

Electronic Supplementary Information for

Silica-bound co-pillar[4+1]arene as a novel supramolecular stationary phase

Subbareddy Mekapothula,¹ Matthew A. Addicoat,¹ David J. Boocock,¹ John D. Wallis,¹
Peter J. Cragg,² and G. W. V. Cave^{1*}

a) School of Science and Technology, Nottingham Trent University, Clifton Campus,
Nottingham, NG11 8NS, United Kingdom

b) School of Pharmacy and Biomolecular Sciences, Huxley Building, University of Brighton,
Brighton BN2 4GJ, UK.

*Email: gareth.cave@ntu.ac.uk

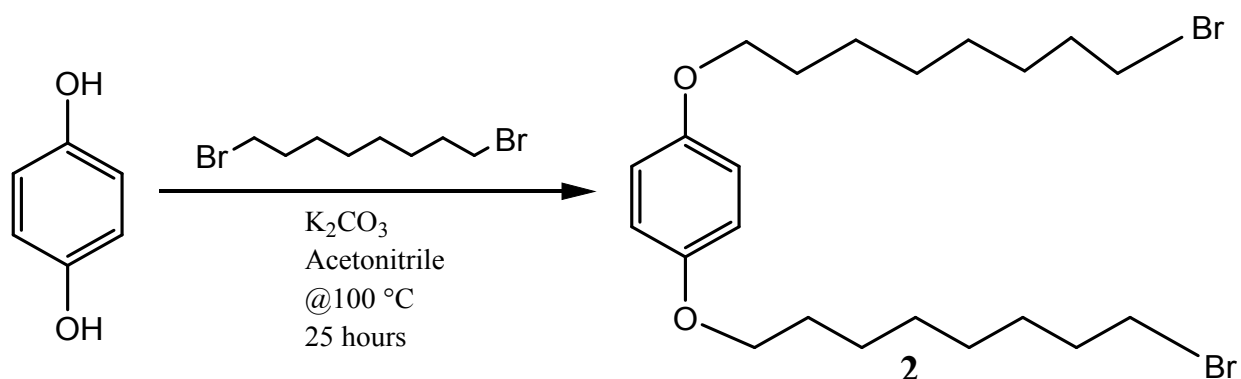
Contents

A. Materials and methods	S2
B. Synthesis of 1,4-bis((8'-bromooctyl)oxy)benzene (compound 2).....	S3
C. Synthesis of co-pillar[4+1]arene by microwave irradiation and condensation method (compound 3).....	S4
D. Synthesis of co-pillar[4+1]arene bonded silica gel stationary phase (compound 4).....	S8
E. Flash column conditioning.....	S8
F. Separation of xylene isomers and toluene on co-pillar[4+1]arene bonded-silica gel stationary phase 4 by flash column chromatography	S11
G. Validation of co-pillar[4+1]arene bonded-silica gel stationary phase 4 using Fisher's Scientific xylenes and toluene, in comparison with normal phase silica column.....	S13
H. Characterization of xylene flash column fraction by ¹H NMR.....	S14
I. Computational studies.....	S19
J. References.....	S20

A. Materials and methods

All chemicals and solvents were purchased as reagent grade, LC-MS and GC-MS grade and used without further purification. Reactions were monitored by TLC plate (pre-coated with 60 Å silica gel, F254) purchased from Merck KGaA and visualized by UV light (254, 365 nm) or iodine. Flash column chromatography was performed using silica gel (silica flash P60 from Fluorochem) as the stationary phase for the purification of synthetic compounds. ¹H and ¹³C NMR spectra were recorded a Jeol 400 MHz NMR ECX-400 spectrometer at 25 °C. Chemical shift presented in ppm and referenced by the residual solvent peak. Mass spectrometric analysis of compounds was performed using a Waters MS XVEOG2XS qTOF through direct infusion spray. A Perkin Elmer thermogravimetric analyser TGA4000 was used for thermogravimetric analysis. Flash column chromatography was carried out using an automated PuriFlash®5.125 flash system with pre-packed and dry self-packed flash cartridges. Interchim® silica cartridges packed with 50µm silica particles with a surface area of 500 m²/g, were used for flash column chromatographic studies. Flash chromatographic fractions were characterized by Jeol 400 MHz NMR ECX-400 spectrometer at 25 °C.

B. Synthesis of 1,4-bis((8'-bromooctyl)oxy)benzene



Scheme 1: Synthesis of 1,4-bis((8'-bromooctyl)oxy)benzene¹

Hydroquinone (1.78 g, 0.0153 mol) and 1,8-dibromooctane (11.5 ml, 0.061 mol) were dissolved in acetonitrile (*ca.*50 ml). K_2CO_3 (6.33 g, 0.045 mol) was added and the reaction mixture was heated to reflux at $100\text{ }^\circ\text{C}$ for 25 hours. The reaction mixture was cooled, filtered and extracted into dichloromethane (*ca.*200 mL). The filtrate was concentrated and the residue was purified by column chromatography (hexane/ethyl acetate 9:1) to afford the desired product **2** as a white solid (6.40 g, 0.01305 mol, 81 %). The ^1H NMR spectrum of **2** is shown in Figure S1 (400 MHz, CDCl_3) δ 6.80 (s, 4H), 3.85 (t, $^3J=6.8\text{ Hz}$, 4H) 3.42 (t, $^3J=6.8\text{ Hz}$, 4H), 1.84 (m, 4H) 1.73 (m, 4H) 1.38 (m, 16H) ppm. ^{13}C NMR (100 MHz, CDCl_3 , $25\text{ }^\circ\text{C}$): δ 151.6, 115.3, 68.7, 33.7, 32.6, 29.6, 28.0, 25.9, 28.6 ppm. Melting point: $82\text{--}83\text{ }^\circ\text{C}$

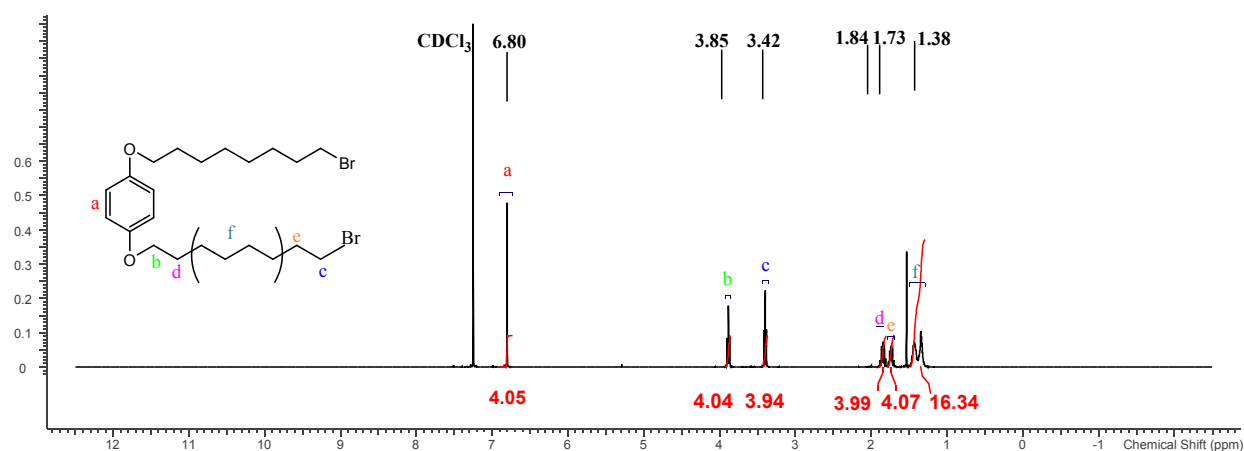
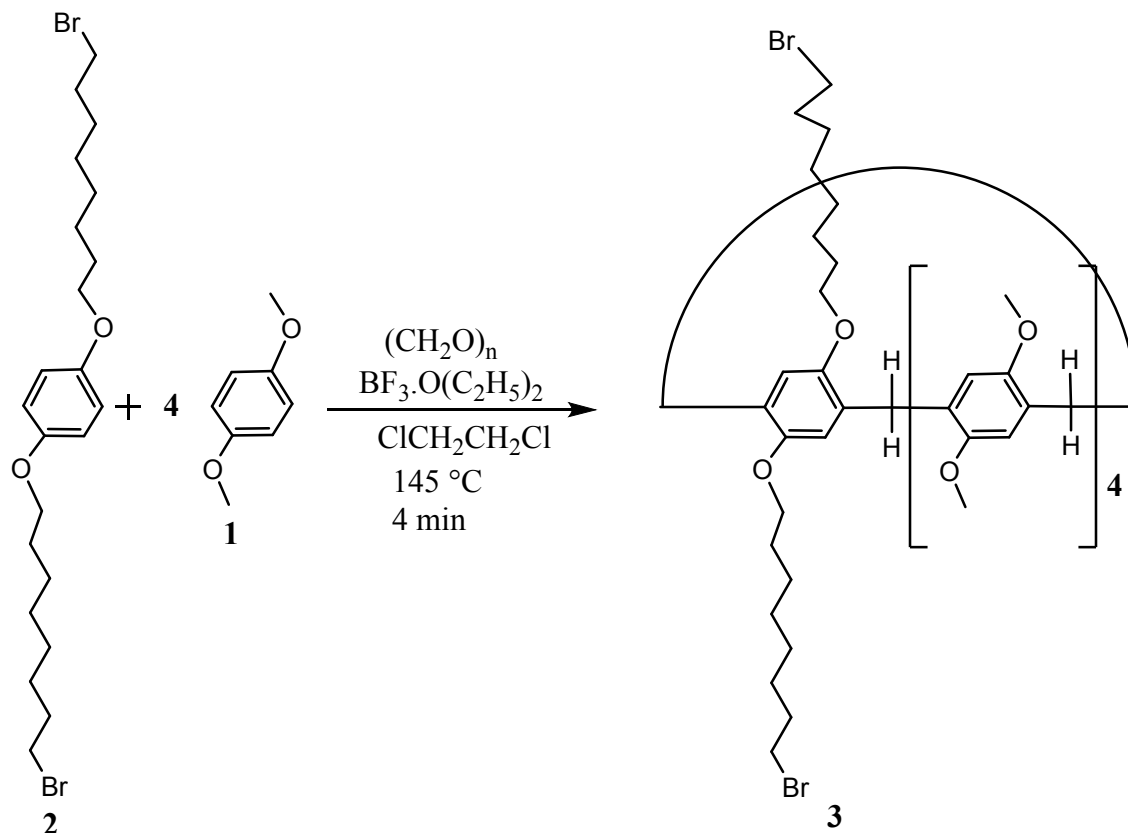


