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Synthesis and gelation properties of a new class of α-amino acid-based sector block dendrons

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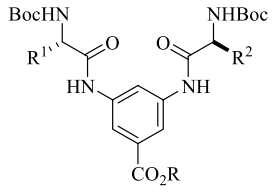
Abstract—A new series of α-amino acid-based sector block dendrons containing alanine, phenylalanine, and valine dendritic sectors was prepared by a solution phase peptide coupling methodology. The structures of the dendrons were fully characterized by nuclear magnetic resonance and mass spectroscopy and by optical polarimetry, and their purities were determined by size exclusion chromatography. Some of the dendrons, especially those containing phenylalanine residues, were found to form strong physical gels with aromatic solvents. The gelation mechanism was further investigated by infra-red and circular dichroism spectroscopy. It was found that both inter-molecular hydrogen bonding and aromatic π–π stacking interactions were the main driving forces for gelation.

1. Introduction

The use of α-amino acids as ingredients for the construction of dendrimers has become a topic of current interest. Such compounds can be used as biomimetic models toward the property studies of artificial proteins and enzymes.[1] Furthermore, such materials are also envisaged to possess better bio-compatibility[2] because of their compositions are consisted of naturally occurring amino acids. Depending on how the amino acids are linked together, such dendrimers can be classified as peptide-based or amino acid-based dendrimers. In the former category the amino acid units are directly connected to each other, while in the latter they are linked to each other through non-amino acid spacers. Typical peptide-based dendrimers are the poly(lysine) dendrimers,[3] poly(ornithine) dendrimers,[4] and poly(glutamate) dendrimers,[5] and some of their derivatives have shown very interesting biological and gelation properties.[3b–3e] Interest in amino acid-based dendrimers and dendrons has also appeared recently.[6] However, most of the peptide-based and amino acid-based dendrimers reported to date are synthesized from only one type of amino acid. Hence, if more than one kind of amino acids are used in their constructions, such as those encountered in natural proteins and peptides, dendrimers with diverse structural variety can then be resulted. In this context Reymond recently reported a combinatorial approach to the synthesis of a library of catalytically active layer block peptide-based dendrimers.[7] One of the most intriguing findings was that the catalytic reactivity was shown to be dependent on the nature of the amino acid in the different concentric layers. This suggested that the amino acid arrangement within the dendrimers, similar to the amino acid sequence of enzymes, has a profound effect on their properties. Our group recently reported the synthesis of a combinatorial series of amino acid-based layer block dendrons using alanine, phenylalanine, and valine as the ingredients.[8] We also found that gelation properties of these dendrons were strongly influenced by their layer block amino acid sequence. Herein, we wish to describe the synthesis of a new series of G1-G2 amino acid-based sector block dendrons **1**–**4**. In addition, we also show that such amino acid-based dendrimers possess rich structural diversities and exhibit amino acid dependent gelation properties.



1 R = Et, [G1-(aa^1)(aa^2)-CO_2Et]
2 R = H, [G1-(aa^1)(aa^2)-CO_2H]
aa^{1}aa^2 = AF, AV and FV

Keywords: Dendrimers; Amino acid-based dendrimers; Organogelators.
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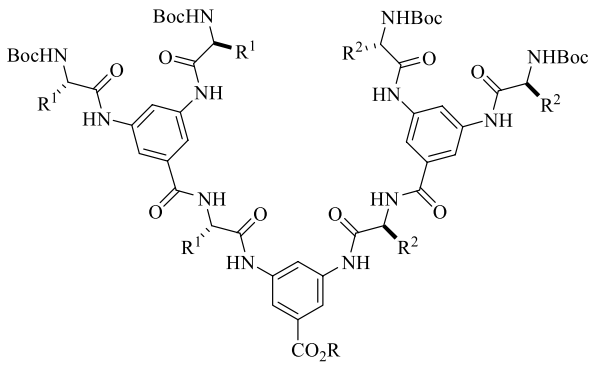


3 R = Et, [G2-(aa^1)$_3$(aa^2)$_3$-CO_2Et]
4 R = H, [G2-(aa^1)$_3$(aa^2)$_3$-CO_2H]
(aa^1)$_3$(aa^2)$_3$ = A_3F_3, A_3V_3 and F_3V_3

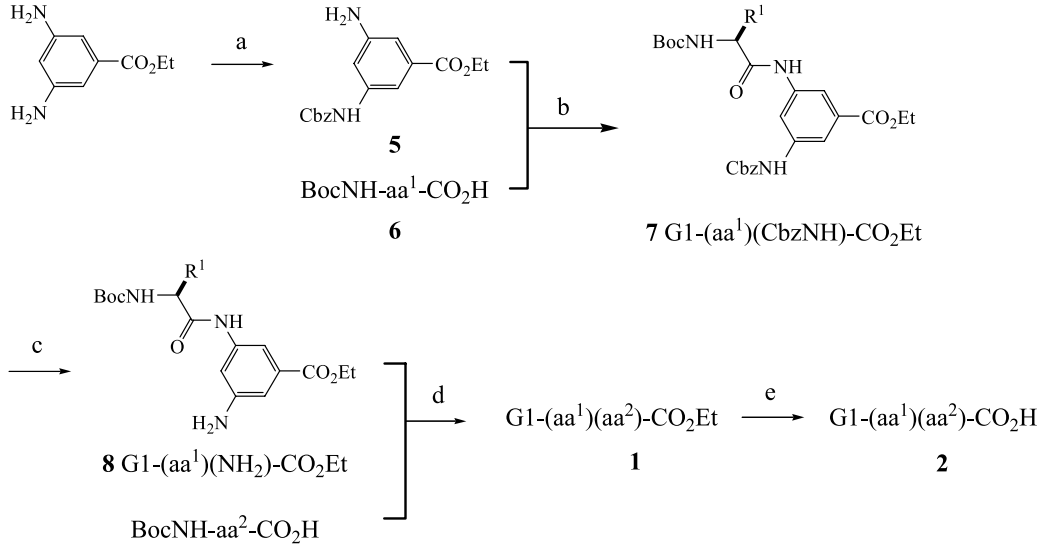
R^1 = Me, Bn or *i*-Pr
R^2 = Me, Bn or *i*-Pr
and $R^1 \neq R^2$

