The synthesis of immobilised chiral dendrimers

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A one pot synthetic approach to amino acid based chiral dendrimers was shown to give good yields for dendrimers with peptide linkages. Using this synthetic strategy, modification of the peripheral functionality is possible, as is the substitution of other amino acids to give mixed amino acid based dendrimers. Immobilization of our dendrimers was accomplished using a coupling method involving the carboxy derivatised dendrimers with an aminopropyl modified silica. The use of 2-ethoxy-1-ethoxy-1,2-dihydroquinoline in a one pot protocol gave enhanced reaction conditions from which silicas bearing very high levels of dendrimer were obtained. Solid state NMR analysis substantiated that the dendrimers were bound to the silica and that they appeared to be structurally intact. These immobilised chiral dendrimers constitute a new class of chiral stationary phases for use in HPLC.

Introduction

Nature provides a convenient and accessible supply of non-racemic chiral materials that can be utilised in chromatography to effect optical resolution of natural and synthetic chiral compounds. One of the first approaches in liquid chromatography for the separation of enantiomers involved the use of chiral mobile phase additives.[1] An alternative strategy has since developed that involves the immobilisation of chiral molecules to create chiral stationary phases (CSPs). Many chiral stationary phases have been developed from natural products and a full range has been investigated, ranging from amino acids and amino acid derivatives to entire biological polymers such as proteins and polysaccharides.[2]

Dendrimers represent a class of highly ordered oligomeric and polymeric structures synthesised using iterative reaction sequences starting from smaller, simple monomer molecules.[3] Dendrimeric molecules are extremely branched three-dimensional macromolecules with a branch at every monomer unit. The structure of a dendrimer molecule can be divided into defined regions, an exterior surface, an interior layer and a central core onto which all the covalent bonds converge.[4] It has been shown that dendrimers display supramolecular behaviour, including both host–guest interactions to give supermolecules, as well as spontaneous assembly, to form well-defined microscopic and mesoscopic organisations.[5] Of particular interest is the ability of these molecules to sequester or act as hosts for organic molecules. The overall size, shape and surface chemistry can be readily controlled during synthesis to provide highly defined surfaces and interfaces. Dendrimers with chiral interior cavities and exterior surfaces can be synthesised using chiral monomers. This chirality may manifest itself as an ability to exhibit selective host–guest complexation or receptor properties based on chiral recognition.

The immobilisation of man-made dendrimers onto chromatographic supports for use as LC stationary phase selectors has not been reported in the literature. Dendrimers have been used as selectors in separation science in electrokinetic chromatography as pseudo-stationary phases, but such phases are not chemically immobilised onto a solid support.[6a–g] Dendrimers have been immobilised onto surfaces for other purposes, the most common being monolayer and multilayer assemblies for use as sensors.[5,7] The dendrimers involved in these studies are invariably non-chiral PAMAM (polyamidoamine) dendrimers. Chiral and non-chiral dendrimers have also been grown from surfaces or have been surface grafted but for non-chromatographic purposes.[8a–c]

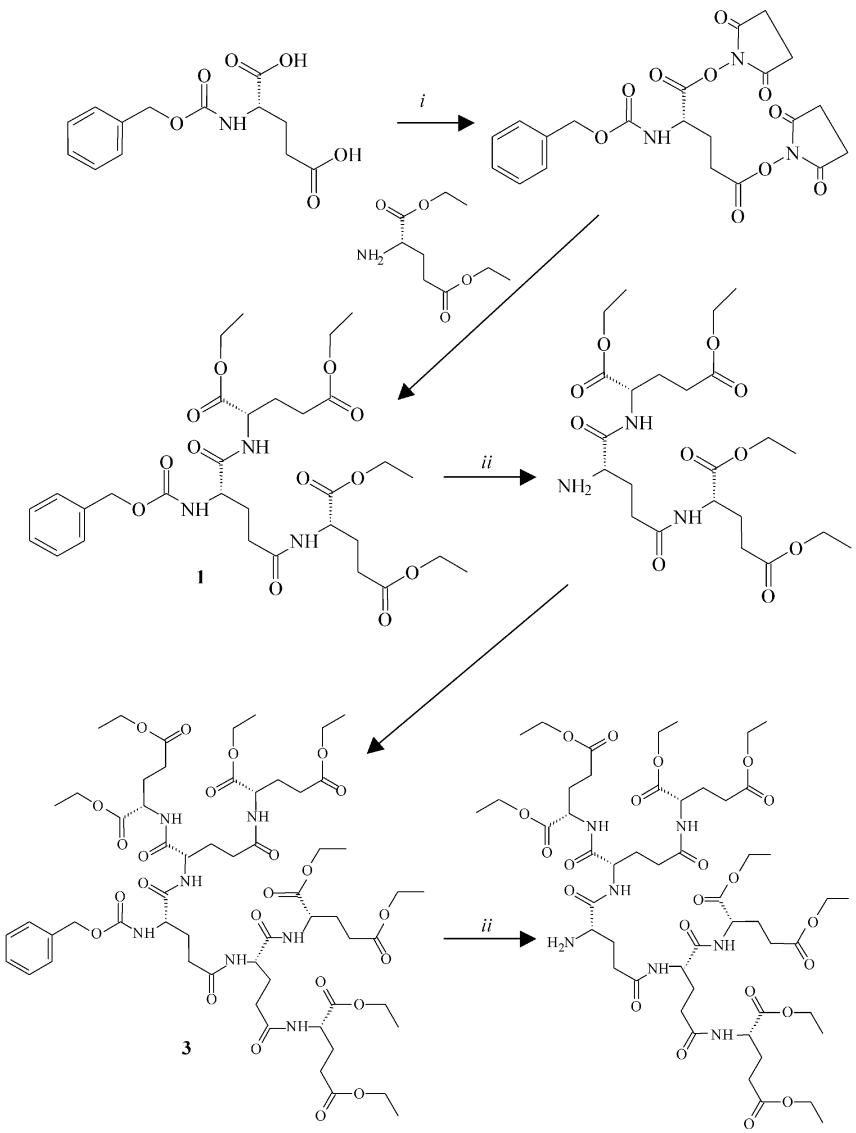
Our aim was to use chiral natural products in the synthesis of supramolecular host molecules.[9a,b] Non-racemic amino acids, in particular L-glutamic acid derivatives, were to be used to construct chiral dendrimers. We report here our efforts in the synthesis and immobilisation chemistry for the formation of dendrimer/silica based materials.

Results and discussion

Synthesis of chiral dendrimers

A strategy for the total synthesis of chirally pure dendrimers based on L-glutamic acid monomers has been developed in this laboratory and described by Mitchell and Twyman *et al.*[10] The dendrimers were constructed *via* peptide bond formation using a convergent synthesis approach. That is, smaller dendrons were synthesised and these were then reacted with a core molecule to produce the next generation dendrimer. The dendrimers are characterised by a high proportion of chiral centres, 3, 7 and 15 for the first, second and third generation dendrimers respectively, all of L-conformation. Construction of the first generation L-glutamic acid dendrimer ([G1]-CBZ-L-glutamic acid ethyl ester **1**; CBZ = carbobenzyloxy) can be achived by the reaction of two equivalents of L-glutamic acid ethyl ester with a single equivalent of the CBZ-L-glutamic acid (see Scheme 1). The next generation dendrimer, [G2]-CBZ-L-glutamic acid ethyl ester (**3**) can be built by reacting two equivalents of the free amine of the first generation dendrimer with CBZ-L-glutamic acid, and so on. It was found that dendrimers up to the third generation could be synthesised.[11a]

The reaction between the primary amine and the carboxylic acid functions of glutamic acid monomers results in the creation of amide (peptide) bonds. In previous reports we have

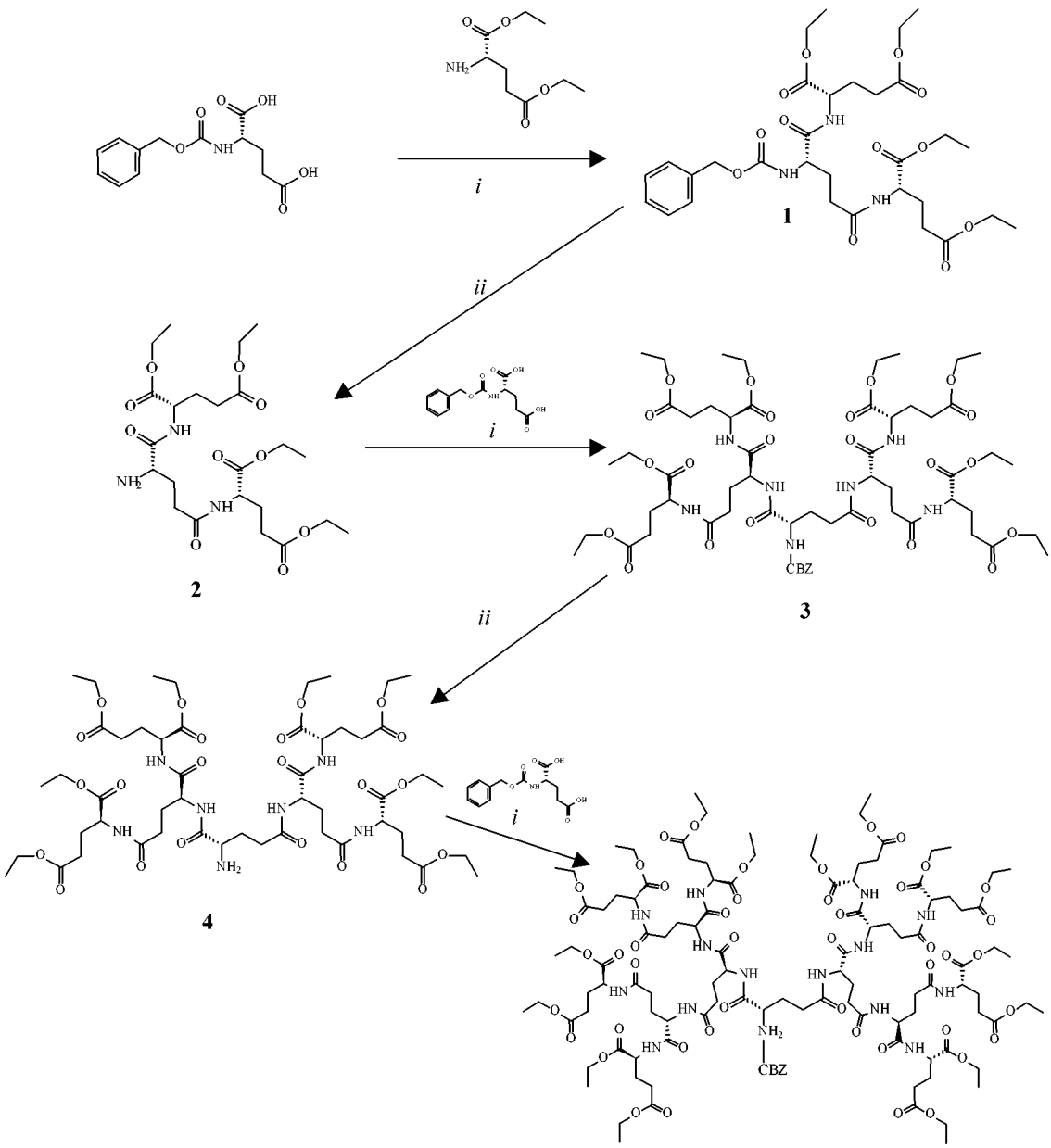


Scheme 1 The synthesis of the L-glutamic acid ethyl ester dendrimers using NHS active esters; (*i*) NHS, DCC, DMAP; (*ii*) catalytic hydrogenolysis.