Figure S1. ^1H NMR spectrum (400 MHz, chloroform-d, room temperature) of 1,4-bis((8'-bromooctyl)oxy)benzene.¹

C.1 Synthesis of co-pillar[4+1]arene **3** using microwave irradiation synthesis



Scheme 2: Synthesis of co-pillar[4+1]arene **3** by microwave irradiation synthesis

A mixture of 1,4-bis((8'-bromooctyl)oxy)benzene (1.5 g, 0.00306 mol), 1,4-dimethoxybenzene (g, 0.01224 mol), 1,2-dichloroethane (50 mL) and paraformaldehyde (0.459 g, 0.0153 mol) was placed in a 10-20 mL Biotage® microwave reaction vial. $\text{BF}_3 \cdot \text{O}(\text{C}_2\text{H}_5)_2$ (1.88 mL, 0.0153 mol) was added to the mixture and the reaction vial sealed. The mixture was pre-stirred (30 s) and heated to $145\text{ }^\circ\text{C}$ in a Biotage Initiator 60 instrument by microwave irradiation (130 W, 2 min power cycle, a total of 4 min). Upon cooling to the room temperature, the crude reaction mixture was triturated with acetone after 1,2-dichloromethane evaporation and collected as a white solid product *via* vacuum filtration. Yield 2.98 g (0.0027 moles, 88.5%). Melting point: $192\text{-}193\text{ }^\circ\text{C}$.

The ^1H NMR spectrum of **3** is shown in Figure S2 (400 MHz, CDCl_3) δ 6.62 (m, 10H), 3.91 (m, 10H), 3.79 (m, 4H), 3.65 (m, 24H), 3.35 (m, 4H), 1.84 (m, 4H), 1.68 (m, 4H), 1.38 (m, 8H), 1.29 (m, 8H) ppm. ^{13}C NMR (100 MHz, CDCl_3 , $25\text{ }^\circ\text{C}$): δ 150.62, 146.71, 129.23, 128.96, 113.81, 113.56, 69.09, 56.17, 33.73, 32.65, 30.1, 29.68, 28.69, 25.91 ppm.

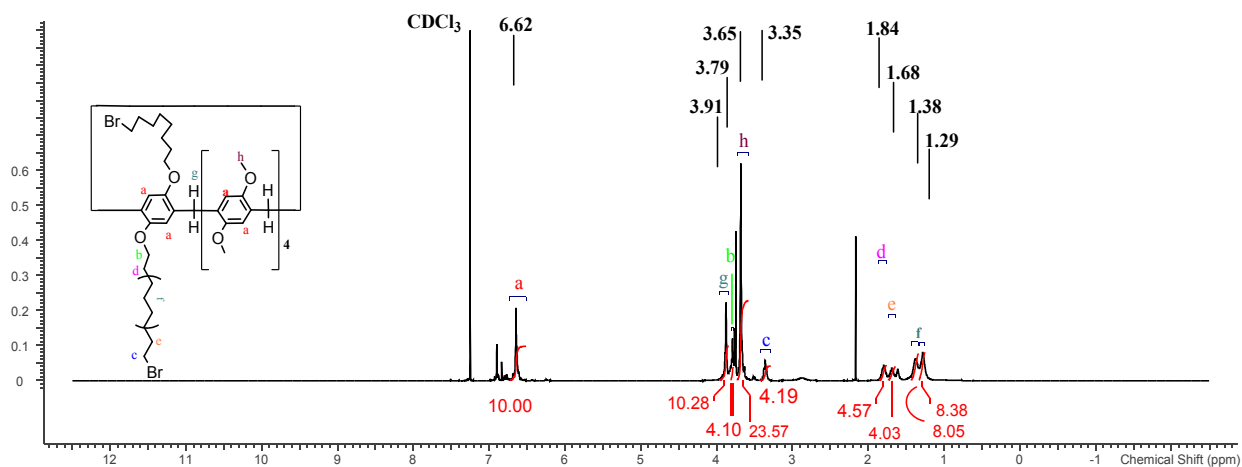


Figure S2. ^1H NMR spectrum (400 MHz, chloroform-d, room temperature) of co-pillar[4+1]arene **3** by microwave irradiation.

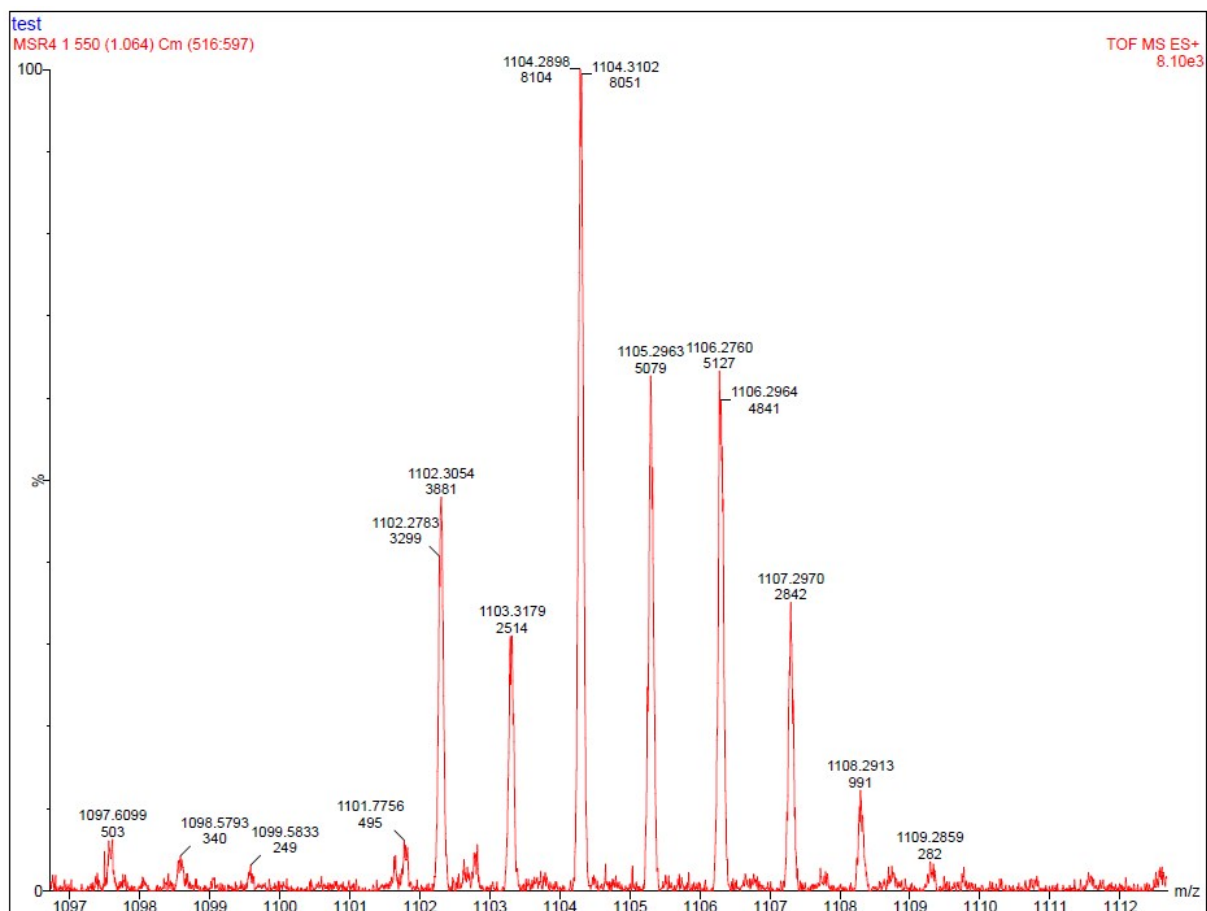
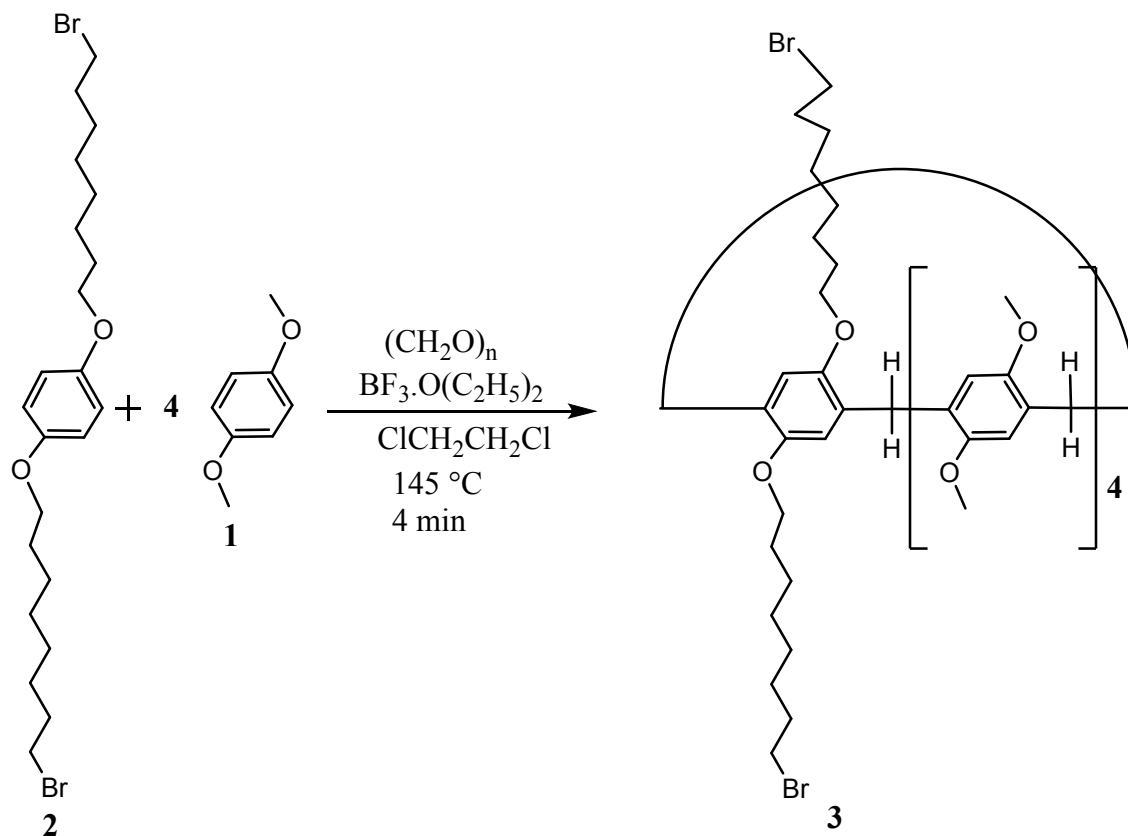


