Synthesis, characterization, and chromatographic evaluation of polyether stationary phases

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Experimental conditions

Additional Synthetic conditions

Ultrasound reactions were conducted by placing the reaction flask in a water-filled Ultrasonik 28x cleaner, manufactured by Ney Dental Inc., Connecticutt, USA, and irradiating with a frequency of 45-49 kHz.

Thin-layer chromatography (TLC) was carried out using silica gel (60 F254) on aluminium sheets, purchased from Merck KGaA, Darmstadt, Germany and visualised using an ultraviolet light source at 254 or 365nm as appropriate. The solvent systems are detailed below for each reaction mixture.

Column chromatography was performed using silica gel 60, 220-440 mesh (0.035-0.070mm diameter) obtained from Fluka (a subsidiary of Sigma-Aldrich).

Sample Characterisation

1H NMRs were run on a Bruker-Spectrospin 300MHz spectrometer and processed with Bruker Xwin-NMR software.

Solid-state 13C and 29Si NMR measurements were performed in the group of Professor Klaus Albert and co-workers at the Institut für Organische Chemie, Tübingen, Germany on a Bruker ASX 300 spectrometer (29Si) and on a Bruker DSX 200 spectrometer (13C), using cross polarization and magic-angle spinning (CP/MAS). For the 29Si nucleus, a contact time of 5 ms and a pulse repetition time of 1.5 s were employed. For 13C, the contact time was 3 ms and the repetition time 2 s. Representative samples for 29Si measurements of 200 to 250 mg were spun at 4 kHz using 7-mm double bearing ZrO2 rotors (for 13C: ca. 150 mg in 4mm rotors at a spinning rate of 10 kHz). Typically 60-80k for 13C and 6k transients for 29Si were recorded at
room temperature in total. All spectra were multiplied by an exponential line broadening function of 30 Hz prior to Fourier transformation. Spectrum processing was performed using Bruker TOPSPIN 2.0 software. Numerical values are detailed in tables 3 and 4.

Microanalyses were performed by the UCC Microanalytical Laboratory using a CE440 elemental analyzer (Exeter Analytical Inc., North Chelmsford, Mass, USA). Infra–red and Diffuse Reflectance Infra–red Fourier Transform (DRIFT) spectra were run on a Bio–rad Excalibur Series IR spectrometer, using 32 scans per sample at resolution 16, and processed using Merlin version 2.97 software in a plot of wavenumber against % transmittance. Silica samples were run in DRIFT mode, mixed with KBr, liquid samples were run using NaCl plates while organic solids were run as KBr discs.

Karl-Fischer titrations were carried out on a 684 KF-coulometer from Mettromh AG, Herisau, Switzerland.

Thermogravimetric data was collected on a Star® TGA/DSC1 instrument, purchased from Mettler-Toledo AG, Switzerland, over a temperature range from 30 to 900°C at a heating rate of 40°C/min under a nitrogen flow of ca. 25 ml/min and analysed using Star® Excellence software.

Modelling of dendrimer conformations was carried out by Dr. Jean-Marie Prat using Hyperchem software, version 7.52, created by Hypercube, Inc., Gainesville, Florida, USA.

### Chromatographic conditions

Deionised water was prepared on a Milli-Q water purification system (Millipore, Milford, Mass, USA) to a resistivity of 18.2 MΩ/cm. Chromatography was performed on an Agilent 1100 series LC connected to a quaternary pump and diode array detector. The chromatographic system was manufactured by Agilent Technologies Deutschland GmbH, Waldbronn, Germany. Spectral data was typically collected at 254nm (bandwidths 4 and 16nm), 210, 230 and 280nm (bandwidth 8, 16 and 16nm respectively) and analysed using Agilent’s Chemstation software. Test conditions are as outlined in tables 1 and 2. Residual air was removed from mobile phases before use by sonication for ca. 5 minutes at 42Hz (approx.). Peak identity was confirmed by injection of the individual standard solutes, if required.

Kinetic plot data was calculated using the kinetic plot method template supplied by Desmet and co-workers [1]. The calculated maximal pressure was determined by the system’s operational cut-off pressure of $4 \times 10^7$ Pa. An arbitrary value of $10^{-3}$ kg s m$^{-1}$ was used for viscosity. While this may differ slightly from the true experimental value, it should not affect the relative performance ranking of the columns.