2. Results and discussion

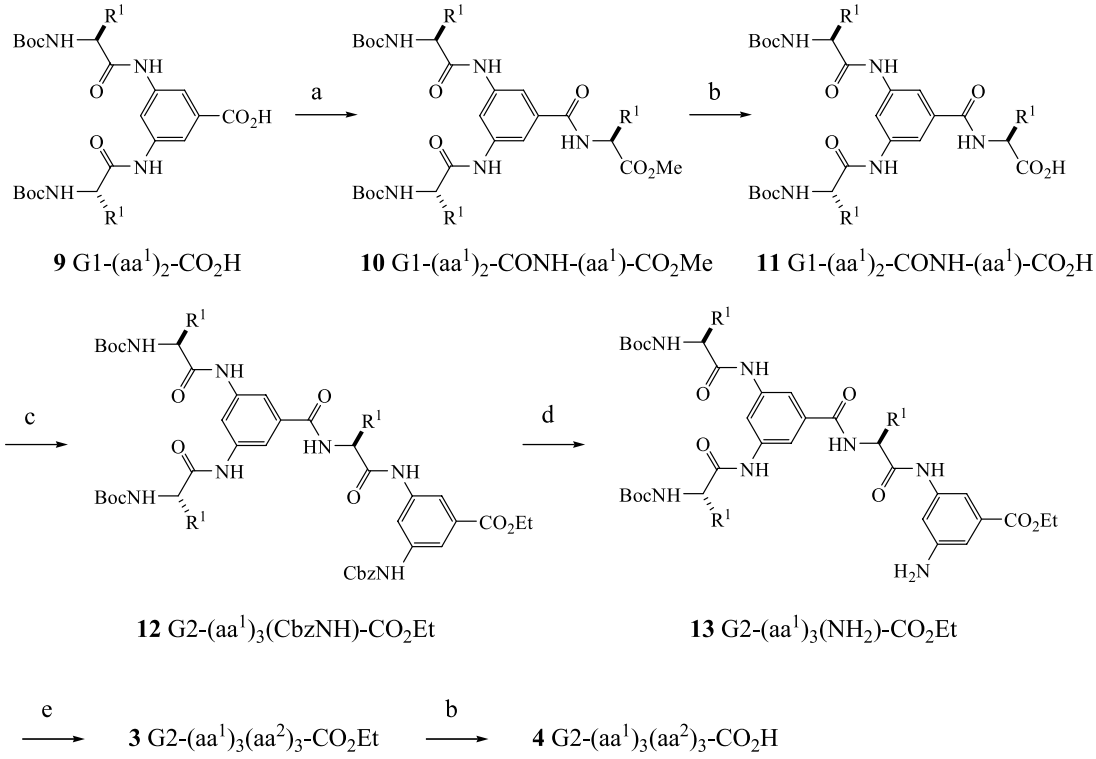
2.1. Synthesis

We employed 3,5-diaminobenzoic acid as the branching agent in this class of sector block dendrimers. In contrast to the synthesis of layer block dendrimers, the synthesis of sector block dendrimers or dendrons requires a high degree of control over the number and placement of functional groups within the same dendritic layer. Therefore, one needs to differentiate the two amino groups in our branching agent. Hence, one of the amino groups was selectively protected as the Cbz derivative **5** in 71% yield by reacting ethyl 3,5-diaminobenzoate with 1 equiv of benzyl chloroformate (Scheme 1). The other amino group was subsequently coupled to either BocNH-L-alanine **6** (aa^1=A) or BocNH-L-valine **6** (aa^1=V) in the presence of 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) to furnish G1-A(CbzNH)-CO_2Et **7** (aa^1=A) or G1-V(CbzNH)-CO_2Et **7** (aa^1=V), respectively, in excellent yields. The Cbz protective group in compounds **7** was then removed by catalytic hydrogenation to afford G1-(aa^1)(NH_2)-CO_2Et **8** in about 95% yields. The different products **8** were then coupled to a second amino acid BocNH-aa^2-CO_2H in the presence of EEDQ to give the three sector block dendrons G1-(aa^1)(aa^2)-CO_2Et **1** (aa^{1}aa^2=AF, AV, and VF) in 87–98% yield as white solids. Alkaline hydrolysis of the ethyl ester group then gave the corresponding carboxylic acid dendrons G1-(aa^1)(aa^2)-CO_2H **2** (aa^{1}aa^2=AF, AV, and VF) in nearly quantitative yields.

The starting materials for the preparation of the three different G2 sector block dendrons **3** were the known layer block dendrons G1-(aa^1)$_2$-CO_2H **9** reported earlier by us.[8] Hence, coupling of compounds **9** (aa^1=A, F, or V) with H_3N^+-(aa^1)-CO_2Me Cl^- bearing the same aa^1 amino acid residue in the presence of dicyclohexylcarbodiimide (DCC), 4-methylmorpholine, and 1-hydroxybenzotriazole (HOBt) afforded the three G1-(aa^1)$_2$-CONH-(aa^1)-CO_2Me (aa^1=A, F, or V) esters **10** in 62–80% yields (Scheme 2). The methyl ester functionality was then removed by alkaline hydrolysis to produce the corresponding acid dendrons G1-(aa^1)$_2$-CONH-(aa^1)-CO_2H (aa^1=A, F, or V) **11** in nearly quantitative yields. The acid dendrons **11** were then anchored to the mono-protected branching unit **5** using either DCC or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide (EDCI) as the coupling agent and HOBt to give the hemi-substituted G2-(aa^1)$_3$(CbzNH)-CO_2Et dendrons **12** containing the aa^1 amino acid dendritic sector. The Cbz protecting group was then dismantled by catalytic hydrogenolysis (10% Pd on C, MeOH) to release the free amino group. The product **13** was then subsequently coupled (DCC or EDCI, HOBt, THF) to an aa^2 containing acid dendron G1-(aa^2)$_2$-CONH-(aa^2)-CO_2H **12** to give the target sector block dendrons G2-(aa^1)$_3$(aa^2)$_3$-CO_2Et **3** (aa^{1}aa^2=AF, AV, FV) in 35–46% yield as white solids. Finally, base hydrolysis (1.0 M aqueous KOH in MeOH) of the ethyl ester group produced the sector block acid dendrons G2-(aa^1)$_3$(aa^2)$_3$-CO_2H **4** (aa^{1}aa^2=AF, AV, FV) in 94–99% yields.



Scheme 1. Reagents and conditions: (a) BnOCOCl, NaOH (1 M), THF, 5–25 °C, 3 h; (b) EEDQ, CH_2Cl_2, 0–25 °C, 12 h; (c) H_2, 10% Pd on C, MeOH, 25 °C, 1 h; (d) EEDQ, THF/CH_2Cl_2, 0–25 °C, 12 h; (e) KOH (1.0 M), MeOH/H_2O, 25 °C.



Scheme 2. Reagents and conditions: (a) $Cl^{-}\ H_3N^{+}$-(aa^1)-CO_2Me, DCC, HOBt, 4-methylmorpholine, THF, −10–25 °C, 22 h; (b) KOH (1.0 M), MeOH/H_2O, 25 °C; (c) **5**, DCC or EDCI, HOBt, THF, −10–25 °C, 22 h; (d) H_2, 10% Pd on C, MeOH, 25 °C, 1 h; (e) G1-(aa^2)$_2$-CONH-(aa^2)-CO_2H, DCC or EDCI, HOBt, THF, −10–25 °C, 50 h.