Figure S3. Direct Infusion Electrospray ionization mass spectrum of co-pillar[4+1]arene **3** in chloroform. m/z calculated for $\text{C}_{59}\text{H}_{76}\text{O}_{10}\text{Br}_2$ $[\text{M}+\text{H}]^+$: 1104.38; found: 1104.2898 along with other isotopes 1102.3054, 1103.3179, 1105.2963, 1106.2760, 1107.2970 and 1108.2913,

C.2. Synthesis of co-pillar[4+1]arene **3** by a conventional condensation method



Scheme 3: Synthesis of co-pillar[4+1]arene **3** by a condensation method

A mixture of 1,4-bis((8'-bromooctyl)oxy)benzene (3.68 g, 0.0074 mol), 1,4-dimethoxybenzene (4.089 g, 0.0296 mol), 1,2-dichloromethane (*ca.* 50 mL) and paraformaldehyde (1.111 g, 0.0370 mol) was added to a 250 mL round bottom flask under nitrogen. $\text{BF}_3 \cdot \text{O}(\text{C}_2\text{H}_5)_2$ (4.57 mL, 0.0370 mol) was then injected. After the reaction mixture was stirred for 4 h at room temperature, the solvent was evaporated *in vacuo*. The solid residue was triturated with acetone to give a white solid product **3**. yield 2.417 g (0.002192 moles, 26.3%). The ^1H NMR spectrum of **3** is shown in Figure S4 (400 MHz, CDCl_3) δ 6.63 (m, 10H), 3.89 (m, 10H) 3.77 (m, 4H), 3.65 (m, 24H), 3.41 (m, 4H), 1.82 (m, 4H), 1.65 (m, 4H), 1.37 (m, 8H), 1.29 (m, 8H) ppm. ^{13}C NMR (100 MHz, CDCl_3 , 25 $^\circ\text{C}$): δ 150.68, 146.69, 129.21, 128.97, 113.82, 113.58, 69.12, 56.19, 33.72, 32.64, 30.1, 29.67, 28.69, 25.91 ppm. Melting point: 192-193 $^\circ\text{C}$

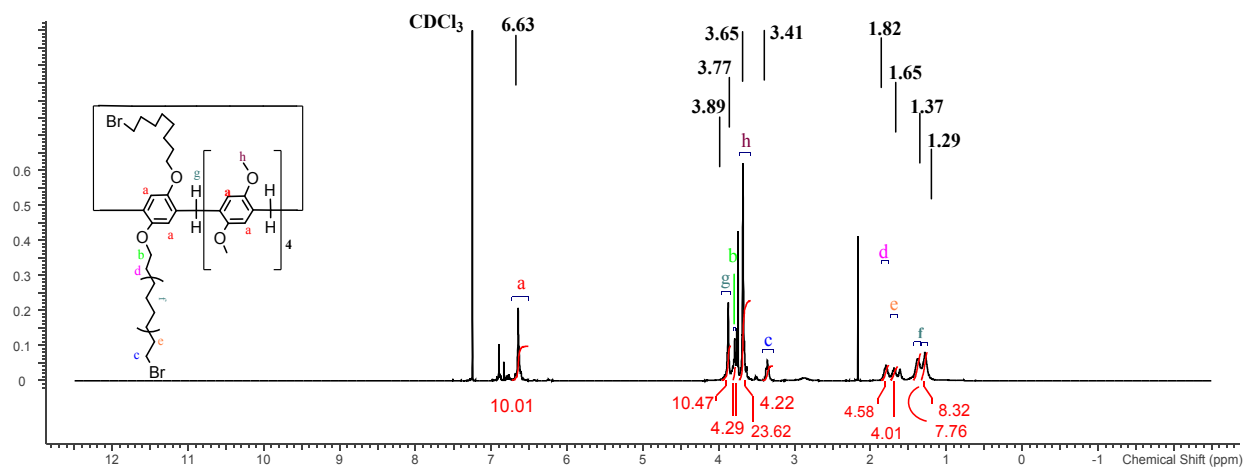


Figure S4. ¹H NMR spectrum (400 MHz, chloroform-d, room temperature) of copillar[4+1]arene **3** by conventional condensation method.