Values for column efficiency and resolution were calculated automatically by the Chemstation software. Tailing factors were derived manually using the relevant USP method at 5% of maximum peak height.

$$\text{TF} = \frac{W_{\text{total}}}{2W_{\text{front}}} = \frac{c-a}{2(b-a)} \quad \text{Equation 1}$$

### Synthesis of Frechet polyethers

The naming convention, outlined in figure 1, is used for dendrimers in generations 1-3 with the prefix 1.,2. or 3. indicating the appropriate generation of each structural type.

Figure S1: Synthesis route towards 1st and 2nd generation polyethers
Preparation of first generation dendrimer

Preparation of 3,5-dihydroxy methyl benzoate, di n-hexyl ether (1.2)

Methyl 3,5-dihydroxybenzoate ([1.1], 5.2mmol, 1eq); potassium carbonate (1.84g, 2.6eq); 18-crown-6 (0.28g, 0.2eq)\(^1\), and 1-bromohexane (1.5ml, 2.05eq) were mixed together in dry THF (10ml) under nitrogen and stirred at reflux overnight. TLC: SiO\(_2\) (1:1 v/v EtOAc:hexane) shows (1.2) with faint traces of (1.1) and of monoalkylated (1.1). The mixture was concentrated \textit{in vacuo} and the resultant white solid separated between DCM (10ml) and DI water (10ml). The aqueous phase was washed with DCM (10ml) and the combined organics concentrated to give a yellow liquid (2g) which was then columned on silica, eluting with hexane followed by 2:1 hexane:EtOAc, to give the desired product as a yellow liquid in 98\%th recovery.

TLC: SiO\(_2\) (heptane) \(R_f\) 0.4; visualisation by uv at 254 nm; TLC: SiO\(_2\) (1:1 v/v EtOAc:heptane) \(R_f\) 0.8; \(R_f\) (1.1) 0.4; \(R_f\) (monoalkylated 1.1) 0.6; visualisation by uv at 254 nm.

\(^1\)H NMR (CDCl\(_3\)): 0.83-0.86 (t, 6H), 1.20-1.40 (m, 12H), 1.66-1.75 (m, 4H), 3.82 (s, 3H), 3.89-3.99 (t, 4H), 6.56-6.57 (t, 1H), 7.08-7.09 (d, 2H).

\(^1\)H NMR of monoalkylated product (CDCl\(_3\)): 0.82-0.85 (t, 3H), 1.20-1.40 (m, 6H), 1.67-1.72 (m, 2H), 3.82 (s, 3H), 3.85 (t, 2H), 5.60 (s, br, 1H), 6.57 (s, 1H), 7.06 (s, 2H).

IR (NaCl plates) found stretches at 2954, 2925, 2856, 1726, 1597 cm\(^{-1}\).

Preparation of 3,5-dihydroxy methyl benzoic acid, di n-hexyl ether (1.3)

5.05mmol (1.2) was dissolved in THF (25ml). A solution of potassium hydroxide (1.5eq) in DI water (4ml) was added followed by MeOH\(^2\) (8ml). TLC shows reaction complete within 2 hours. The mixture was concentrated to low volume \textit{in vacuo} and partitioned between 1N HCl (30ml) and DCM (20ml). The aqueous phase was extracted with fresh DCM (20ml) and the combined organics concentrated \textit{in vacuo} to give (1.3) as a white solid in 93\%th yield.

TLC: SiO\(_2\) (1:1 v/v hexane:EtOAc) \(R_f\) 0.6; \(R_f\) (1.2) 0.75; visualisation by uv at 254 nm, (1.2) was also visible at 365nm (faint blue by uv).

Microanalysis found: C 70.62\%; H 9.36 \%w/w

C\(_{19}\)H\(_{30}\)O\(_4\) requires: C 70.81\%; H 9.32 \%w/w

\(^1\)H NMR (CDCl\(_3\)): 0.82-0.86 (t, 6H), 1.24-1.29 (m, 12H), 1.32-1.35 (m, 4H), 3.82 (s, 3H), 3.89-3.94 (t, 4H), 6.60 (s, br, 1H), 7.15 (s, 2H).

Preparation of 3,5-dihydroxy N-allyl benzamide, di n-hexyl ether (1.4)

The acid (1.3), (1.44g, 4.47mmol) was dissolved in dry THF (5ml). CDI (784mg, 4.84mmol, 1.08eq) was added and the solution stirred for 1 hour. TLC shows a new high running spot (visualisation at 254nm). Allylamine (0.35ml, 4.67mmol, 1.05eq) was added and the mixture stirred for 3 hours. The phases were separated and organic phase washed successively with 1N NaOH (10ml); 1N HCl (10ml); DI water (10ml). The organics were concentrated \textit{in vacuo} to give initially a yellow liquid which on standing solidified to a pale yellow solid (1.5g, 94\%th).