2.2. Characterization

2.2.1. NMR spectroscopy. The structural identities of all the intermediates and target compounds were characterized by ^{1}H NMR and ^{13}C NMR spectroscopy. In contrast to the layer block dendrons reported earlier,[8] the sector block dendrons **1**–**4** are devoid of a *pseudo*-C_2 axis and their ^{1}H NMR spectra are slightly more complex. Nonetheless, the characteristics of the 'finger print' peaks due to the protons on the amino acid side chains were readily diagnosed. For example, the ^{1}H NMR spectrum of G1-AF-CO_2Et showed the presence of the alanine methyl side chain as a doublet at δ 1.26 and the diastereotopic benzylic protons of the phenylalanine side chain as a multiplet at δ 2.72–3.05 (Fig. 1). The two anilide protons were chemically non-equivalent and appeared as two separate singlets at δ 10.18 and 10.29. The two Boc groups were also different and showed up as two singlets at δ 1.32 and 1.38. On the other hand, the isopropyl side chain of the valine residue in the dendron G1-AV-CO_2Et appeared as a doublet at δ 0.89 and a multiplet at δ 1.85–2.08. The chemical shift values of the amino acid side chain protons remained essentially the same on going from the G1 to the G2 dendrons when they were located on the surface layer. Hence, in the ^{1}H NMR spectrum of G2-A_3F_3-CO_2Et, the surface alanine methyl groups appeared as a doublet at δ 1.26 and the surface

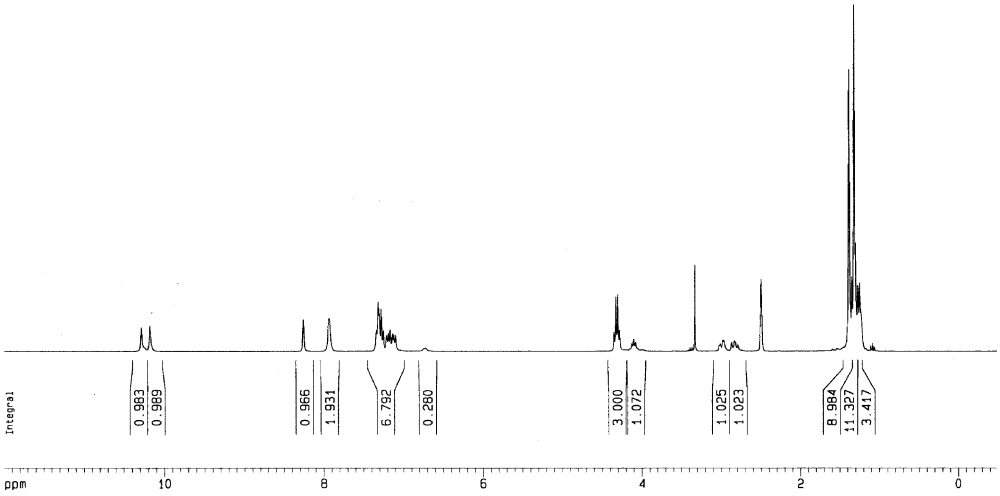


Figure 1. ^{1}H NMR spectrum of G1-AF-CO_2Et in d_6-DMSO.

benzylic protons of the phenylalanine side chains appeared as a multiplet at δ 2.70–3.08. On the other hand, the chemical shift values of the interior amino acid side chain protons were slightly downfield shifted due to a change of the *N*-linking group from the Boc to the benzamide functionality.

The ^{13}C NMR spectra of the target sector block dendrons share a common spectral feature; the chemical shift values of the carbon nuclei due to the dendritic backbone and the aromatic 3,5-diaminobenzamide branching unit are nearly the same for the different dendrons of the same generation. Hence, the primary and tertiary carbons of the Boc groups resonated at δ 29 and 79, respectively, while the peaks for the ethyl ester focal point group were located at δ 15 and 61. Due to a broken down of the C_2 symmetry, up to six aromatic carbon signals originated from the interior branching unit could be identified (δ 115–140). Likewise, the carbon signals due to the surface aromatic branching units were also found to scatter between the same spectral regions. The carbonyl ^{13}C signals appeared at the most downfield region of the spectra. The ^{13}C signal(s) at $\sim\delta$ 156 corresponded to the Boc carbamate group(s) while the one(s) at $\sim\delta$ 167 could be attributed to the focal point carbonyl ester/acid or to the benzamide carbonyl moieties. On the other hand, the signal(s) located at $\sim\delta$ 172 corresponded to the anilide C=O(s). The chemical identities of the amino acid side chains could be similarly assigned by their respective 'fingerprint' signals as in the case of ^{1}H NMR spectroscopic analysis.

2.2.2. Mass spectroscopic analysis. The structures of all the G1 and G2 sector block dendrons were also characterized by FAB or ESI mass spectrometry. For both the G1 and G2 series of compounds, molecular ions appeared in forms of M^+, $(M+H)^+$, and/or $(M+Na)^+$ could be identified. It was found that ESI was a superior ionization technique than FAB in order to obtain the molecular mass data for the higher molecular weight G2 series. The exact masses of the molecular ion peak were measured and the results also matched well with the theoretical values.

2.2.3. Size exclusion chromatography. Due to the presence of a large number of amide and carbamate functional groups, the amino acid-based dendrons prepared here were found to form hydrates.[8,9] As a result, elemental analysis data could not be used to assess their structural purity. Furthermore, it has been noted that elemental analysis data are not reliable in assessing dendrimer purities due to the extremely small variation of the analysis data across a series of dendrimers bearing the same architectural elements. Most often their homogeneities were assessed by size exclusion chromatography (SEC). Hence, all the dendrons as well as the intermediates were subjected to SEC analysis and they were found to produce a symmetrical peak with a narrow polydispersity (PDI$\leq$1.03) in their SEC chromatogram (Table 1 and Fig. 2). Therefore, all the dendrons were determined to be >95% pure by SEC analysis. More interestingly, the G2 acid and ester dendrons all have nearly the same retention volume ($\sim$16.1 min), suggesting that they possess similar hydrodynamic radius, irrespective of the amino acid composition and the focal point functionality.

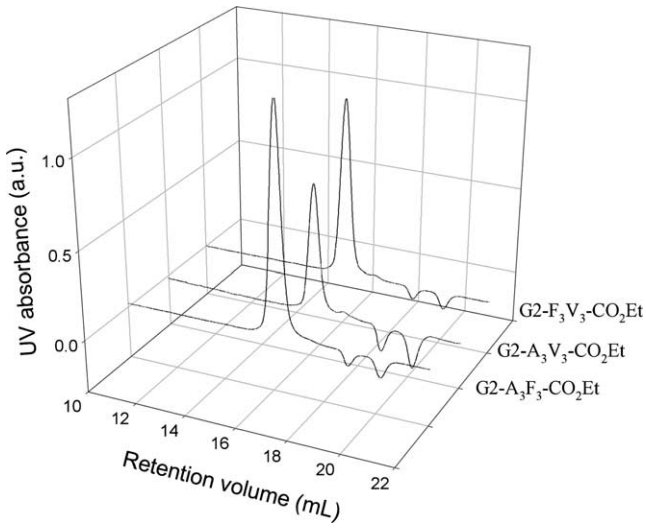


Figure 2. SEC chromatograms of G2 ester dendrons (flow rate: 1 mL min^{-1}; temperature = 40 °C; solvent = 5% HOAc/DMF). The negative absorption peaks are due to signals of water and acetic acid.