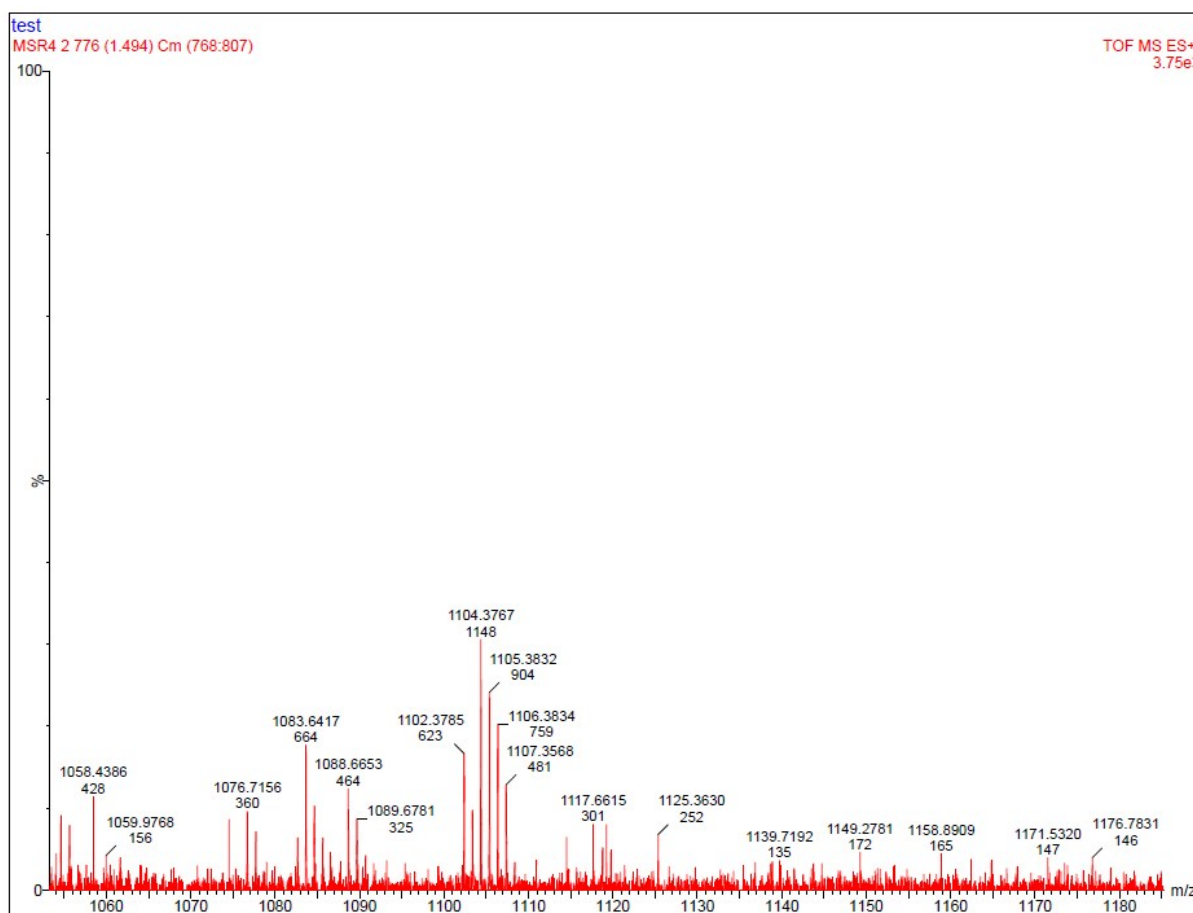


Figure S5. Direct Infusion Electrospray ionization mass spectrum of copillar[5]arene **3** in chloroform. *m/z* calculated for C₅₉H₇₆O₁₀Br₂ [M+H]⁺: 1104.38; found: 1104.3767 and its isotopes 1105.3832, 1106.3834, 1107.3568, 1102.3785 and 1103.3689.

D. Synthesis of co-pillar[4+1]arene bonded silica gel stationary phase (compound 4)

Interchim® flash (50 µm) silica was stirred overnight in a basic solution of tetrahydrofuran and triethylamine (1:1) and filtered under vacuum until dry. The activated silica was then capped with the co-pillar[4+1]arene **3** by stirring overnight in a solution of acetone and triethylamine. The stationary phase was then separated from the solution by vacuum filtration, washed with water/methanol and allowed to dry at room temperature. A 10 µg sample of the silica functionalized co-pillar[5]arene **4** was removed from the sample and analysed by thermogravimetric analysis and the results of which indicate a mass loading of the co-pillar[4+1]arene **3** at 16% w/w for the flash stationary phase with a 50 µm silica particle size.

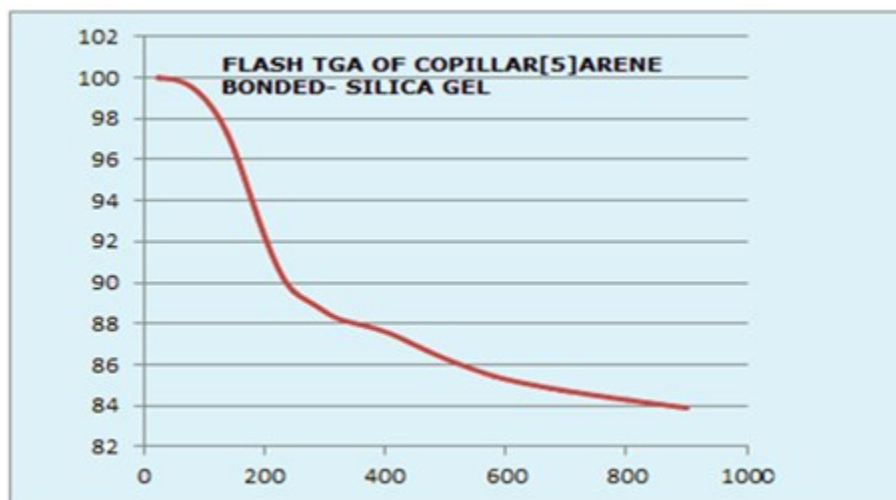


Figure S6. TGA analysis of co-pillar[4+1]arene silica bound **4** silica bound stationary phase for flash column chromatography.

E. Flash Column Conditioning

Co-pillar[4+1]arene bonded-silica gel **4** was dry-packed into an empty flash column and primed with cyclohexane (100%), ethyl acetate (100%) and finally with methanol (100%). The flash column eluent was collected to run mass spectrometry to identify elution of co-pillar[5]arene from the silica-bound co-pillar[5]arene. There was no observation of co-pillar[4+1]arene eluting from the column even after 1000 column volumes.

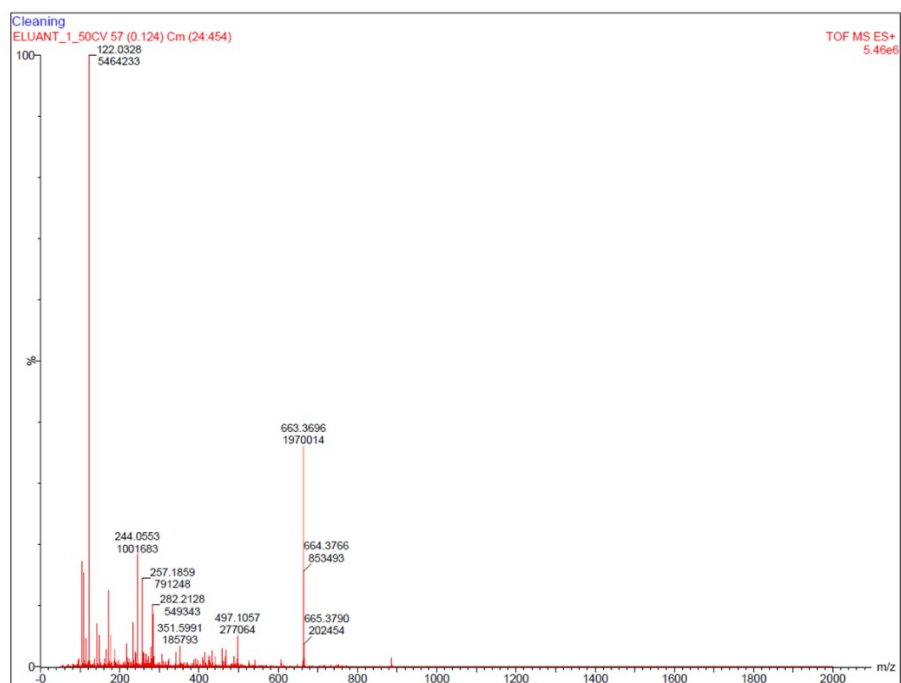


Figure S. LC-MS spectrum of eluent from co-pillar[4+1]arene bonded-silica flash column at 50 column volumes.

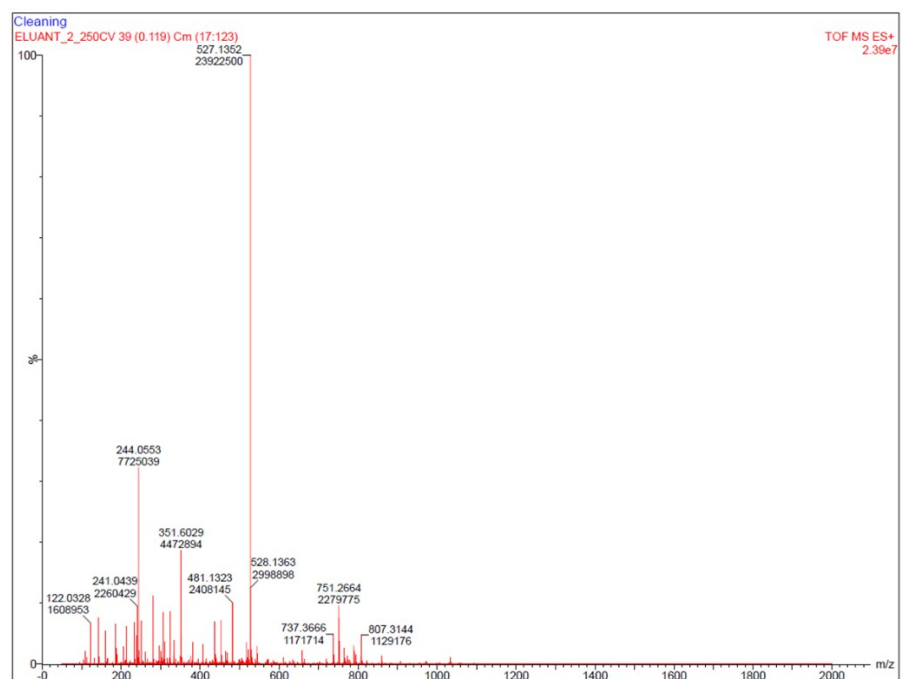


Figure S. LC-MS spectrum of eluent from co-pillar[4+1]arene bonded-silica flash column at 250 column volumes.

