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Research Article

# Synthesis of the chiral selector heptakis (6-*O*-methyl)-β-cyclodextrin by phase-transfer catalysis and hydrazine-mediated transfer-hydrogenation

The exhaustive primary-side alkylation of cyclodextrins has never been achieved directly. The undesired and simultaneous derivatization of the secondary hydroxyl moieties generates intricate isomeric mixtures that are challenging to purify, analyse and characterize. The aim of this study was to develop a chromatography-free and up-scalable strategy towards the preparation of per-6-*O*-methylated cyclodextrin and to test the compound as potential chiral selector. The target molecule was prepared according to a five-step synthesis by using methyltriphenylphosphonium bromide as catalyst under heterogeneous conditions. The removal of benzyl moieties, used as temporary secondary-side protecting groups, was attained by applying hydrazine-carbonate in the presence of Pd/C. All the intermediates were obtained in high yields, thoroughly characterized and their purity was assessed by *ad-hoc* developed HPLC methods. The per-6-*O*-methylated β-cyclodextrin showed promising chiral recognition ability as background electrolyte additive in cyclodextrin-modified capillary electrophoresis using the recreational drug methylene-dioxypyrovalerone as model compound. Additionally, a model for the inclusion geometry between the single isomer host and the selected drug was developed based on the extensive 2D NMR analysis. The versatility of the proposed synthetic strategy opens the way to the industrial production of homogeneously primary-alkylated cyclodextrins and to their wide application in chiral separation of various drugs.

**Keywords:**
Enantioseparation / Methylated-cyclodextrin / NMR / Single isomer
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Additional supporting information may be found online in the Supporting Information section at the end of the article.

## 1 Introduction

Cyclodextrins (CDs) are water-soluble cyclic oligosaccharides presenting a truncated-cone shape limiting a hydrophobic cavity. These sugars have been extensively utilized as excipients in pharmaceutical and food industry [1] and due to their specific 3D arrangement they have been used as analytical tools for enantioseparation [2], proposed as artificial enzyme [3] and applied as catalyst for organic reactions [4]. Particularly, CDs have been exploited as mass transfer additives for decades [5] and their role as inverse phase transfer catalyst has been widely investigated [6]. However, the derivatization of CDs based on phase transfer catalysis (PTC) is a field poorly investigated and only a few works deal with this topic. Szejtli et al. [7] investigated methylation of unmodified CDs with dimethylsulfate under PTC conditions. In their optimization work the influence of solvent, catalyst and base has been explored. In the general reaction procedure, native CDs and the selected base were suspended in a poorly soluble solvent and the methylating agent was added subsequently. According to the Hungarian team, THF was the best performing solvent, KOH powder the optimal base and Aliquat® the most efficient catalyst. To the best of our knowledge, no other study has been reported on the modification of CDs under PTC. However, Ciucanu and Kerek previously reported an in-depth study about permethylation of sugars in dipolar aprotic

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**Abbreviations**: **6-Me-β-CD**, heptakis(6-*O*-methyl)-β-CD; **MDPV**, methylenedioxypyrovalerone; **PTC**, phase transfer catalysis; **TBDMS-Cl**, *tert*-butyldimethylsilyl chloride

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solvents with methyl-iodide and solid base such as NaOH, KOH or *tert*-BuOH/NaOH [8]. Their investigation showed that the combination of methyl iodide, solid alkali-metal hydroxide and dimethylsulfoxide is a rapid and high-yielding method for the complete methylation of carbohydrates.

The selective modification of CDs is an evergreen challenge for synthetic chemist. For example, direct per-6-*O*-alkylation has not been accomplished yet. The primary-side homogeneous substitution of CDs can be only obtained through "the long method" where the primary face is first protected and the secondary side is temporary blocked. The primary face is then deprotected and reacted with alkyl halide and the protecting groups on the secondary side are finally removed. The most common and effective way of protecting the primary face of the CDs is based on *tert*-butyldimethylsilyl chloride (TBDMS-Cl). Two main procedures are available for the exhaustive per-6-silylation of CDs: the approach introduced by Takeo [9] that utilizes DMF-imidazole as environment for the *tert*-butyldimethylsilylation and the method based on pyridine [10]. Regardless of the upgrades introduced by other groups to the two main silylating procedures [11, 12], improvements are needed concerning the simplicity of equipment and scale-up, easiness of work-up and purification.

Additionally, the removal of secondary side protecting groups such as benzyl moieties from CD scaffolds is usually carried out by hydrogenolysis over metals, palladium derivatives most commonly [13, 14]. However, hydrogenolysis has one main drawback, particularly on the large scale that is the danger and inconvenience of handling highly flammable hydrogen gas in high pressure explosion-proof vessel. The strict requirements needed for the equipment make this approach not economically advantageous.

On the contrary, catalytic transfer hydrogenation, using *in-situ* hydrogen production, has emerged as an appealable alternative solution during the last century. Hydrazine-carbonate with its inherent advantages such as stability, convenience of use, cost effectiveness and ease of availability, is a practical source of hydrogen. The combination of hydrazine carbonate with transition metal catalyst such as palladium generates a mild and efficient catalytic transfer hydrogenation system conveniently used in industrial scale. The use of hydrazine-mediated transfer-hydrogenation for the debenzylation of CDs has never been reported before. Originally, this method was introduced by Jicsinszky [15] for the effective reduction of primary-substituted azido CD to amino derivatives.

The synthesis of the single isomer, heptakis(6-*O*-methyl)-β-CD (6-Me-β-CD), has been only described in the pioneering work of Takeo et al. [16] and in the sophisticated work of Uccello-Barretta et al. [17]. The first procedure, based on extensive chromatographic purification, resulted in low yields for each compound, while the use of hazardous reagents in the second approach makes challenging the industrial scale-up.

In this work, we developed and proposed an alternative and efficient synthesis for heptakis(6-*O*-methyl)-β-CD (and in general for 6-*O*-alkylated CDs), amenable of industrial scale-up and completely chromatography-free at each step. The current procedure allowed the 100–500 g scale preparation of each intermediate and resulted in 50 g batch production of the final compound. The single isomer methylated CD has been additionally investigated as chiral selector toward the psychoactive drug methylenedioxypyrovalerone (MDPV).

