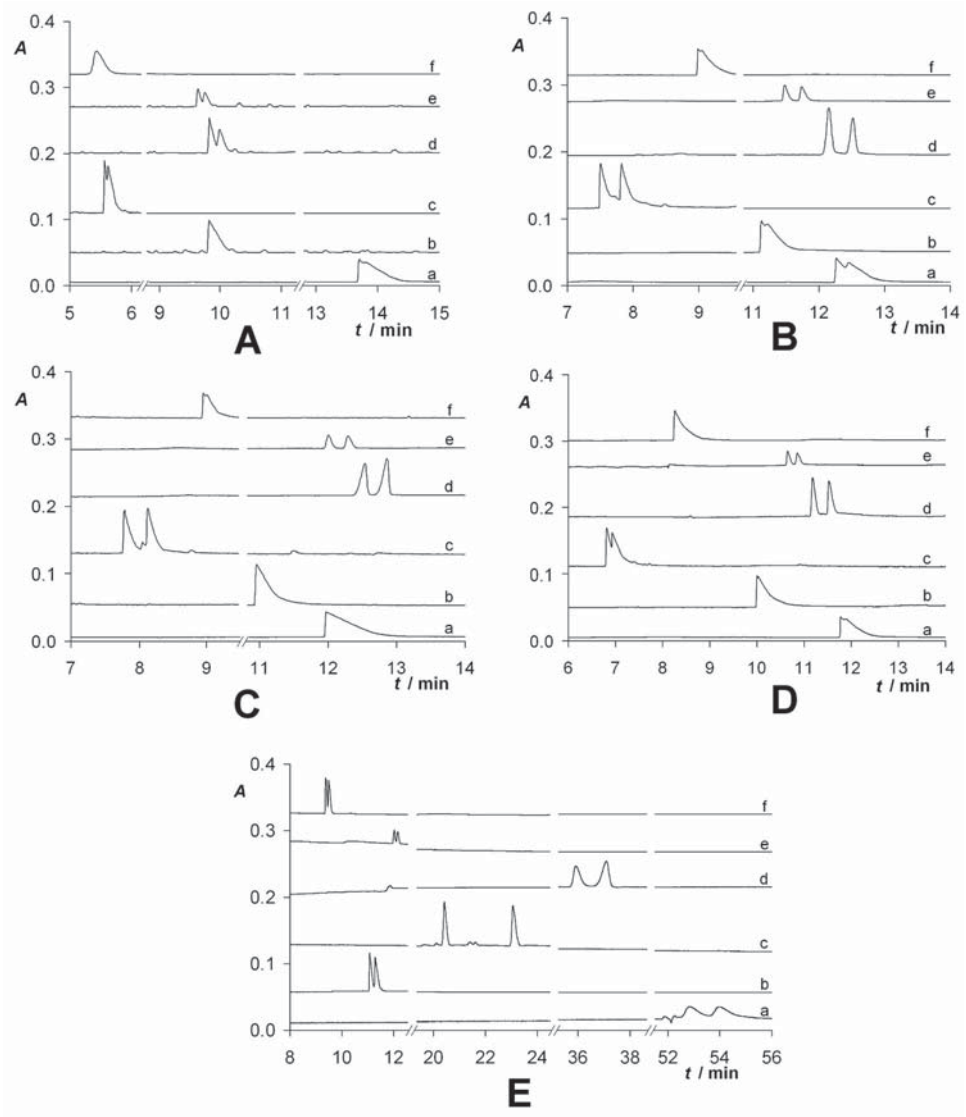

Figure 2. Electropherograms of the analyzed racemic mixtures of the studied compounds; Dependences of absorbance at 207 nm on electromigration time. Separated analytes: (a) diperodon, (b) mefloquine, (c) primaquine, (d) TB1, (e) TB2, (f) tryptophan methyl ester; used chiral selectors: (A) native γ-CD, (B) – 2^{I}-*O*-CMGCD, (C) 3^{I}-*O*-CMGCD, (D) 6^{I}-*O*-CMGCD, (E) RCMGCD; BGE: 20 mmol/L sodium phosphate; pH 2.5, 10 mmol/L chiral selector; fused-silica capillary: od/id = 375/75 μm; total/effective length 58.5/50.0 cm; voltage 20 kV; temperature 25°C.

(anode at the injection capillary end) with a voltage ramp time of 12 s. Detection was carried out at 207 nm and the capillary was thermostatted at 25°C during the analyses.

3 Results and discussion

In the present work, all the enantioseparations of the investigated compounds were carried out at pH 2.5. These conditions were intentionally chosen as at such pH we obtained the best enantioseparation for similar analytes using monosubstituted carboxymethyl-α- and β-CDs in our previous studies [21, 22]. At pH 2.5, the analytes are positively charged, and CMGCDs are only partially dissociated. It looks like that electrostatic interaction does not play a significant role in the enantiorecognition. This seems to be a valid conclusion as it has been also shown previously that at higher pH, when carboxymethylated CDs are dissociated completely, no separation of enantiomers has been obtained [22].

