Copolymerization preparation of cationic cyclodextrin chiral stationary phases for drug enantioseparation in chromatography

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Method Article

Keywords: chiral separation, chiral stationary phases, cyclodextrin, radical copolymerization

Posted Date: June 6th, 2012

DOI: https://doi.org/10.1038/protex.2012.023

Abstract

We described a facile and effective protocol wherein radical copolymerization is employed to covalently bond cationic β-cyclodextrin \(β-CD) onto silica particles with extended linkage, resulting in a chiral stationary phase \(IMPCSP) that can be used for the enantioseparation of racemic drugs in both high-performance liquid chromatography \(HPLC) and supercritical fluid chromatography \(SFC). Starting from commercially available chemicals, the IMPCSP is prepared in several steps: \(i) reaction of β-CD with 1-\(p-toluenesulfonyl)-imidazole to afford mono-6A-\(p-toluenesulfonyl)-6A-deoxy-β-cyclodextrin \(B); \(ii) nucleophilic addition between B and 1-vinylimidazole and followed by treatment with anionic-exchange resin to give mono-vinylimidazolium-CD chloride \(C); \(iii) electrophilic addition between C and phenyl isocyanate to generate 6A-\(3-vinylimidazolium)-6-deoxyperphenylcarbamate-β-CD chloride \(D); \(iv) reaction of silica gel with 3-methacryloxypropyltrimethoxysilane to engender vinylized silica \(E); \(v) immobilization of C onto vinylized silica via radical copolymerization with 2,3-dimethyl-1,3-butadiene in the presence of 2,2'-azobis\(2-methylpropionitrile) \(AIBN) to afford the desired chiral stationary phases. The overall IMPCSP preparation and column packing protocol requires ~2 weeks.

Introduction

Chromatographic methodologies have been extensively explored for accurate sample analyses, online monitoring of reaction progress and purifying of synthesized products[1]. Especially, modern chromatographic techniques have been developed as powerful tools for chiral separation and preparation of enantiomers, most of which are of biological and pharmaceutical interests. Whatever chiral chromatographic techniques are used, chiral selectors either dissolved as mobile phases or mobilized onto supporting materials as stationary phases are crucial for successful and robust enantioseparations. Among the chiral selectors used so far, cyclodextrins \(CDs) and their derivatives have been widely used in chiral chromatography since its first introduction by Armstrong et al.[2]. In order to obtain a better solubility, the charged moieties are favorably introduced onto CD rims for capillary electrophoresis \(CE). The self-mobility of CD in the ionized form enhances the separation ability when it is opposite to the electrophoretic mobility of the analytes[3]. The hydrophobic moieties on the analytes could be included or adsorbed into the chiral cavity of CDs. Meanwhile, the analytes could also interact with the substituents on the CD rims[4,5]. The native CDs and their chemically-modified derivatives afford fine-tuned hydrophobicities, charges and cone shapes etc., which ultimately result in different tightness and interactions for CD-analytes complexes selectors and thus chemically-manipulated diving forces for chiral recognitions[6-14]. The charged CD-based chiral mobile phases and chiral stationary phases \(CSPs) have been extensively explored in drug chiral analyses[15-20]. A sulfated β-CD based CSP was developed which enabled great enantioseparations towards 33 racemic drugs in high-perfomace liquid chromatography \(HPLC)9. Recently, ionic-liquids featured positively-charged CDs have been covalently bonded onto silica gels to prepare novel CSPs, which exhibited dual selectivity in HPLC for the enantioseparation of both polar and non-polar compounds[18]. The additional electrostatic interactions are significant to achieve separations of polar analytes which may interact with the neutral CDs too weakly,