## 2 Materials and methods

The β-cyclodextrin was the product of Wacker Chemie AG (München, Germany); syntheses solvents such as pyridine (Pyr), tetrahydrofuran (THF) and ethanol (EtOH, 96%) were of reagent grade and were sourced from Molar Chemicals (Halásztelek, Hungary); methyltriphenylphosphonium bromide (98%), benzyl bromide (Bn-Br, 98%), tetrabutylammonium fluoride (TBAF, 98%), palladium on activated charcoal (10%), potassium hydroxide (KOH, 85%), *tert*-butyldimethylsilyl chloride (TBDMSCl, 97%), methyl iodide (ReagentPlus®, 99%), hydrazine carbonate (70% in water, ca. 7.3 M), $D_2O$ (99.9% D atom), DMSO-d6 (99.9 atom % D) and $CDCl_3$ (99.8 atom % D) were sourced from Sigma Aldrich (St. Louis, MO, USA). Silica gel coated aluminum sheets were from Merck. Acetonitrile (HiPerSolv for HPLC-SuperGradient) was the product of VWR International (Radnor, PA, USA). Methanol (MeOH), ethyl acetate (EtOAc), hexane, chloroform ($CHCl_3$), 1-propanol, triethylamine, formic acid, 1,4-dioxane, ethanol (96%), $H_2SO_4$ (96%), ammonia (25%) used for preparation of HPLC and TLC solutions, as well as $H_3PO_4$ (85%), NaOH, $KH_2PO_4$ anhydrous and DCl (≥99% D atom) used for the preparation of CE and NMR buffer solutions were of analytical grade and purchased from Molar Chemicals (Halásztelek, Hungary) . All reagents were used without further purification. Purified water (Millipore-Synergy) was used throughout the capillary electrophoretic study. The chiral test analytes used in this study were obtained from commercial suppliers.

TLC was performed on silica gel coated aluminum sheets DC-Alufolien Keiselgel 60 F254 (Merck, Germany). Plates were developed in a saturated chamber in a EtOAc:EtOH (96%):$H_2O$ = 30:5:4 (intermediate I), Hexane:EtOAc = 9:1 (intermediate II), $CHCl_3$:MeOH = 9:1 (intemediate III), chloroform containing 0.5-1.0% ethanol (intermediate IV) or 1,4-dioxane:ammonia (25%):1-propanol = 10:7:3 (6-Me-β-CD). Visualization of the CD derivatives was achieved under UV light at 254 nm and by dipping the TLC plates in 50% $H_2SO_4$-ethanol solution and subsequent carbonization using a heat gun. Quantitative analysis of TLC plates was performed with the software JustQuantify Free.

Melting points were measured on a Büchi B-21 450 melting point apparatus and are uncorrected. Optical rotations were recorded on a Jasco P-1030 22 polarimeter at room temperature. [α]D values are given in $10^{-1}$ deg/cm/g. MALDI-TOF mass spectra were recorded on a 4800 Plus AB SCIEX spectrometer with 2,5-dihydroxybenzoic acid (DHB) as the matrix. Infrared spectra were recorded as KBr disk on a Perkin Elmer FTIR spectrometer Spectrum RX1. HPLC measurements were performed on an Agilent 1260 Infinity HPLC

 

system equipped with UV detector (Agilent 1200 Series Diode Array Detector, G1315B) coupled to refractive index (RI) detector (Agilent 1260 Infinity Refractive Index Detector + 8 μL flowcell, G1362A) or with UV detector coupled to evaporative light scattering (ELS) detector (Agilent 385 Evaporative Light Scattering Detector, G4261A). These setups were used to determine the purity of all cyclodextrin derivatives.

**HPLC method 1** Purity assessment of intermediate **1** (6-TBDMS-β-CD) was obtained on a Luna C18 250 × 4.6 mm, 5 μm (Phenomenex, Torrance, CA, USA) analytical column with the mobile phase of methanol:ethylacetate (78:22), isocratic elution at a flow rate of 1.8 mL/min and RI detection.

**HPLC method 2** Purity assessment of intermediate **2** (6-TBDMS-2,3-Bn-β-CD) was obtained on a Luna C18 250 × 4.6 mm, 5 μm analytical column with the mobile phase of (methanol:acetonitrile (9:1): ethyl acetate (80:20), isocratic elution at a flow rate of 1.8 mL/min and ELS detection.

**HPLC method 3** Purity assessment of intermediate **3** (2,3-Bn-β-CD) was obtained on a Kinetex C18 100 × 4.6 mm, 2.6 μm (Phenomenex, Torrance, CA, USA) analytical column with a gradient elution of methanol:water at a flow rate of 1 mL/min and DAD detection at 254 nm.

**HPLC method 4** Purity assessment of intermediate **4** (6-Me-2,3-Bn-β-CD) was obtained on a Luna C18 250 × 4.6 mm, 5 μm analytical column with the mobile phase of (methanol:acetonitrile (9:1):ethyl acetate (40:60), isocratic elution at a flow rate of 1.8 mL/min and ELS detection.

**HPLC method 5** Purity assessment of compound **5** (6-Me-β-CD) was obtained on a Kinetex C18 100 × 4.6 mm, 2.6 μm analytical column with a gradient elution of methanol:water at a flow rate of 0.7 mL/min and ELS detection.

CE measurements were carried out on an Agilent 7100 instrument (Agilent Technologies, Waldbronn, Germany), equipped with a DAD and the Chemstation software for data handling. Measurements were performed in untreated fused silica capillaries (33.5 cm total and 25 cm effective length and 50 μm id) purchased from Agilent Technologies. Prior to all runs the capillary was preconditioned by rinsing with 0.1 M NaOH (2 min), water (2 min) and the appropriate BGE (3 min). The temperature of the capillary was set to 20°C. During measurements 20 kV was applied, UV detection was performed at 200 nm. Samples were injected hydrodynamically (40 mbar x 3 s). The running buffer was 20 mM phosphoric acid (85%) adjusted to pH 2.5 with 1 M NaOH. The BGE contained 6-Me-β-CD at 1 mM and 2.5 mM concentrations. Stock solution of MDPV (1 mg/mL) was prepared in methanol and its 50-fold dilution with water was used to prepare working solutions for CE analysis.

NMR experiments were carried out on a 600 MHz Varian DDR NMR spectrometer equipped with a 5 mm inverse-detection gradient (IDPFG) probehead. Standard pulse sequences and processing routines available in VnmrJ 3.2 C/Chempack 5.1 were used for structure identifications. The complete resonance assignments were established from direct $^{1}H$–$^{13}C$, long-range $^{1}H$–$^{13}C$, and scalar spin–spin connectivities derived from 1D $^{1}H$, $^{13}C$, 1D TOCSY, $^{1}H$–$^{1}H$ gCOSY, zTOCSY, $^{1}H$–$^{13}C$ gHSQCAD, $^{1}H$–$^{13}C$ gHMBCAD experiments, respectively. The probe temperature was maintained at 298 K and standard 5 mm NMR tubes were used. The $^{1}H$ chemical shifts were referenced to the applied NMR solvent ($CDCl_3$ ($\delta_{1H\ ref.} = 7.24$ ppm, $\delta_{13C\ ref.} = 77.23$ ppm), DMSO-$d_6$ ($\delta_{1H\ ref.} = 2.50$ ppm, $\delta_{13C\ ref.} = 39.52$ ppm) or $D_2O$ ($\delta_{1H\ ref.} = 4.79$ ppm)).