As can be seen in Table 1 and Fig. 2, the application of 2^{I}-*O*-CMGCD as the additive to the BGE allowed for at least partial enantioseparation of all six investigated compounds. This γ-CD derivative provided three baseline separations ($R \geq 1.5$), both TBs' and primaquine, and partial separations ($R < 1.5$) for the three other analytes. Three baseline separations and one partial separation were achieved with 3^{I}-*O*-CMGCD and in comparison to 2^{I}-*O*-CMGCD similar resolutions were found for TBs and primaquine. The utilization of 6^{I}-*O*-CMGCD as a chiral selector led to the baseline enantioseparation for two racemates only, TBs, and a slight indication of the separation for diperodon and primaquine was achieved. Two other analytes showed no enantioseparation. Comparing the resolutions reached for TBs with the assistance of 2^{I}-*O*-CMGCD, it can be clearly seen that the enantioseparation improved with respect to the application of the native γ-CD as the chiral BGE additive.

Although in our previous studies 6^{I}-*O*-carboxymethyl-α- and β-CD derivatives provided similar resolution to corresponding native CDs [21, 22], this time the resolution for the TB 1, 2, and primaquine in the presence of 6^{I}-*O*-CMGCD was slightly higher than for the native γ-CD itself. This effect might be related to the possible partial penetration of the analytes into the large γ-CD cavity from both sides accompanied with the formation of specific interactions. Thus, it seems that in the case of γ-CD the substitution at position 6 has more significant influence on the resulting enantioselectivity than in α- and β-CD with smaller cavities where the enantioselective interactions occurred mainly at the wider rim of CDs bearing secondary hydroxyl groups at C2 and C3.

The commercial RCMGCD provided better resolutions for diperodon, mefloquin, primaquine, and tryptophan methyl ester than the single-isomer CMGCDs probably due to its higher DS (3–6) or some other reasons [31]. On the other hand, for TB1 and 2 the level of the enantiomer separation was comparable. Moreover, as already mentioned in the Introduction, the significant drawback of the application of the commercial RCMGCD consists a limited reproducibility of its synthesis leading to a significant variability of the composition of the resulting product, which may result in fluctuation of migration times and resolution values obtained for the chiral analytes with different batches of RCMGCD [20].

4 Concluding remarks

In this work, six biologically important chiral compounds were used for the study of the influence of the position of carboxymethyl group in mono-CMGCD on the enantioselectivity of the mentioned compounds. The analyses were carried out in 20 mmol/L phosphate buffer at pH 2.5 with the addition of the specific CD at 10 mmol/L concentration. The results clearly demonstrate a significant influence of the position of the carboxymethyl group in the CD molecule on the enantioseparation. The addition of 2^{I}-*O*-CMGCD to the BGE resulted in at least partial enantioseparation of all six investigated compounds. Although the separation abilities of 3^{I}-*O*-CMGCD and 6^{I}-*O*-CMGCD were slightly worse (only four compounds were at least partially enantioseparated) still these two modified CDs provided better enantioselectivity than the native γ-CD.

Together with our previous studies [21, 22], this work documents a significant influence of both the substituent location of the carboxymethyl group in the monosubstituted CD as well as the size of the CD cavity on chiral separation of biologically important compounds. Although the analyzed compounds were partially different within these studies to respect the difference in the CD cavity size, the influence of both the mentioned attributes can be demonstrated in the example of TB1. Considering exclusively the native CDs the best resolution ($R = 1.9$) was achieved for β-CD. However, taking into account all of the studied CDs, the best resolution ($R = 3.6$) was obtained with 3^{I}-*O*-carboxymethyl-α-CD. The substitution in position 6 of the CD has a negligible effect on enantioseparation with α-CD and β-CD derivatives but for 6^{I}-*O*-carboxymethyl-γ-CD a slightly higher resolution than for native γ-CD was achieved probably due to the fact that the analytes can interact and partially penetrate both the sides of the large γ-CD cavity.

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The authors have declared no conflict of interest.

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Molecular Recognition of Phenylalanine Enantiomers onto a Solid Surface Modified with Electropolymerized Pyrrole-β-Cyclodextrin Conjugate

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Abstract: We report the electrochemical deposition of a β-cyclodextrin pyrrole conjugate (Py-β-CD) on an electrode surface including i) characterization based on surface-enhanced Raman scattering and field-emission scanning electron microscopy; ii) studies of the molecular recognition of enantiomers of phenylalanine methyl ester hydrochlorides (Phe) based on linear sweep voltammetry and a quartz crystal microbalance. The PPy-β-CD polymeric layer on a metallic substrate is distinguished by its inhomogeneity, in which both highly ordered β-CD units and highly disordered polymer chains are observed. The voltammetric recognition results showed that PPy-β-CD exhibited a higher sensitivity for D-Phe $(138\pm15)\times10^3$ than for L-Phe $(6\pm1)\times10^3$ within the concentration range 0.1–0.75 mM (n = 3) despite the differences in the polymer arrangement on the surface. A possible mechanism of molecular recognition of phenylalanine enantiomers is discussed.