where16 aromatic alcohol racemates and 2 drugs were achieved in polar organic mobile phases19. In our previous report, a series of coated CSPs based on fully derivatized cationic β-CD were prepared. These cationic β-CD CSPs have shown strong enantioseparation abilities and moderate retention times towards a series of α-phenyl alcohols in both supercritical fluid chromatography \(SFC) and HPLC20. However, polar organic solvents in the mobile phase could cause damage to the coated CSPs. Besides, the efficiencies of the coated CSPs are usually low as the surface thickness of chiral selectors is hard to control [21]. Figure-1 \(R Wang) Syntheses of Ts-CD \(B), compounds C and D. The immobilization of functionalized CD onto silica gel is generally achieved by chemical reactions between highly reactive substituent on CD and the functional groups on silica gel[14,16-18]. Tedious synthetic approaches with possible protecting and de-protecting steps are usually required for successful grafting. Comparatively, the facile co-polymerization method is more accessible and reliable, universally employed in producing non-silica fillings for columns applicable for size-exclusion chromatography \(SEC) or ion-exchange applications[22]. The polymerized materials used for chromatography have wide applicable pH ranges and improved retentions for polar analytes, although lower mechanic strength and efficiency are often observed than the silica-based CSPs. The co-polymerization approach was also explored in the immobilizing of polysaccharide or enantiopure small molecules onto silica gel for the preparation of CSPs[23,24]. Figure-2 \(R Wang) Preparation of IMPCSP via radical copolymerization. In view of the our continuous and successful research endeavors in developing powerful cationic CDs as chiral selectors for both CE and HPLC[12,25,26], we recently developed the facile copolymerization methodology in preparing novel covalently-bonded cationic β-CD CSPs via co-polymerization approach \(see Figs. 1 and 2). These as-prepared CSPs have successfully expanded the enatioseparation windows towards a broader range of chromatographic conditions for both HPLC and SFC application[27,28]. The linkage between CD selector and silica-support was built by using diene \(ca. 2,3-dimethyl-1,3-butadiene) as the third monomer for the dual copolymerization system with vinyllated CD and silica gel. The extended linkage can effectively improve the surface loading issue of CD onto silica gel, a challenge faced when direct immobilizing CD derivatives onto silica surface with short spacers due to steric hindrance[29]. The as-developed CDs CSPs exhibited great potential in drug enantioseparations in both HPLC and SFC applications. The protocol describes herein the synthesis of imidazolium-based IMPCSP. This methodology can also be applied for the synthesis of other ammonium-based cationic CD CSPs[27,28], which may find wide applications for both drug enantioseparations and NOM assessments.

Reagents

• β-Cyclodextrin \(β-CD; >95%; TCI, cat. no. C0900) • Imidazole \(99%; Merck, cat. no. 436151) • Sodium hydroxide \(NaOH, 97%; Sigma-Aldrich, cat. no. 138701) • Ammonium chloride \(99.5%; Fluka, cat. no. 09725) • p-Toluenesulphonyl chloride \(99%; Fluka, cat. no. 89730) \!CAUTION p-toluenesulfonyl chloride is very smelly and highly corrosive. It is recommended that it be weighed in a glovebox and transferred with sealed bottles or directly weighted out in reaction flask. Please refer to the MSD sheet of this compound for safety information. • Dichloromethane \(CH2Cl2, 99.6%, ACS reagent; Sigma-Aldrich, cat. no. 443484) • 1-Vinylimidazole \(≥ 99%; Sigma-Aldrich, cat. no. 235466) • Ethyl acetate \(99.5%, ACS

reagent; Sigma-Aldrich, cat. no. 141786) • n-Hexane \(98.5%; Sigma-Aldrich, cat. no. 178918) • Phenyl isocyanate \(≥ 98%; Sigma-Aldrich, cat. no. 185353) • Amberlite IRA-900 ion-exchange resin \(Sigma-Aldrich, cat. no. 216585) • N,N-Dimethylformamide \(DMF, 99.8%; Sigma-Aldrich, cat. no. 319937) • Pyridine \(99.5%, Extra dry, Fisher, cat. no. AC33942) \! CAUTION Pyridine is very harmful to eyes and skin. Goggle and gloves must be worn in handling pyridine and conduct experiments in well ventilated fumehood to avoid inhalation. • Chloroform \(99.8%, ACS reagent; Fisher, cat. no. AC40463) • Magnesium sulphate \(≥ 97%, anhydrous, reagent grade; Sigma-Aldrich, cat. no. 208094) • Nitrogen gas \(ALPHAGAZ™; SOXAL) • Liquid nitrogen \(SOXAL) • Paraffin oil \(puriss.; Sigma-Aldrich, cat. no. 18512) • Silica gel \(5 µm, Kromasil) • 3-Methacryloxypropyltri-methoxysilane \(98%; Sigma-Aldrich, cat. no. 440159) • 2,2’-Azobis\(2-methyl-propionitrile) \(AIBN, ≥ 98%, purum; Sigma-Aldrich, cat. no. 11630) • Toluene \(99.8%, anhydrous, reagent grade; Sigma-Aldrich, cat. no. 244511) • 2,3-Dimethyl-1,3-butadiene \(98%; Sigma-Aldrich, cat. no. 145491)