outlined the use of several methods for the synthesis of the L-glutamic acid ethyl ester dendrimers, the most fruitful example being the strategy employing NHS (*N*-hydroxysuccinimide) active esters as shown in Scheme 1.[11a,12,13] It is, however, worth noting that for this strategy, additional reaction steps for the formation and purification of active esters are required and yields obtained from the use of these coupling reagents tend to be low.[14,15]

A one pot procedure for peptide bond formation would be advantageous. Such a method would involve the production of an active species as an intermediate during a reaction which resulted in amide bond formation. Protocols involving the use of the reagent benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium[12] (HBTU), to form the active ester *in situ*, are now routinely used in solid phase peptide synthesis. *N*-Hydroxybenzotriazole (HOBt) is used as an additive with HBTU because it keeps racemisation to a minimum. Treatment of CBZ-L-glutamic acid with HBTU and HOBt followed by the addition of L-glutamic acid ethyl ester hydrochloride rapidly afforded [G1]-CBZ-L-glutamic acid ethyl ester (**1**, see Scheme 2). TLC analysis was used to follow the reaction and indicate when it was complete. Analysis by NMR, MS and TLC established that the desired product had been formed and that it was pure. The procedure proved simpler and quicker than the previous method and gave improved yields: 92% in this case compared with an overall yield of 58% for [G1]-CBZ-L-glutamic acid ethyl ester prepared using NHS esters. [G1]-CBZ-L-glutamic acid ethyl ester (**1**) was deprotected as before using catalytic hydrogenolysis. The resultant [G1]-NH_2-L-glutamic acid ethyl ester (**2**) was then used to synthesise the second generation dendrimer, [G2]-CBZ-L-glutamic acid ethyl ester (**3**, see Scheme 2), again using HBTU and HOBt coupling reagents. The solid product was purified by trituration from methanol upon addition of water to give the product as a transparent solid in 50% yield. TLC indicated the presence of only one compound. Analysis using NMR and MS compared favourably with the reference data[15] and confirmed the compound to be [G2]-CBZ-L-glutamic acid ethyl ester (**3**). Again the reaction method proved to be better than the previous (NHS) method: the overall yield of [G2]-CBZ-L-glutamic acid ethyl ester using the HBTU strategy being 50%, compared with 10% using the NHS protocol.

Deprotection of the second generation dendrimer was carried out under the same conditions as for the first generation dendrimers, using catalytic hydrogenolysis the NMR verified that deprotection had occurred and, along with FAB-MS, corroborated the proposed structure. Both NMR and MS were in accordance with the reference results. The deprotected dendrimer, [G2]-NH_2-L-glutamic acid ethyl ester (**4**, Scheme 2) was obtained as an opaque solid in 90% yield. The third generation dendrimer, [G3]-CBZ-L-glutamic acid ethyl ester (Scheme 2) can also be easily formed by this



Scheme 2 The synthesis of the L-glutamic acid ethyl ester dendrimer using the HBTU/HOBt active ester coupling technique; (*i*) HBTU, HOBt, DIPEA; (*ii*) catalytic hydrogenolysis.

method. CBZ-glutamic acid was activated with HBTU and HOBt and reacted with [G2]-NH_2-L-glutamic acid ethyl ester (**4**). A thick colourless oil was recovered and purified by trituration from methanol by the addition of water in 43% yield. NMR and FAB-MS analysis revealed that the reaction had produced the desired third generation dendrimer, [G3]-CBZ-L-glutamic acid ethyl ester. The synthesis of the next generation dendrimer was not attempted.

Chiroptical data for the dendrimers were identical to those reported using the NHS active ester approach.[11a,15] In those reports we noted how the optical rotations of the higher generation dendrimeric species varied only slightly from that of the L-glutamic acid based first generation dendrimer. Other workers have explored similar trends in optical rotations of complex dendrimer structures.[11b–e]

Assigning the ^{13}C NMR signals to the fine structure of the dendrimers

The gross δC shift values of the dendrimers were in accordance with the expected values and readily assigned. However, every carbon atom gave a discrete signal in the spectra for the smaller dendrimers, *i.e.* carbon atoms in analogous environments (*e.g.* those of residue 1 and residue 2, see Fig. 1) gave separate signals in the spectra. The spectra for the different generations of the same dendrimer showed obvious similarities and became more complicated as the dendrimers grew in size. An attempt was made to assign these signals to the individual carbon atoms within the dendrimer structure. The δC shift values for [G1]-L-glutamic acid ethyl ester were calculated using the InfoSpec database.[14]

Some general trends for the relative shift positions of the similar carbons in the dendrimers can be suggested using reported shift values and the calculated shifts for the [G1]-L-glutamic acid ethyl ester dendrimers. Starting at high frequency, amide carbonyl carbons are normally found at higher frequency than ester carbonyl carbons.[16] This was apparent in the calculated δC values, where the ester carbonyl carbons had lower frequencies than the amide carbonyl carbons of the core residue (r_3): with the exception of the α carbonyl carbon of [G1]-NH_2-L-glutamic acid ethyl ester which was shifted to a lower frequency in this case by the primary amine. The reported δC positions[16] for the carbonyl carbons of L-glutamic acid place the α carbonyl carbon at a lower frequency than the γ carbonyl carbon. These relative positions were reflected in the calculated δC shifts of the α and γ amide

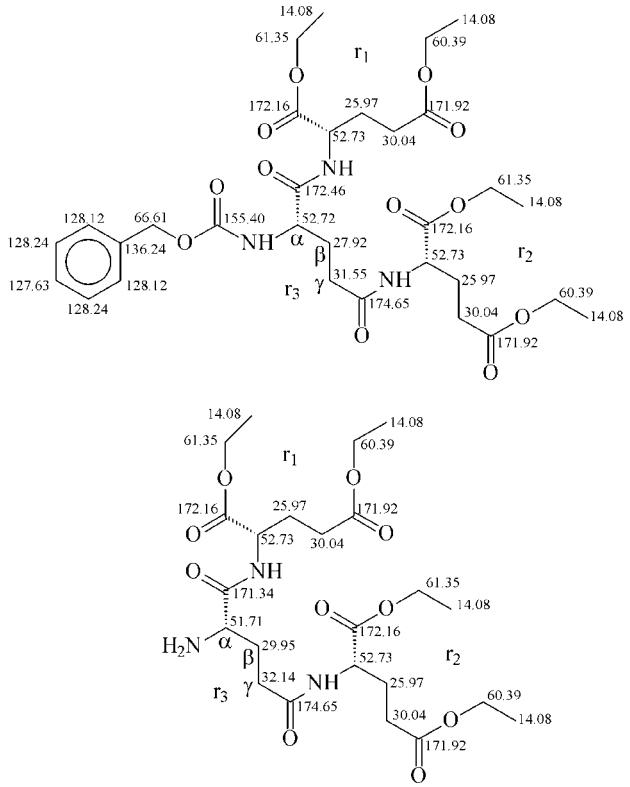


Fig. 1 The calculated δC values for the [G1]-CBZ-L-glutamic acid ethyl ester (**1**) and [G1]-NH_2-L-glutamic acid ethyl ester (**2**) dendrimers: the three L-glutamic acid residues in the dendrimers are marked r_1, r_2 and r_3.

carbonyl carbons of r_3 in the [G1]-L-glutamic acid dendrimer shown above. However, the calculated shifts suggest that this was reversed for the ester carbonyl carbons of r_1 and r_2 within the dendrimer, *i.e.* their α carbonyl carbons occurred at higher frequency than their γ carbonyl carbons.

Modification at the periphery

The existing synthetic protocol can be used to manufacture new dendrimers with varying properties. A more hydrophobic dendrimer could be synthesised by substituting the L-glutamic acid diethyl ester for another ester of glutamic acid during the dendrimer synthesis. An interesting choice for the production of a hydrophobic glutamic acid dendrimer was the use of L-glutamic acid di-*tert*-butyl ester hydrochloride. An advantage of this amino acid derivative is that the *tert*-butyl esters are readily removed using TFA (trifluoroacetic acid) yielding carboxylic acid groups which can be exploited in subsequent

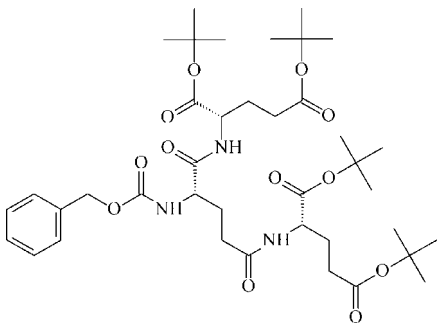


Fig. 2 [G1]-CBZ-L-Glutamic acid *tert*-butyl ester dendrimer (**5**).

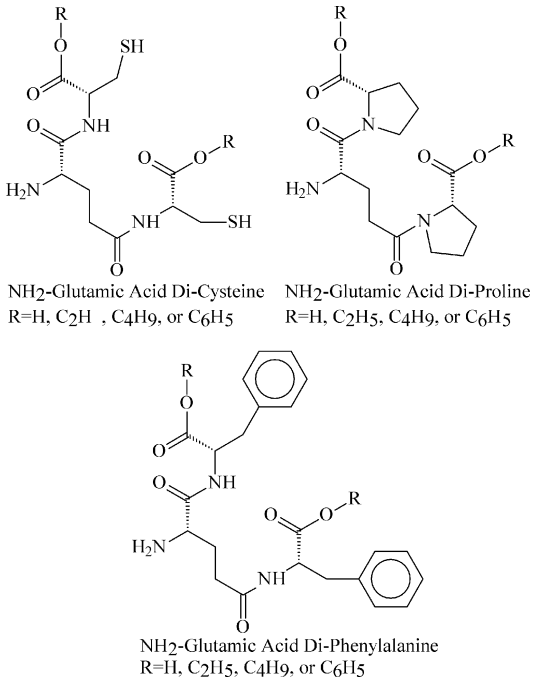


Fig. 3 Three examples of the many glutamic acid based dendron fragments with different amino acid termination that are theoretically possible.

reactions. The synthesis of the first generation glutamic acid *tert*-butyl ester dendrimer (**5**, Fig. 2) was attempted by the reaction of two equivalents of L-glutamic acid *tert*-butyl ester hydrochloride with one equivalent of CBZ-L-glutamic acid using HBTU and HOBt activation chemistry. The product was obtained as a thick oil in 86% yield. NMR and FAB-MS supported the proposed structure.