Keywords: β-cyclodextrin pyrrole conjugate · electrochemical polymerization · enantiomer recognition · voltammetry · quartz crystal microbalance

1 Introduction

Cyclodextrins (CDs) are able to form inclusion complexes with a number of organic or inorganic analytes, whose stability depends on non-covalent interactions [1] as well as the steric properties of the CD cavity [2]. The complexing properties of CDs are interesting with respect to the development of sensors [3–4]. In order to transfer the recognizing process onto the sensor surface, various approaches for anchoring CD molecules have been proposed and used. β-CDs can be deposited on an electrode surface by polycondensation with dialdehyde [5–7], covalent linking to hyperbranched poly(acrylic acid) films capped with a chemically grafted, ultrathin polyamine layer [8] or nucleophilic attack of the highly oxidized conducting poly(*N*-acetylaniline) polymer by the hydroxyl group of β-CD [9]. Electrochemical polymerization is another surface modification option. Zhang et al. [10] modified a carbon paste electrode with β-CD mixed with L-Arg using electrochemical polymerization. A number of authors described the attachment of β-CD onto the electrode surface during the electrochemical polymerization of pyrrole or aniline by doping them with substituted CDs as counter-anions (sulfonated CD) [11–13]. There were attempts to prepare the composite films based on functionalized poly(pyrrole/β-cyclodextrin) using electropolymerization of a 20:1 mixture of β-CD and the pyrrole monomer [14]. An alternative way to attach β-CD onto the electrode surface can be based on the electrochemical polymerization of β-CD derivatives bearing polymerizable units. The polymerizable unit could be a suitable pyrrole which can guarantee the stability of the polymer in both air and water [15]. In this case, it is interesting to look into the contribution of nonspecific and specific interactions during the process of molecular recognition occurring at the solid-liquid interface.

Here we examine the electrochemical deposition, characterization and recognition properties of a solid surface modified with a poly(pyrrole-β-cyclodextrin conjugate) (PPy-β-CD) towards phenylalanine methyl ester hydrochlorides (Phe).

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2 Materials and Methods

2.1 Materials

The following chemicals were used for the experiments: a pyrrole-β-cyclodextrin conjugate (Py-β-CD, Figure 1) was synthesized as described previously in [16], pyrrole (Py, Aldrich, USA), lithium perchlorate (Aldrich, USA), potassium hexacyanoferrate(II) and hexacyanoferrate (III) (Lachema, Czech Republic), potassium chloride (Eutech, Netherlands), L-phenylalanine methyl ester hydrochloride (L-Phe, Fluka, Switzerland) and D-phenylalanine methyl ester hydrochloride (D-Phe, Fluka, Switzerland). Prior to use, pyrrole was distilled repeatedly under vacuum until a colourless liquid was obtained. The distilled monomer was stored in the absence of light. The aqueous solutions were prepared with doubly distilled and demineralised water for electrochemical and spectroscopic measurements, respectively.

2.2 Electrochemical Modification of Electrode Surface

Electrochemical modifications of electrode surfaces were performed using cyclic voltammetry (CV) with a Palmsens 3 (PalmSens BV, Netherlands) in a three-electrode system. The working electrodes used were a glassy carbon electrode (GCE) (Electrochemical detectors, Czech Republic), gilded platinum plate (Pt/Au) and chrome/gold (Cr/Au) coated quartz crystals (Stanford Research Systems) for voltammetric, spectroscopic and quartz crystal microbalance (QCM) measurements, respectively. Ag/AgCl (3 M KCl) and a Pt foil were served as the reference and the counter electrodes, respectively.

Electrochemical deposition of Py-β-CD was carried out onto the surfaces of each working electrode from the supporting electrolyte with 1.85 mM Py-β-CD and 0.1 M $LiClO_4$: the potential window was from 0.0 V up to 1.8 V, with a scan rate of 25 mV/s, 14 cycles. For comparison purposes, we conducted the electrochemical deposition of a native pyrrole on the surfaces of GCE from the supporting electrolyte with 0.14 mM Py and 0.1 M $LiClO_4$. Before each modification, the surface of GCE was primarily cleaned mechanically using filter paper and then treated electrochemically by cycling the potential from −0.3 to 1.8 V in 0.5 M H_2SO_4 and rinsed with doubly distilled water.