## 2.1 Synthesis

### 2.1.1 Heptakis(6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (6-TBDMS-β-CD, Intermediate 1)

Dry β-cyclodextrin (312.5 g, 0.27 mol) was solubilized in dry pyridine (5 L) under inert atmosphere and tert-butyldimethylsilyl-chloride (347.5 g, 2.31 mol) was added portionwise. The resulting suspension was stirred at room temperature for 6 h. The reaction was monitored by TLC (EtOAc:EtOH (96%):$H_2O$ = 30:5:4), showing three spots at $R_f = 0.7$, 0.6 and 0.3. Portions of TBDMSCl (41.5 g, 0.27 mol) were added every 6 h until the spot with $R_f = 0.3$ disappeared. The mixture was gradually poured into water (30 L) under vigorous stirring and the obtained white precipitate was filtered (sintered glass filter porosity G3). The solid was washed with water (5 × 3 L) and dried into a vacuum drying box. The crude product (~ 560 g) was solubilized in DMF (1.5 L) acetone (375 mL) mixture, hot filtered and precipitated with water (9.3 L). The white solid was filtered and washed with water (3 × 3 L). The precipitation procedure based on DMF-acetone-water was repeated twice until the spot with $R_f = 0.7$ disappeared. The white solid was dried until constant weight at 50°C into a vacuum drying box (437 g, 0.23 mol, 82%).

The purity of the compound was estimated ≥98.5% based on HPLC method 1.

m.p. 296–300°C (decomp.), lit. values 299–318°C [1-3]; $[\alpha_D{}^{25}] = +113.22°$ ($c$ = 1, $CH_2Cl_2$), lit. values +(105.7-115.0)°C [2-4]; $R_f = 0.6$ (EtOAc:EtOH (96%):$H_2O$ = 30:5:4); IR $\nu/cm^{-1}$ 3392, 2954, 2930, 1253, 1156, 1085, 835, 778.$^{1}H$ NMR (600 MHz, $CDCl_3$, 298 K) δ(ppm) 4.87 (d, $J$ = 3.5 Hz, 7H, H1), 4.02 (t, $J$ = 9.2 Hz, 7H, H3), 3.88 (dd, $J$ = 11.4 Hz, 3.1 Hz, 7H, H6a), 3.69 (d, $J$ = 10.6 Hz, 7H, H6b), 3.62 (dd, $J$ = 9.7, 3.4 Hz, 7H, H2), 3.60 (m, 7H, H5), 3.54 (t, $J$ = 9.3 Hz 7H, H4), 0.85 (s, 63H, H8), 0.02 (s, 21H, H7), 0.01 (s, 21H, H7'); $^{13}C$ NMR (151 MHz, $CDCl_3$, 298 K) δ(ppm) 102.24 (C1), 82.01 (C4), 73.84 (C2), 73.63 (C3), 72.78 (C5), 61.86 (C6), 26.13, (C8), 18.50 (C9), -4.84 (C7'), -4.96 (C7). MALDI-TOF m/z $[M + Na]^+$, found: 1957.799, calculated for $C_{84}H_{168}O_{35}Si_7Na$: 1957.799.

### 2.1.2 Heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-benzyl)-β-cyclodextrin (6-TBDMS-2,3-Bn-β-CD, Intermediate 2)

Heptakis(6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (487.6 g, 0.25 mol) was dissolved in THF (5 L). The solution was

 

cooled-down with an ice-water bath to 10°C and KOH (1.29 kg, 23 mol) was added portionwise under vigorous stirring. The obtained white suspension at first slightly gelified then became more stirrable. Methyltriphenylphosphonium bromide (50.4 g, 0.14 mol) was added to the reaction mixture and the white suspension was stirred for 3 h. Benzyl bromide (460.8 mL, 662.6 g, 3.87 mol) was slowly and carefully added to the heterogeneous mixture (2 h addition), by keeping the temperature always below 25°C. After 1 h stirring the reaction mixture became of a pearly white colour showing a milk-like consistence. The reaction was stirred at room temperature overnight. The proceeding of the reaction was monitored by TLC (Hexane:EtOAc = 9:1), the reaction mixture was cooled-down to 10°C and a second portion of KOH (129.6 g, 2.31 mol) and BnBr (46.1 mL, 66.30 g, 0.39 mol) was added. After 2 h, a third portion of KOH (64.8 g, 1.15 mol) and BnBr (23 mL, 33.1 g, 0.19 mol) was added and the reaction mixture was additionally stirred for 3 h. The heterogeneous mixture was filtered on a sintered glass filter (porosity 4) and the solid was thoroughly washed with THF (3 × 1.8 L). The filtrate was concentrated at rotavapor (~ 180 mL) and poured to MeOH (7.2 L) under vigorous stirring. The resulting yellowish, gel-like material was separated by decantation. The solid was extensively washed with $H_2O$ (5 × 9 L) and with MeOH:$H_2O$ = 1:9 (3 × 3.6 L) and finally dried until constant weight in a vacuum drying box in the presence of $P_2O_5$ and KOH as desiccants (753 g, 0.24 mol, 93%).

The purity of the compound was estimated ≥98.3% based on HPLC method 2.

m.p. 87–90°C; $[\alpha_D{}^{25}] = +42.39°$ ($c$ = 1, $CH_2Cl_2$); $R_f$ = 0.55 (Hexane:EtOAc = 9:1); IR $\nu$/cm$^{-1}$ 2954, 2928, 2856, 1252, 1143, 1094, 1034, 834, 696. $^1$H NMR (600 MHz, $CDCl_3$, 298 K) δ(ppm) 7.19-7.00 (m, 70H, Ph), 5.30 (br d, $J$ = 2.3 Hz, 7H, H1), 5.06 (d, $J$ = 10.6 Hz, 7H, O3C$\underline{H}_2$Ph), 4.69 (d, $J$ = 10.6 Hz, 7H, O3C$\underline{H}_2$Ph), 4.49 (qd, $J$ = 12.2, 3.8 Hz, 14H, O2C$\underline{H}_2$Ph), 4.24 (d, $J$ = 11.1 Hz, 7H, H6a), 4.04 (t, $J$ = 9.2 Hz, 7H, H3), 3.99 (t, $J$ = 9.1 Hz, 7H, H4), 3.72 (br d, $J$ = 9.2 Hz, 7H, H5), 3.69 (d, $J$ = 11.4 Hz, 7H, H6b), 3.37 (dd, $J$ = 9.6, 3.4 Hz, 7H, H2), 0.86 (br s, 63H, H8), 0.01 (br s, 21H, H7), 0.00 (br s, 21H, H7'); $^{13}$C NMR (151 MHz, $CDCl_3$, 298 K) δ(ppm) 139.50 (quaternary 3OBn, 1C), 138.49 (quaternary 2OBn, 1C), 127.86 (2OBn, 2C), 127.68 (3OBn, 2C), 128.21 (OBn, 3C), 128.05 (OBn, 3C), 127.43 (OBn, 4C), 126.98 (OBn, 4C), 98.09 (C1), 81.05 (C3), 79.43 (C2), 77.86 (C4), 75.64 (O3$CH_2$Ph), 72.73 (O2CH2Ph), 72.67 (C5), 62.54, (C6), 26.12 (C8), 18.49 (C9), -4.63 (C7'), -4.99 (C7). MALDI-TOF m/z [M + Na]$^+$, found: 3218.530, calculated for $C_{182}H_{252}O_{35}Si_7Na$: 3219.515.