The CBZ protecting group was removed by catalytic hydrogenolysis to give the free amine first generation glutamic acid *tert*-butyl ester dendrimer, [G1]-NH_2-L-glutamic acid *tert*-butyl ester (**6**) as a thick oil (73% yield). FAB-MS and ^{1}H NMR verified that deprotection was complete and substantiated the proposed dendrimer structure.

Substitution of the peripheral amino acid residues

The synthesis of the first generation *tert*-butyl terminated glutamic acid dendrimer (**5**) used the same protocol as with the ethyl ester terminated dendrimer by replacing L-glutamic acid ethyl ester with L-glutamic acid *tert*-butyl ester. Taking this principle one step further, the outermost amino acid residue could be substituted for any amino acid. This theoretically

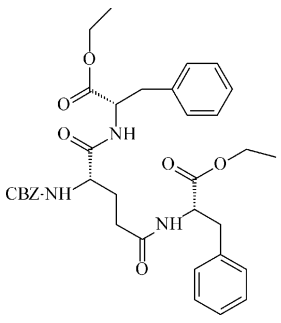


Fig. 4 [G0.5]-CBZ-L-Glutamic acid di-L-phenylalanine ethyl ester (**7**).

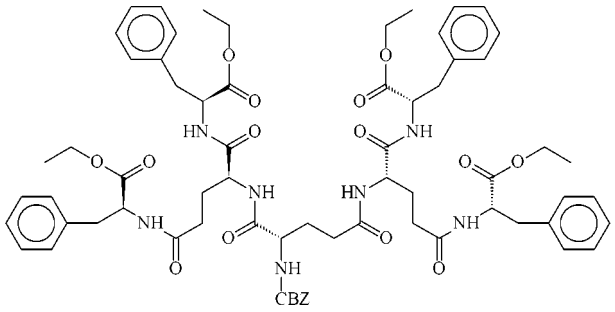


Fig. 5 [G1]-CBZ-L-Glutamic acid L-phenylalanine ethyl ester dendrimer.

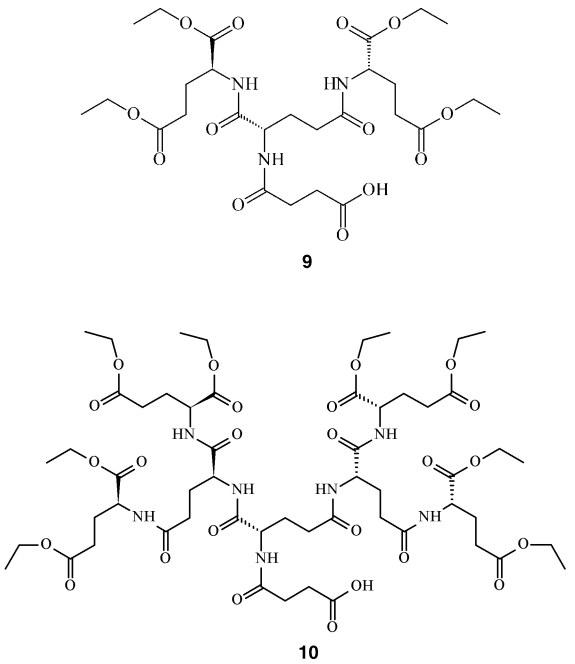


Fig. 6 The first (**9**) and second (**10**) generation ethyl ester terminated alkylcarboxy derivatised L-glutamic acid dendrimers.

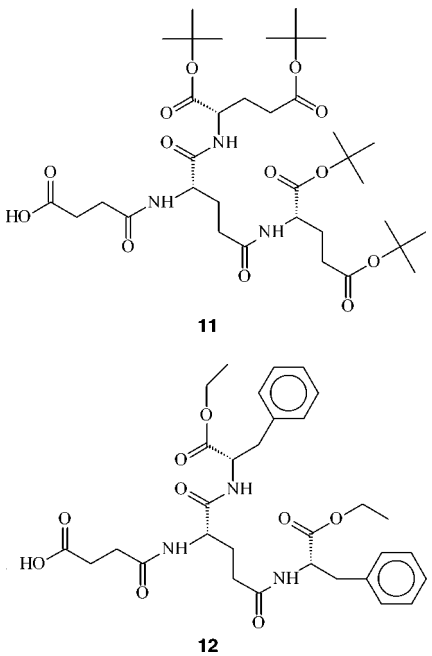


Fig. 7 The alkylcarboxy-[G1]-L-glutamic acid *tert*-butyl ester (**11**) and alkylcarboxy-[G0.5]-L-glutamic acid di-L-phenylalanine ethyl ester (**12**) dendrimers.

opens up a vast range of amino acid terminated glutamic acid dendrimers, of which a few examples stand out as most intriguing materials with regard to their application as chiral stationary phases for use in chromatography (see Fig. 3).

Among these is phenylalanine ethyl ester, a well-known hydrophobic amino acid derivative which can be used as a chiral stationary phase under normal phase conditions. In this case it is the phenyl ring which is involved in the formation of enantioselective inclusion complexes, *via* π–π interactions. Dendrimer structures containing these amino acids may have the propensity to form inclusion complexes under normal and reversed phase conditions and hence, they could be used as reversed phase chiral stationary phases. Thus, L-phenylalanine ethyl ester hydrochloride and triethylamine were dissolved in DCM (dichloromethane) and cooled over ice. A second solution containing CBZ-L-glutamic acid, HBTU, HOBt and DIPEA (NPr^i_2Et) in DCM was made at 0 °C and stirred for 20 min to allow activation of the carboxylic acids to occur. The solution containing the L-phenylalanine was then added to the activated CBZ-L-glutamic acid mixture and the reaction mixture was stirred for 45 min at 0 °C. The crude product was purified by flash chromatography giving [G0.5]-CBZ-L-glutamic acid di-L-phenylalanine ethyl ester (**7**, see Fig. 4) as an opaque solid in 90% yield. FAB-MS and NMR established that the product was the desired compound.

A hydrogenation reaction was used to remove the CBZ protecting group affording the free amine derivative [G0.5]-NH_2-L-glutamic acid di-L-phenylalanine ethyl ester (compound **8**). The reaction gave the product in 98% yield. NMR and FAB-MS analysis confirmed that deprotection was complete and verified the structure of the product. Compounds **7** and **8** are best classed as tripeptides rather than dendrons. The synthesis of the first generation phenylalanine terminated dendrimer was attempted. CBZ-L-Glutamic acid was activated with HBTU and HOBt followed by the addition of a solution containing [G0.5]-NH_2-L-glutamic acid di-L-phenylalanine ethyl ester (**8**). Work up produced [G1]-CBZ-L-glutamic acid L-phenylalanine ethyl ester (see Fig. 5) in 76% yield.

Modification at the central core: derivatisation of the central primary amine

The dendrimers were intended for immobilisation onto suitably modified chromatographic silica. The L-glutamic acid dendrimers possessed a single primary amine reactive function that could be bonded with a suitably modified solid phase. However, during the synthesis of the dendrimer series it became clear that the core primary amine is sterically masked to some degree depending on the size of the dendrimer. As such, the derivatisation of the dendrimer's primary amine with a linker molecule was appealing.

Using succinic anhydride the primary amine was modified to yield the alkylcarboxy derivative bearing a reactive carboxylic acid function. [G1]-NH_2-L-Glutamic acid ethyl ester (**2**) was dissolved in DCM with triethylamine followed by the addition of succinic anhydride. N^{α}-Alkylcarboxy-[G1]-L-glutamic acid ethyl ester (**9**, see Fig. 6) was obtained as a white solid in 45% yield. NMR and FAB-MS confirmed the proposed structure. Hence, the second generation NH_2-L-glutamic acid ethyl ester dendrimer (**4**) was derivatised using the same method yielding the corresponding alkylcarboxy compound (**10**, see Fig. 6). NMR and FAB-MS established that the desired product had been formed.

Additionally, the [G1]-L-glutamic acid *tert*-butyl ester dendrimer (**6**) was derivatised with succinic anhydride and the alkylcarboxy-[G1]-L-glutamic acid *tert*-butyl ester (**11**) was obtained. Likewise, the phenylalanine terminated compound, [G0.5]-NH_2-L-glutamic acid di-L-phenylalanine ethyl ester (**8**) afforded the N^{α}-alkylcarboxy-[G0.5]-L-glutamic acid di-L-

phenylalanine ethyl ester (**12**). NMR and FAB-MS corroborated the structure for both compounds **11** and **12** (shown in Fig. 7).

These four compounds (**9**, **10**, **11** and **12**) represent a series of dendrimeric compounds with less sterically hindered reactive (carboxylic acid) functions compared with their primary amine derivatives. This was found to be beneficial when these materials were used in the synthesis of chiral stationary phases.[14]

The immobilisation of chiral dendrimers

The amino acid based chiral dendrimers described above were covalently immobilised onto chromatographic silica (5 μm Spherisorb) to produce dendrimeric chiral stationary phases. This was achieved *via* reaction of chromatographic silica modified with 3-aminopropyltrimethoxysilane with the *N*-alkylcarboxy derivatised dendrimers **9**, **10**, **11** and **12**, yielding compounds [G1]-L-glutamic acid ethyl ester-AC-silica (**13**), [G2]-L-glutamic acid ethyl ester-AC-silica (**14**), [G1]-L-glutamic acid *tert*-butyl ester-AC-silica (**15**, see Fig. 8) and [G0.5]-L-glutamic acid L-phenylalanine ethyl ester-AC-silica (**16**, see Fig. 9), respectively.

Synthesis of 3-aminopropyltrimethoxysilane modified silica

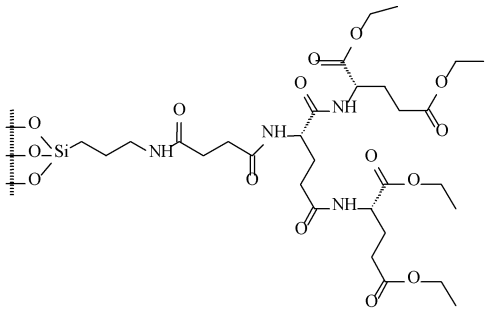
Spherisorb silica was dried and added to 3-aminopropyltrimethoxysilane. The product, 3-aminopropyl-silica (AP-silica), was collected and elemental analysis (see Table 1) verified that a sufficient quantity (2.4% w/w, 429 μmol g^{-1}) of the reagent had bound to the silica. The process was repeated to provide a second and third batch of amine terminated silica and was found to be reliably reproducible (correlating to 2.5% and 2.45% w/w, 443 μmol g^{-1} and 435 μmol g^{-1}, respectively).