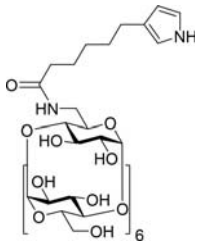


Fig. 1. Structure of a pyrrole-β-cyclodextrin conjugate used in this study.

2.3 Characterization of the Electrochemically Modified Electrode Surface

Surface-enhanced Raman scattering (SERS) measurements were carried out on a gilded platinum (Pt/Au) plate prepared according to [17]. Surface-enhanced Raman spectra were collected in an FT-Raman spectrometer (FT-NIR spectrometer EQUINOX 55, Raman module FRA 106/S, Nd:YAG laser (excitation line 1064 nm, laser power ca. 300 mW) with a Ge diode detector cooled with liquid nitrogen (Bruker Optics)). A standard 4 cm^{-1} spectral resolution was used for all data accumulation. 2048 scans were co-added to obtain spectra of a reasonable S/N ratio. The FT-Raman spectrometer was equipped with a thin-layer chromatography (TLC) mapping stage (Bruker Optics), 8 points were selected to perform a simple spectral mapping. The distance between points was set to 500 μm, because the diameter of the laser beam was ca. 300 μm. Collected spectra of modified Pt/Au-plate were processed in the software package OMNIC 9 (Thermo Scientific, USA).

The morphology studies were carried out by field-emission scanning electron microscopy (FE-SEM Mira, Tescan Orsay Holding, a.s.) using an in-beam secondary electron detector and an accelerating voltage of 10 kV on the Cr/Au surface. Before each measurement, the tested Au surfaces were rinsed in demineralized water and dried at room temperature. Imaging of the bare and PPy-β-CD-modified electrode (Cr/Au) surface was additionally performed by means of AFM and C-AFM Bruker Dimension Icon microscope. The AFM scanning was done using two types of tips namely, Bruker scan asyst air (tip diameter 2 nm, spring constant 0.4 N/m) and Bruker scm-tip conductive tips covered by platinum/iridium (diameter 20 nm, spring constant 2.8 N/m).

2.4 Phenylalanine Methyl Ester Enantiomers Binding Studies

The linear sweep voltammetric (LSV) measurements were performed at bare, PPy- and PPy-β-CD-modified GCE (0.28 cm^2) with the supporting electrolyte (5.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) in 0.1 M $LiClO_4$) in the absence and presence of the amino acid methyl ester hydrochloride in question using a Palmsens 3 device over the potential range of −0.5 to +1.4 V at a scan rate of 100 mV/s. Before each LSV measurement, the supporting electrolyte was purged with N_2. To estimate the sensitivity of the experimental electrodes and at the same time eliminate electrode-to-electrode variation in the background signal, we used the following equation:

$$Response = [(I - I_0)/(I_0)] \times 100\%,$$

where I_0 and I were the current response of the tested GCEs recorded in the supporting electrolyte before and after adding the different concentrations of amino acid methyl ester hydrochlorides.

The QCM experiments were carried out at bare and PPy-β-CD-modified quartz crystals (AT-cut, 1-inch diameter, base frequency 5 MHz, Cr/Au electrodes, polished surface) using a QCM200 equipped with a Kylar QCM head (SRS Stanford Research Systems, USA). Before application, the quartz crystals were purified with piranha solution and rinsed with demineralised water. Before starting the QCM measurements, the solutions were degassed and stabilised to the temperature of the QCM200 probe head. The frequency was recorded with a resolution of 0.1 Hz. Mass changes were computed using the Sauerbrey equation.

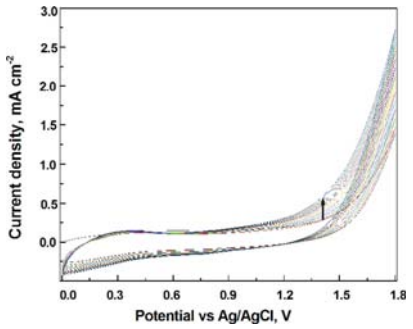


Fig. 2. Cyclic voltammograms of electrochemical polymerization of pyrrole-β-cyclodextrin conjugate in 0.1 M $LiClO_4$ aqueous supporting electrolyte onto the surface of glassy carbon electrode.