2.3. Properties

2.3.1. Chiroptical properties. The chiroptical properties of the sector block dendrons were measured and tabulated (Table 2). To avoid complications due to self aggregation,[8,9] the data were acquired in 5% HOAc in 1,2-dichloroethane. Previously, it was found that the sign of the specific rotations of the layer block dendrons generally did not change from the dendritic esters to their corresponding carboxylic acids and that the molar rotation of the resulting dendron was the simple sum of the molar rotations of all the constituted amino acid chiral units resided within the dendron.[8] Such observations were consistent with the notion that the focal point functionality had little effect on the chiral conformation of the layer block dendrons and that they adopted an open and conformationally flexible

Table 1. SEC data of sector block dendrons

Dendron	Retention time (min)	PDI	Dendron	Retention time (min)	PDI
G1-AF-CO_2Et	17.92	1.02	G1-A_2-CONH-A-CO_2H	16.81	1.02
G1-AV-CO_2Et	18.00	1.02	G1-F_2-CONH-F-CO_2H	16.79	1.01
G1-FV-CO_2Et	17.61	1.01	G1-V_2-CONH-V-CO_2H	16.98	1.02
G1-AF-CO_2H	17.36	1.01	G2-A_3F_3-CO_2Et	16.10	1.01
G1-AV-CO_2H	17.42	1.01	G2-A_3V_3-CO_2Et	16.18	1.01
G1-FV-CO_2H	17.52	1.02	G2-F_3V_3-CO_2Et	16.09	1.02
G1-A_2-CONH-A-CO_2Me	17.01	1.02	G2-A_3F_3-CO_2H	15.92	1.02
G1-F_2-CONH-F-CO_2Me	17.23	1.03	G2-A_3V_3-CO_2H	15.98	1.02
G1-V_2-CONH-V-CO_2Me	17.40	1.02	G2-F_3V_3-CO_2H	16.03	1.02

Table 2. Chiroptical data of sector block dendrons

Dendrons	$[\alpha]_D$[a]	$[\Phi]_D$[b]	Dendrons	$[\alpha]_D$[a]	$[\Phi]_D$[b]
G1-AF-CO_2Et	−12.1	−72	G1-A_2-CONH-A-CO_2H	−43.9	−248
G1-AV-CO_2Et	−50.8	−279	G1-F_2-CONH-F-CO_2H	+42.0	+333
G1-FV-CO_2Et	+8.6	+54	G1-V_2-CONH-V-CO_2H	−8.9	−58
G1-AF-CO_2H	−14.1	−80	G2-A_3F_3-CO_2Et	+25.8	+388
G1-AV-CO_2H	−57.7	−301	G2-A_3V_3-CO_2Et	+38.5	+523
G1-FV-CO_2H	−7.3	−44	G2-F_3V_3-CO_2Et	+78.3	+1242
G1-A_2-CONH-A-CO_2Me	−50.8	−294	G2-A_3F_3-CO_2H	+22.9	+338
G1-F_2-CONH-F-CO_2Me	+46.6	+376	G2-A_3V_3-CO_2H	+27.9	+371
G1-V_2-CONH-V-CO_2Me	−15.3	−101	G2-F_3V_3-CO_2H	+63.8	+995

[a] Specific rotation (10^{-1} degrees cm^2 g^{-1}).
[b] Molar rotation (10 degrees cm^2 mol^{-1}).

architecture in such a solvent system. However, for the sector block dendrons reported here, some anomalies to these trends were found. Hence, the absolute signs of G1-FV-CO_2H and G1-FV-CO_2Et were different. In addition, while the specific rotations of G1-A_2-CONH-A-CO_2R (R=H or Me) and G2-V_2-CONH-V-CO_2R (R=H or Me) are negative, the values of G2-A_3V_3-CO_2R (R=H or Et) are positive. We speculated that these abnormalities were probably due to a change of the chiral conformations on going from G1 to G2 dendrons, but were unable to offer an explanation at the present moment.

2.3.2. Gelation properties. Similar to the strong gelation property of the layer block dendrons[8] reported by us, the sector block dendrons described here also exhibited very excellent gelation behavior in aromatic solvents (Fig. 3).

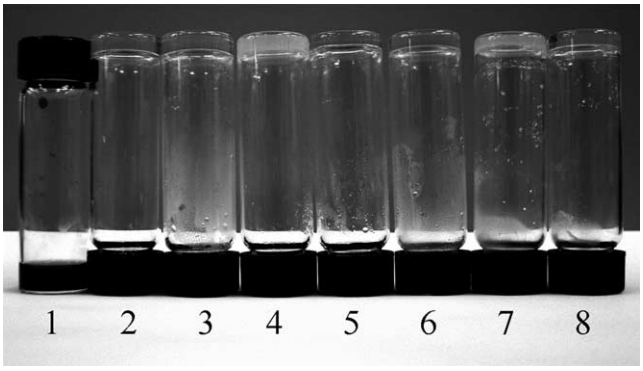


Figure 3. Gels (concentration = 10 mg mL^{-1}) formed from G1-AF-CO_2H in (2) toluene, (3) nitrobenzene, (4) *o*-xylene, (5) anisole, (6) G1-FV-CO_2H in nitrobenzene, G2-A_3V_3-CO_2Et in (7) *o*-xylene and (8) toluene. The first one on the left is $KMnO_4$ solution.

The gel was prepared by heating a weighted amount of an organogelator in a specified solvent inside a septum-capped vial until dissolution, and the sample was allowed to stand at room temperature for 1 day and the state of the sample was then examined. A stable gel was formed when a homogeneous phase exhibited no gravitation flow by inverting the sample. The gel state could be further classified as transparent gel (CG) or opaque gel (OG) according to its transparency. If part of the sample formed a gel and part of it remained in solution, it was considered as a partial gel (PG). The sample was regarded as soluble (S) when the solution remained clear and no gelation occurred. For the transparent gels described here, they were stable up to several months when stored in screw-capped vials.

Generally, the G1 acid dendrons are better organogelators than the G1 ester dendrons. This observation was similar to that observed with the G1 layer block dendrons.[8] It was also noted that the phenylalanine containing dendron G1-AF-CO_2H was the best organogelator with minimum gelation concentration (mgc) below 10 mg mL^{-1} in nearly all aromatic solvent tested (Table 3). The other phenylalanine containing dendron, G1-FV-CO_2H, was also a good organogelator with slightly inferior mgc values (<100 mg mL^{-1}). Again, these observations were in line with the fact that G1-F_2-CO_2H was found to be the best gelating agent among the layer block dendrons.[8] All these findings strongly suggested that the aromatic phenylalanine side chain was responsible for stabilizing the gel via π–π stacking interaction with the aromatic solvents.