Coupling of alkylcarboxy functionalised dendrimers to AP-silica

As before, a one pot reaction would be ideal because an excess of activating agent could be used to minimise the effects of hydrolysis of the active species and this could result in better ligand loading. A coupling procedure reported in the literature[17] employs the reagent 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) in a one pot reaction protocol.[18,19] The dendrimeric compounds that were suitably functionalised in order to be compatible with this method of immobilisation onto silica were, alkylcarboxy-[G1]-L-glutamic acid ethyl ester (**9**), alkylcarboxy-[G2]-L-glutamic acid ethyl ester (**10**, see Fig. 6), alkylcarboxy-[G1]-L-glutamic acid *tert*-butyl ester (**11**) and the alkylcarboxy-[G0.5]-L-glutamic acid di-L-phenylalanine ethyl ester (**12**) compound (shown in Fig. 7). For example, compound **9** was dissolved in DME into which AP-silica had been suspended. EEDQ was then added and the reaction mixture was sonicated briefly followed by swirling for one week at room temperature. Elemental

Table 1 Elemental analysis results for 3-aminopropyl modified silica

	Run 1	Run 2	Run 3	Bare Silica
C%	1.82	2.17	2.00	0.35
H%	0.56	1.48	0.57	0.23
N%	0.60	0.62	0.61	0.00
Quantity of reagent bound to silica/μmol g^{-1}	429	443	435	



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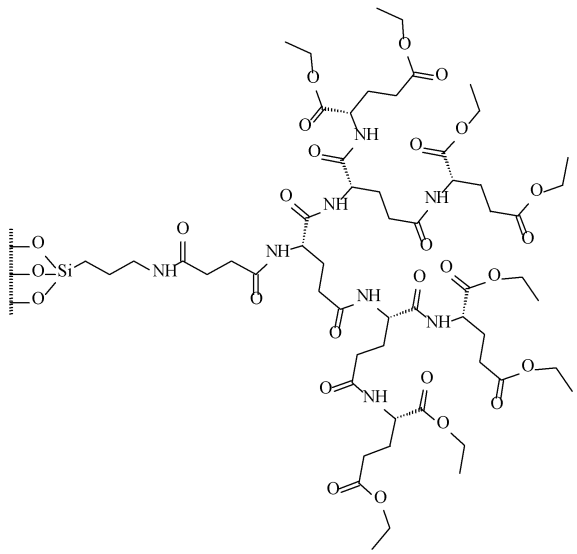

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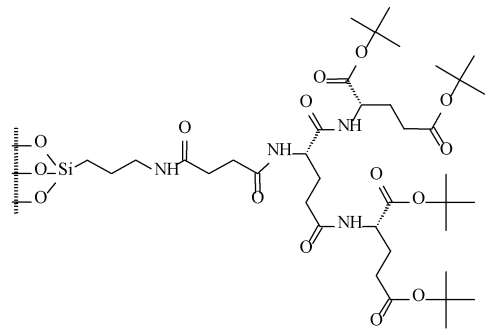

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Fig. 8 The dendrimer based chiral stationary phases, [G1]-L-glutamic acid ethyl ester-AC-silica (**13**), [G2]-L-glutamic acid ethyl ester-AC-silica (**14**) and the first generation *tert*-butyl ester terminated dendrimer phase [G1]-L-glutamic acid *tert*-butyl ester-AC-silica (**15**).

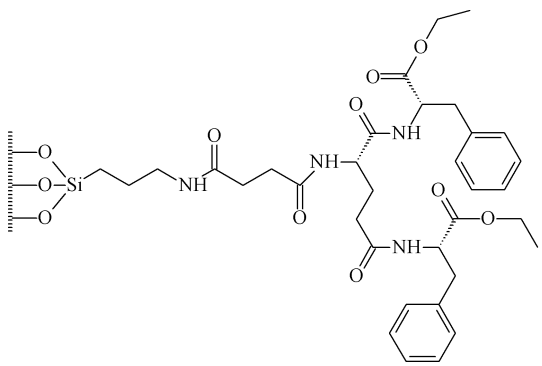

Fig. 9 [G0.5]-L-Glutamic acid di-L-phenylalanine ethyl ester-AC-silica dendrimer CSP (**16**).

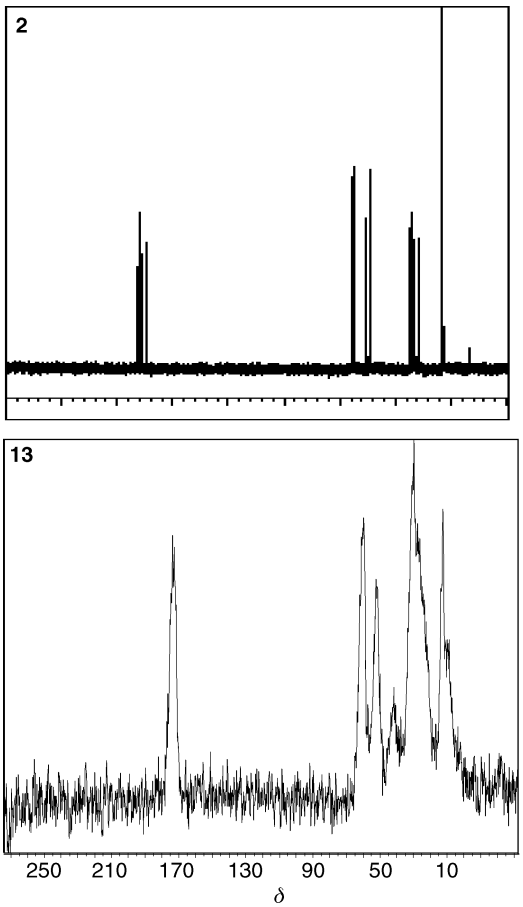

Fig. 10 The solution ^{13}C NMR spectrum for the [G1]-NH_2-L-glutamic acid ethyl ester (**2**) dendrimer and the ^{13}C solid state NMR spectrum for the [G1]-L-glutamic acid ethyl ester-AC-silica compound **13**. Note, solvent peaks have been removed from the solution NMR for ease of comparison with the solid state NMR.

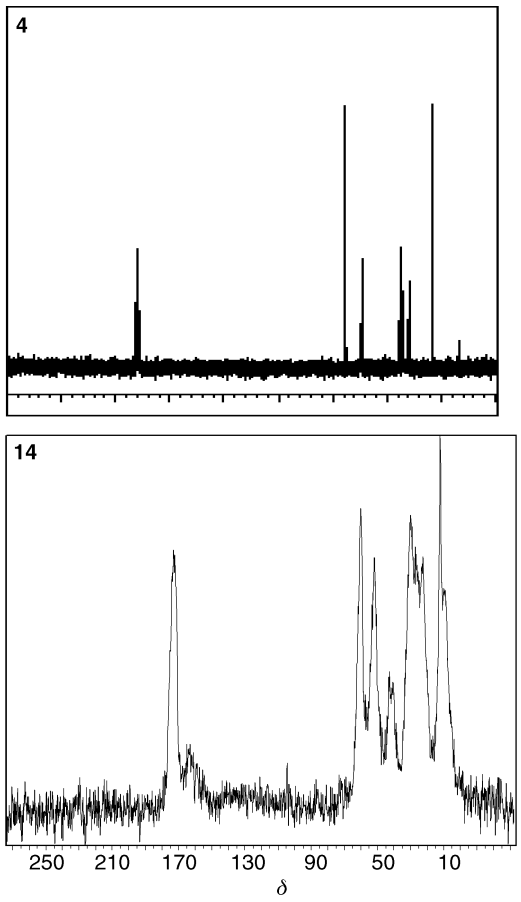

Fig. 11 The solution ^{13}C NMR spectrum for the [G2]-NH_2-L-glutamic acid ethyl ester (**4**) dendrimer and the ^{13}C solid state NMR spectrum for the [G2]-L-glutamic acid ethyl ester-AC-silica compound **14**. Note, solvent peaks have been removed from the solution NMR for ease of comparison with the solid state NMR.

analysis (see Experimental section) equated to a loading of the alkylcarboxy-dendrimer on the silica of between 12.95 and 11.9% (210 and 195 μmol g^{-1}), calculated using the increase in carbon and nitrogen content respectively. The quantity of unreacted dendrimer, recovered from the washings, substantiated this level of binding. The quantity of dendrimer on the silica (minimum 195 μmol g^{-1}) is pleasing as it is near commercial levels of silica coverage with linear molecules. Yields can also be calculated with respect to the total number of amino residues present on the AP-silica (*i.e.* between 435 and 475 μmol g^{-1}).

Reaction of the second generation dendrimer, alkylcarboxy-[G2]-L-glutamic acid ethyl ester (**10**) with AP-silica in the presence of EEDQ was monitored for 34 days after which time a sample taken for elemental analysis showed no further increase in organic matter on the silica. The elemental analysis results (see Experimental section) correlated to the addition of 7.0–5.7% (57 and 46 μmol g^{-1}) of the dendrimer onto the silica, calculated from carbon and nitrogen content respectively. The smaller quantity of dendrimer that was able to bond to the silica in this case reflects the increased steric hindrance of the second generation dendrimer and possibly its decreased solubility in the solvents compatible with the coupling chemistry.

The alkylcarboxy-[G1]-L-glutamic acid *tert*-butyl ester dendrimer (**11**) was also coupled to AP-silica in the same manner as above. The reaction was complete after two weeks. Elemental analysis of the product (silica **15**, see Experimental section) corresponded to between 13.5 and 11.5% (185 and 156 μmol g^{-1}) alkylcarboxy-dendrimer on silica, calculated from the carbon and nitrogen content respectively. The intermediate quantity of ligand bound in this case may be caused by the steric hindrance of the dendrimer ligand, which should be in between those of the dendrimers used to produce the two phases described immediately above.

The remaining carboxy functionalised compound, alkylcarboxy-[G0.5]-L-glutamic acid di-L-phenylalanine ethyl ester (**12**), was then reacted with AP-silica using EEDQ. The reaction mixture was swirled for two weeks at room temperature. The product was collected and washed to give silica **16** (Fig. 9). Elemental analysis (see Experimental section) revealed that between 3.5 and 4.8% (60 and 80 μmol g^{-1}) of the ligand had coupled to the silica.

Solid state NMR characterisation of the dendrimer-AC-silica CSPs

While elemental analysis provides valuable information on the quantity of bound organic material, the absolute identity of this material cannot be established with this method of analysis alone. Thus, solid state NMR was used to characterise the organic matter on the silica. Solid state ^{13}C NMR was conducted upon dendrimer-AC-silica compounds **13**, **14** and **15** (see Fig. 10, 11 and 12): solution state ^{13}C NMR spectra of corresponding dendrimers are shown alongside the ^{13}C NMR spectra for comparative purposes.