3 Results and Discussion

3.1 Electrochemical Modification of Electrode Surface

Electrochemical polymerizations of Py-β-CD were performed onto the surface of the GCE using cyclic voltammetry (Figure 2). The polymerization process of Py-β-CD proceeded with difficulty. Recently, the steric and electronic influence of various substituents on the electropolymerization of five-membered heterocycles were extensively discussed by Lemaire et al. [18] and Waltman [19]. The oxidation process of Py-β-CD was probably significantly affected by the steric hindrance of the bulky β-CD cavity. The bulky substituents attached onto polymerizable units (β-cyclodextrin pyrrole conjugate) or present in the polymerization system are able to reduce the rate of polymer formation. Taking into account this fact, the potential was swept between 0.0 V up to 1.8 V at a scan rate of 25 mV/s [20–21]. The increase in the currents occurring at 1.5 V after 7 cycles during the electrochemical oxidation of PPy-β-CD implies that the polymer was deposited onto the GCE surface. The electrosynthesis was stopped after 14 cycles when the current changes were not observed. An increasing current at 1.5 V was recently observed by Arjomandi et al. during the electrochemical oxidation of pyrrole and 2,6-dimethyl-β-cyclodextrin complex and was taken as confirmation of the formation of the polymeric layer onto the GCE surface [22].

3.2 Characterization of the Electrochemically Modified Electrode Surface

Surface-enhanced Raman scattering (SERS) allows the observation of structural details of films with very small thicknesses deposited on the enhancing substrates. To confirm the formation of the polymer derived from Py-β-CD, the SERS spectra of the monomer (Figure 3A) and

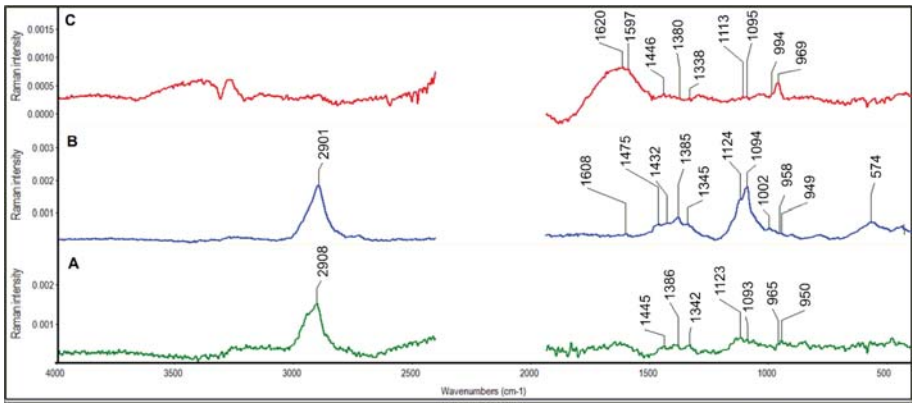


Fig. 3. Raman spectrum of pyrrole-β-cyclodextrin conjugate monomer (A), polymer spectrum type I (B) and type II (C).

the polymer (Figure 3B, C) prepared by electrochemical polymerization onto a Pt/Au-plate were compared. Because of possible inhomogeneity of the polymeric layer on the metallic substrate, simple spectral mapping (8 points) was used. The obtained spectra can be divided into two groups exhibiting distinct spectral patterns. The first group, designated as spectrum type I, (Figure 3B) shows a similar band in the range of the C–H stretching vibration to the spectrum of the monomer (Figure 3A) while the second group, designated as spectrum type II (Figure 3C), differs very significantly. Interpretation of the bands in all collected Raman spectra was performed empirically based on their assignment to the characteristic bands of functional groups/skeletons using literature data [23–24]. We should note that the spectra of both groups informed us about the very distinct arrangement of the polymeric layer derived from PPy-β-CD onto the metallic substrate. The polymer spectrum type I exhibited a relatively narrow band characteristic of β-CD at 2901 cm^{-1} assigned to the CH groups of β-CD (Figure 3B). The clearly distinguishable bands at 1124, 1094, 1475, 1432 and 1385 cm^{-1} were assigned to the several ν(C–O) and δ(CH_2) modes, respectively. Hence, the spectra confirmed the presence of β-CD. On the other hand, the band appearing at ca. 1608 cm^{-1} was assigned to the plane vibrations of the pyrrole rings. The out-of-plane modes of the pyrrole ring were observed as weak bands at ca. 958 cm^{-1} and 949 cm^{-1}, which can be attributed to almost parallel orientation of pyrrole ring with the metallic surface [25–26].

The polymer spectrum type II differed substantially from both the spectrum of the monomer and the polymer spectrum type I. In the spectrum type II (Figure 3C), the broad bands characteristic of in-plane vibrations of the pyrrole ring, namely, 1597 cm^{-1} and 1620 cm^{-1}, were slightly shifted and relatively intensified compared to the monomer (Figure 3A). Further pyrrole ring bands (e.g. at 1380 cm^{-1} of pyrrole C–N group and at 969 cm^{-1} attributed to out-of-plane vibration) were more intense than the bands characteristic of β-CDs indicating the close vicinity of pyrrole rings to the metal surface. The evident band shifts and changes of band shapes for pyrrole vibrational modes compared both to monomer and polymer type I are explained by an interaction of adsorbed pyrrole units with Au surface in the case of polymer type II.