Microanalysis found: C, 72.52; H 9.68; N 3.97 \%w/w

C\(_{22}\)H\(_{35}\)NO\(_3\) requires: C, 73.13; H 9.70; N 3.88 \%w/w

\(^1\) Mixture develops orange tint on addition of crown ether.

\(^2\) This promoted phase mixing – the original reaction mixture (pre-MeOH) was cloudy.
\( ^1 \)H NMR (CDCl\(_3\)): 0.88-0.93 (t, 6H), 1.34-1.35 (m, 8H) 1.40-1.47 (m, 4H), 3.94-3.99 (m, 4H), 4.05-4.09 (t, 2H), 5.17-5.21 (dd, 2H), 5.23-5.29 (dd, 1H), 6.13, (br, s, 1H), 6.56-6.57 (t, 1H), 6.87-6.88 (d, 2H).

**Preparation of 3,5-dihydroxy benzylic alcohol, di n-hexyl ether (1.5)**

KBH\(_4\) (4.99g, 3.1eq) and LiCl (1.41g, 1.1eq) were placed in a dry flask under N\(_2\) atmosphere. Dry THF (30ml) was cautiously added, followed by the ester (1.2)(11.4g, 30mmol). The mixture was warmed to reflux – tlc after 6hrs showed ca. 30% conversion but no change on reflux for a further 18 hours. The mixture was cooled to room temperature and further charges of KBH\(_4\) (4.99g, 2.9eq) and LiCl (1.39g, 1.1eq) made. After a further 12 hours of reflux, reaction was complete. The mixture was cooled and cautiously quenched with DI water (40ml) followed by 1N HCl (30ml). Once effervescence was complete, the phases were separated and the aqueous extracted with EtOAc (30ml). The combined organics were washed (DI water, 10ml), dried (MgSO\(_4\)) and concentrated \textit{in vacuo} to give a yellow liquid in near quantitative yield.

Tlc: SiO\(_2\) (2:1 v/v EtOAc:hexane) shows \( R_f \) (1.2) 0.7, \( R_f \) (1.5) 0.5; visualisation by uv at 254nm.

\( ^1 \)H NMR (CDCl\(_3\)): 0.83-0.85 (t, 6H), 1.23-1.27 (m, 8H), 1.36-1.40 (m, 4H), 1.62-1.71 (m, 4H), 3.84-3.88 (m, 4H), 4.54 (s, 2H), 6.29-6.31 (t, 1H), 6.42-6.43 (d, 2H).

IR (NaCl plates) found stretches at 3429 (br), 2954, 2925, 2856, 1738 (weak), 1598 cm\(^{-1}\).

**Preparation of 3,5-dihydroxy benzylalcohol, di n-hexyl ether (1.5) from dihydroxybenzyl alcohol**

3,5-dihydroxybenzyl alcohol ((1.7), 0.965g, 6.9mmol) was dissolved in dry THF (10ml); pre-dried potassium carbonate (2.42g, 2.54eq), 18-crown-6 (0.42g, 0.23eq), and 1-bromohexane (2ml. 2.07eq) were added. The mixture was stirred at reflux under nitrogen for 32 hours – loosing most of the solvent during this time.

The mixture was separated between EtOAc (10ml) and DI water (10ml). The aqueous phase was washed with EtOAc (10ml) and the combined organics washed with water and concentrated to give a brown oil (1.9g) which was then columned on silica, eluting with hexane followed by 25\%v/v EtOAc in hexane, to give the desired product as a yellow liquid in 25\%th.

Tlc: SiO\(_2\) (1:1 v/v EtOAc:hexane) shows \( R_f \) (1.7) 0.2, \( R_f \) (1.5) 0.8; visualisation by uv at 254nm.

\( ^1 \)H NMR (CDCl\(_3\)): 0.83-0.86 (t, 6H), 1.35-1.40 (m, 8H), 1.64-1.74 (quin, 4H), 3.89-3.84 (t, 4H), 4.55 (s, 2H), 6.30-6.32 (t, 1H), 6.37-6.38 (d, 2H).