### 2.1.3 Heptakis(2,3-di-*O*-benzyl)-β-cyclodextrin (2,3-Bn-β-CD, Intermediate 3)

Heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-benzyl)-β-cyclodextrin (125 g, 39.1 mmol) was solubilized in THF (5 L) under inert atmosphere and tetrabutylammonium fluoride trihydrate (197.5 g, 0.62 mol) was added portionwise. The yellowish solution was stirred overnight at room temperature. The desilylation was followed by TLC ($CHCl_3$:MeOH = 9:1) and it was completed overnight. The reaction crude was concentrated at rotavapor, methanol was added (1.2 L) and the solution was once more concentrated at rotavapor. The azeotropic distillation procedure was repetead three times (3 × 1.2 L methanol) and the crude was finally concentrated until dryness. The residual yellowish material was suspended in water (2.4 L), filtered on a sintered glass filter (porosity 4) and extensively washed with water (5 × 6.2 L) and with a mixture of MeOH:$H_2O$ = 1:9 (3 × 2.5 mL) until a white, odourless, solid was obtained. The white solid was dried until constant weight into a vacuum drying box in the presence of $P_2O_5$ and KOH as desiccants (86 g, 36 mmol, 92%).

The purity of the compound was estimated ≥98.3% based on HPLC method 3.

m.p. 159–161°C, lit. values 163–164°C [5]; $[\alpha_D{}^{25}] = +52.89°$ ($c$ = 1, $CH_2Cl_2$); $R_f$ = 0.16 ($CHCl_3$:MeOH = 9:1) as final, purified compound while during the reaction monitoring the $R_f$ is higher due to the presence of tetrabutylammonium fluoride ($R_f$ = 0.42). IR $\nu$/cm$^{-1}$ 3356, 2927, 1497, 1454, 1161, 1099, 1027, 733, 696.$^1$H NMR (600 MHz, DMSO-$d_6$, 298 K) δ(ppm) 7.22-7.06 (m, 70H, Ph), 5.27 (br d, $J$ = 3.2 Hz, 7H, H1), 4.92 (d, $J$ = 11.5 Hz, 7H, O3CHaHPh), 4.63 (br m, 14H, O3CHHbPh/(C6)$H_2$-OH), 4.52 (dd, $J$ = 12.6 Hz, 14H, O2C$H_2$Ph), 3.91 (t, $J$ = 8.6 Hz, 7H, H3), 3.86 (m, 7H, H6a), 3.83 (m, 7H, H4), 3.78 (m, 7H, H5), 3.66 (br d, $J$ = 11.1 Hz, 7H, H6b), 3.41 (overlapped with HOD signal, 7H, H2); $^{13}$C NMR (151 MHz, DMSO-$d_6$, 298 K) δ(ppm) 139.01 (quaternary Ph, 1C), 138.38 (quaternary Ph, 1C) 128.00 (Ph, 3C), 127.88 (Ph, 3C), 127.36 (Ph, 2C), 127.28 (Ph, 4C), 127.17 (Ph, 2C), 127.03 (Ph, 4C), 96.65 (C1), 80.49 (C3), 78.66 (C2), 77.12 (C4), 74.52 (O3$CH_2$Ph), 72.21 (C5), 71.60 (O2$CH_2$Ph), 60.19 (C6). MALDI-TOF m/z [M + Na]$^+$, found: 2419.090, calculated for $C_{140}H_{154}O_{35}Na$: 2419.688.

### 2.1.4 Heptakis(6-*O*-methyl-2,3-di-*O*-benzyl)-β-cyclodextrin (6-Me-2,3-Bn-β-CD, Intermediate 4)

Heptakis(2,3-di-*O*-benzyl)-β-cyclodextrin (350 g, 0.15 mol) was solubilized in THF (5 L). The slightly greenish solution was cooled-down with an ice-water bath to 10°C and KOH (390 g, 6.95 mol) was added portionwise under vigorous stirring. Methyltriphenylphosphonium bromide (24.5 g, 68.6 mmol) was added to the slightly greenish suspension and the reaction mixture was stirred for 1 h. Methyl iodide (87.5 mL, 199.5 g, 1.4 mol) was slowly and carefully added to the heterogeneous mixture (30 min addition) by keeping the temperature below 25°C. After 1 h stirring the reaction mixture became of a pearly white colour and it showed a milk-like consistence. The reaction was stirred at room

 