Similar to the G2 layer block dendrons, the G2 sector block dendrons generally possess weaker gelation ability. Another

Table 3. Gelation behavior of sector block dendrons in aromatic solvents[a]

Dendrons	Toluene	*o*-Xylene	Anisole	Nitrobenzene	*o*-Dichlorobenzene
G1-AF-CO_2Et	S	S	S	S	S
G1-AV-CO_2Et	PG	PG	PG	PG	PG
G1-FV-CO_2Et	S	S	S	S	S
G1-AF-CO_2H	CG (3)	OG (4)	CG (10)	CG (4)	CG (40)
G1-AV-CO_2H	OG (40)	OG (50)	OG (50)	CG (100)	PG
G1-FV-CO_2H	OG (100)	OG (100)	OG (100)	CG (5)	OG (100)
G2-A_3F_3-CO_2Et	OG (50)	OG (50)	S	S	PG
G2-A_3V_3-CO_2Et	CG (30)	OG (40)	PG	PG	CG (100)
G2-F_3V_3-CO_2Et	PG	PG	PG	PG	PG
G2-A_3F_3-CO_2H	OG (50)	OG (50)	S	S	PG
G2-A_3V_3-CO_2H	PG	PG	CG (100)	PG	OG (100)
G2-F_3V_3-CO_2H	OG (50)	OG (50)	PG	PG	CG (100)

[a] CG, transparent gel; OG, opaque gel; PG, partial gel; S, soluble (>100 mg mL^{-1}). The values given in parentheses are the minimum concentration (mg mL^{-1}) to achieve gelation at 25 °C.

interesting observation was that none of the phenylalanine containing G2 dendrons showed strong gelating ability. In fact, the most efficient organogelator among the G2 dendrons was G2-A_3V_3-CO_2Et, highlighting the intriguing influence of the amino acid side chain on the gelation ability. Hence, the amino acid side chains have a subtle yet determining role on the gelation property, and only those possessing favorable hydrophobic and steric parameters can stabilize the physical gel.

2.4. Gelation mechanism

The gelation mechanism of the organogelators was investigated by infra-red (IR) spectroscopy. The FT-IR spectra of a 10 mg mL^{-1} $CHCl_3$ solution of G1-AF-CO_2H and a 10 mg mL^{-1} clear transparent gel in *o*-xylene were recorded and the data were tabulated (Table 4). The IR spectrum in $CHCl_3$ exhibited two peaks at 3439 and 3329 cm^{-1} in the ν_{N-H} region, which could be attributed to the stretching bands of the carbamate and anilide N–H, respectively. In addition, one broad peak at 1691 cm^{-1}, assignable to C=O stretching frequency was noted. On the other hand, the IR spectrum of the *o*-xylene gel showed a broad N–H absorption peak at 3313 cm^{-1} and another broad C=O absorption at peak 1668 cm^{-1}, both of which were significantly red-shifted. Hence, in addition to aromatic π–π stacking, intermolecular hydrogen bonding is also an important driving force for gelation.

Table 4. FT-IR data of G1-AF-CO_2H (concentration = 10 mg mL^{-1}) in the solution and gel states[a]

Conditions	Absorption frequency (cm^{-1})	
	ν (carbamate/anilide N–H)	ν (acid/anilide/carbamate C=O)
$CHCl_3$ solution	3429, 3329	1691
o-Xylene gel	3313	1668

[a] All spectra were recorded at 25 °C.

To further investigate the chirality of the gel aggregates, circular dichroism (CD) spectra of G1-AF-CO_2H in *o*-xylene (10 mg mL^{-1}) were recorded at different temperatures (Fig. 4). At a temperature above the melting temperature, the solution sample exhibited a positive Cotton effect at 338 nm. As the temperature decreases below the melting temperature, the molar ellicipity of the gel sample increases, indicating the emergence of secondary chiral architecture in the gel state. Furthermore, a gradual shift (~2 nm) of the λ_{max} to the blue region was also noted on cooling of the sample, suggesting the gradual transition from an isotropic solution to an anisotropic environment in the gel state.

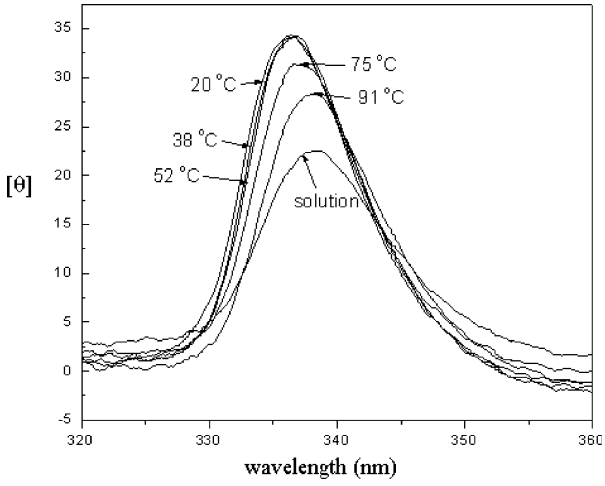


Figure 4. Temperature dependent CD spectra of G1-AF-CO_2H in *o*-xylene (10 mg mL^{-1}).

3. Conclusions

A new series of G1 and G2 α-amino acid-based sector block dendrons based on alanine, phenylalanine, and valine was synthesized by a convergent synthesis strategy involving selective functionalization and protection of a branching agent. Such dendrons were found to exhibit very strong gelation property (mgc down to 3 mg mL^{-1}) with aromatic solvents through aromatic π–π stacking, intermolecular hydrogen bonding, and more interestingly, hydrophobic interactions among the amino acid side chains. As a result, only those dendrons with the optimal amino acid constitution and focal point functionality can form strong physical gels. This new library of sector block dendrons, together with the layer block dendrons reported earlier, provide a new repertoire of biomimetic dendrimers with rich structural diversity and interesting physico–chemical properties that can be used as new bio-compatible materials.

4. Experimental

4.1. General

THF was distilled from sodium benzophenone ketyl and CH_2Cl_2 from P_2O_5 prior to use. Silica gel for flash chromatography is Macherey Nagel 60 M (230–400 mesh) silica gel. *N*-Boc-protected L-amino acids [BocNH-(aa^1)-CO_2H], L-amino acid methyl ester hydrochlorides [H_3N^+-(aa^1)-CO_2Me Cl^-] and other reagents were used as supplied from Aldrich or Sigma. All reactions were conducted under dry N_2 unless otherwise stated. All NMR spectra were recorded in d_6-DMSO (dried over molecular sieve 4 Å) on a Brüker DPX spectrometer at 300 MHz for ^{1}H and 75.5 MHz for ^{13}C nucleus at 25 °C. The residual proton or carbon signals of d_6-DMSO (δ_H=2.50; δ_C=40.5) were used as internal references. All chemical shifts are reported in ppm (δ) and coupling constants in Hz. Positive ion ESI and FAB spectra were carried out on a Thermo Finnigan MAT 95XL mass spectrometer. Melting points were measured on an Electrothermal IA9100 Digital Melting Point Apparatus and were uncorrected. Melting temperatures (T_m) were recorded on a Perkin-Elmer DCS6 differential scanning meter and are referred to the onset of the transition. IR spectra were recorded on a Nicolet 420 FT-IR spectrophotometer. Optical rotations were taken on a Perkin Elmer 341 Polarimeter at 589 nm and at 20 °C, in a solvent mixture of 1,2-dichloroethane/HOAc (*v*/*v* 95:5). CD spectra were recorded on a JASCO J-715 spectropolarimeter connected to a NESLAB RTE-211 temperature controller. Size exclusion chromatography (SEC) analyses were performed on Waters® Styragel columns (HR 1 and HR 3 in serial) at