Equipment

• Magnetic stirrer with thermal and speed controller \(Heidolph) • Rotary evaporator \(Büchi, R205) • Teflon-coated magnetic stirring bars • Vacuum pump • Balance • Round-bottomed flask • Conical flask • Pressure-equalizing addition funnel • Büchner funnel • Soxhlet extractor • Liebig condenser • Dewar dish • Glass and plastic syringes \(polypropylene) • Disposable hypodermic syringe needles • NMR spectrometer \(300 MHz; Brüker, cat. no. ACF300) • FTIR spectrometer \(FTS165) • Vario EL universal CHNOS elemental analyzer • MALDI-TOF-MS \(Shimadzu, AXIMA Confidence) • Stainless steel HPLC column \(15 cm length, 2.1 mm in inner diameter; Isolation Technologies) • HPLC pump \(LabAlliance-Scientific) • Filter membrane used for syringe \(0.45 µm pore size; Millipore) • Membrane Filter \(0.45 µm pore size; Millipore) • Agilent HPLC \(HP 1100) equipped with a variable-wavelength detector \(190–300 nm) • Jasco SFC \(SF 2000) equipped with a variable-wavelength detector \(190–900 nm) and a back pressure regulator \(0-30 MPa)

Procedure

Synthesis of compound C 1| Fit a 100-ml double-necked round-bottomed flask containing a Teflon-coated magnetic stir bar with a rubber septum and a Liebig condenser. Fit the condenser a rubber septum with inlet of dry N2 and an outlet towards a bubbler containing paraffin oil, in order to prevent the ingress of moisture and air. 2| Weigh out 6A-toluenesulfonyl-β-CD B 12.91 g \(0.01 mol) into the flask. 3| Turn on the circulating water in the condenser. 4| Add 20 ml DMF into the flask and switch on the magnetic stirrer and heater. 5| Inject 1-vinylimidazole 3 ml \(0.03 mol) into the flask though a plastic syringe. ? TROUBLESHOOTING 6| Allow the reaction to proceed at 90°C for 48 h under reflux. Cool down to room temperature. 7| Precipitate the product in acetone \(200 ml). Collect the precipitate by filtration. Wash the raw product with acetone. 8| Dissolve the solid into water/methanol \(200 ml/50 ml) \! CAUTION The solution could be heated towards 50°C to afford a clear solution. 9| Fill a column \(I.D. 30 × 250 mm) with Amberlite IRA-900 ion-exchange resin and washed with MilliQ water till the effluent pH going neutral. 10|