Simultaneously, these spectral features indicate some disordering of the polymer chain system deposited on the Pt/Au metallic substrate.

We can summarize that the layer is composed of two different forms, the polymer arrangement of type I exhibits i) a higher degree of ordering and interaction of β-CD units with Pt/Au in the deposited polymeric film and ii) relatively less significant contribution of pyrrole units compared to the arrangement of type II, which exhibit i) a low degree of order of polymer chains and ii) a significant interaction of pyrrole rings with the Pt/Au metallic substrate (see above).

The morphology of the Au surface before (Figure 4A) and after the deposition of PPy-β-CD (Figure 4B) was characterized by SEM. Due to the very low roughness of the Au surface and the proper grounding, it was possible to suppress charging effects and take micrographs of the bare and PPy-β-CD-modified Cr/Au surface without any additional deposition of the conducting film. As shown in Figure 4A, the Cr/Au surface is a very dense flat structure with visible surface texture with dimensions of ~30 nm. After the deposition of PPy-β-CD (Figure 4B), cone/ball-shaped structures with diameters from 50 to 200 nm were observed on the surface. These structures are homogeneously distributed across the whole surface and are distant from each other ~400 nm (Figure 4C). The AFM imaging results showed the same morphology as SEM. The structures of PPy-β-CD on the gold surface differ in adhesion, conductivity, and surface potential (see Supporting Information, Figures 1s, 2s, 3s).

In summary, the spectroscopic results independently confirm that the PPy-β-CD polymeric layer was present as the cone/ball-shaped structures uniformly arranged over the entire surface.

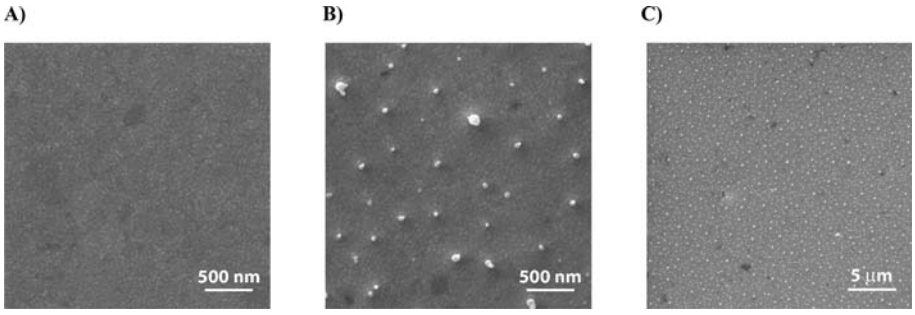


Fig. 4. SEM micrographs of gold surface before (A; magnification 100 k×) and after electrochemical deposition of pyrrole-β-cyclodextrin conjugate (B and C with 100 k× and 10 k× magnification, respectively).

3.3 Molecular Recognition

In order to assess the recognizing properties of PPy-β-CD deposited onto a solid surface towards enantiomers of Phe, LSV and QCM experiments were additionally carried out. According to the obtained spectroscopic results, the recognizing process could be affected by both the β-CD units arranged on the solid electrode surface and the areas uncoated by the polymeric layer. From our perspective, combining LSV and QCM techniques should provide insight into the possible mechanism of binding.

Figure 5 depicts the LSV responses towards L-Phe (left column) and D-Phe (right column) of a bare GCE before and after the electrodeposition of native (PPy) and substituted (PPy-β-CD) pyrrole. Here it is important to note that voltammetric discrimination can be based both on the change in peak current and the difference between peak potentials observed in the solutions of enantiomers [27–29].

For the bare GCE, the peak current intensity and the difference between the peak potentials of the redox marker were insignificant (Figure 5A–B). For the PPy-modified GCE, it was difficult to compare the intensity of the broad and not easily distinguishable peak, whose intensity visibly lost height with every addition of L- and D-Phe (Figure 5C–D). For the PPy-β-CD-modified GCE (Figure 5E–F), we observed both improvements in the peak form and concentration dependences towards Phe enantiomers compared to the PPy-modified GCE. The PPy-β-CD-modified GCE fulfilled our expectations in terms of the changes in current signal and shift in peak potentials for the redox marker.

As shown in Table 1, the PPy and PPy-β-CD-modified GCE responded in different ways to the changes in concentration of the added amino acid methyl ester. Differences were first of all observed between the signs and values of sensitivity towards Phe enantiomers for the tested GCEs. Firstly, the sensitivity for PPy-β-CD-modified GCE was positive, while the sensitivity for the PPy-modified GCE was negative. Secondly, while the sensitivity for L- and D-Phe was the same for the PPy-modified GCE, a higher sensitivity toward D-Phe than for L-Phe was obtained with the PPy-β-CD-modified GCE. With the PPy-modified GCE, the observed signal results from unspecific sorption that can not guarantee the discrimination of the amino acid methyl esters in question.