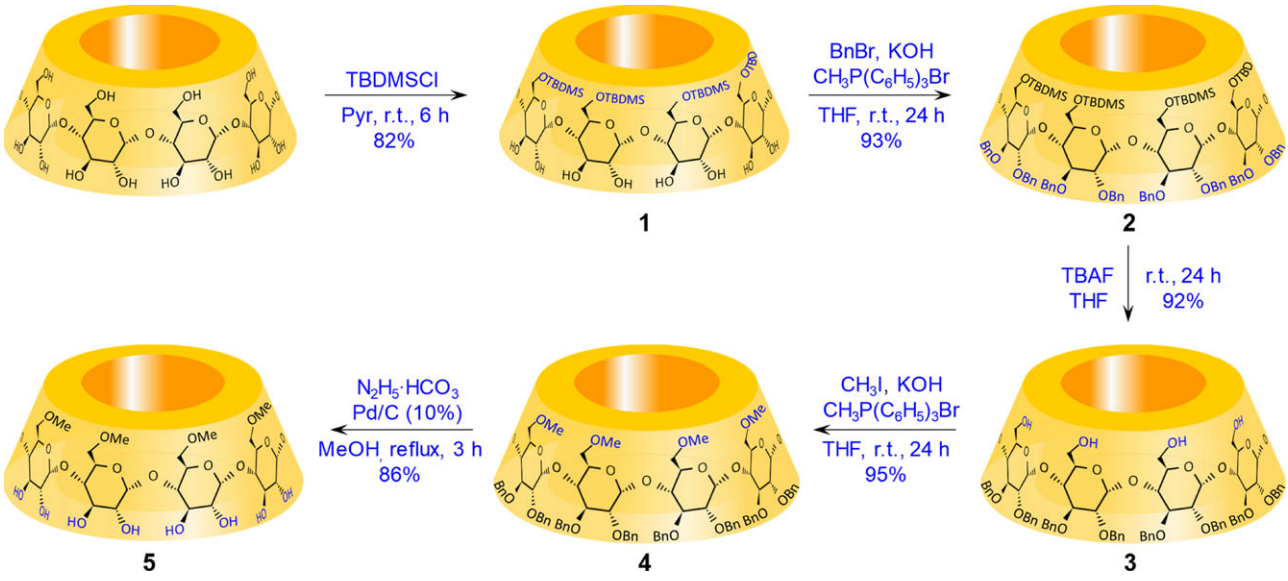


**Figure 1.** Synthesis of heptakis(6-*O*-methyl)-β-CD (compound **5**) with reagents, conditions and yields.

temperature overnight. The proceeding of the reaction was monitored by TLC (chloroform containing 0.5–1% ethanol as stabilizer was used as eluent). The reaction mixture was filtered on a sintered glass filter (porosity 4) and the solid was thoroughly washed with THF (3 × 1.75 L). The filtrate was concentrated at rotavapor (~ 180 mL) and poured to MeOH (1.8 L) under vigorous stirring. The resulting yellowish, gel-like material was separated by decantation, resuspended in water (3.5 L), filtered and extensively washed with $H_2O$ (5 × 1.8 mL) and with a mixture MeOH:$H_2O$ = 1:9 (3 × 1.8 mL) until a white solid was obtained. The white solid was dried until constant weight in a vacuum drying box in the presence of $P_2O_5$ and KOH as desiccants (346 g, 0.14 mmol, 95%).

The purity of the compound was estimated ≥98.6% based on HPLC method 4.

m.p. 88–89°C; $[\alpha_D{}^{25}]$ = +3.25° ($c$ = 1, $CH_2Cl_2$); $R_f$ = 0.25 (chloroform). IR $\nu$/cm$^{-1}$ 2925, 2891, 1497, 1454, 1356, 1197, 1139, 1094, 1040, 734, 696. $^1$H NMR (600 MHz, DMSO-$d_6$, 298 K) δ(ppm) 7.24-7.08 (m, 70H, Ph), 5.09 (br d, $J$ = 1.8 Hz, 7H, H1), 4.93 (d, $J$ = 11.1 Hz, 7H, O3CHaHPh), 4.61 (d, $J$ = 11.1 Hz, 7H, O3CHHbPh), 4.56 (d, $J$ = 12.3 Hz, 7H, O2CHaHPh), 4.47 (d, $J$ = 12.3 Hz, 7H, O2CHHbPh), 3.88 (m, 7H, H3), 3.87 (m, 7H, H5), 3.81 (dd, $J$ = 11.2, 4.4 Hz, 7H, H6a), 3.71 (br t, $J$ = 8.7 Hz, 7H, H4), 3.50 (d, $J$ = 10.5 Hz, 7H, H6b), 3.44 (dd, $J$ = 9.5, 3.3 Hz, 7H, H2), 3.23 (s, 21H, OC$\underline{H}_3$). $^{13}$C NMR (151 MHz, DMSO-$d_6$, 298 K) δ(ppm) 138.90 (quaternary 3OBn, 1C), 138.33 (quaternary 2OBn, 1C), 127.93 (2OBn, 3C), 127.82 (3OBn, 3C), 127.45 (2OBn, 2C), 127.25 (2OBn, 4C), 127.11 (3OBn, 2C), 126.96 (3OBn, 4C), 97.20 (C1), 80.24 (C3), 78.54 (C2), 77.86 (C4), 74.46 (O3C$H_2$Ph), 71.82 (O2C$H_2$Ph), 71.09 (C6), 70.69 (C5), 58.19, (O$CH_3$). MALDI-TOF m/z [M + Na]$^+$, found: 2517.934, calculated for $C_{147}H_{168}O_{35}Na$: 2517.875.

### 2.1.5 Heptakis(6-*O*-methyl)-β-cyclodextrin (6-Me-β-CD, compound 5)

Heptakis(6-*O*-methyl-2,3-di-*O*-benzyl)-β-cyclodextrin (125 g, 50 mmol) was solubilized in methanol (5 L). The reaction mixture was heated at 40°C, Pd/C (31.3 g) was added under vigorous stirring and hydrazine carbonate (1.2 L) was added dropwise to the vessel (3 h addition). The mixture was heated at gentle reflux for 3 h and the proceeding of the reaction was monitored by TLC (1,4-dioxane:$NH_3$ (25%):1-propanol = 10:7:3). The reaction mixture was cooled-down to room temperature, filtered on a sintered glass filter (porosity 3) and the Pd/C pad was thoroughly washed with MeOH (3 × 6.5 L), $H_2O$ (3 × 6.5 L) and MeOH:$H_2O$ = 50:50 (3 × 6.5 L). The filtrate was evaporated until dryness at rotavapor (60°C). The residual solid was solubilized in water (12.5 L), treated with ion exchange resins and clarified with charcoal. The obtained solution was then filtered through a pad of celite and finally evaporated until dryness. The white solid was dried until constant weight in a vacuum drying box in the presence of $P_2O_5$ and KOH as desiccating agents (52.5 g, 42.6 mmol, 86%).

The purity of the compound was estimated ≥98.2% based on HPLC method 5.

m.p. 299–303°C (decomp.), lit. values 303–310°C [6]; $[\alpha_D{}^{25}]$ = +164.24° ($c$ = 1, $H_2O$), lit. value +166°C [1]; $R_f$ = 0.6 (1,4-dioxane:$NH_3$ (25%):1-propanol = 10:7:3 (v/v/v)). IR $\nu$/cm$^{-1}$ 3379, 2931, 1156, 1087, 1041. $^1$H NMR (600 MHz, $D_2O$, 298 K) δ(ppm) 5.06 (d, $J$ = 3.7 Hz, 7H, H1), 3.97 (t, $J$ = 9.6 Hz, 7H, H3), 3.98 (dd, $J$ = 10.0, 2.9 Hz, 7H, H5), 3.77 (m, 14H, H6), 3.65 (dd, $J$ = 9.9, 3.6 Hz, 7H, H2), 3.60 (t, $J$ = 9.5 Hz, 7H, H4), 3.42 (s, 21H, OC$\underline{H}_3$).$^{13}$C NMR (151 MHz, $D_2O$, 298 K) δ(ppm) 104.44 (C1), 83.84 (C4), 75.60 (C3), 74.61 (C2), 73.21 (C6), 73.02 (C5), 60.99, (O$\underline{C}H_3$). MALDI-TOF m/z [M + Na]$^+$, found: 1255.925, calculated for $C_{49}H_{84}O_{35}Na$: 1256.159.