**Preparation of 3,5-dihydroxy benzyl bromide, di n-hexyl ether (1.6)**

3,5-dihydroxy benzylalcohol, di n-hexyl ether (1.5) (0.98g, 3.18mmol) was stirred in neat PBr\(_3\) (0.5ml, 4.3mmol, 1.66eq) overnight – producing a clear yellow solution. DI H\(_2\)O (5ml – slight fuming observed) and EtOAc (10ml) were added. The phases were separated and the aqueous (pH 2) extracted with EtOAc (5ml). The combined organics were washed with 10ml portions of K\(_2\)CO\(_3\) (aq) until the aqueous phase reached pH 9 (or greater), followed by a wash with DI water (10ml). The organic phase was concentrated \textit{in vacuo} to give a yellow liquid (1.102g, 93\%th).

Tlc: SiO\(_2\) (2:1 v/v hexane:EtOAc) \( R_f \) 0.8, \( R_f \) (1.5) 0.7; visualisation by uv (254nm).

\( ^1 \)H NMR (CDCl\(_3\)): 0.81-0.86 (t, 6H), 1.23-1.28 (m, 4H), 1.35-1.40 (m, 4H), 1.64-1.74 (quin, 4H), 3.84-3.88 (t, 4H), 4.34 (s, 2H), 6.31-6.36 (t, 1H), 6.44 (d, 2H).
Preparation of second generation dendrimer

**Preparation of 2nd generation methyl ester (2.2) – original route**

Alkyl bromide ((1.6), est. 7.14mmol, 2eq); Methyl 3,5-dihydroxybenzoate ((1.1), 600mg, 3.57 mmol); 18-crown-6 (400mg, 0.4mmol, 0.4eq) and potassium carbonate (1.5g, 10.8mmol, 3eq) were stirred together in dry THF (25ml) at reflux under N₂ for ca. 64 hours, then cooled and the residue partitioned between EtOAc (20ml) and DI water (20ml). The aqueous phase was extracted with EtOAc (2x20ml) and the combined organics concentrated *in vacuo*. The residue was columned on silica, eluting with hexane followed by 20%v/v EtOAc in hexane, to give, on concentration of appropriate fractions, a clear liquid which became a mobile wax on standing. Yield = 0.74g, 28%th of (2.2) 0.38g (23%th) monoalkylated product was also recovered.

Tlc: SiO₂ (10%v/v EtOAc in hexane) shows Rₜ(2.2) 0.5, Rₜ(monoalkylated 1.1) 0.1, Rₜ(1.6) 0.8; visualisation by uv.

Tlc: SiO₂ (1:2 v/v EtOAc:hexane) shows Rₜ(2.2) 0.8, Rₜ(monoalkylated 1.1) 0.5.

¹H NMR (CDCl₃): (monoalkylated species) 0.90-0.95 (t, 6H), 1.33-1.37 (m, 4H), 1.42-1.49 (m, 4H), 1.76-1.83 (quin, 4H), 3.92 (s, 3H), 3.93-3.98 (t, 4H), 5.01 (s, 2H), 6.40-6.43 (t, 1H), 6.56 -6.57 (d, 2H), 6.68-6.69 (t, 1H), 7.15 (d, 1H), 7.26-7.27 (t, 2H).

**Preparation of 2nd generation methyl ester, (2.2), by Mitsunobu route**

(1.5) (5.36mmol, 1.65g), 3,5-dihydroxy methyl benzoate (1.1) (454mg, 2.69mmol, 0.5eq), and triphenylphosphine (1.41g, 5.37mmol, 1eq) were mixed with THF (1ml, dry, water content ca 43ppm). DIAD (di-isopropyl azodicarboxylate, 1.14g, 5.6mmol, 1eq) was added and the solution sonicated for 4 hours. The reaction mixture was concentrated *in vacuo* and the residue subjected to column chromatography on SiO₂, eluting with 10%v/v EtOAc in hexane to give the desired product in 67%th yield as a yellow liquid.

¹H NMR (CDCl₃): 0.88, 0.90, 0.92 (t, 12H), 1.30-1.35 (m, 16H), 1.40-1.51 (m, 8H), 1.72-1.82 (quin, 8H), 3.90 (s, 3H), 3.92, 3.94, 3.96 (t, 8H), 4.98 (s, 4H), 6.40, 6.41, 6.42 (t, 2H), 6.54, 6.55 (d, 4H), 6.78, 6.79, 6.80 (t, 1H), 7.27, 7.28 (d, 2H).

¹H NMR (CDCl₃) of monoalkylated intermediate: 0.88, 0.90, 0.92 (t, 6H), 1.31-1.35 (m, 8H), 1.42-1.51 (m, 4H), 1.71-1.81 (m, 4H), 3.89 (s, 3H), 3.89-3.96 (m, 2H), 4.99 (s, 2H), 6.40, 6.405, 6.41 (t, 1H), 6.54, 6.55 (d, 2H), 6.65, 6.66, 6.67 (t, 1H), 7.11, 7.11, 7.12, 7.12 (dd, 1H), 7.24, 7.24, 7.25, 7.25 (dd, 1H).