The effects observed on a PPy-β-CD-modified GCE can be considered to be confirmation of the presence and functionality of selective β-CD units attached by electrochemical oxidation of pyrrole onto the electrode surface. Moreover, our statement can be supported by the results of Gao et al. who investigated L- and D-Phe recognition using β-CD deposited onto a sp^3-to-sp^2 converted regenerative graphene/diamond (G/D) electrode [30]. However, it is difficult to agree with the statements in [30], that the electrochemical signal is the result of the oxidation of Phe enantiomers (E_{ox}(L-Phe) = 576 mV, E_{ox}(D-Phe) = 550 mV). Unfortunately, the authors did not discuss the mechanism of binding of the Phe enantiomers by β-CD onto the G/D surface and the group responsible for the observable electrochemical signal. Our attempts to obtain an electrochemical signal without a redox marker were unsuccessful. Therefore, we proposed that the redox marker is playing an important role in the formation of the signal as a result of the recognition process that occurred at the PPy-β-CD-modified GCE.

Further, this was followed by a series of QCM experiments to provide quantitative information on the amount of adsorbed enantiomers of phenylalanine methyl ester hydrochlorides on the bare and PPy-β-CD-modified surface. When comparing the adsorbed mass (Table 2), L-Phe has a higher affinity towards the electrode area, where it is able to combine both nonspecific (present at the uncoated area) and specific interactions (present at PPy-β-CD-modified area). Otherwise, L-Phe acts as a surface insulator and electron transfer inhibitor.

3.4 Mechanism of Recognition

Spectroscopic and electrochemical studies showed that a surface modified with PPy-β-CD has both uncoated and coated areas. We propose that highly ordered β-CD units distributed near the surface form inclusion complexes with enantiomers, while uncoated areas of the surface support electron exchange of the redox marker (Scheme 1). Recently, special attention was paid to the $[Fe(CN)_6]^{4-}/[Fe(CN)_6]^{3-}$ non-ideal heterogeneous charge transfer mechanism [31–32]. This has led to the proposal of the existence of an activated complex formed between the cation and the complex anion which is already paired to at least one other cation. As can be observed, the increasing concentration of D-Phe led to an increase in the electrochemical signal. This phenomenon should occur if a negatively charged redox marker compensates the

Table 1. Comparison of sensitivity obtained with bare and modified glassy carbon electrodes in supporting electrolyte (5.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1 : 1) + 0.1 M $LiClO_4$) plus the different concentrations of the amino acid methyl ester hydrochloride in question.

Analyte	GCE electrode sensitivity ($\times 10^3$) (n = 3)		
	Bare	PPy-modified	PPy-β-CD-modified
L-Phe	11	−22	6 ± 1
D-Phe	18	−28	138 ± 15

Table 2. Sorption of enantiomers of phenylalanine methyl ester hydrochlorides onto bare and PPy-β-CD-modified surface studied using quartz crystal microbalance.

Analyte	Bare Mass (ng/cm^2)	PPy-β-CD-modified Mass (ng/cm^2)
L-Phe	350	6406
D-Phe	17	2016