Each of the solid state ^{13}C NMR spectra bears signals that correspond well to the structure of the bound dendrimers. The relative heights of the signals within the solution ^{13}C NMR

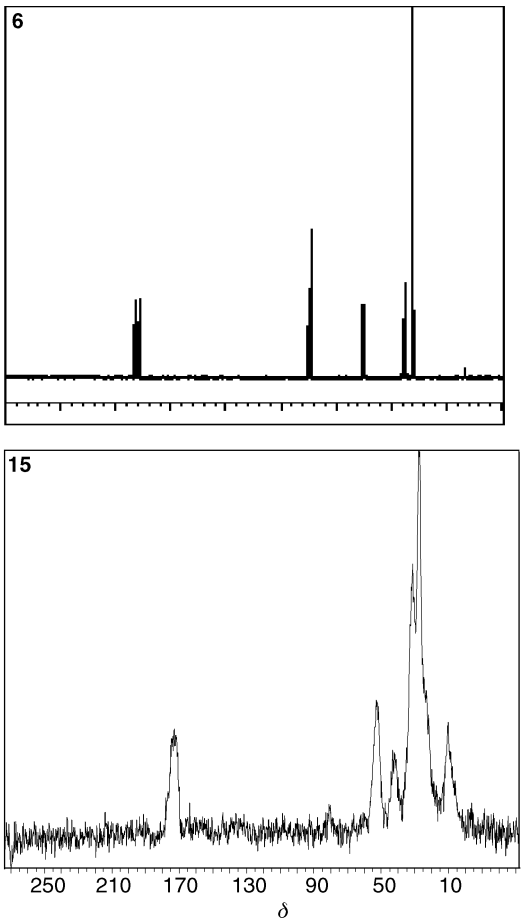

Fig. 12 The solution ^{13}C NMR spectrum for the [G1]-NH_2-L-glutamic acid *tert*-butyl ester (**6**) dendrimer and the ^{13}C solid NMR spectrum for the [G1]-L-glutamic acid *tert*-butyl ester-AC-silica compound **15**. Note, solvent peaks have been removed from the solution NMR for ease of comparison with the solid state NMR.

spectra compare well with those of the solid state spectra. Signals for the AP-linker are also apparent in the solid state spectra. Furthermore, the absence of signals in the regions of the spectra where aromatic moieties are typically observed indicates that the EEDQ reagent, used to couple the dendrimers to the AP-silica, was not bound (or adsorbed) onto the silica surface. Hence, solid state ^{13}C NMR gave compelling evidence that the organic materials on compounds **13**, **14** and **15** were indeed the desired dendrimer materials.

Conclusions

A one pot synthetic approach to amino acid based chiral dendrimers was shown to give good yields for dendrimers with peptide linkages. Glutamic acid based dendrimers can be easily synthesised up to three generations. Using this synthetic strategy, modification of the peripheral functionality is possible, as is the substitution of other amino acids to give mixed amino acid based dendrimers.

Immobilization of our dendrimers was accomplished using a coupling method involving the carboxy derivatised dendrimers with an aminopropyl modified silica. The use of 2-ethoxy-1-ethoxy-1,2-dihydroquinoline in a one pot protocol gave enhanced reaction conditions from which silicas bearing very high levels of dendrimer were obtained. Solid state NMR analysis substantiated the fact that the dendrimers were bound to the silica and that they appeared to be structurally intact. Hence, the [G1]-L-glutamic acid ethyl ester-AC-silica (**13**), the [G2]-L-glutamic acid ethyl ester-AC-silica (**14**), the [G1]-L-glutamic acid *tert*-butyl ester-AC-silica (**15**) and the [G0.5]-L-glutamic acid di-L-phenylalanine ethyl ester-AC-silica (**16**) dendrimeric CSPs (chiral stationary phases) were successfully produced. These constituted the desired CSPs that were required for future investigation into the potential of chiral hydrophobic dendrimer stationary phases in HPLC.

Experimental

Solvents

All reaction solvents were of analytical grade, anhydrous and freshly distilled unless otherwise stated. Anhydrous dichloromethane was prepared by distillation from calcium hydride under a nitrogen atmosphere. Dimethylformamide was prepared by distillation under a nitrogen atmosphere. Benzene was dried by standing on sodium for 24 h. Anhydrous methanol, anhydrous dimethoxyethane and anhydrous dimethyl sulfoxide were purchased from Aldrich. Water was distilled. Solvents used for TLC were of analytical reagent grade.

Reagents

All reactions were carried out under a nitrogen atmosphere unless otherwise stated or unless aqueous solvents were used. Liquid reagents were dried as necessary by standing over $MgSO_4$ (anhyd.) or molecular sieves. Spherisorb silica was dried at 150 °C prior to use. All other reagents were used as supplied. *N*-Benzyloxycarbonyl-L-glutamic acid, 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ), L-glutamic acid, L-glutamic acid diethyl ester hydrochloride and L-phenylalanine ethyl ester hydrochloride were purchased from Lancaster Chemicals. Benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU), *N*-hydroxy benzotriazole (HOBt) and Spherisorb (5 μm, hydroxylated) chromatographic silica were supplied by Phase Separations/Waters Corporation. Ammonium formate and L-glutamic acid di-*tert*-butyl ester hydrochloride were purchased from Sigma Chemicals.

Waters Spherisorb 5 μm: physical properties

Spherisorb silica was characterised as having a mean particle diameter of 5.26 μm, a specific surface area of 175.5 $m^2\ g^{-1}$, a pore volume of 0.45 ml g^{-1} and an average pore diameter of 100 nm.

Analytical

^1H NMR spectra were measured on a JEOL GSX270 270 MHz spectrometer. Solution state ^{13}C NMR were measured using the same instrument with proton noise decoupling. All NMR solvents were deuterated and all δ values are given in ppm relative to tetramethylsilane. Solid state ^{13}C NMR spectra were measured on a Bruker 300 MHz spectrometer. FAB-MS was conducted by the Swansea Mass Spectroscopy Service Centre at the University of Wales, Swansea. Elemental analysis was conducted by the Analytical Services at the University of Kent, Canterbury. In all but two cases elemental analysis proved inappropriate for the determination of the purity of the higher molecular weight dendrimers. MS evidence of molecular weight coupled with proton and carbon NMR evidence of compound structure is presented in all cases.

Note: ligand loading on silica expressed in μmol g^{-1} refers to the number of μmol of ligand coupled per gram of silica. Ligand loading on silica expressed in μmol m^{-2} refers to the number of μmol coupled per m^2 of silica surface.

Synthesis of dendrimers

[G1]-CBZ-L-Glutamic acid ethyl ester (1). A solution containing L-glutamic acid diethyl ester hydrochloride (15.00 g, 62.6 mmol) and triethylamine (5.06 ml, 6.97 g, 68.9 mmol) dis-

solved in DCM (100 ml) was prepared and cooled over ice. A second solution containing CBZ-L-glutamic acid (8.00 g, 28.4 mmol), HBTU (23.73 g, 62.6 mmol), HOBt (9.58 g, 62.6 mmol), and DIPEA (12.0 ml, 16.17 g, 125 mmol) in DCM (150 ml) was made at 0 °C and stirred for 20 min. The first solution was added to the second and the reaction mixture was stirred for 45 min at 0 °C. The reaction mixture was allowed to warm to room temperature with stirring. The reaction was followed using TLC (eluent: 5% methanol in ethyl acetate) and this indicated that the reaction was complete after another 3 h. The volume of the solvent was reduced to approximately 50 ml under vacuum and HCl (5% soln., 50 ml) was added. The solid was removed by filtration and washed with DCM. The filtrate and the washings were combined and washed with water (3 × 40 ml), HCl (5% solution, 3 × 40 ml), water (2 × 30 ml), $NaHCO_3$ (sat. soln., 3 × 40 ml), water (2 × 30 ml) and brine (sat. soln., 50 ml). The DCM was removed under vacuum and the residue redissolved in methanol. The product was purified by trituration from methanol upon the addition of water. The solid was collected by filtration and partitioned between DCM and water. The DCM was collected and washed with brine (sat soln., 30 ml). The solvent was removed under vacuum yielding the product as a white solid. The product may be purified further by flash chromatography (eluent: ethyl acetate : hexane 3 : 1) if required. Yield: 17.12 g, 26.26 mmol, 92%. ^{1}H NMR ($CDCl_3$) δH (270 MHz); 1.19–1.30 (12H, m, CHCH_3), 1.79–2.52 (12H, m, CHCH_2CH_2), 4.09 (5H, m, CH_2CH$_3$ and CHCH$_2$CH$_2$), 4.19 (4H, q, CH_2CH$_3$), 4.65 (2H, m, CHCH$_2$CH$_2$), 5.04 (2H, d, CH_2Ar), 5.49 (1H, d, ArCH$_2$CO$_2$NH), 7.30 (5H, s, Ar), 7.65 (1H, d, NH), 8.02 (1H, d, NH); ^{13}C NMR ($CDCl_3$) δC; 13.94 (γ CH$_2$CH$_3$, r$_1$/r$_2$), 14.07 (α CH$_2$CH$_3$, r$_1$/r$_2$), 26.52 (β CH$_2$, r$_1$ or r$_2$), 26.67 (β CH$_2$, r$_1$ or r$_2$), 29.04 (β CH$_2$, r$_3$), 30.27 (γ CH$_2$, r$_1$ or r$_2$), 30.53 (γ CH$_2$, r$_1$ or r$_2$), 31.90 (γ CH$_2$, r$_3$), 51.41 (α CH, r$_1$ or r$_2$), 51.59 (α CH, r$_1$ or r$_2$), 53.44 (α CH, r$_3$), 60.44 (γ CH_2CH$_3$, r$_1$ or r$_2$), 60.54 (γ CH_2CH$_3$, r$_1$ or r$_2$), 62.01 (α CH_2CH$_3$, r$_1$ or r$_2$), 62.09 (α CH_2CH$_3$, r$_1$ or r$_2$), 66.66 (Ar-CH_2), 127.66 (*p*-ArC), 127.84 (*o*-ArC), 128.29 (*m*-ArC), 136.17 (*ipso*-ArC), 155.31 (Ar-CH$_2$-O-*C*O), 171.77 (CO), 172.16 (CO), 172.26 (CO), 172.47 (CO), 173.67 (CO), 173.80 (CO); FAB-MS found MH$^+$ 652 and [M + Na]$^+$ 674; $C_{31}H_{45}N_3O_{12}$ requires 651.30.