**Preparation of 2nd generation carboxylic acid (2.3)**

Methyl ester (2.2) (1.335g, 1.78mmol) was taken up in THF (10ml). A solution of KOH (177mg, 3.15mmol, 1.8eq) in DI water (3ml) was added, giving a biphasic mixture. MeOH (ca. 2ml) was added until the phases combined. This was refluxed for 2 hours, concentrated *in vacuo* and the residue partitioned between EtOAc (15ml) and DI water (10ml) – although the aqueous phase was basic it contained no organic material. The organic phase was concentrated *in vacuo* and the residue columned on SiO₂, eluting with hexane, 10%v/v EtOAc in hexane and finally 20%v/v EtOAc in hexane, to give the desired product as a white solid in 70%th yield.

Tlc: SiO₂ (20%v.v EtOAc in hexane) Rₜ 0.25; visualisation by uv at 254nm

¹H NMR (CDCl₃): 0.88, 0.90, 0.92 (t, 12H), 1.30-1.35 (m, 16H), 1.43-1.47 (m, 8H), 1.72-1.82 (quin, 8H), 3.90 (s, 3H), 3.92, 3.94, 3.96 (t, 8H), 5.00 (s, 4H), 6.40, 6.41, 6.42 (t, 2H), 6.55, 6.56 (d, 4H), 6.83, 6.84, 6.85 (t, 1H), 7.33, 7.34 (d, 2H).

IR (KBr disc) found stretches at 2954, 2926, 2856, 1687, 1598 cm⁻¹.
Preparation of 2\textsuperscript{nd} generation allyl amide (2.4)

2.3 (1.24mmol) was dissolved in dry THF (3ml). CDI (240mg, 1.48mmol, 1.2eq) was added and the mixture stirred for 2 hours. Allyl amine (0.15ml, 2mmol, 1.6eq) was added and the mixture stirred for a further 4 hours, then partitioned between EtOAc (8ml) and 0.1N HCl (8ml). The aqueous was extracted with fresh EtOAc (8ml) and the combined organics dried (MgSO\textsubscript{4}) to give an off white solid in 98%th yield.

Tlc: SiO\textsubscript{2} (20\%v/v EtOAc in hexane) R\textsubscript{f} 0.4; visualization by uv at 254nm
Microanalysis found: C 73.60, H 9.18, N 1.69 \%w/w
Theory for C\textsubscript{48}H\textsubscript{71}O\textsubscript{7}N: C 74.48, H 9.25, N 1.81 \%w/w

1\textsuperscript{H} NMR (CDCl\textsubscript{3}): 0.88, 0.90, 0.92 (t, 12H), 1.30-1.35 (m, 16H), 1.40-1.50 (m, 8H), 1.69-1.81 (quin, 8H), 3.92, 3.94, 3.96 (t, 8H), 4.04, 4.06, 4.08 (tt 2H), 4.97 (s, 4H), 5.16-5.17, 5.20-5.21 (dq, 1H), 5.22-5.23, 5.27-5.28, (dq, 1H), 5.86-5.99 (m, 1H), 6.13, 6.15, 6.17 (br t, 1H), 6.40, 6.41, 6.42 (t, 2H), 6.71, 6.72, 6.83 (t, 1H), 6.54, 6.55 (d, 4H), 6.99, 7.00 (d, 2H).
IR (KBr disc) found stretches at 3273, 2956, 2928, 2858, 1638, 1595, 1539 cm\textsuperscript{-1}.

Preparation of 2\textsuperscript{nd} generation benzyl alcohol (2.5)

Alkyl bromide ((1.6), 0.75g, 2.02mmol, 2eq); 3,5-dihydroxybenzylalcohol ((1.7), 143mg, 1.02mmol); 18-crown-6 (54mg, 0.2mmol, 0.2eq) and potassium carbonate (400mg, 2.9mmol, 2.9eq) were stirred together in dry THF (20ml) at reflux under N\textsubscript{2} for 28 hours, then cooled, concentrated \textit{in vacuo} and the residue partitioned between EtOAc (10ml) and DI water (10ml). The aqueous phase was washed with EtOAc (10ml) and the combined organics concentrated \textit{in vacuo}. The residue was columned on silica, eluting with hexane followed by 20\%v/v EtOAc in hexane, to give a clear liquid which became a mobile wax on standing.
Yield = 0.458g, 62%th