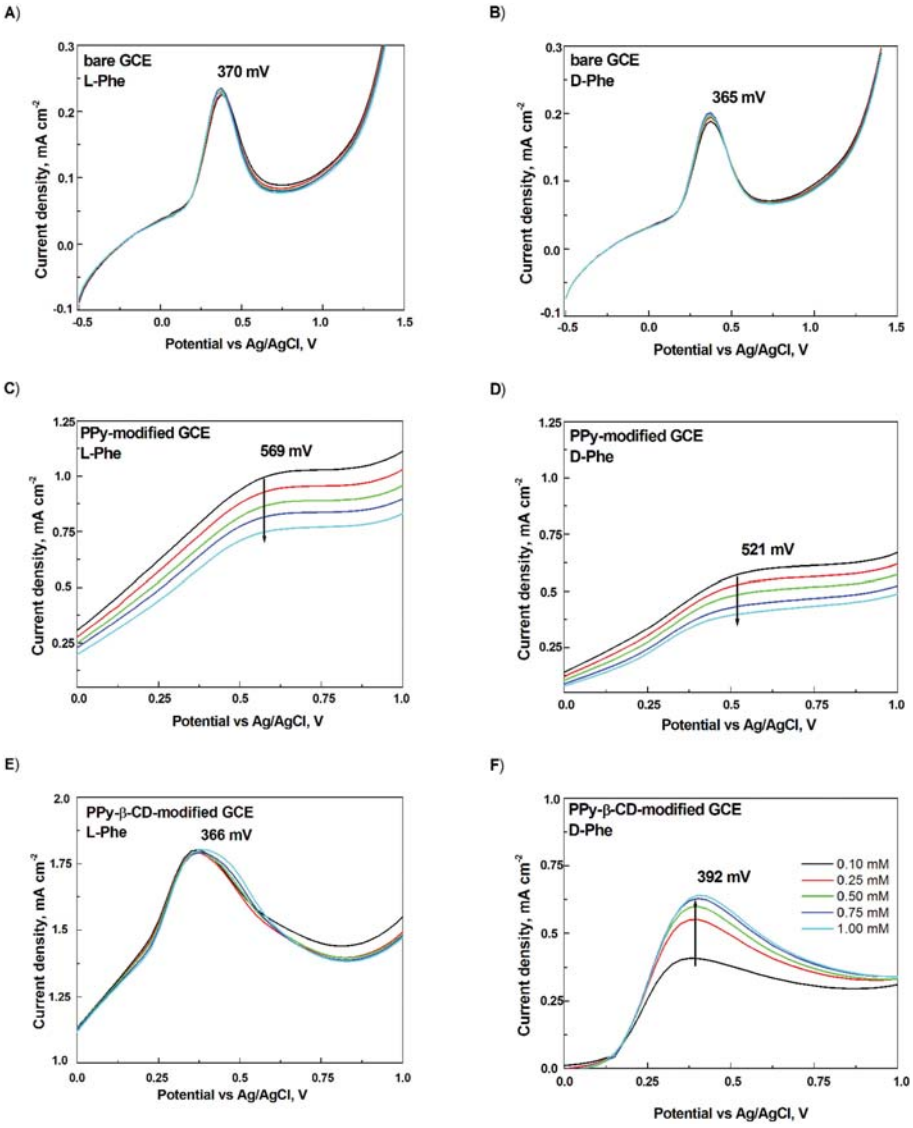
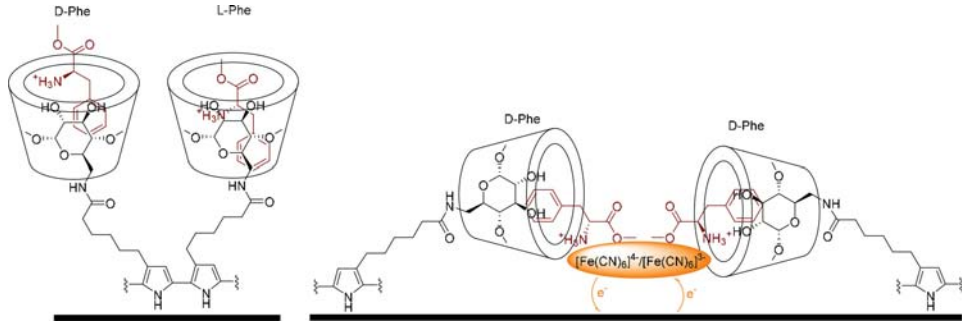


Fig. 5. Linear sweep voltammetry measurement obtained with glassy carbon electrode before modification (A, B) and after modification with unsubstituted pyrrole (PPy; C–D), a poly(pyrrole-β-cyclodextrin conjugate) (PPy-β-CD; E–F) in the supporting electrolyte containing 5.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) changes the concentration of phenylalanine methyl ester enantiomers (L-Phe left side; D-Phe right side).

positive charge located on the amino group of the bound D-Phe methyl ester hydrochloride. The situation in which the electrochemical signal does not change with the increasing concentration of L-Phe is likely if the positively charged $-NH_3^+$ of L-Phe methyl ester hydrochloride is hidden in the inner β-CD cavity [29] and is, therefore,



Scheme 1. Possible mechanism of molecular recognition of methyl ester hydrochloride of phenylalanine onto surface modified with pyrrole-β-cyclodextrin conjugate.

unavailable for the redox marker (Scheme 1). This observation is in agreement with previously published results [33–35].

4 Conclusion

In this work, a polymer layer based on PPy-β-CD was deposited onto solid surfaces using cyclic voltammetry. The results from both the SERS and SEM measurements confirm that the PPy-β-CD polymeric layer is comprised of cone/ball-shaped structures uniformly arranged over the entire surface. Moreover, the PPy-β-CD polymeric layer consists of differently ordered β-CD units and pyrrole rings near the solid surface that are responsible for its ability to react by various means towards enantiomers of phenylalanine methyl ester hydrochlorides. The combination of LSV and QCM measurements provided insight into the mechanism binding enantiomers onto the PPy-β-CD modified solid surface. While L-Phe acts as a surface insulator and electron transfer inhibitor, D-Phe creates a complex with $[Fe(CN)_6]^{4-}/[Fe(CN)_6]^{3-}$ and facilitates electron transfer. We are currently working on improving the electroanalysis by taking advantage of the possibilities of chemometrics.

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