[G1]-NH$_2$-L-Glutamic acid ethyl ester (2). [G1]-CBZ-L-Glutamic acid ethyl ester (26.8 g, 41 mmol) and ammonium formate (114 g, 180 mmol) were dissolved in methanol (200 ml). Palladium (5%) on carbon catalyst (5 g) was added and the reaction flask fitted with a balloon containing hydrogen gas. The reaction mixture was sonicated for 6 h not allowing the temperature to rise above 40 °C: the reaction was followed by TLC. The carbon was filtered off and washed with methanol. The washings were combined with the filtrate and the solvent removed under vacuum. The residue was partitioned between DCM (30 ml) and $NaHCO_3$ (sat. soln., 30 ml). After removing the aqueous phase the organic layer was washed twice more with base (30 ml each) and then with water (3 × 30 ml), briefly with HCl (5% soln., 15 ml), water (30 ml) and finally brine (sat soln., 30 ml). The DCM was removed under vacuum and the residue purified by flash chromatography (eluent; methanol (5%) in ethyl acetate) to yield a very pale yellow oil which solidified overnight under high vacuum. Yield: 18.7 g, 36 mmol, 88%. ^{1}H NMR (CD_3OD) δH (270 MHz); 1.23 (6H, t, CHCH_3), 1.29 (6H, t, CH$_2$CH_3), 1.70–2.57 (14H, m, CHCH_2CH_2 and NH_2), 4.11 (5H, m, CH_2CH$_3$ and CHCH$_2$CH$_2$), 4.20 (4H, q, CH_2CH$_3$), 4.60 (2H, m, CHCH$_2$CH$_2$), 7.45 (1H, d, NH), 7.88 (1H, d, NH); ^{13}C NMR (CD_3OD) δC; 14.48 (γ CH$_2$CH$_3$, r$_1$/r$_2$), 14.53 (α CH$_2$CH$_3$, r$_1$/r$_2$), 27.58 (β or γ CH$_2$), 30.13 (β or γ CH$_2$), 30.60 (β or γ CH$_2$), 30.98 (β or γ CH$_2$), 31.27 (β or γ CH$_2$), 32.16 (β or γ CH$_2$), 53.11 (α CH, r$_3$), 51.17 (α CH, r$_1$ or r$_2$), 55.32 (α CH, r$_1$ or r$_2$), 61.68 (γ CH_2CH$_3$, r$_1$ or r$_2$), 61.71 (γ CH_2CH$_3$, r$_1$ or r$_2$), 62.44 (α CH_2CH$_3$, r$_1$ or r$_2$), 62.74 (α CH_2CH$_3$, r$_1$ or r$_2$), 169.99 (α COO, r$_1$ or r$_2$), 170.09 (α COO, r$_1$ or r$_2$), 173.13 (α CONH, r$_3$), 174.12 (γ COO, r$_1$ or r$_2$), 174.26 (γ COO, r$_1$ or r$_2$), 174.80 (γ CONH, r$_3$); FAB-MS found MH$^+$ 518 and [M + Na]$^+$ 540; $C_{23}H_{39}N_3O_{10}$ requires 517.26.

[G2]-CBZ-L-Glutamic acid ethyl ester (3). A solution containing [G1]-NH$_2$-L-glutamic acid ethyl ester hydrochloride (13.10 g, 25.3 mmol, compound **2**) and triethylamine (2.03 ml, 2.80 g, 27.7 mmol) dissolved in DCM (50 ml) was prepared and cooled over ice. A second solution containing CBZ-L-glutamic acid (3.38 g, 12.0 mmol), HBTU (11.38 g, 30.0 mmol), HOBt (4.60 g, 30.0 mmol), and DIPEA (5.75 ml, 7.75 g, 60.0 mmol) in DCM (70 ml) was made at 0 °C and stirred for 20 min. The first solution was added to the second and the reaction mixture was stirred for 45 min at 0 °C. The reaction mixture was allowed to warm to room temperature with stirring overnight. The reaction mixture was transferred to a beaker and HCl (5% soln., 50 ml) was added. The solid was collected by filtration and washed with DCM. The filtrate and the washings were combined and washed with water (3 × 40 ml), HCl (5% solution, 3 × 40 ml), water (2 × 30 ml), $NaHCO_3$ (sat. soln., 3 × 40 ml), water (2 × 30 ml) and brine (sat. soln., 50 ml). The DCM was removed under vacuum and the residue redissolved in methanol. The product was purified by trituration from methanol upon the addition of water. The precipitate was collected by filtration, washed with water and then partitioned between DCM and water. The DCM was collected and washed with brine (sat soln., 30 ml). The organic solvent was removed under vacuum yielding the product as a transparent solid. Yield: 7.78 g, 6.07 mmol, 50%. Elemental analysis found C: 55.3, H: 7.0, N: 7.6, $C_{59}H_{89}N_7O_{24}$ requires C: 55.3, H: 7.0, N: 7.7%. ^{1}H NMR ($CDCl_3$ [or CD_3OD]) δH (270 MHz); 1.24 (12H, t, CHCH_3), 1.28 (12H, t, CH$_2$CH_3), 1.85–2.50 (28H, m, CHCH_2CH_2), 4.12 (8H, q, CH_2CH$_3$), 4.20 (11H, q, CH_2CH$_3$ and CHCH$_2$CH$_2$), 4.63 (4H, m, CHCH$_2$CH$_2$), 5.09 (2H, s, CH_2Ar), 5.58 (1H, d, ArCH$_2$CO$_2$NH), 7.01 (1H, d, NH), 7.30 (5H, s, Ar), 7.66 (1H, d, NH), 7.83 (1H, d, NH), 8.10 (1H, d, NH), 8.27 (1H, d, NH), 8.70 (1H, d, NH); ^{13}C NMR ($CDCl_3$) δC; 13.90 (γ CH$_2$CH$_3$), 14.00 (α CH$_2$CH$_3$), 26.39 (β CH$_2$), 26.50 (β CH$_2$), 26.55 (β CH$_2$), 26.65 (β CH$_2$), 26.78 (β CH$_2$), 26.96 (β CH$_2$), 27.38 (β CH$_2$), 30.19 (γ CH$_2$), 30.24 (γ CH$_2$), 30.42 (γ CH$_2$), 30.55 (γ CH$_2$), 31.00 (γ CH$_2$), 31.68 (γ CH$_2$), 31.83 (γ CH$_2$), 51.52 (α CH), 51.62 (α CH), 52.27 (α CH), 52.87 (α CH), 53.03 (α CH), 60.24 (γ CH_2CH$_3$), 60.47 (γ CH_2CH$_3$), 60.52 (γ CH_2CH$_3$), 60.57 (γ CH_2CH$_3$), 61.79 (α CH_2CH$_3$), 61.87 (α CH_2CH$_3$), 61.95 (α CH_2CH$_3$), 62.13 (α CH_2CH$_3$), 66.58 (Ar-CH_2), 127.74 (*p*-ArC), 127.82 (*o*-ArC), 128.27 (*m*-ArC), 136.12 (*ipso*-ArC), 161.25 (Ar-CH$_2$-O-*C*O), 172.36 (CO), 172.45 (CO), 172.67 (CO), 172.96 (CO), 173.41 (CO), 173.49 (CO), 173.59 (CO); FAB-MS found MH$^+$ 1281 and [M + Na]$^+$ 1303; $C_{59}H_{89}N_7O_{24}$ requires 1279.60.

[G2]-NH$_2$-L-Glutamic acid ethyl ester (4). [G2]-CBZ-L-Glutamic acid ethyl ester (6.78 g, 5.3 mmol) and ammonium formate (1.33) were dissolved in methanol (50 ml). Palladium (5%) on carbon catalyst (1 g) was added and the reaction flask fitted with a balloon containing hydrogen gas. The reaction mixture was sonicated for 6 h not allowing the temperature to rise above 40 °C. The carbon was filtered off and washed with methanol. The washings were combined with the filtrate and the solvent removed under vacuum. The residue was partitioned between DCM (30 ml) and $NaHCO_3$ (sat. soln., 30 ml). After removing the aqueous phase the organic layer was washed twice more with base (30 ml each) and then with water (3 × 30 ml), briefly with HCl (5% soln., 15 ml), water (30 ml) and finally brine (sat soln., 30 ml). The DCM was removed

under vacuum and the residue purified by flash chromatography (eluent; methanol (10%) in ethyl acetate) to yield an opaque solid which was dried under high vacuum. Yield: 5.47 g, 4.77 mmol, 90%. ^{1}H NMR ($CD_3OD/CDCl_3$ 1 : 1) δH (270 MHz); 1.26 (12H, t, $CHCH_3$), 1.29 (12H, t, CH_2CH_3), 1.92–2.51 (30H, m, $CHCH_2CH_2$ and NH_2), 4.13 (8H, q, CH_2CH_3), 4.20 (8H, q, CH_2CH_3), 4.24–4.40 (3H, m, $CHCH_2CH_2$), 4.54 (4H, m, $CHCH_2CH_2$), 8.10 (1H, d, NH), 8.23 (1H, d, NH), 8.53 (1H, d, NH), 8.73 (1H, d, NH), 9.01 (1H, d, NH); ^{13}C NMR (CD_3OD) δC; 14.45 (γ CH_2CH_3), 14.50 (α CH_2CH_3), 27.23 (β or γ CH_2), 27.39 (β or γ CH_2), 27.52 (β or γ CH_2), 28.18 (β or γ CH_2), 28.56 (β or γ CH_2), 30.86 (β or γ CH_2), 31.04 (β or γ CH_2), 31.14 (β or γ CH_2), 32.24 (β or γ CH_2), 32.36 (β or γ CH_2), 32.42 (β or γ CH_2), 52.73 (α CH), 52.80 (α CH), 52.83 (α CH), 53.21 (α CH), 53.47 (α CH), 53.90 (α CH), 61.45 (γ CH_2CH_3), 61.49 (γ CH_2CH_3), 62.51 (α CH_2CH_3), 62.54 (α CH_2CH_3), 172.92 (CO), 173.00 (CO), 173.13 (CO), 173.40 (CO), 173.44 (CO), 173.68 (CO), 173.73 (CO), 173.76 (CO), 174.22 (CO), 174.33 (CO), 174.44 (CO), 174.82 (CO); FAB-MS found MH^+ 1146 and $[M + Na]^+$ 1168; $C_{51}H_{83}N_7O_{22}$ requires 1145.56.