Microanalysis found: C, 74.83; H 9.46 \%w/w
C\textsubscript{22}H\textsubscript{35}NO\textsubscript{3} requires: C, 74.96; H 9.51; \%w/w

Tlc: SiO\textsubscript{2} (20\%v/v EtOAc in hexane) shows R\textsubscript{f} 0.3, R\textsubscript{f}(1.6) 0.8, R\textsubscript{f}(1.1) 0.05 – visualisation by uv (254nm)

1\textsuperscript{H} NMR (CDCl\textsubscript{3}): -0.81-0.85 (t, 12H), 1.23-1.28 (m, 16H), 1.35-1.40 (m, 8H), 1.64-1.73 (quin, 8H), 3.84-3.88 (t, 8H), 4.56 (s, 2H), 4.88 (s, 4H), 6.32-6.34 (t, 2H), 6.46 (d, 1H), 6.48-6.47 (d, 2H), 6.48-6.47 (d, 2H).

Third generation dendrimer

Figure S2: Preparation of 3\textsuperscript{rd} generation dendrimer
Ph₃P/DIAD/THF
Ultrasound
67%th

R = n-C₆H₁₃
Preparation of 3\textsuperscript{rd} generation methyl ester (3.2)

2\textsuperscript{nd} generation alcohol dendrimer (2.5), (0.525g/0.7mmol), 3,5-dihydroxy methyl benzoate (60mg, 0.36mmol, 0.5eq), triphenylphosphine (141mg, 0.537mmol, 0.76eq) were mixed with THF (dry, ca 43ppm water content, 1ml). DIAD (0.11ml, 0.52mmol, 0.75eq) was added and the solution sonicated for 6hours. Tlc shows good conversion. The reaction mixture was concentrated and chromatographed on SiO\textsubscript{2}, eluting with 20\%v/v DCM in hexane followed by 50:50 DCM:hexane to give the desired product as a yellow oil in 15\%th.

Tlc: SiO\textsubscript{2} (DCM) R\textsubscript{f} 0.2 – visualisation by uv (254nm)

\textsuperscript{1}H NMR(CDCl\textsubscript{3}): 0.87, 0.90, 0.92 (t, 24H), 1.29-1.35 (m, 32H), 1.39-1.49 (m, 16H), 1.71-1.80 (quin, 16H), 3.91, 3.93, 3.95 (t, 19H), 4.95 (s, 8H), 5.00 (s, 4H), 6.39, 6.40, 6.41 (t, 4H), 6.55, 6.56 (d, 8H), 6.57, 6.58 (t, 2H), 6.67, 6.68 (d, 4H), 6.79, 6.80, 6.81 (t, 1H), 7.29, 7.28 (d, 2H).

Dendrimer Modelling

The size and shape of the silica-bonded dendrimer is a significant issue. Firstly, it is important that the ligands are not so large as to block silica pores – this would restrict access by analytes, reduce the effective surface area and diminish chromatography efficiency. Secondly, knowledge of the likely confirmation can aid interpretation of the selectivity data and deduction of retention mechanisms. Modelling of key ligands (1.4) and (2.4) were carried out by Dr. Jean-Marie Prat to examine the range of conformations and molecular dimensions.

These structures, which bear the allyl amide unit used as a point of attachment to the silica surface, were felt to be the closest simple analogues to the actual silica-bonded dendrimer. Modelling was done for an idealised scenario of a molecule isolated in vacuum, which was deemed an acceptable approximation for supercritical carbon dioxide (a non-polar, low density solvent). These structures may, of course, have slightly different orientations when bound to silica surface and when surrounded by other groups and by solvent molecules.

For both molecules, preliminary simulations allowed us to ascertain permanent conformational features that could be hypothesized from the structure. Furthermore, definite trends also arose in the configuration of other functional elements of the molecules, indicating that the final conformation of the molecule was likely to be driven by only a limited number of bond angle orientations.

For each structure, a series of conformations were randomly produced by varying the dihedral (torsion) angle around each of these critical bonds to a value comprised between 0 and ±180\°. Each configuration was subsequently optimised to an energy minimum using a force-field method (MM+). Each stable configuration obtained was then compared to those already obtained to remove duplicates.

For each conformer, the largest dimension was measured and retained as representative of the system’s size.

Preliminary simulations confirmed that for the first generation allyl amide (1.4), a large delocalization of electrons expanded from the aromatic ring to the amide functionality. Consequently, it could be assumed that this section of the molecule, as illustrated in structure 3, would remain essentially planar without conformational changes.