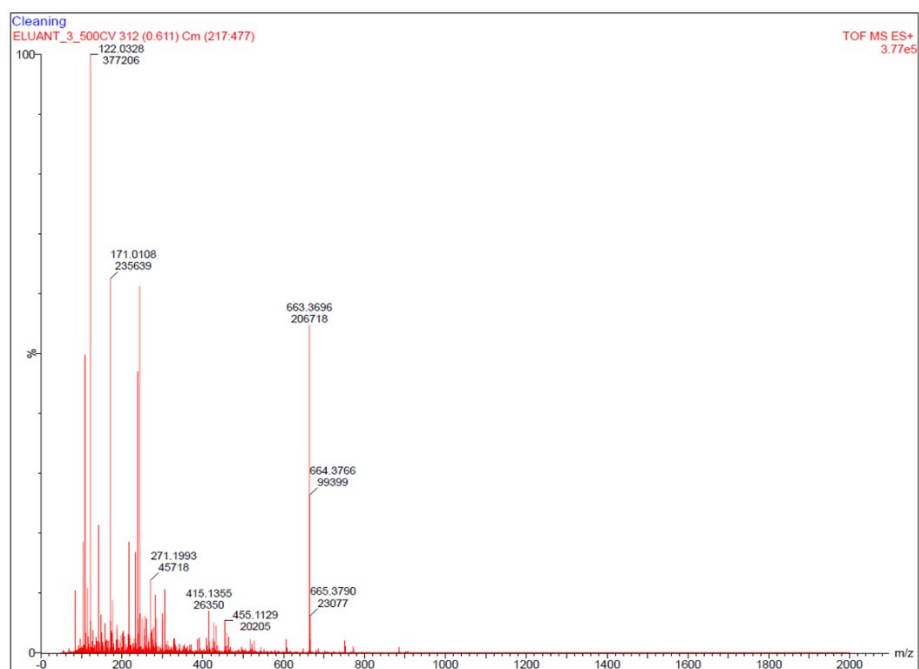


Figure S. LC-MS spectrum of eluent from co-pillar[4+1]arene bonded-silica flash column at 500 column volumes.

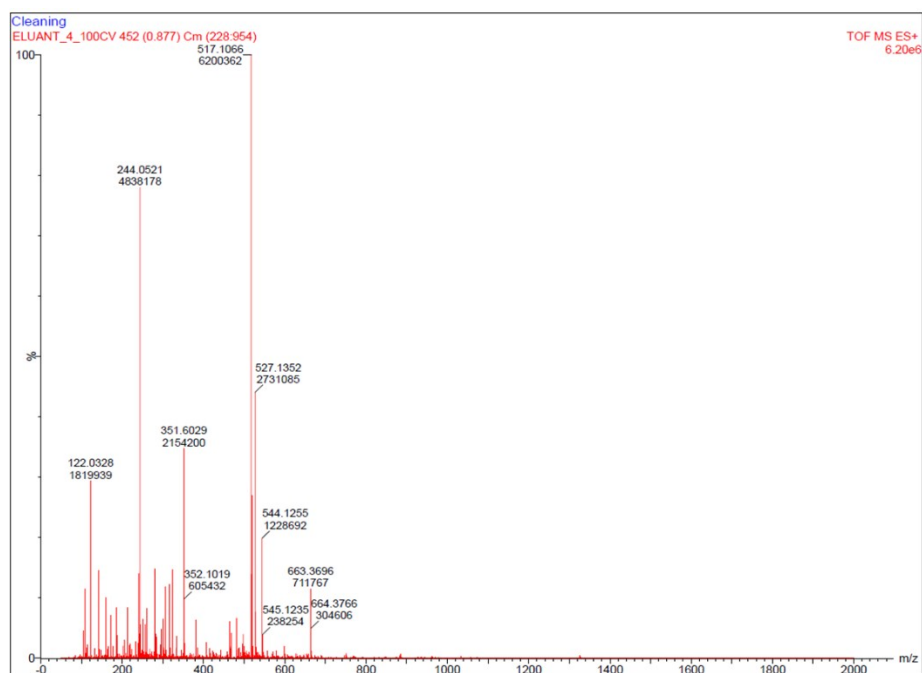


Figure S. LC-MS spectrum of eluent from co-pillar[4+1]arene bonded-silica flash column at 1000 column volumes.

F. Separation of xylene isomers and toluene on co-pillar[4+1]arene bonded-silica gel stationary phase 4 by flash column chromatography

Co-pillar[4+1]arene bonded-silica gel stationary phase **4** was dry packed into an empty flash column cartridge and primed with cyclohexane (100%), ethyl acetate (100%) and finally with methanol (100%). The analytes was prepared by dissolving in methanol: ethyl acetate (1:1) ratio. The mobile phase used for the analysis was methanol and ethyl acetate in isocratic conditions. The flow rate was set to 21 mL/min and UV detection was set to 200-800 nm. Fraction collection volume was set to 13 mL. The column was equilibrated by in-built optimization conditions depending on the column and particle size. Flash column chromatography automated PuriFlash®5.125 flash system on both normal phase and co-pillar[4+1]arene bonded-silica phase were carried out under the same conditions, using automated PuriFlash®5.125 flash system. The flash column fractions were collected and characterized using ¹H-NMR. The flash column chromatography was carried out at room temperature.

F.1 Separation of individual xylene isomers and toluene on co-pillar[4+1]arene bonded-silica gel stationary phase 4 using flash column chromatography

Analyte: a. *m*-xylene

b. toluene

c. *o*-xylene

d. *p*-xylene

Flow rate: 21 mL/min

Mobile Phase: methanol: ethyl acetate (10:90)

UV detection: 200-800 nm

Injection volume: 2 mL

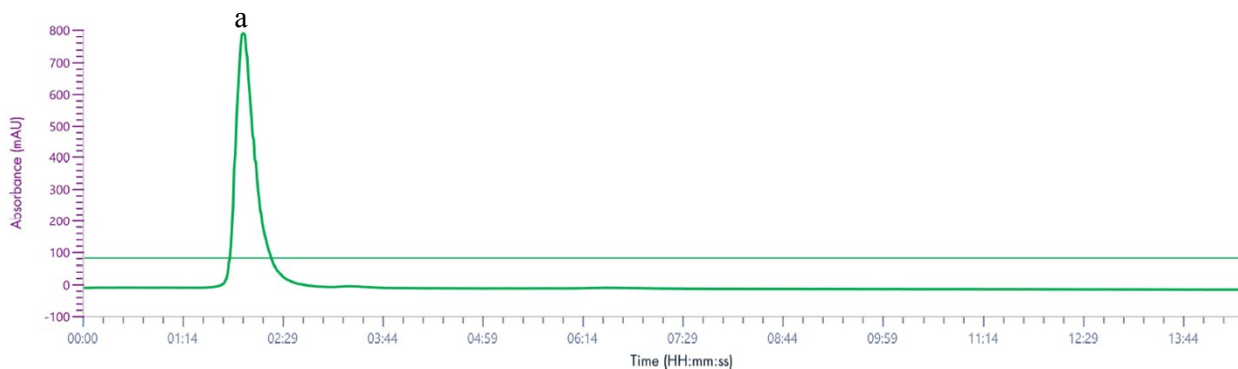


Figure S8. Separation of *m*-xylene on co-pillar[4+1]arene bound silica flash column cartridge.

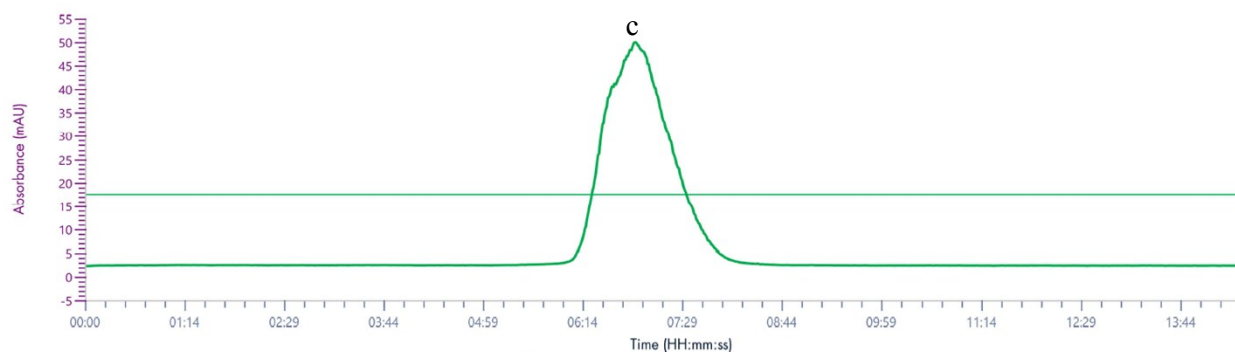


Figure S9. Separation of *o*-xylene on co-pillar[4+1]arene bound silica flash column cartridge.