[G1]-CBZ-L-Glutamic acid *tert*-butyl ester (5). A solution containing L-glutamic acid di-*tert*-butyl ester hydrochloride (5 g, 16.9 mmol) and DIPEA (2.94 ml, 2.18 g, 16.9 mmol) dissolved in DCM (50 ml, dist.) was prepared and cooled over ice. A second solution containing CBZ-L-glutamic acid (2.27 g, 8.05 mmol), HBTU (7.33 g, 19.32 mmol), HOBt (2.96 g, 19.32 mmol) and DIPEA (6.7 ml, 5.0 g, 38.7 mmol) in DCM (70 ml) was made at 0 °C and stirred for 20 min. The first solution was added to the second and the reaction mixture was stirred for 45 min at 0 °C. The reaction mixture was allowed to warm to room temperature whilst stirring for a further 3 h. The volume of the solvent was reduced to approximately 50 ml under vacuum and water was added. The DCM layer was washed with water (3 × 40 ml), HCl (5% solution, 3 × 40 ml), water (2 × 30 ml), $NaHCO_3$ (sat. soln., 3 × 40 ml), water (2 × 30 ml) and brine (sat. soln., 50 ml). The DCM was removed under vacuum and the residue was purified by flash chromatography (eluent: hexane : ethyl acetate 1 : 1). TLC (eluent: methanol (5%) in ethyl acetate) indicated the presence of only one compound. Yield: 5.25 g, 6.88 mmol, 86%. ^{1}H NMR ($CDCl_3$) δH (270 MHz); 1.43 (18H, s, $CHCH_3$), 1.47 (18H, s, CH_2CH_3), 1.79–2.38 (12H, m, $CHCH_2CH_2$), 4.07 (1H, m, $CHCH_2CH_2$), 4.58 (2H, m, $CHCH_2CH_2$), 5.07 (2H, d, CH_2Ar), 5.53 (1H, d, $ArCH_2CO_2NH$), 7.31 (5H, s, Ar), 7.83 (1H, d, NH), 8.16 (1H, d, NH); ^{13}C NMR ($CDCl_3$) δC; 26.60 (β CH_2, r_1 or r_2), 26.83 (β CH_2, r_1 or r_2), 27.80 (γ CH_3), 27.88 (α CH_3), 29.13 (β CH_2, r_3), 31.34 (γ CH_2, r_1 or r_2), 31.59 (γ CH_2, r_1 or r_2), 31.85 (γ CH_2, r_3), 51.93 (α CH, r_1 or r_2), 52.16 (α CH, r_1 or r_2), 53.29 (α CH, r_3), 66.51 (Ar-CH_2), 80.36 (γ $C(CH_3)_3$, r_1 or r_2), 80.46 (γ $C(CH_3)_3$, r_1 or r_2), 82.83 (α $C(CH_3)_3$, r_1 or r_2), 82.93 (α $C(CH_3)_3$, r_1 or r_2), 127.79 (*p*-ArC), 128.23 (*o*/*m*-ArC), 136.17 (*ipso*-ArC), 155.10 (Ar-CH_2-O-CO), 171.46 (CO), 171.56 (CO), 171.69 (CO), 172.52 (CO), 173.09 (CO), 173.33 (CO); FAB-MS found MH^+ 764 and $[M + Na]^+$ 1786; $C_{39}H_{61}N_3O_{12}$ requires 763.43.

[G1]-NH_2-L-Glutamic acid *tert*-butyl ester (6). [G1]-CBZ-L-Glutamic acid *tert*-butyl ester (10.12 g, 13.32 mmol) and ammonium formate (3.4 g, 54 mmol) were dissolved in methanol (100 ml) containing DCM (15 ml). A 5% palladium on carbon catalyst (1 g) was added and the solution was sonicated for 3 h under a hydrogen atmosphere. The carbon was filtered off and the filtrate evaporated to dryness under vacuum. The residue was partitioned between DCM (100 ml) and water (50 ml). The DCM layer was washed with water (2 × 40 ml), HCl (5% solution, 2 × 40 ml), water (2 × 30 ml), $NaHCO_3$ (sat. soln., 3 × 40 ml), water (2 × 30 ml) and brine (sat. soln., 50 ml). The DCM was removed under vacuum and the residue dried in a vacuum oven yielding a very thick honey coloured oil. TLC (methanol (5%) in ethyl acetate) followed by development with iodine and ninhydrin indicated the presence of only one compound and that it contained a primary amine function. Yield: 6.085 g, 9.67 mmol, 73%. ^{1}H NMR ($CDCl_3$) δH (270 MHz); 1.43 (18H, s, $CHCH_3$), 1.47 (18H, s, CH_2CH_3), 1.78–2.37 (14H, m, $CHCH_2CH_2$ and NH_2), 4.58 (3H, m, $CHCH_2CH_2$), 8.16 (1H, d, NH); ^{13}C NMR (CD_3OD) δC; 27.68 (β CH_2, r_1 or r_2), 27.91 (β CH_2, r_1 or r_2), 28.31 (γ CH_3), 28.40 (α CH_3), 32.42 (γ CH_2, r_1 or r_2), 32.52 (γ CH_2, r_1 or r_2), 32.65 (β CH_2, r_3), 33.01 (γ CH_2, r_3), 53.48 (α CH, r_1 or r_2), 53.66 (α CH, r_1 or r_2), 54.96 (α CH, r_3), 81.68 (γ $C(CH_3)_3$, r_1/r_2), 83.47 (α $C(CH_3)_3$, r_1 or r_2), 83.65 (α $C(CH_3)_3$, r_1 or r_2), 173.10 (CO), 173.14 (CO), 173.44 (CO), 173.63 (CO), 175.19 (CO), 176.78 (CO); FAB-MS found MH^+ 630 and $[M + Na]^+$ 652; $C_{31}H_{55}N_3O_{10}$ requires 629.39.

[G0.5]-CBZ-L-Glutamic acid di-L-phenylalanine ethyl ester (7). A solution containing L-phenylalanine ethyl ester hydrochloride (25 g, 109 mmol) and triethylamine (8.8 ml, 12.1 g, 120 mmol) dissolved in DCM (150 ml) was prepared and cooled over ice. A second solution containing CBZ-L-glutamic acid (14.56 g, 51.8 mmol), HBTU (45.51 g, 120 mmol), HOBt (18.36 g, 120 mmol) and DIPEA (31 g, 240 mmol) in DCM (200 ml) was made at 0 °C and stirred for 20 min. The first solution was added to the second and the reaction mixture was stirred for 45 min at 0 °C. The reaction mixture was allowed to warm to room temperature whilst stirring for a further 6 h and placed in a refrigerator overnight. The volume of the solvent was reduced to approximately 100 ml under vacuum and then transferred to a beaker and HCl (5% soln., 50 ml) was added. The solid was collected by filtration and washed with DCM. The filtrate and the washings were combined and washed with water (3 × 40 ml), HCl (5% solution, 3 × 40 ml), water (2 × 30 ml), $NaHCO_3$ (sat. soln., 3 × 40 ml), water (2 × 30 ml) and brine (50 ml). The DCM was removed under vacuum and the residue redissolved in methanol. The product was purified by trituration from methanol upon the addition of water. The precipitate was collected by filtration, washed with water and then partitioned between DCM and water. The DCM was collected and washed with brine (sat soln., 30 ml). This crude product was purified by flash chromatography (eluent: ethyl acetate) to yield a opaque solid. TLC (eluent: ethyl acetate) indicated the presence of only one compound. Yield: 29.29 g, 46.1 mmol, 90%. Elemental analysis found C: 66.5, H: 6.5, N: 6.7, $C_{35}H_{41}N_3O_8$ requires C: 66.6, H: 6.5, N: 6.7%. ^{1}H NMR ($CDCl_3$) δH (270 MHz); 1.17–1.28 (6H, m, $CHCH_3$), 1.75–2.28 (4H, m, $CHCH_2CH_2$), 2.80–3.12 (4H, m, $CHCH_2$-Ar), 4.14 (5H, m, CH_2CH_3 and $CHCH_2CH_2$), 4.84 (2H, q, $CHCH_2Ar$), 5.04 (2H, d, $ArCH_2CO_2$), 5.57 (1H, d, $ArCH_2CO_2NH$), 7.14–7.30 (15H, m, ArH), 7.33 (1H, d, NH), 7.90 (1H, d, NH); ^{13}C NMR ($CDCl_3$) δC; 13.86 (CH_2CH_3), 13.92 (CH_2CH_3), 29.10 (β CH_2, r_3), 31.72 (γ CH_2, r_3), 37.19 (β CH_2, r_1 or r_2), 37.50 (β CH_2, r_1 or r_2), 53.21 (α CH, r_3), 53.57 (α CH, r_1/r_2), 61.61 (CH_2CH_3), 61.75 (CH_2CH_3), 66.53 (Ar-CH_2-O-), 126.80 (*p*-ArC), 127.76 (*p*-ArC), 128.24 (*o*-ArC), 128.32 (*o*-ArC), 128.99 (*m*-ArC), 129.09 (*m*-ArC), 135.86 (*ipso*-ArC), 136.08 (*ipso*-ArC), 136.23 (*ipso*-ArC), 155.21 (Ar-CH_2-O-CO), 171.51 (CO), 172.32 (CO), 173.01 (CO), 173.20 (CO); FAB-MS found MH^+ 632 and $[M + Na]^+$ 654; $C_{35}H_{41}N_3O_8$ requires 631.29.

[G0.5]-NH_2-L-Glutamic acid di-L-phenylalanine ethyl ester (8). [G0.5]-CBZ-L-Glutamic acid di-L-phenylalanine ethyl ester (28.12 g, 44.5 mmol, compound **7**) and ammonium formate (11.7 g, 186 mmol) were dissolved in methanol (150 ml) containing DCM (10 ml). A 5% palladium on carbon catalyst (3 g) was added and the solution was stirred for 6 h at room temperature. The carbon was removed by filtration and washed with methanol. The filtrate was combined with the

washings and the methanol was removed under vacuum. The residue was partitioned between DCM (100 ml) and water (50 ml). The DCM layer was washed with water (3 × 100 ml), $NaHCO_3$ (sat. soln., 3 × 50 ml), water (2 × 30 ml), HCl (5% solution, 3 × 30 ml), water (2 × 30 ml) and brine (sat. soln., 75 ml). The DCM was removed under vacuum and the residual oil redissolved in hot ethyl acetate. The solution was filtered and the filtrate collected. The product was triturated from the filtrate by the addition of hexane. TLC (eluent: ethyl acetate) followed by development with iodine and ninhydrin indicated the presence of only one compound and that it contained a primary amine function. Yield: 21.75 g, 43.8 mmol, 98%. ^{1}H NMR (CD_3OD) δH (270 MHz); 1.17–1.26 (6H, m, $CHCH_3$), 1.75–2.25 (4H, m, $CHCH_2CH_2$), 2.79–3.14 (4H, m, $CHCH_2$-Ar), 4.15 (5H, m, CH_2CH_3 and $CHCH_2CH_2$), 4.68 (2H, m, $CHCH_2$Ar), 7.23 (12H, m, Ar*H* and 2N*H*); ^{13}C NMR (CD_3OD) δC; 14.42 (CH_2CH_3), 31.77 (β CH_2, r_3), 32.55 (γ CH_2, r_3), 38.25 (β CH_2, r_1 or r_2), 38.30 (β CH_2, r_1 or r_2), 54.60 (α CH, r_3), 55.28 (α CH, r_1 or r_2), 55.36 (α CH, r_1 or r_2), 62.65 (CH_2CH_3), 62.74 (CH_2CH_3), 127.96 (*p*-ArC), 128.01 (*p*-ArC), 129.52 (*o*-ArC), 129.57 (*o*-ArC), 130.23 (*m*-ArC), 137.85 (*ipso*-ArC), 138.01 (*ipso*-ArC), 173.53 (CO), 175.06 (CO), 175.58 (CO); FAB-MS found MH^+ 498 and $[M + Na]^+$ 520; $C_{27}H_{35}N_3O_6$ requires 497.25.