 

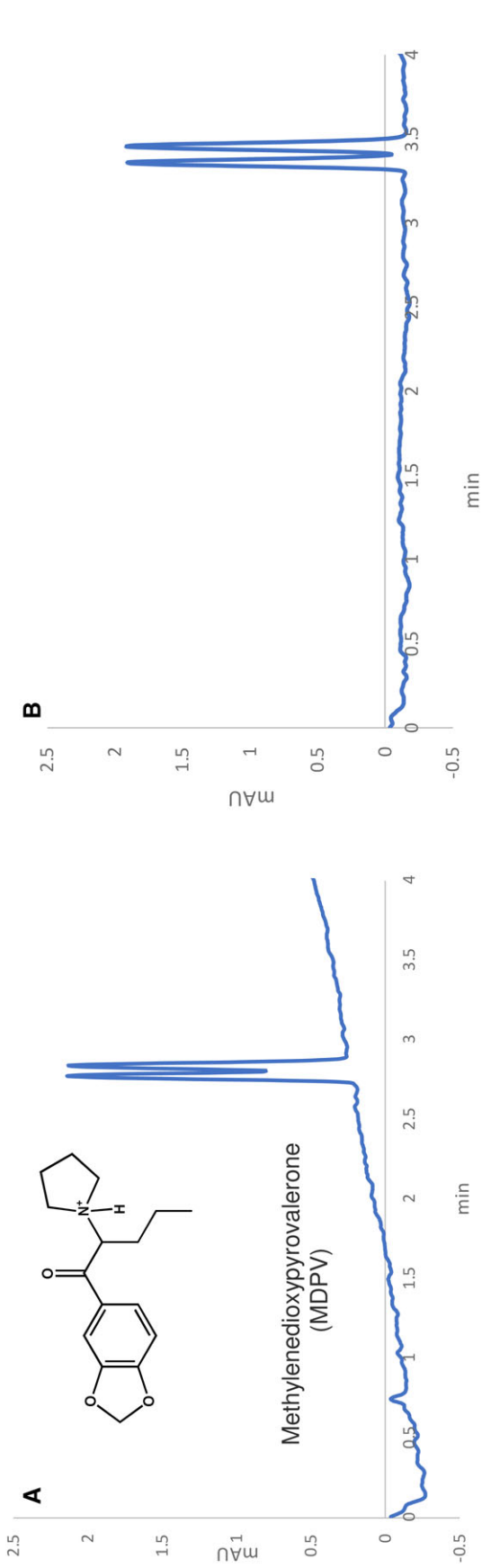


**Figure 2.** CE electropherograms applying 6-Me-β-CD demonstrating the effect of the CD concentration on the enantioseparation of MDPV. a) 1 mM 6-Me-β-CD and b) 2.5 mM 6-Me-βCD.

## 3 Results and discussion

### 3.1 Synthesis and characterization

The primary hydroxyl groups of the native β-CD were selectively protected with TBDMS-Cl in pyridine. The use of pyridine instead of the combination of DMF-imidazole proposed by Takeo made the reaction crude composition simpler (three components *versus* four components) and cost-effective (exclusion of imidazole). In order to obtain a crude which can be purified by sole crystallization, the reaction conditions were tuned towards a mixture made from intermediate **1** (see Fig. 1) and a discrete amount of over-silylated CDs (see Supporting Information Fig. 1).

These latter CD-related impurities are more soluble in DMF-acetone mixture than the symmetric per 6-substituted silyl-β-CD derivative and could be effectively removed by precipitation of the intermediate **1**. The crystallization cycles were repeated until the purity was optimal (≥98%, with two crystallization cycles). The second step, the benzylation by PTC, was the key point of the whole procedure. In Takeo's approach, the secondary side's temporary protection was achieved under relative harsh conditions (acetic anhydride in pyridine at 100°C) and the product was isolated by chromatography. On the contrary, the heterogeneous catalytic set-up allowed exhaustive protection of the secondary rim under mild conditions, without the need of rigorous dry environment and generated a reaction crude that was purified by simple precipitation with excellent yields. It is worth to mention that reported methods for partially or complete benzylation of CDs require strict anhydrous conditions [18], are based on sodium hydride [13, 19–21] and usually need chromatography for isolation of the products [9]. The scale-up based on sodium hydride is precarious, it needs explosion-proof reactors and it is not economically advantageous. Additionally, the chromatography needed for purification is time-consuming, challenging and expensive. Contrary to that, the method developed herein uses a readily up-scalable benzylation/alkylation based on an easily accessible organic phosphonium salt as catalyst. Among the tested catalysts (see Supporting Information Table 1) triphenylmethyl phosphonium bromide was uniquely effective in promoting fully secondary-side substitution with a large variety of alkylating agents (such as methyl-iodide, ethyl-iodide, benzyl-bromide/chloride and allyl bromide) and independently from the anion utilized. Furthermore, this molecule and its by-products are highly water soluble and hence promptly and effectively removed by precipitation. It should be also emphasized that under PTC conditions, the reaction rate and the exothermicity intimately connected to the alkylation process, are simply controlled by adjusting the stirring speed without extra cooling; this is particularly advantageous when large reaction batches are handled. In the eventuality that 2,3-di-*O*-alkylation of the primary-side protected CD did not occur exhaustively, with the current method, the reaction can be pushed to completeness by extra additions of calculated amount of base (KOH) and/or alkyl halide. On the contrary,

 