40 °C in 5% HOAc/DMF as eluent (flow rate = 1.0 mL min^{-1}) on a Waters HPLC 515 pump equipped with a Waters 486 tunable UV absorbance detector. Molecular weights obtained from SEC measurements were based on a calibration curve derived from polystyrene standards. Elemental analyses were performed at MEDAC LTD, Brunel Science Centre, Cooper's Hill Lane, Englefield Green, Egham, Surrey TW20 0JZ, UK.

4.1.1. Ethyl 3-amino-5-(*N*-benzyloxycarbonylamino) benzoate 5. A mixture of benzyl chloroformate (14.4 mL, 100 mmol) and aqueous NaOH (100 mL, 1.0 M) in THF (200 mL) was added dropwise to a stirred solution of ethyl 3,5-diaminobenzoate[9] (18.0 g, 100 mmol) in THF (500 mL) at 5 °C. After the mixture had been kept at 25 °C for 3 h, the solvent was evaporated in vacuo. The residue was dissolved in $CHCl_3$ (300 mL) and washed with water (2×75 mL). The organic layer was dried ($MgSO_4$), filtered and evaporated in vacuo. The residue was chromatographed on silica gel (eluent: EtOAc/hexane 1:2) to afford the title compound as a white solid (22.3 g, 71%). R_f 0.53 (EtOAc/hexane 2:3). Mp 160–161 °C. ^{1}H NMR: 1.28 (3H, t, J=7.1 Hz, CH_2CH_3), 4.24 (2H, q, J=7.1 Hz, CH_2Me), 5.13 (2H, s, $PhCH_2$), 5.39 (2H, s, NH_2), 6.85 (1H, s, ArH), 6.98 (1H, s, ArH), 7.26 (1H, s, ArH), 7.28–7.49 (5H, m, PhH), 9.67 (1H, s, CONHAr). ^{13}C NMR: 15.2, 61.3, 66.6, 107.8, 108.6, 110.0, 129.0, 129.2, 129.4, 131.7, 137.7, 140.9, 150.3, 154.2, 167.1. MS (FAB): 315 [$(M+H)^+$, 58%]. HRMS (FAB): calcd for $C_{17}H_{18}N_2O_4+H^+$, 315.1339; found, 315.1340. Anal. Calcd for $C_{17}H_{18}N_2O_4$: C, 64.96; H, 5.77; N, 8.91; found: C, 65.20; H, 5.88; N, 8.89.

4.2. General procedure for the preparation of G1-(aa^1)(CbzNH)-CO_2Et 7

EEDQ (1.0 equiv) was added to a stirred mixture of BocNH-(aa^1)-CO_2H **6** (1 equiv) and ethyl 3-amino-5-(*N*-benzyloxycarbonylamino)benzoate **5** (1 equiv) in dry CH_2Cl_2 at 0 °C. The mixture was kept at 25 °C for 12 h after which the solvent was evaporated in vacuo. The residue was redissolved in EtOAc (200 mL), and washed successively with saturated $NaHCO_3$ solution (50 mL), 10% citric acid solution (2×50 mL), saturated $NaHCO_3$ solution (2×50 mL), and water (2×50 mL). The organic layer was dried ($MgSO_4$), filtered and evaporated in vacuo to give the target compound, which was further purified by flash chromatography on silica gel.

4.2.1. G1-A(CbzNH)-CO_2Et (7, aa^1=A). Starting from BocNH-A-CO_2H (1.02 g, 5.4 mmol), ethyl 3-amino-5-(*N*-benzyloxycarbonylamino)benzoate **5** (1.70 g, 5.4 mmol) and EEDQ (1.34 g, 5.4 mmol), the titled compound was obtained as a white solid (2.37 g, 90%) after flash chromatography (eluent: EtOAc/hexane 2:3). R_f 0.29 (EtOAc/hexane 2:3). Mp 153–155 °C. $[\alpha]_D^{20}$ −42.5 (*c* 1.16). ^{1}H NMR: 1.25 (3H, d, J=7.1 Hz, $CHCH_3$), 1.31 (3H, t, J=7.1 Hz, CH_2CH_3), 1.38 (9H, s, $C(CH_3)_3$), 4.02–4.18 (1H, m, NCHMe), 4.30 (2H, q, J=7.1 Hz, CH_2Me), 5.17 (2H, s, $PhCH_2$), 7.10 (1H, d, J=7.1 Hz, NHCHMe), 7.28–7.49 (5H, m, PhH), 7.81 (1H, s, ArH), 7.95 (1H, s, ArH), 8.05 (1H, s, ArH), 10.03 (1H, s, CONHAr), 10.15 (1H, s, CONHAr). ^{13}C NMR: 15.1, 18.8, 29.2, 51.4, 61.8, 66.8, 79.0, 114.1, 114.6, 115.0, 129.0, 129.4, 131.6, 137.5, 140.7, 154.3, 156.1, 166.4, 173.1. MS (FAB): 486 [$(M+H)^+$, 21%]. HRMS (FAB): calcd for $C_{25}H_{31}N_3O_7+H^+$, 486.2235; found, 486.2234.

4.2.2. G1-V(CbzNH)-CO_2Et (7, aa^1=V). Starting from BocNH-V-CO_2H (1.46 g, 6.7 mmol), ethyl 3-amino-5-(*N*-benzyloxycarbonylamino)benzoate **5** (2.10 g, 6.7 mmol) and EEDQ (1.66 g, 6.7 mmol), the target compound was obtained as a white solid (2.90 g, 85%) after flash chromatography (eluent: EtOAc/hexane 1:4 gradient to 1:3). R_f 0.20 (EtOAc/hexane 1:4). Mp 154–156 °C. $[\alpha]_D^{20}$ −29.4 (*c* 0.97). ^{1}H NMR: 0.89 (6H, d, J=6.5 Hz, $CH(CH_3)_2$), 1.31 (3H, t, J=7.1 Hz, CH_2CH_3), 1.39 (9H, s, $C(CH_3)_3$), 1.85–2.08 (1H, m, $CHMe_2$), 3.91 (1H, t, J=7.7 Hz, NCH), 4.30 (2H, q, J=7.1 Hz, CH_2Me), 5.17 (2H, s, $PhCH_2$), 6.92 (1H, d, J=8.5 Hz, NHCH), 7.28–7.49 (5H, m, PhH), 7.81 (1H, s, ArH), 7.97 (1H, s, ArH), 8.06 (1H, s, ArH), 10.02 (1H, s, CONHAr), 10.20 (1H, s, CONHAr). ^{13}C NMR: 15.1, 19.4, 20.1, 29.1, 31.2, 61.6, 61.8, 66.8, 79.0, 114.1, 114.7, 114.9, 129.0, 129.4, 131.6, 137.5, 140.5, 140.8, 154.3, 156.5, 166.4, 172.0. MS (FAB): 514 [$(M+H)^+$, 25%]. HRMS (FAB): calcd for $C_{27}H_{35}N_3O_7+H^+$, 514.2548; found, 514.2557.