***N*-Alkylcarboxy-[G1]-L-glutamic acid ethyl ester (9).** [G1]-NH_2-L-Glutamic acid ethyl ester (2.02 g, 3.9 mmol) was dissolved in DCM (35 ml) containing triethylamine (0.6 ml, 435 mg, 4.2 mmol). To this was added succinic anhydride (781 mg, 7.8 mmol) and the reaction mixture was stirred for 5 h at room temperature. The reaction was followed by TLC (eluent: methanol (5%) in ethyl acetate) and deemed complete at this point. The reaction mixture was washed with water (5 × 50 ml), HCl (5% soln., 4 × 50 ml) and again with water (5 × 20 ml) and the organic layer collected. The DCM was removed under vacuum yielding a white solid. The solid was recystallised from methanol affording a white solid. Yield: 1.08 g, 1.75 mmol, 45%. ^{1}H NMR ($CDCl_3$) δH (270 MHz); 1.20–1.31 (12H, m, $CHCH_3$), 1.90–2.67 (16H, m, $CHCH_2CH_2$), 4.14 (8H, m, CH_2CH_3), 4.32–4.49 (3H, m, $CHCH_2CH_2$), 8.19 (1H, d, N*H*), 8.28 (1H, d, N*H*); FAB-MS found MH^+ 618, $[M + Na]^+$ 640 and $[M + 2Na]^+$ 662; $C_{27}H_{43}N_3O_{13}$ requires 617.28.

***N*-Alkylcarboxy-[G2]-L-glutamic acid ethyl ester (10).** [G2]-NH_2-L-Glutamic acid ethyl ester (500 g, 436 μmol) was dissolved in DCM (5 ml) containing DIPEA (76 μl, 56 mg, 436 μmol), and prepared as in (**9**). Yield: 279 mg, 224 μmol, 51%. ^{1}H NMR δH (270 MHz); 1.23–1.33 (24H, m, $CHCH_3$), 1.89–2.67 (28H, m, $CHCH_2CH_2$), 4.17 (16H, m, CH_2CH_3), 4.47–4.66 (7H, m, $CHCH_2CH_2$), 7.80 (1H, d, N*H*), 7.99 (1H, d, N*H*), 8.32 (1H, d, N*H*), 8.54 (1H, d, N*H*); FAB-MS found MH^+ 1246, $[M + Na]^+$ 1269; $C_{55}H_{87}N_7O_{25}$ requires 1245.

***N*-Alkylcarboxy-[G1]-L-glutamic acid *tert*-butyl ester (11).** [G1]-NH_2-L-Glutamic acid *tert*-butyl ester (2.86 g, 4.55 mmol) was dissolved in DCM (15 ml) containing triethylamine (619 μl, 450 mg, 4.6 mmol) and prepared as for (**9**). Yield: 2.97 g, 4.08 mmol, 90%. ^{1}H NMR (CD_3OD) δH (270 MHz); 1.41 (9H, s, $CHCH_3$), 1.42 (9H, s, $CHCH_3$), 1.44 (9H, s, $CHCH_3$), 1.45 (9H, s, $CHCH_3$), 1.75–2.60 (16H, m, $CHCH_2CH_2$), 4.28 (3H, m, $CHCH_2CH_2$), 8.06 (1H, d, N*H*), 8.17 (1H, d, N*H*); ^{13}C NMR (CD_3OD) δC; 27.55 (β CH_2, r_1 or r_2), 27.71 (β CH_2, r_1 or r_2), 28.28 (γ CH_3), 28.36 (α CH_3), 29.05 (HO_2CCH_2-CH_2CONH), 30.17 (α CH_2, r_3), 31.37 ($HO_2CCH_2CH_2$-CONH), 32.44 (γ CH_2, r_1 or r_2), 32.55 (γ CH_2, r_1 or r_2), 32.81 (β CH_2, r_3), 53.68 (α CH), 53.72 (α CH), 81.72 (γ $C(CH_3)_3$), 83.32 (α $C(CH_3)_3$), 172.85 (CO), 173.10 (CO), 173.13 (CO), 173.37 (CO), 173.49 (CO), 174.35 (CO), 174.96 (CO), 176.16 (CO); FAB-MS found MH^+ 730, $[M + Na]^+$ 752; $C_{35}H_{59}N_3O_{13}$ requires 729.40.

***N*-Alkylcarboxy-[G0.5]-L-glutamic acid di-L-phenylalanine ethyl ester (12).** [G0.5]-NH_2-L-Glutamic acid di-L-phenylalanine ethyl ester (4.59 mg, 9.24 mmol) was dissolved in DCM (200 ml) containing DIPEA (1.6 ml, 1.19 g, 9.24 mmol), and prepared as for (**9**). Yield: 4.79 g, 8.03 mmol, 87%. ^{1}H NMR (CD_3OD) δH (270 MHz); 0.98–1.81 (14H, m, $CHCH_3$ and $CHCH_2CH_2$), 2.90–3.26 (4H, m, $CHCH_2$Ar), 3.76 (1H, dt, $CHCH_2$Ar), 4.12 (4H, m, CH_2CH_3), 4.33 (1H, dt, $CHCH_2$Ar), 4.62 (1H, dd, $CHCH_2CH_2$), 7.03–7.34 (15H, m, Ar*H*), 7.86 (1H, d, N*H*), 8.04 (1H, d, N*H*); ^{13}C NMR δC; 14.16 (CH_2CH_3), 14.21 (CH_2CH_3), 31.14 (CH_2, r_3), 37.98 (β CH_2, r_1 or r_2), 39.63 (β CH_2, r_1 or r_2), 54.29 (α CH, r_1 or r_2), 54.59 (α CH, r_1 or r_2), 56.79 (α CH, r_3), 61.83 (CH_2CH_3), 127.49 (*p*-ArC), 127.93 (*p*-ArC), 129.08 (*o*-ArC), 129.16 (*o*-ArC), 129.83 (*m*-ArC), 131.22 (*m*-ArC), 136.29 (*ipso*-ArC), 137.65 (*ipso*-ArC), 168.09 (CO), 168.55 (CO), 176.2 (CO); FAB-MS found $[M + Na]^+$ 620 and $[M + 2Na]^+$ 642; $C_{31}H_{39}N_3O_9$ requires 597.27.

Synthetic modification of chromatographic silica

3-Aminopropyl-silica. Spherisorb silica (52 g, dried overnight at 150 °C) was suspended in petroleum spirit (bp 80–100 °C) (200 ml). Diethylamine (71 μl, 0.136% w/w) was added followed by 3-aminopropyltrimethoxysilane (14.6 ml, 28% w/w). The reaction mixture was refluxed with gentle overhead stirring for 16 h. The product was collected by filtration and washed with petroleum spirit (100 ml), DCM (250 ml), methanol (500 ml), methanol–water (1 : 1) (500 ml, degassed) and water (500 ml, deionised). The silica was then transferred to a beaker and suspended in water (250 ml) for 1 h, during which time the fines were removed, followed by a final wash with methanol. A sample of the silica was suspended in a ninhydrin solution in methanol–butanol (1 : 1) and heated (80 °C) for 30 min. The silica particles turned blue/purple, while the solvent remained colourless. The product was dried under high vacuum at 150 °C. Elemental analysis; C: 2.00, H: 0.57, N: 0.61%. Ligand loading: 2.45% w/w, 435 μmol g^{-1} AP-spacer on silica.

Synthesis of dendrimer chiral stationary phases

[G1]-L-Glutamic acid ethyl ester-AC-silica (13). Alkylcarboxy-[G1]-L-glutamic acid ethyl ester (700 mg, 1.10 mmol, **9**) was dissolved in DME (25 ml, anhy.). To this solution AP-silica (2.50 g) was added. The mixture was treated with 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (333 mg, 1.35 mmol, vacuum dried), sonicated briefly and then swirled at room temperature for 7 days. The silica was collected by filtration and washed with DME (30 ml), DCM (50 ml), DCM–methanol (1 : 1, 30 ml), methanol (50 ml), methanol–water (1 : 1, 40 ml) and then stood in water (100 ml) for 30 min. During this time the fines were removed by repeatedly decanting the supernatant and replacing it with water. The silica was recovered by filtration and washed further with water (50 ml), methanol (50 ml) and then DCM (30 ml). The silica was dried under high vacuum. Elemental analysis; C: 8.80, H: 1.38, N: 1.42 (reference AP-silica); C: 2.00, H: 0.57, N: 0.61%. Ligand loading: 210 μmol g^{-1}, 1.2 μmol m^{-2}.

[G2]-L-Glutamic acid ethyl ester-AC-silica (14). Alkylcarboxy-[G2]-L-glutamic acid ethyl ester (270 mg, 217 μmol, **10**) was dissolved in DME–DMF mix (1 : 1, 20 ml, anhy.), stirred for 34 days and otherwise prepared as for (**13**). Elemental analysis; C: 5.72, H: 0.98, N: 1.06 (reference AP-silica); C: 2.00, H: 0.57, N: 0.61%. Ligand loading: 57 μmol g^{-1}, 0.32 μmol m^{-2}.

[G1]-L-glutamic acid *tert*-butyl ester-AC-silica (15). Alkylcarboxy-[G1]-L-glutamic acid *tert*-butyl ester (816 mg, 1.1 mmol, **11**) was dissolved in DME (25 ml, anhy.), stirred for 14 days and otherwise prepared as for (**13**). Elemental analysis; C: 9.79, H: 1.55, N: 1.27 (reference AP-silica); C: 2.00, H: 0.57, N: 0.61%. Ligand loading: 185 μmol g^{-1}, 1.05 μmol m^{-2}.

[G0.5]-L-glutamic acid di-L-phenylalanine ethyl ester-AC-silica (16). Alkylcarboxy-[G0.5]-L-glutamic acid di-L-phenylalanine ethyl ester (836 mg, 1.4 mmol, **12**) was dissolved in DME-DMF (1 : 3, 25 ml, anhy.) and prepared as in (**15**). Elemental analysis; C: 4.96, H: 0.86, N: 0.86 (reference AP-silica); C: 2.00, H: 0.57, N: 0.61%. Ligand loading: 80 μmol g^{-1}, 0.46 μmol m^{-2}.

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