Figure S3: Permanent conformational features in the 1\textsuperscript{st} generation allyl amide (bold bonds) and conformation driving bonds (a-e)
For the main study, the dihedral angles around bonds $a$, $b$, $c$, $d$, and $e$ were allowed to freely rotate with all other molecular fragments fixed. This yielded a number of configurations: all essentially retained a triangular structure with the various groups around the aromatic ring extending in opposite directions from each other and with the hexyl chains fully extended along the long axis with a typical cross-section between ca. 15 and 20Å and the dihedral angle around bond $a$ preferentially set at 60° from the reference plane.

Initially modelling of the 2nd generation allyl amide (2.4) followed a similar pattern, as shown in structure 4.

Figure 4: Permanent conformational features in the 2nd generation allyl amide (bold and dashed bonds) and conformation driving bonds (a-e)
Free rotation was allowed to ether C-O linkages \((b, c, d, e)\) and their equivalent within the first generation structure only. The angle around bond \(a\) was set to 60°, which had been shown to be the preferred conformation for the 1st generation analogue. The resulting profile showed a variety of competing structures, most featuring alignment between the 2 terminal benzyl rings on the same side of the reference plane as the allyl amide functionality, presumably due to favourable π-π interactions between the aromatic rings, to form a bucket type structure. As a result, the molecular cross-section remained mainly driven by the size of the first generation elements within the second generation structure, typically ca. 15 and 20Å.

These calculations are based on isolated molecules: actual dendrimer conformation is highly dependent on the solvent [2]. The radius of a polyarylether dendron has been shown to vary by a factor of \(10^4\) – fully extended in toluene but progressively contracting in acetone or acetonitrile – with the sensitivity towards solvent increasing with number of generations [3]. Wooley and co-workers examined the wavelength of maximum absorbance for a number of polyether dendrimers in various solvents to conclude that there was a structural change in non-polar solvents from extended to globular forms between the 3rd and 4th generation [4] – later confirming that viscosity (and hence shape) was invariant for differing generations in chloroform (collapsed architecture) but not in THF (well solvated) [5]. The reverse behaviour was seen for a more polar PEI dendrimer – extending in chloroform but folding in benzene [6].

Wooley et al., studying dendrimer packing by NMR, showed that backfolding of the periphery increased with subsequent generations. They used this information, together with molecular dynamics simulations, to infer the conformation of the dendrimer in the solid state. They also found that density decreases near the periphery of dendrimers because of the inward folding of chain ends. However, some of the properties of well-known dendrimers can be explained as a result of globular shapes with the chain ends accessible to the surface [7]. The actual structure may be highly dependent on the exact nature of the terminal groups [8], as well as the solvent composition and dendrimer concentration. Dendrimer-dendrimer interactions may also become significant.

For Frechet-type dendrimers, the class directly relevant to this study, it should be noted that Wooley estimated that a 1st generation dendrimer has a solid-state diameter of 10-12Å while 3rd to 5th generations possessed a diameter of about 12Å [7]; whereas a study on donor-acceptor electron transfer rates in DCM found an effective chain length of 7.7Å for first generation polybenzylether dendrimers, increasingly incrementally to 8.0, 8.3 and 8.5Å for 2nd, 3rd and 4th generations respectively [9]. These values are less than those estimated here, where the molecular cross-section of an isolated dendrimer was estimated in the range 15-20 Å – the difference indicates that the addition of solvent to the system results in compression of the polyether. The estimated size range is still less than the computed size of 23Å for the C\(_{18}\) reagent octadecyldimethylmethoxysilane and indicated that pore blocking should not be an issue.
<table>
<thead>
<tr>
<th>Test</th>
<th>Mobile phase</th>
<th>Flow rate (ml/min)</th>
<th>Temp (°C)</th>
<th>V&lt;sub&gt;inj&lt;/sub&gt; (μl)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Van Deemter</td>
<td>50:50 MeCN:H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>0.2 (optimum)</td>
<td>20</td>
<td>0.5</td>
<td>[10]</td>
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<td>Kinetic plot</td>
<td>Obtained from Van Deemter data</td>
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<td></td>
<td></td>
<td>[11]</td>
</tr>
<tr>
<td>Engelhardt</td>
<td>55:45 MeOH:H&lt;sub&gt;2&lt;/sub&gt;O</td>
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<td>40</td>
<td>0.3-0.5</td>
<td>[12]</td>
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<tr>
<td>Neue</td>
<td>65:35 MeOH: 20mM K&lt;sub&gt;2&lt;/sub&gt;HPO&lt;sub&gt;4&lt;/sub&gt;/K&lt;sub&gt;2&lt;/sub&gt;HPO&lt;sub&gt;4&lt;/sub&gt;(aq) (pH 7)</td>
<td>0.2</td>
<td>20</td>
<td>0.5</td>
<td>[13]</td>
</tr>
<tr>
<td>SRM869</td>
<td>85:15 MeCN:H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>0.2, 0.4, 1, 2</td>
<td>25</td>
<td>10</td>
<td>[14]</td>
</tr>
<tr>
<td>SRM1647</td>
<td>1</td>
<td>0.2</td>
<td>23</td>
<td>3</td>
<td>[15]</td>
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</tbody>
</table>