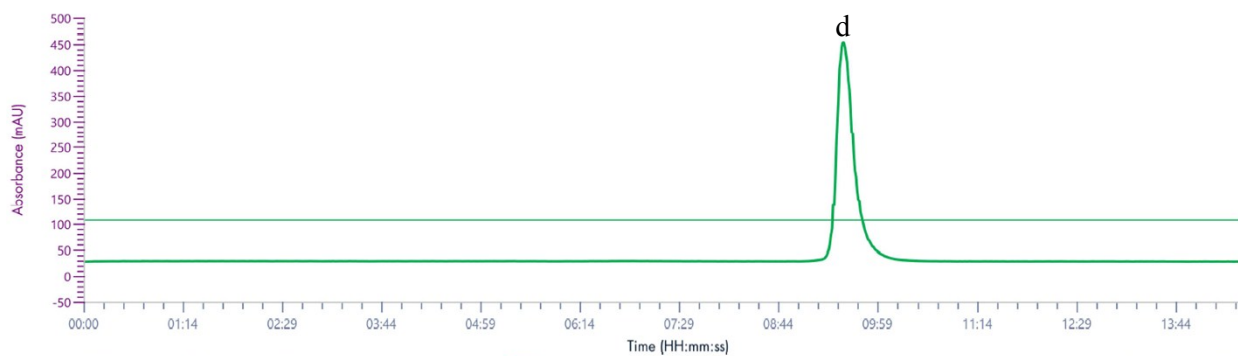


Figure S10. Separation of *p*-xylene on co-pillar[4+1]arene bound silica flash column cartridge.

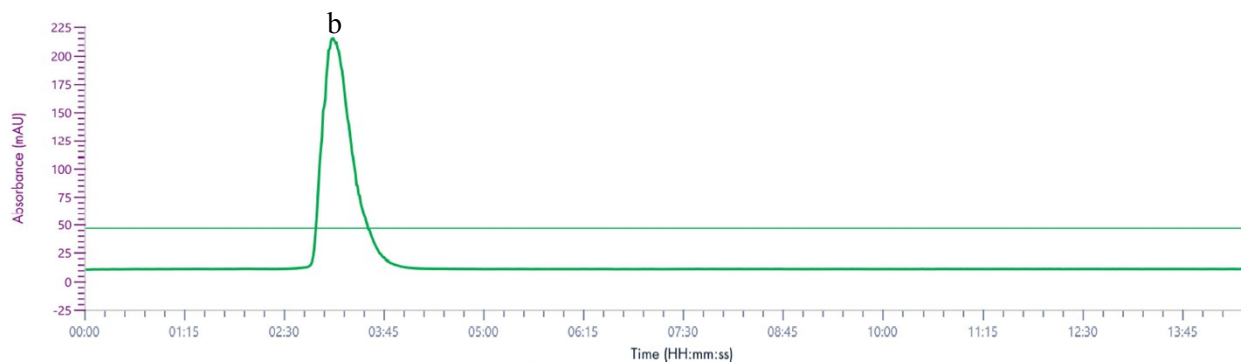


Figure S11. Separation of toluene on co-pillar[4+1]arene bound silica flash column cartridge.

G. Validation of co-pillar[4+1]arene bonded-silica gel stationary phase 4 using Fisher Scientific's xylene and toluene, in comparison with normal phase silica column.

G.1 Separation of Fisher Scientific's xylene and toluene on normal phase flash column chromatography

Analyte: Fisher's xylene and toluene (CAS no: 1330-20-7 and CAS number:108-88-3)

Flow rate: 21 mL/min

Mobile Phase: methanol: ethyl acetate (10:90)

UV detection: 200-800 nm

Injection volume: 2 mL

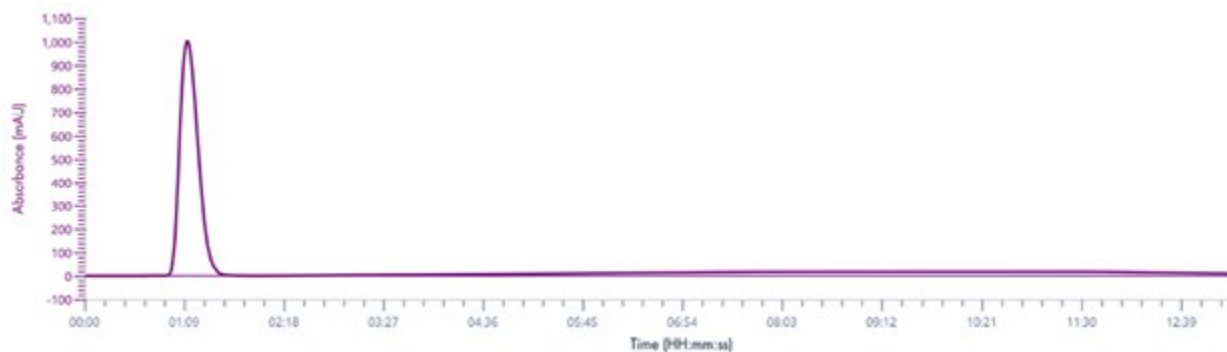


Figure S7. Separation of Fisher Scientific's xylene and toluene mixture on normal phase silica flash column cartridge.

G.2 Separation Fisher Scientific's xylene on co-pillar[4+1]arene bonded-silica flash column chromatography

Analyte: Fisher's Scientific xylene and toluene mixture

Flow rate: 21 mL/min

Mobile Phase: methanol: ethyl acetate (10:90)

UV detection: 200-800 nm

Injection volume: 2 mL

Identified fractions by $^1\text{H-NMR}$:

- a. *m*-xylene
- b. toluene
- c. *o*-xylene,
- d. *p*-xylene,
- e. ethylbenzene

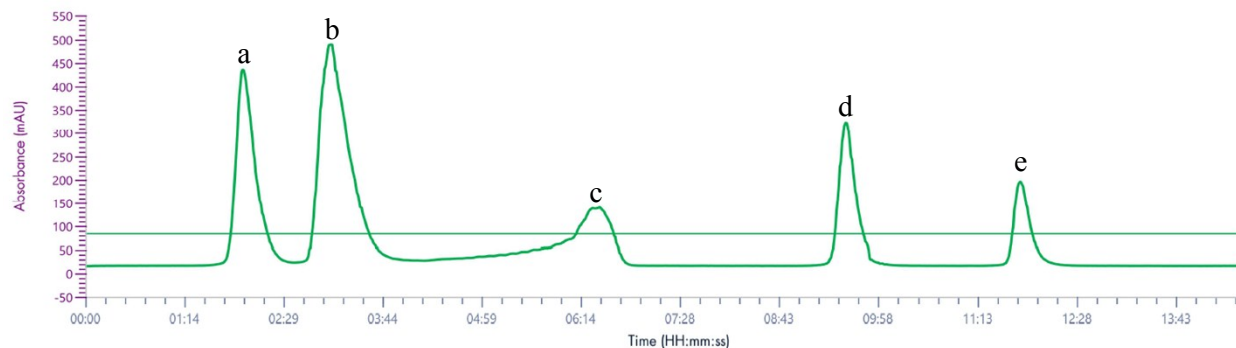


Figure S13. Separation Fisher Scientific's xylene and toluene mixture on co-pillar[4+1]arene bound silica flash column cartridge.

H Characterization of xylene flash column fraction by ^1H NMR

H.1. Characterization of fractions of individual xylene isomers and toluene from silica- bound co-pillar[4+1]arene flash column.

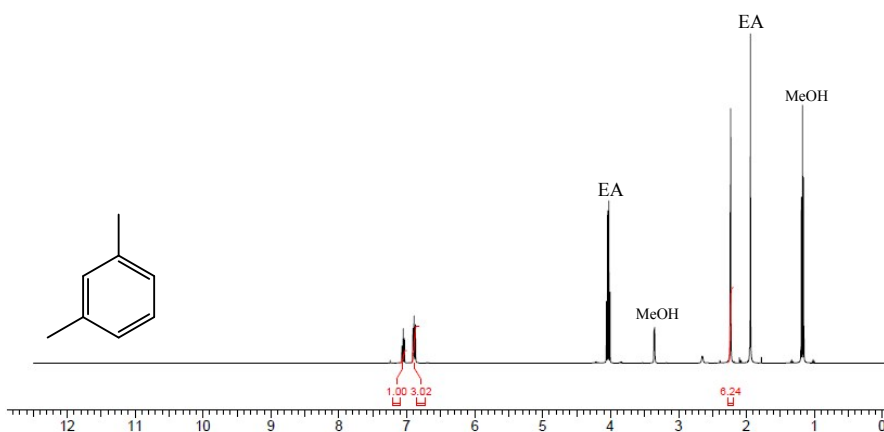


Figure S15. ^1H NMR spectrum (400 MHz, chloroform- d , room temperature) of *m*-xylene fraction from co-pillar[4+1]arene stationary phase.