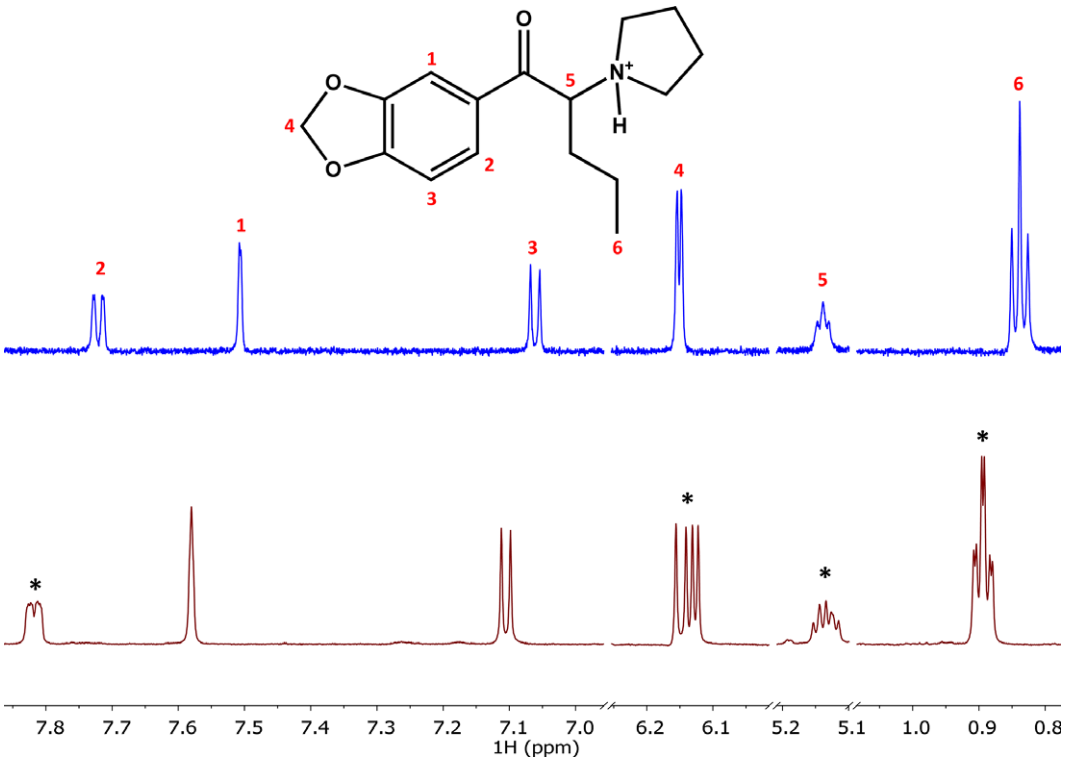


**Figure 3.** The $^{1}$H NMR spectrum of racemic MDPV (blue colour, top) and the $^{1}$H NMR spectrum of racemic MDPV:6-Me-β-CD solution at 1:1.5 molar ratio (brown, bottom). The latter shows remarkable enantioresolution effects as indicated by*.

alkylation reactions based on hydrides need to be reprocessed for yielding a homogeneous product as successive additions of reactants are ineffective for achieving a full conversion. The minimal work-up for the PTC-based benzylation consisted of KOH filtration (KOH is acting as a base as well as a drying agent), azeotropic distillation and precipitation. All these advantageous features make PTC ideal for industrial scale-up.

According to our experience, deprotection of the primary-side (*O*-desilylation) can be exhaustively accomplished with excess of (harmless) TBAF, at room temperature, overnight in THF, while, under the same conditions, the deprotection is incomplete in MeOH. The secondary-side benzylated intermediate was recovered in high purity by washing out the excess of TBAF with methanol:water mixtures. This method is simpler and safer than Takeo's approach, where *O*-desilylation was performed in DCM with (hazardous) boron trifluoride etherate and heptakis(2,3-di-*O*-acetyl)-β-CD was isolated by chromatography.

In the present strategy, per-6-*O*-methylation of the CD ring was also achieved by PTC with methyl iodide. This step clearly shows that the developed procedure is versatile, from every point of view. It has been reported that alkyl-iodide agents are poisonous for bromide-based catalysts, while in the explored conditions, the phosphonium salt did not show any limitation regarding the choice of the halogen counterpart (see Supporting Information Table 1). The methylation resulted in a crude which was easily purified by filtration, azeotropic distillation and precipitation resulting heptakis (6-*O*-methyl)-β-CD in good yield. Methylation with methyl trifluoromethanesulfonate (triflate) and DCM in sealed tube in the presence of 2,6-di-*tert*-butyl-4-methypyridine as catalyst and chromatographic purification afforded heptakis(2,3-di-*O*-acetyl)-β-CD in the work of Takeo.

The current debenzylation procedure requires 25% w/w palladium content and a gentle reflux to achieve exhaustive deprotection. The hydrogen gas evolution caused by the addition of hydrazine carbonate to the heterogeneous mixture is effectively controlled by slowly adding the hydrogen source. Heptakis(6-*O*-methyl)-β-CD is isolated by removing the transition metal on a celite pad and by treating the resulting filtrate with ion exchange resins and charcoal. In the procedure of Takeo [15] the primary-substituted methylated β-CD was obtained by Zemplén *O*-deacetylation, a very effective deprotection method. This is the only step of the all synthetic strategy proposed by the Japanese group that is chromatography-free. Globally, a chromatography-free synthetic strategy towards heptakis(6-*O*-methyl)-β-CD has been developed. The synthetic approach could be potentially extended to the alpha and gamma analogues and used as protocol for the industrial preparation of any per(6-*O*-alkyl)-CD.

All the compounds have been extensively and unambiguously characterized by NMR, MALDI-MS and IR. Optical rotations and melting points have been measured as well. The purity of all the derivatives have been established by *ad-hoc* developed HPLC methods (see Supporting Information).

 