<sup>1</sup> SRM1647 was analysed using the MeCN:H<sub>2</sub>O gradient conditions detailed in Table 2.

---

<table>
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<th>Time (min)</th>
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<th>18</th>
<th>25</th>
<th>26</th>
<th>30</th>
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<tr>
<td>%MeCN</td>
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<td>50</td>
<td>100</td>
<td>100</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Comment</td>
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<td></td>
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<td></td>
<td>Linear gradient</td>
<td></td>
<td>End of data collection</td>
<td>Linear gradient</td>
<td>Column re-equilibration</td>
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</tr>
</tbody>
</table>

Table S1: Chromatographic conditions

Table S2: Chromatographic conditions for SRM1647
### Table S3: $^{13}$C CP/MAS NMR chemical shift assignments of the 1st and 2nd generation dendrimer silicas

<table>
<thead>
<tr>
<th>Residue</th>
<th>1st generation dendrimer silica</th>
<th>2nd generation dendrimer silica</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1</td>
<td>13.8</td>
<td>15.9</td>
</tr>
<tr>
<td>C-2</td>
<td>23.2</td>
<td>24.6</td>
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<tr>
<td>C-3</td>
<td>32.1</td>
<td>33.6</td>
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<td>C-4</td>
<td>29.1</td>
<td>27.8</td>
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<tr>
<td>C-5</td>
<td>29.1</td>
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<tr>
<td>C-6</td>
<td>40.9</td>
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<tr>
<td>C-7</td>
<td>166.8</td>
<td>162.0</td>
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<tr>
<td>C-8</td>
<td>135.6</td>
<td>109.0</td>
</tr>
<tr>
<td>C-9</td>
<td>105.2</td>
<td>162.0</td>
</tr>
<tr>
<td>C-10</td>
<td>160.4</td>
<td>100.5</td>
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<tr>
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<td>105.2</td>
<td>162.0</td>
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<tr>
<td>C-12</td>
<td>160.4</td>
<td>109.0</td>
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<td>C-13</td>
<td>105.2</td>
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<tr>
<td>C-14</td>
<td>68.4</td>
<td>69.5</td>
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<tr>
<td>C-15</td>
<td>29.1</td>
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<tr>
<td>C-16</td>
<td>26.7</td>
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<tr>
<td>C-17</td>
<td>32.1</td>
<td></td>
</tr>
<tr>
<td>C-18</td>
<td>23.2</td>
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<tr>
<td>C-19</td>
<td>13.8</td>
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<tr>
<td>Ar</td>
<td>N.A.</td>
<td>140.6 / 136.4</td>
</tr>
<tr>
<td>-C$<em>5$H$</em>{10}$-</td>
<td>N.A.</td>
<td>24.6</td>
</tr>
</tbody>
</table>

*a* Chemical shifts are in units of ppm referenced to glycine.  
*b* Carbon numbering refers to structures in Figures 5 and 6 in main document text.

### Table S4: $^{29}$Si CP/MAS NMR chemical shift assignments of the 1st and 2nd generation dendrimer silicas

<table>
<thead>
<tr>
<th>Residue</th>
<th>1st generation dendrimer silica</th>
<th>2nd generation dendrimer silica</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-1</td>
<td>-</td>
<td>-47.4</td>
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<tr>
<td>T-2</td>
<td>-56.9</td>
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<tr>
<td>Q-2</td>
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<td>-84.8</td>
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<td>Q-3</td>
<td>-99.8</td>
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<tr>
<td>Q-4</td>
<td>-110.0</td>
<td>-110.5</td>
</tr>
</tbody>
</table>

*a* Chemical shifts are in units of ppm referenced to glycine.  
*b* Silicon numbering refers to structures in Figures 5 and 6 in main document text.