Transfer the solution from step 8 into the column and let it hold for 1 h. Subsequently, collect the effluent drop by drop. Flush the column with equal volume MilliQ water and collect the effluent. 11| Distill off water on rotary evaporator to yield C as a light yellow solid \(9.7 g, 78% yield) ■ PAUSE POINT Compound C can be stored in oven at 80°C for several weeks. **Synthesis of compound D** 12| Fit a 250-ml double-necked, round-bottomed flask containing a Teflon-coated magnetic stir bar with a rubber septum and a Liebig condenser. Fit the condenser a rubber septum with inlet of dry N2 and an outlet towards a bubbler containing paraffin oil, in order to prevent the ingress of moisture and air. 13| Weigh out compound C 2.15 g \(1.72 mmol) into the flask. 14| Add 20 ml dried pyridine into the flask and switch on the magnetic stirrer and heater. \! CAUTION Pyridine is highly toxic solvent. All experiments dealing with pyridine should be operated in fumehood. Goggles, gloves and mask should be worn. 15| Inject phenyl isocyanate 12 ml \(110.32 mmol) into the flask though a plastic syringe. \! CAUTION Phenyl isocyanate has acute toxicity. It may cause severe skin burns and eye damage. It may cause allergy or asthma symptoms or breathing difficulties if inhaled. Adding 12 ml phenyl isocyanate as a whole would cause large extent of side reaction to produce triphenyl isocyanurate as a by-product. It is strongly recommended to add phenyl isocyanate dropwise with pressure equalizing funnel. Goggles, gloves and mask should be worn before experimental operation in fumehood. ▲ CRITICAL STEP Phenyl isocyanate should be added with four equal portions. Add 12 ml as a whole would cause large extent of side reaction to produce triphenyl isocyanurate as a by-product. Add phenyl isocyanate drop by drop with pressure equalizing funnel would end up with its transformation in the funnel and the liquid colour changes to light yellow. 16| Allow the reaction to proceed at 85 °C for 20 h. Set up the vacuum distillation pipeline. 17| Distill off pyridine under reduced pressure at 85 °C. ? TROUBLESHOOTING 18| Dissolve the residue with chloroform 15 ml. 19| Add the solution into silica column. Flush the impurities with n-hexane/ethyl acetate \(70:30 v:v). ▲ CRITICAL STEP The ratio of n-hexane/ethyl acetate was determined by TLC analyses. The flash column separation progress was also monitored by TLC analyses. Lowering the ratio would result in an increase of the amount of solvents used for eluting the impurities completely. 20| Flush the product out of the column with MeOH. 21| Remove the solvent on rotary evaporator to yield D as a dark yellow solid \(6.2 g, 66% yield). ■ PAUSE POINT Compound D can be stored in oven at 80 °C for several weeks. **Synthesis of vinylized silica E** 22| Dry spherical silica gel particles \(5 μm, 5g) in vacuum \(10 mm Hg) at 150 °C for 24 h. Cool down to room temperature. 23| Fit a 250-ml double-necked, round-bottomed flask containing a Teflon-coated magnetic stir bar with a rubber septum and a Liebig condenser. Fit the condenser a rubber septum with inlet of dry N2 and an outlet towards a bubbler containing paraffin oil, in order to prevent the ingress of moisture and air. 24| Add dry toluene 100 ml into the flask. Switch on the magnetic stirrer and heater. 25| Inject 3-methacryloxypropyltrimethoxysilane \(2.3 ml) into the flask. 26| Add dried silica gel from step 22 into the flask. ? TROUBLESHOOTING 27| Allow the reaction to stand at 90 °C for 18 h. Product was collected by filtration through 0.45 μm pore size membrane and washed with MeOH in Soxhlet apparatus overnight. 28| Collect the product by filtration through 0.45 μm pore size membrane and washed with MeOH in Soxhlet extractor overnight. 29| Dry the product overnight in an oven at 60°C in vacco to afford the vinylized silica E. ■ PAUSE POINT Vinylized silica E can be stored at room temperature for several months. **Co-polymerization for preparation of IMPCSP** 30| Dissolve compound D \(0.7 g) in chloroform \(30 ml). Filter the solution through 0.45 μm

pore size membrane. 31| Transfer the filtrate and drop onto vinylized silica gel E \(1.4 g) evenly with a glass syringe. \! CAUTION A glass syringe was preferred to avoid any introduction of siloxal impurities from plastic syringes. Chloroform could corrode the rubber piston of the plastic syringe. 32| Dry the colloid-like mixture in vacuum \(10 mm Hg) at 25 °C. 33| Fit a 100-ml double-necked, round-bottomed flask containing a Teflon-coated magnetic stir bar with two rubber septums. 34| Add the solid from step 32 into the flask. Add AIBN \(3 mg) into the flask. ▲ CRITICAL STEP The amount of AIBN should be controlled. A less amount of AIBN would lead to slow reaction rate but a great amount resulted in rapid radical reactions and short chain growth. In both conditions, successful immobilized CD amounts were low. 35| Inject anhydrous toluene \(20 ml) and 2,3-dimethyl-1,3-butadiene \(2.6 ml) into the flask. ▲ CRITICAL STEP 2,3-Dimethyl-1,3-butadiene should be added before heating started. It was initiated together with the vinyl groups in compound D and vinylized silica E. Therein, 2,3-dimethyl-1,3-butadiene could act as a interlink. A delayed addition led to a lower CD amount on IMPCSP, since the radicals formed on D and E could have been annihilated before 2,3-dimethyl-1,3-butadiene was added. 36| Freeze the mixture in liquid N2 in a Dewar dish and degas the reaction system in vacuum \(10 mm Hg) for 0.5 h. \! CAUTION Liquid N2 37| Take the flask out of the Dewar dish and thaw at room temperature. 38| Repeat step 36 and 37 for three times. ? TROUBLESHOOTING 39| Fit the flask with a Liebig condenser. Fit the condenser a rubber septum with inlet of dry N2 and an outlet towards a bubbler containing paraffin oil, in order to prevent the ingress of moisture and air. 40| Switch on the magnetic stirrer and heater. Leave the reaction to proceed at 60 °C for 18 h. 41| Cool down the reaction mixture and collect the product by filtration through 0.45 μm pore size membrane. 42| Wrap the filter cake with filter paper. Extract the solid in a Soxhlet extractor overnight. 43| Stop heating and collect the silica from the Soxhlet extractor. Dry the product in an oven at 60 °C for 24 h. 44| Pack IMPCSP into an empty stainless steel column with MeOH at 8,000 psi for 30 min. ■ PAUSE POINT IMPCSP can be stored at room temperature for several months.