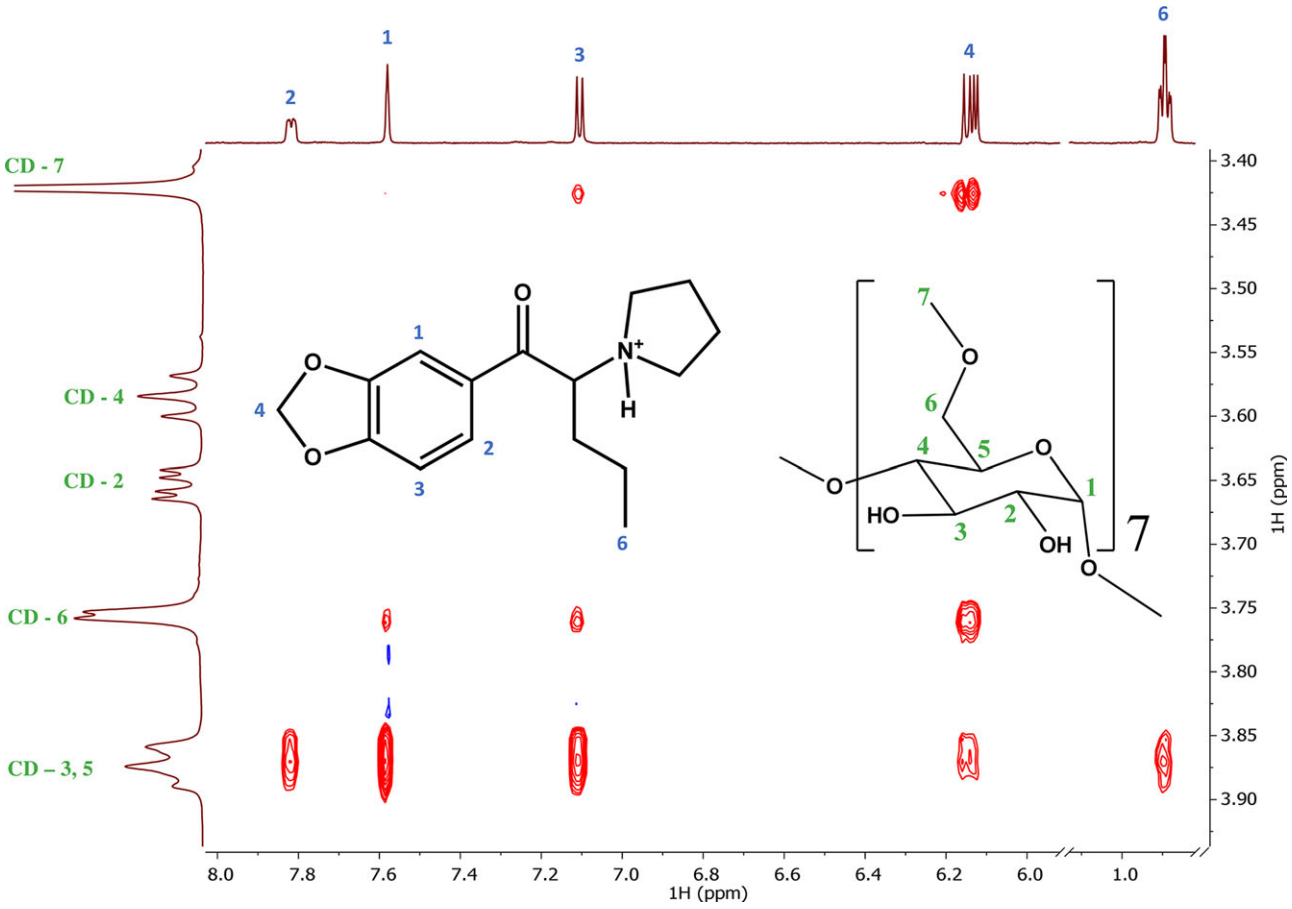


**Figure 4.** Partial 2D ROESY NMR spectrum of MDPV and 6-Me-β-CD showing intense cross-peaks between the inner CD protons (H3, H5) and MDPV.

### 3.2 CE separation of racemic MVPD using 6-Me-β-CD as chiral selector

Chiral CE mediated by CDs is continuously flourishing, as it still offers efficient separations at a reasonable costs and it is completely suited for the rapid screening of optimal conditions in an environmentally friendly fashion. Recent trends reflect that CDs with well-defined structures ("single isomer CDs") are preferred as background electrolyte additives in order to overcome batch-to-batch reproducibility issues as a result of poorly characterized structures of isomeric mixtures. Additionally, it has been reported that exhaustive substitution (methylation in particular) at the primary positions significantly improves the binding potential of cyclodextrin derivatives [22]. The applicability of 6-Me-β-CD as chiral selector was tested in CD-modified capillary electrophoresis using the racemic MDPV as a model compound. The synthetic cathinone, 3,4-MDPV is a popular and at the same time dangerous recreational drug. Despite its similar structure to methamphetamine, MDPV acts by potent inhibition of dopamine reuptake rather than inducing dopamine release like methamphetamine. While this drug is only available as a racemic mixture so far, its (S)-enantiomer shows much greater pharmacological activity than the (R)-MDPV [23, 24]

Figure 2 shows the representative chiral CE electropherograms on the separation of racemic MDPV using 6-Me-β-CD at two different concentrations. It is demonstrated, that enantioselectivity occurred in a reasonable concentration range, as 2.5 mM selector concentration resulted in the baseline separation of the enantiomers in less than 4 minutes, providing rather symmetric peak shapes without any additional optimization. These data support that 6-Me-β-CD can enter into the CD-based chiral selector market as a new single isomer entity.

### 3.3 Structural characterization of the host-guest system by NMR

In order to characterize the host-guest interactions at the molecular level and to get a deeper insight into the molecular interactions between the 6-Me-β-CD and the MDPV enantiomers $^{1}$H and 2D ROESY NMR experiments were performed according to previous works [25, 26]. The enantioselectivity of 6-Me-β-CD was monitored at neutral pH,

 

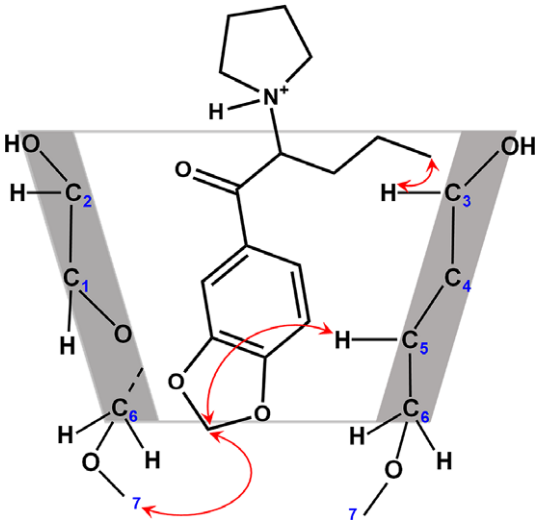


**Figure 5.** Proposed geometric arrangement of MDPV in the cavity of 6-Me-β-CD, based on the ROESY experiment.

with racemic MDPV. In the $^{1}$H NMR spectrum of the racemic guest and the 6-Me-β-CD host, complexation induced chemical shift changes could be observed for the H-2, H-4, H-5 and H-6 resonances of MDPV (see Fig. 3).

2D ROESY NMR spectrum was acquired in order to confirm the hypothesized substantial interactions between 6-Me-β-CD and MDPV at the atomic level. A partial ROESY spectrum of the MDPV/6-Me-βCD system is shown in Fig. 4.

Intense cross-peaks can be observed between the aromatic moiety of MDPV and the inner cavity protons of 6-Me-β-CD, suggesting that the phenyl moiety is fully immersed into the cavity. As the resonances of the methylenedioxy moiety show intense cross-peaks exclusively with CD protons H7, H6 and H5, an inclusion arrangement in which the phenyl ring is located in the "extended cavity" surrounded by the methoxy groups at the primary side of the CD can be hypothesized. This geometrical arrangement can be supported by nonpolar interactions between the methylenedioxy moiety of MPDV and the nonpolar methoxy groups of the host. The inclusion complex of MDPV is schematically shown in Fig. 5, using key ROESY interactions for the determination of MDPV orientation in the cavity. This arrangement may also be favoured by the polar interactions between the positively charged tertiary amine and the secondary OH groups of the host.

## 4 Concluding remarks

Heptakis(6-*O*-methyl)-β-CD has been effectively prepared through a five-step synthetic strategy. Each intermediate has been isolated in high purity without the need of chromatography and thoroughly characterized. The methylated single isomer CD acts as a promising chiral selector in CE and can separate the two enantiomers of the drug MDPV. A 2D model for the host-guest spatial interactions has been proposed based on the extensive NMR characterization. Additionally, the developed synthetic approach is versatile, amenable of scale-up and applicable to any per-6-substituted CD. The preparation of primary-side alkylated CDs adds a powerful tool to the analytical separation of chiral drugs, furthermore industrial production of these selectors might open new avenues towards preparative-scale resolution of a large variety of enantiomers.

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*The authors have declared no conflict of interest.*

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