4.3. General procedure for the preparation of G1-(aa^1)(NH_2)-CO_2Et 8

A suspension of G1-(aa^1)(CbzNH)-CO_2Et **7** and 10% palladium on charcoal in MeOH was stirred under H_2 for 1 h. The catalyst was removed by filtration and the solvent evaporated in vacuo to give the target compound G1-(aa^1)(NH_2)-CO_2Et **8**.

4.3.1. G1-A(NH_2)-CO_2Et (8, aa^1=A). Starting from G1-A(CbzNH)-CO_2Et (2.00 g, 4.1 mmol), the target compound was obtained as a white solid (1.38 g, 95%). Mp 142–144 °C. $[\alpha]_D^{20}$ −49.5 (*c*=0.77). ^{1}H NMR: 1.22 (3H, d, J=7.1 Hz, $CHCH_3$), 1.29 (3H, t, J=7.0 Hz, CH_2CH_3), 1.38 (9H, s, $C(CH_3)_3$), 3.90–4.12 (1H, m, NCHMe), 4.25 (2H, q, J=7.1 Hz, CH_2Me), 5.42 (2H, s, NH_2), 6.89 (1H, s, ArH), 7.03 (1H, d, J=7.1 Hz, OCONH), 7.18 (1H, s, ArH), 7.31 (1H, s, ArH), 9.80 (1H, s, CONHAr). ^{13}C NMR: 15.2, 18.9, 29.1, 51.4, 61.3, 78.9, 108.7, 109.6, 110.5, 131.6, 140.8, 150.3, 156.1, 167.1, 172.8. MS (FAB): 351 (M^+, 26%). HRMS (FAB): calcd for $C_{17}H_{25}N_3O_5^+$, 351.1789; found, 351.1795.

4.3.2. G1-V(NH_2)-CO_2Et (8, aa^1=V). Starting from G1-V(CbzNH)-CO_2Et (1.62 g, 3.2 mmol), the titled product was obtained as a white solid (1.13 g, 94%). Mp 88–90 °C. $[\alpha]_D^{20}$ −34.1 (*c* 0.63). ^{1}H NMR: 0.88 (6H, d, J=6.3 Hz, $CH(CH_3)_2$), 1.28 (3H, t, J=7.3 Hz, CH_2CH_3), 1.38 (9H, s, $C(CH_3)_3$), 1.85–2.08 (1H, m, $CHMe_2$), 3.88 (1H, t, J=7.8 Hz, NCH), 4.25 (2H, q, J=6.9 Hz, CH_2Me), 5.42 (2H, s, NH_2), 6.84 (1H, d, J=8.1 Hz, OCONH), 6.89 (1H, s, ArH), 7.19 (1H, s, ArH), 7.32 (1H, s, ArH), 9.85 (1H, s, CONHAr). ^{13}C NMR: 15.2, 19.4, 20.1, 29.1, 31.3, 61.3, 61.6, 79.0, 108.7, 109.7, 110.6, 131.6, 140.6, 150.3, 156.5, 167.0, 171.6. MS (FAB): 379 (M^+, 100%). HRMS (FAB): calcd for $C_{19}H_{29}N_3O_5^+$, 379.2102; found, 379.2112.

4.4. General procedure for the preparation of G1-(aa^1)(aa^2)-CO_2Et 1

EEDQ (1 equiv) was added to a stirred mixture of BocNH-(aa^2)-CO_2H (1 equiv) and G1-(aa^1)(NH_2)-CO_2Et (1 equiv) in dry THF/CH_2Cl_2 (*v*/*v* 1:1) at 0 °C. The mixture was kept at 25 °C overnight and the solvent was evaporated in vacuo. The residue was redissolved in EtOAc (200 mL), and washed successively with saturated $NaHCO_3$ solution (50 mL), 10% citric acid solution (2×50 mL), saturated $NaHCO_3$ solution (2×50 mL), and water (2×50 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated in vacuo to give the target compound that was purified by flash chromatography on silica gel.

4.4.1. G1-AF-CO_2Et (1, aa^1aa^2=AF). Starting from BocNH-F-CO_2H (0.75 g, 2.9 mmol), G1-A(NH_2)-CO_2Et (1.00 g, 2.9 mmol) and EEDQ (0.70 g, 2.9 mmol), the target compound was obtained as a white solid (1.48 g, 87%) after flash chromatography (eluent: EtOAc/hexane 1:2). R_f 0.23 (EtOAc/hexane 1:2). Mp 167–169 °C. $[\alpha]_D^{20}$ −12.1 (*c* 1.04). ^{1}H NMR: 1.26 (3H, d, *J*=7.1 Hz, CHC*H*$_3$), 1.25–1.32 (12H, m, CH_2CH_3 and C(C*H*$_3$)$_3$), 1.38 (9H, s, C(C*H*$_3$)$_3$), 2.72–2.91 (1H, m, C*H*HPh), 2.91–3.05 (1H, m, CH*H*Ph), 4.00–4.15 (1H, m, NC*H*Me), 4.26–4.37 (3H, m, C*H*$_2$Me and NC*H*Bn), 7.03–7.39 (7H, m, Ar*H* and OCON*H*), 7.93 (2H, s, Ar*H*), 8.26 (1H, s, Ar*H*), 10.18 (1H, s, CON*H*Ar), 10.29 (1H, s, CON*H*Ar). ^{13}C NMR: 15.1, 18.8, 29.1, 29.2, 38.2, 51.4, 57.6, 61.8, 79.0, 79.1, 115.1, 115.6, 127.3, 129.0, 130.2, 131.5, 138.9, 140.5, 140.6, 156.1, 156.3, 166.4, 172.1, 173.2. MS (FAB): 599 [(M+H)$^+$, 31%]. HRMS (FAB): calcd for $C_{31}H_{42}N_4O_8+H^+$, 599.3075; found, 599.3067.