Timing

Synthesis of **B**: ~40 h include synthesis of A. Steps 1-11 Synthesis of **C**: Steps 1-5, 1 h; Step 6, 48 h; Steps 7-8, 1 h; Steps 9-10, 1 h; Step 11, 12 h. Steps 12-21 Synthesis of **D**: Steps 12-14, 1 h; Step 15, 2 h; Step 16, 20 h; Step 17, 5 h; Steps 18-20, 12 h; Step 21, 12 h. Steps 22-29 Preparation of **E**: Step 22, 12 h; Steps 23-25, 4 h; Steps 26-27, 18 h; Steps 28-29, 20 h. Steps 30-44 Preparation of **IMPCSP**: Steps 30-32, 6 h; Steps 33-35, 1 h; Steps 36-38, 1 h; Steps 39-40, 20 h; Steps 41-42, 20 h; Step 43, 24 h; Step 44, 2 h.

Troubleshooting

? TROUBLESHOOTING Troubleshooting advices can be found in Table 1.

Anticipated Results

The success of immobilizing CD onto silica gel could be characterized by typical FT-IR vibration bands of phenyl-groups in phenylcarbamate substituents in CSP. The amount of immobilized CD derivatives on

silica gel could be calculated based on elemental analyses results \(%N exclusively from CD derivative). The representative analytical data of organic compounds: **C**, **D**, **E** and **IMPCSP** are given below. **Organic synthesis:** Compound **C**: m.p. 254-268°C. 1H NMR \(300 MHz, DMSO-d6, δ ppm) 2.73 \(m, 1H H-2), 2.88 \(m, 1H, H-4), 3.00-3.15 \(m,1H, H-5), 3.32-3.45 \(overlap with solvent peak, 12H, H-2,4), 3.20-3.80 \(overlap with solvent peak, 27H, H-3,5,6) 4.30-4.40 \(m, 1H, OH-6), 4.48-4.59 \(m, 6H, OH-6, =CH2vinyl) 4.84-4.86 \(m, 6H, H-1) 5.00 \(d, 1H, H-1) 5.40-5.50 \(m, 1H, -CHvinyl) 5.64-5.84 \(m, 13H, OH-2,3) 5.95-6.10 \(d, 1H, OH-2), 7.87 \(s, 1H, =CH-4im) 8.18 \(s, 1H, CH-5im) 9.42 \(s, 1H, =CH-2im) Compound **D**: m.p. 197-199°C. MALDI-TOF-MS \[M+]: \(expected) 3592.16; \(found) 3592.07. 1H NMR \(300 MHz, CDCl3, δ ppm) 3.00-6.00 \(m, 52H, H-CD, H-vinyl) 6.00-7.80 \(m, 100H, H-phenyl). Microanalysis for $C_{187}H_{175}ClN_{22}O_{54}$ \(expected) C: 61.87%, H: 4.86%, N: 8.49%, \(found) C: 60.25%, H: 5.13%, N: 9.11%. Surface modified silica gel E: Obvious vibration bands in FT-IR spectrum of 2964, 2855 cm-1 \(C-H) 1705 cm-1 \(C=O) 1635 cm-1 \(C=C) and 1130 cm-1 \(C-O and Si-O) represent the successful surface modification with mathacryloyl-groups. Microanalyses data give the surface double bond loading of 5 μm silica as 2.16 μmol/m2 based on the carbon content \(Table 2)[30]. **Prepared IMPCSP** The characteristic peaks in FT-IR spectrum at 1720 cm-1 \(C=O), 1647, 1558, 1458 cm-1 \(C=C phenyl group) and 1130 cm-1 \(C-O and Si-O) show the CD derivative has been successfully bonded onto silica surface. The cyclodextrin derivatives' grafting coverage was calculated based on the nitrogen content \(%N), to be 0.09 μmol m-2 \(Table 2)[30]. **Chromatographic separation results:** The packed column with IMPCSP was applied for enantiomeric separations in RP-LC, NP-LC and SFC respectively. The cationic β-CD exhibited good enantioselectivity and stability towards four representative racemic analytes in Figure 3.