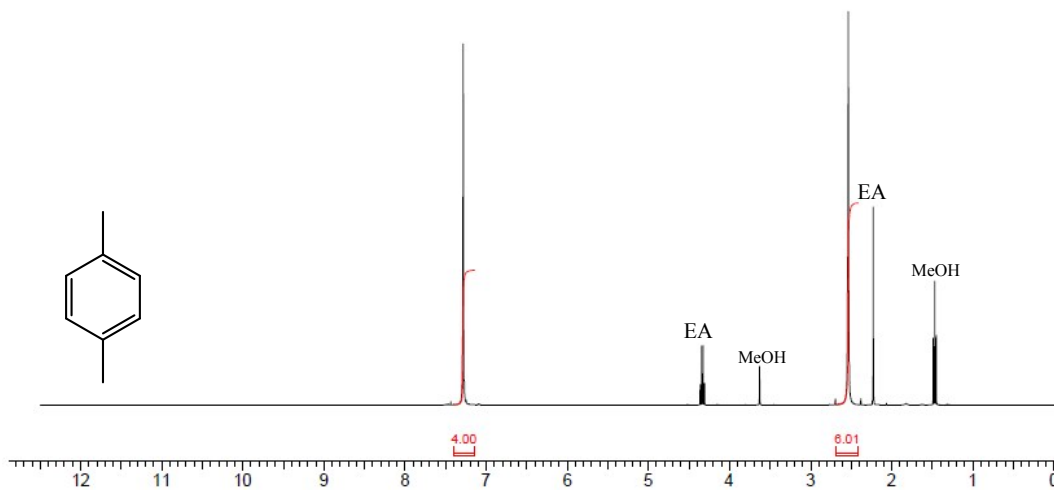


Figure S11. ^1H NMR spectrum (400 MHz, chloroform-d, room temperature) of *p*-xylene fraction from co-pillar[4+1]arene stationary phase.

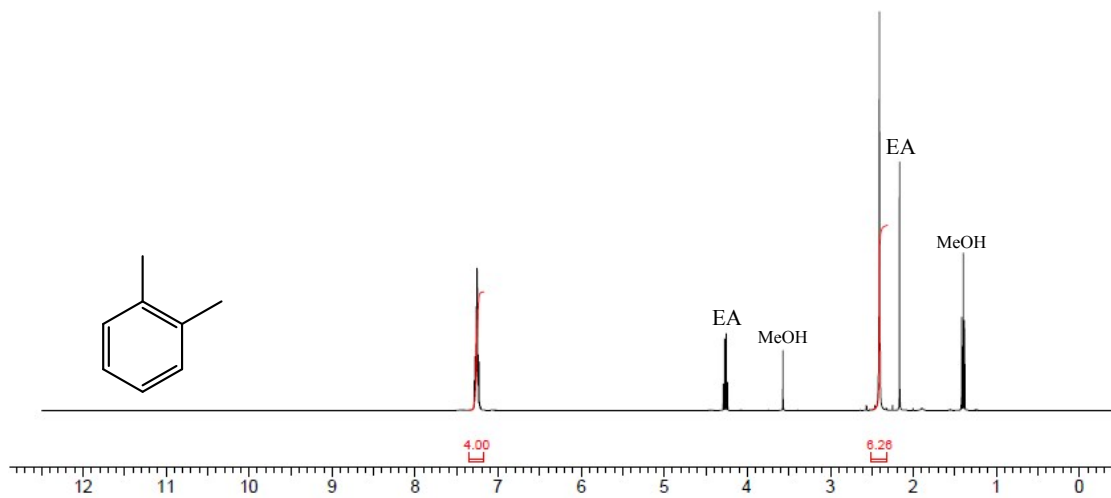


Figure S12. ^1H NMR spectrum (400 MHz, chloroform-d, room temperature) of *o*-xylene fraction from co-pillar[4+1]arene stationary phase.

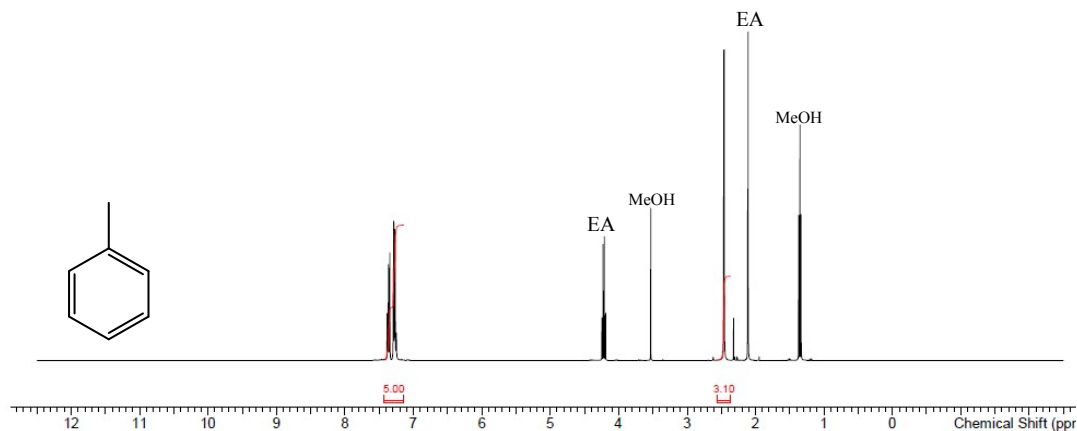


Figure S13. ¹H NMR spectrum (400 MHz, chloroform-d, room temperature) of *toluene* fraction from co-pillar[4+1]arene stationary phase.

H.3. Characterization of fractions of Fisher Scientific's xylene and toluene mixture from normal phase and silica-bound co-pillar[4+1]arene flash column.

H.3.1 Characterization of Fisher Scientific's xylene fraction from normal phase flash column

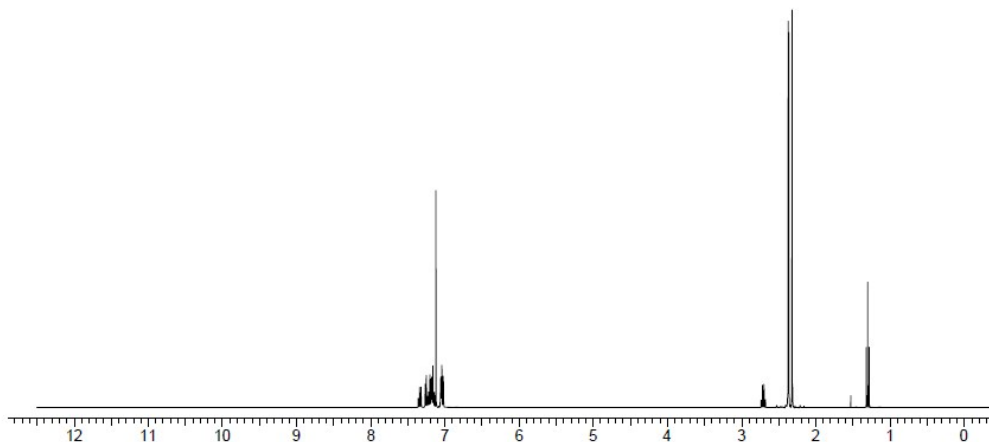


Figure S1. ¹H NMR spectrum (400 MHz, chloroform-d, room temperature) of xylene mixture fraction from normal phase.

H.3.2 Characterization of Fisher Scientific's xylene fraction from silica-bound co-pillar[4+1]arene flash column

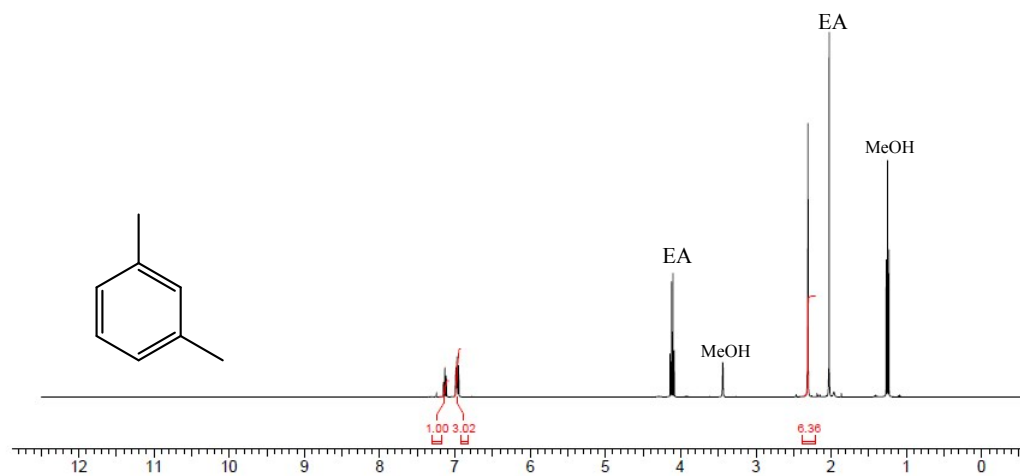


Figure S16. ^1H NMR spectrum (400 MHz, chloroform- d , room temperature) of fraction 1 from the mixture of Fisher Scientific's xylene and toluene and confirmed as *m*-xylene

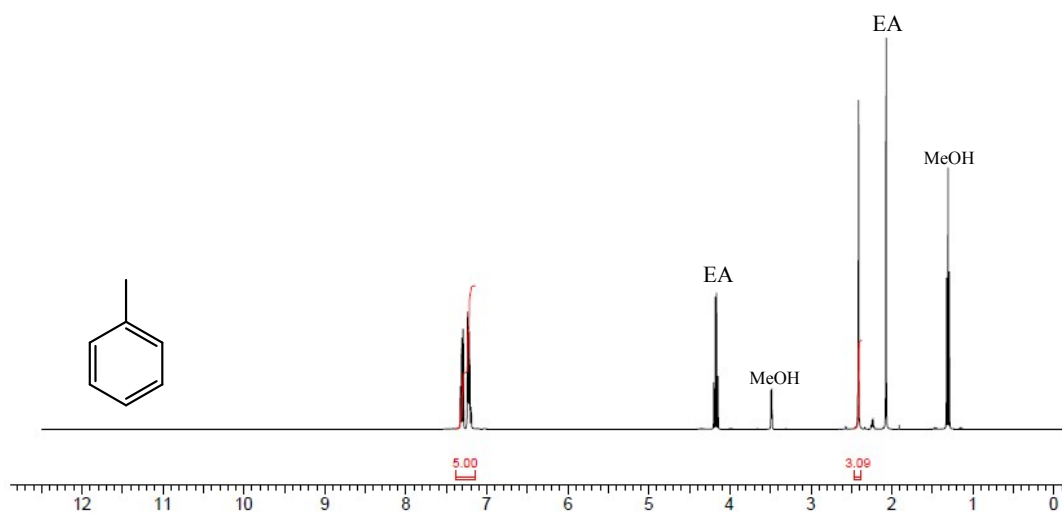


Figure S17. ^1H NMR spectrum (400 MHz, chloroform- d , room temperature) of fraction 2 from the mixture of Fisher Scientific's xylene and toluene and confirmed as toluene.

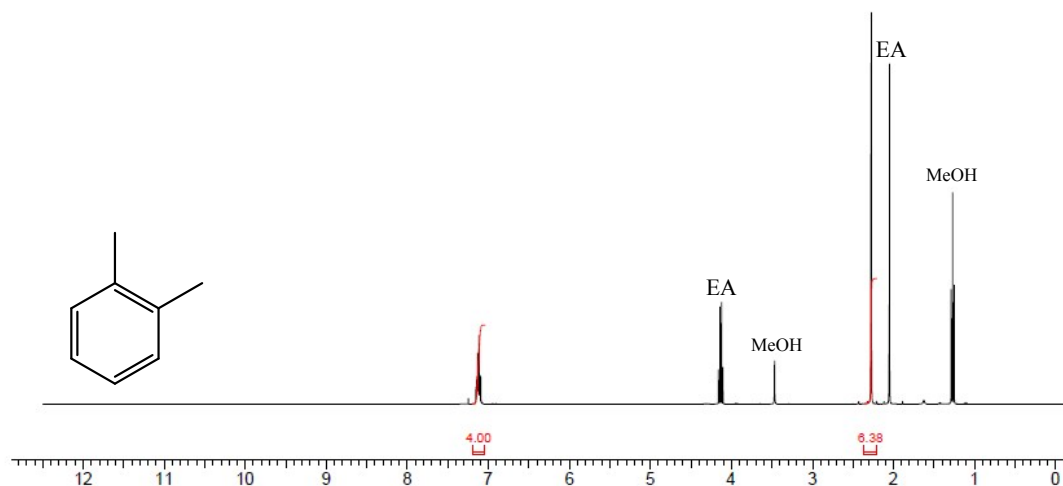


Figure S18. ^1H NMR spectrum (400 MHz, chloroform- d , room temperature) of fraction 3 from the mixture of Fisher Scientific's xylene and toluene and confirmed as *o*-xylene.

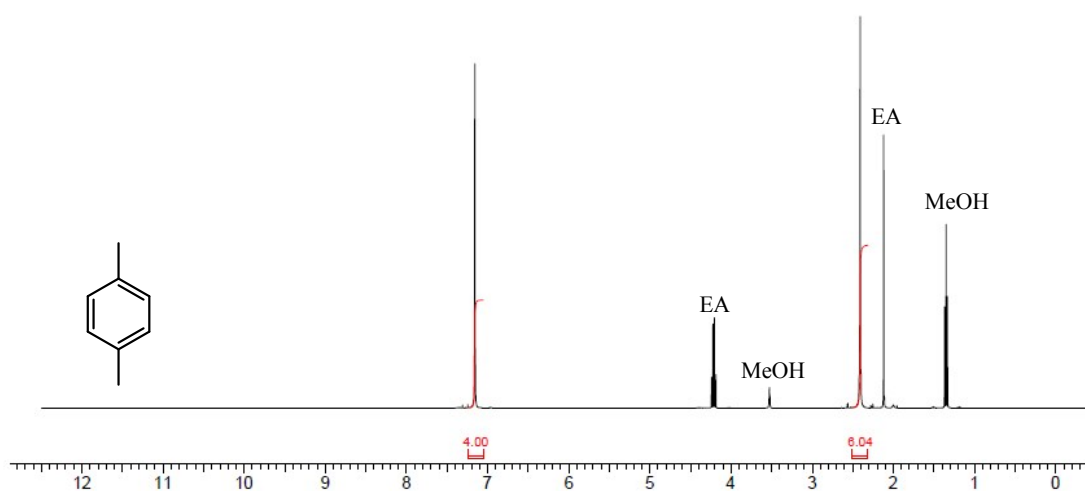


Figure S19. ^1H NMR spectrum (400 MHz, chloroform- d , room temperature) of fraction 4 from the mixture of Fisher Scientific's xylene and toluene and confirmed as *p*-xylene.

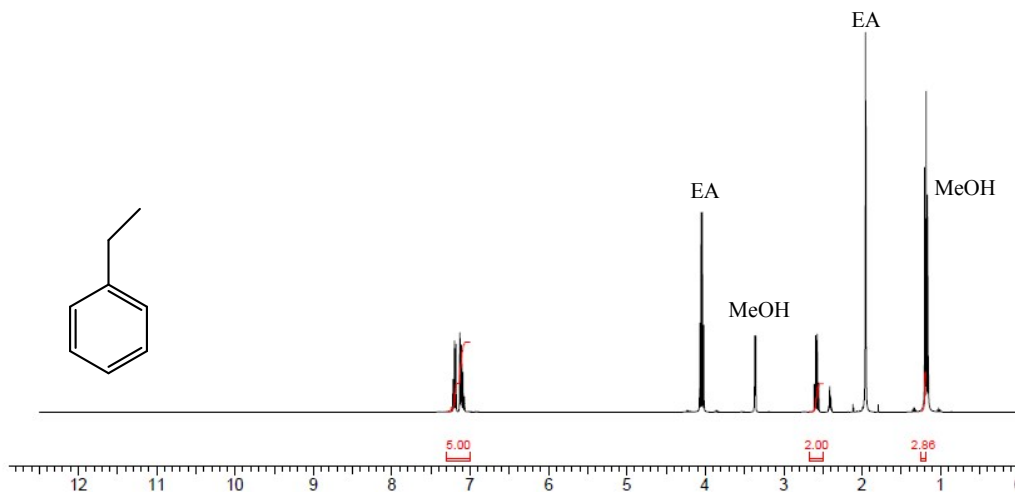


Figure S20. ^1H NMR spectrum (400 MHz, chloroform- d , room temperature) of fraction 5 from the mixture of Fisher Scientific's xylene and toluene and confirmed as ethylbenzene.

I. Computational studies

The structure of a single molecule of the dimethoxy-pillar[5]arene was extracted from a crystal structure.³ 500 random geometries each of ethylbenzene \square dimethoxy-pillar[5]arene, toluene \square dimethoxy-pillar[5]arene, *ortho*-xylene \square dimethoxy-pillar[5]arene, *meta*-xylene \square dimethoxy-pillar[5]arene and *para*-xylene \square dimethoxy-pillar[5]arene were generated using Kick^{3,4} and optimised using Density Functional Tight Binding (DFTB) as implemented in DFTB+ 1.3. DFTB calculations employed the 3ob-3-1 parameter set⁵ and DftD3 dispersion. The lowest energy structures of each guest \square dimethoxy-pillar[5]arene were reoptimized using TPSSTPSS/Def2TZVP in Gaussian 16.⁶

J. References

1. J. Chang, Q. Zhao, L. Kang, H. Li, M. Xie and X. Liao, *Macromolecules*, 2016, **49**, 2814-2820.
2. Z. Zhang, B. Xia, C. Han, Y. Yu and F. Huang, *Org. Lett.*, 2010, **12**, 3285-3287; L. Liu, D. Cao, Y. Jin, H. Tao, Y. Kou and H. Meier, *Org. & Biomole. Chem.*, 2011, **9**, 7007-7010.
3. T. Ogoshi, S. Kanai, S. Fujinami, T. Yamagishi and Y. Nakamoto, *J. Am. Chem. Soc.*, 2008, **130**, 5022-5023
4. M. A. Addicoat, S. Fukuoka, A. J. Page, S. Irle, *J. Comput. Chem.*, 2013, **30**, 2591-2600.
5. M. Gaus, A. Goez, M. Elstner, *J. Chem. Theory Comput.*, 2012, **9**, 338-354.
6. Gaussian 16, Revision A.02, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. V. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. J. Bearpark, J. J. Heyd, E. N. Brothers, K. N. Kudin, V. N. Staroverov, T. A. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. P. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2016.