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Acknowledgements

We acknowledge funding from the A*STAR \(SERC Grant No.: 0921010056) in support of this project. R.-Q.W. is grateful for the award of a research scholarship by NTU and helpful discussions with Dr A. Rajendran of NTU SCBE.

Figures

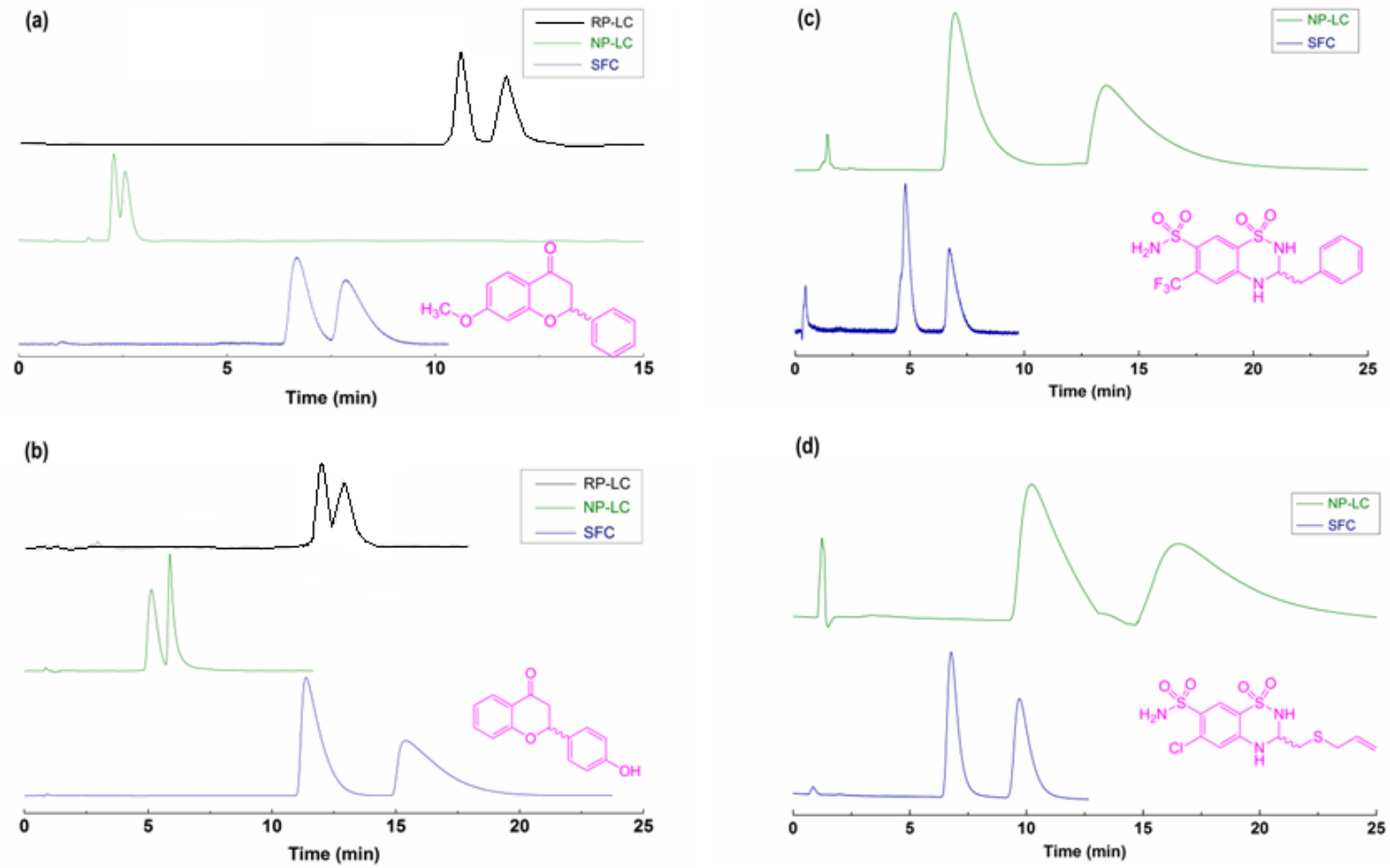

Figure 1

Figure 3 Figure-3 Enantioseparations of racemic drugs using IMPCSP with multiple channel UV detector detection at 254 nm. Flow rate is 0.4 ml min-1 in normal-phase HPLC (NP-LC), 0.5 ml min-1 in reverse-phase HPLC (RP-LC) and 1.0 ml min-1 in SFC. Separation conditions are as follows: (a) 7-methoxyflavanone, reverse-phase HPLC buffer (0.1% TEAA pH 4.3)/MeOH (30/70); NP-LC n-hexane/2-propanol (97/3, v/v); SFC CO2/2-propanol (99/1, v/v); (b) 4'-hydroxyflavanone, RP-LC buffer (0.1% TEAA pH 4.3)/MeOH (40/60); NP-LC n-hexane/2-propanol (97/3, v/v); SFC CO2/2-propanol (99/1, v/v); (c) bendroflumethiazide, NP-LC n-hexane/2-propanol (85/15, v/v); SFC CO2/2-propanol (70/30, v/v); (d) althiazide, NP-LC n-hexane/2-propanol (70/30, v/v); SFC CO2/2-propanol (70/30, v/v).

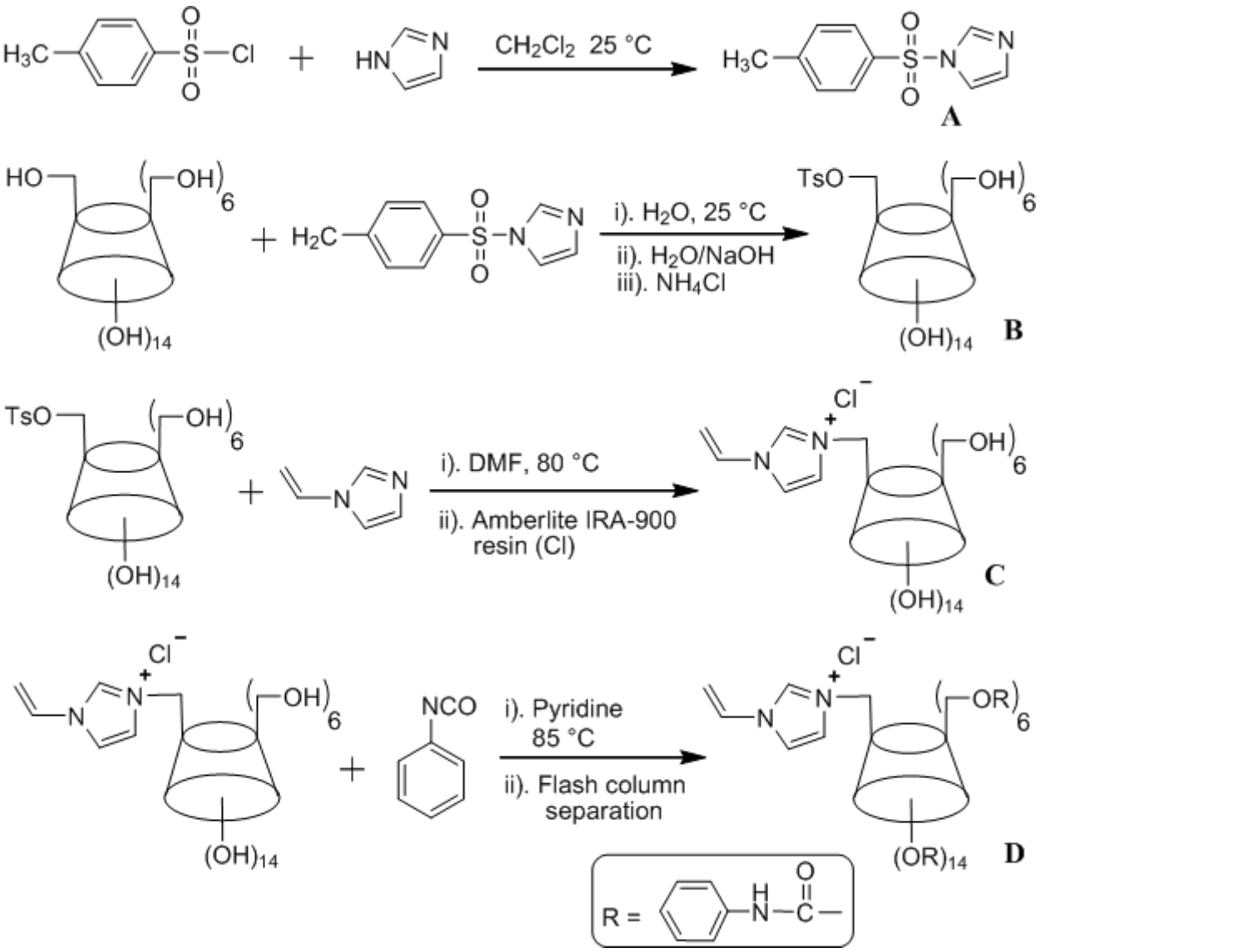

Figure 2

Figure 1 Figure-1 (R Wang) Syntheses of Ts-CD (B), compounds C and D.

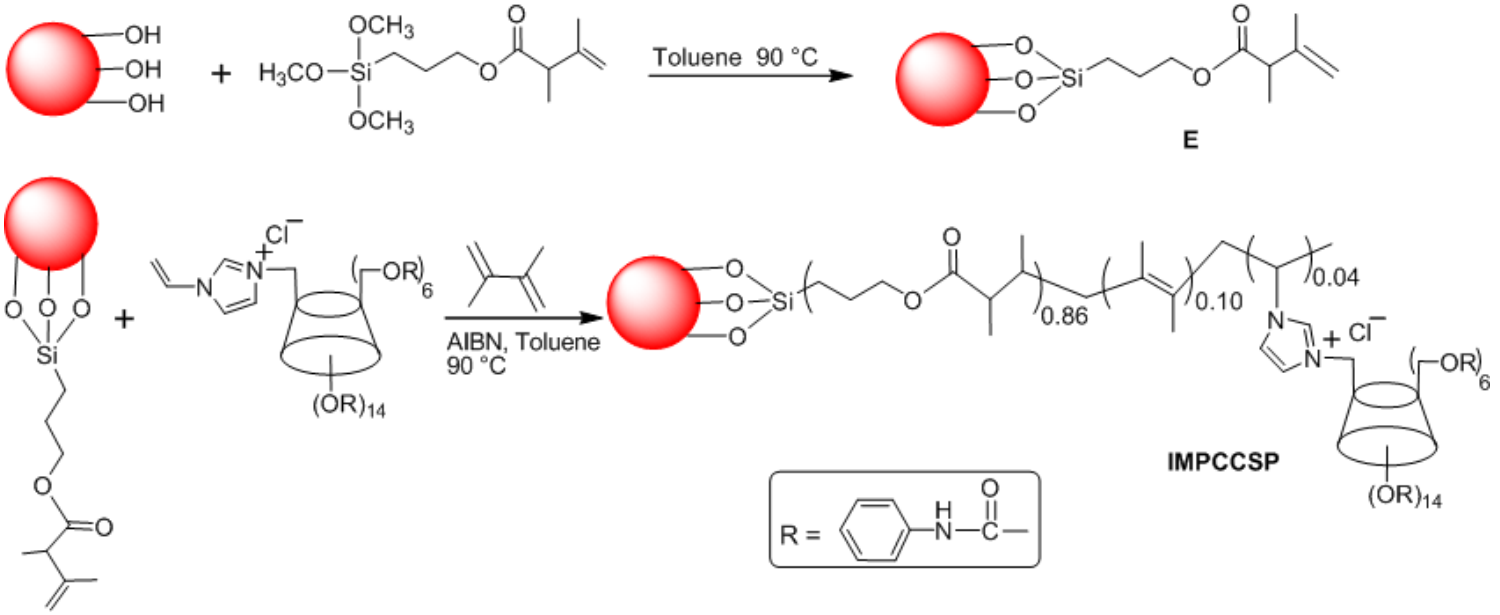

Figure 3

Figure 2 Figure-2 (R Wang) Preparation of IMPCSP via radical copolymerization.

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