4.4.2. G1-AV-CO_2Et (1, aa^1aa^2=AV). Starting from BocNH-V-CO_2H (0.41 g, 1.9 mmol), G1-A(NH_2)-CO_2Et (0.67 g, 1.9 mmol) and EEDQ (0.47 g, 1.9 mmol), the target compound was obtained as a white solid (1.0 g, 98%) after flash chromatography (eluent: EtOAc/hexane 1:2). R_f 0.17 (EtOAc/hexane 1:2). Mp 150–151 °C. $[\alpha]_D^{20}$ −50.8 (*c* 1.05). ^{1}H NMR: 0.89 (6H, d, *J*=6.5 Hz, CH(C*H*$_3$)$_2$), 1.25 (3H, d, *J*=7.1 Hz, CHC*H*$_3$), 1.32 (3H, t, *J*=7.1 Hz, CH_2CH_3), 1.38 (18H, s, C(C*H*$_3$)$_3$), 1.85–2.08 (1H, m, C*H*Me$_2$), 3.90 (1H, t, *J*=7.8 Hz, NC*H*CH), 4.05–4.16 (1H, m, NC*H*Me), 4.31 (2H, q, *J*=7.0 Hz, C*H*$_2$Me), 6.93 (1H, d, *J*=8.3 Hz, OCON*H*), 7.11 (1H, d, *J*=7.0 Hz, OCON*H*), 7.94 (2H, s, Ar*H*), 8.25 (1H, s, Ar*H*), 10.17 (1H, s, CON*H*Ar), 10.21 (1H, s, CON*H*Ar). ^{13}C NMR: 15.1, 18.8, 19.4, 20.1, 29.2, 31.2, 51.4, 61.6, 61.8, 79.0, 115.1, 115.5, 115.6, 131.5, 140.4, 140.7, 156.1, 156.5, 166.4, 172.0, 173.2. MS (FAB): 551 [(M+H)$^+$, 30%]. HRMS (FAB): calcd for $C_{27}H_{42}N_4O_8+H^+$, 551.3075; found, 551.3073.

4.4.3. G1-FV-CO_2Et (1, aa^1aa^2=FV). Starting from BocNH-F-CO_2H (0.70 g, 2.6 mmol), G1-V(NH_2)-CO_2Et (1.00 g, 2.6 mmol) and EEDQ (0.65 g, 2.6 mmol), the product was obtained as a white solid (1.43 g, 87%) after flash chromatography (eluent: EtOAc/hexane 1:3). R_f 0.27 (EtOAc/hexane 1:2). Mp 114–116 °C. $[\alpha]_D^{20}$ +8.6 (*c* 1.09). ^{1}H NMR: 0.90 (6H, d, *J*=6.6 Hz, CH(C*H*$_3$)$_2$), 1.17–1.35 (12H, m, CH_2CH_3 and C(C*H*$_3$)$_3$), 1.39 (9H, s, C(C*H*$_3$)$_3$), 1.85–2.08 (1H, m, C*H*Me$_2$), 2.72–2.91 (1H, m, C*H*HPh), 2.91–3.05 (1H, m, CH*H*Ph), 3.91 (1H, t, *J*=7.9 Hz, NC*H*), 4.26–4.37 (3H, m, NC*H*Bn and C*H*$_2$Me), 6.94 (1H, d, *J*=8.4 Hz, OCON*H*), 7.09–7.43 (6H, m, Ar*H* and OCON*H*), 7.93 (1H, s, Ar*H*), 7.96 (1H, s, Ar*H*), 8.26 (1H, s, Ar*H*), 10.23 (1H, s, CON*H*Ar), 10.30 (1H, s, CON*H*Ar). ^{13}C NMR: 15.1, 19.5, 20.1, 29.1, 31.2, 38.2, 57.6, 61.7, 61.9, 79.1, 115.1, 115.7, 127.3, 129.0, 130.2, 131.5, 138.9, 140.4, 140.5, 156.4, 156.5, 166.4, 172.1. MS (FAB): 626 (M$^+$, 26%). HRMS (FAB): calcd for $C_{33}H_{46}N_4O_8^+$, 626.3310; found, 626.3302.

4.5. General procedure for the preparation of G1-(aa^1)(aa^2)-CO_2H 2

An aqueous KOH solution (1.0 M) was added to a solution of G1-(aa^1)(aa^2)-CO_2Et **1** in MeOH. The reaction mixture was stirred at 25 °C until completion of the reaction as monitored by TLC analysis. The solvent was evaporated in vacuo and the residue was poured into large amount of water. The precipitate formed was collected by filtration, washed with water and dried in vacuo.

4.5.1. G1-AF-CO_2H (2, aa^1aa^2=AF). Starting from G1-AF-CO_2Et (0.50 g, 0.84 mmol) in MeOH (10 mL) and aqueous KOH solution (2 mL, 1.0 M), the target compound was obtained as a white solid (0.47 g, 98%). T_m 160 °C. $[\alpha]_D^{20}$ −14.1 (*c* 1.07). ^{1}H NMR: 1.26 (3H, d, *J*=7.2 Hz, CHC*H*$_3$), 1.32 (9H, s, C(C*H*$_3$)$_3$), 1.38 (9H, s, C(C*H*$_3$)$_3$), 2.72–2.91 (1H, m, C*H*HPh), 2.91–3.05 (1H, m, CH*H*Ph), 3.90–4.15 (1H, m, NC*H*Me), 4.22–4.36 (1H, m, NC*H*Bn), 7.03–7.39 (7H, m, Ar*H* and OCON*H*), 7.92 (2H, s, Ar*H*), 8.20 (1H, s, Ar*H*), 10.13 (1H, s, CON*H*Ar), 10.24 (1H, s, CON*H*Ar), 12.95 (1H, br s, COO*H*). ^{13}C NMR: 18.8, 29.1, 29.2, 38.3, 51.5, 57.7, 79.0, 79.1, 114.8, 116.0, 127.3, 129.0, 130.2, 132.6, 138.9, 140.3, 140.5, 156.1, 156.4, 168.0, 172.0, 173.1. MS (FAB): 571 [(M+H)$^+$, 100%]. HRMS (FAB): calcd for $C_{29}H_{38}N_4O_8+H^+$, 571.2762; found, 571.2770.

4.5.2. G1-AV-CO_2H (2, aa^1aa^2=AV). Starting from G1-AV-CO_2Et (0.79 g, 1.4 mmol) in MeOH (10 mL) and aqueous KOH solution (3.0 mL, 1.0 M), the titled compound was obtained as a white solid (0.72 g, 96%). T_m 202 °C. $[\alpha]_D^{20}$ −57.7 (*c* 0.22). ^{1}H NMR: 0.89 (6H, d, *J*=6.6 Hz, CH(C*H*$_3$)$_2$), 1.25 (3H, d, *J*=7.1 Hz, CHC*H*$_3$), 1.38 (18H, s, C(C*H*$_3$)$_3$), 1.85–2.08 (1H, m, C*H*Me$_2$), 3.91 (1H, t, *J*=7.7 Hz, NC*H*CH), 4.00–4.19 (1H, m, NC*H*Me), 6.91 (1H, d, *J*=8.4 Hz, OCON*H*), 7.10 (1H, d, *J*=7.1 Hz, OCON*H*), 7.92 (2H, s, Ar*H*), 8.19 (1H, s, Ar*H*), 10.12 (1H, s, CON*H*Ar), 10.16 (1H, s, CON*H*Ar), 12.98 (1H, br s COO*H*). ^{13}C NMR: 18.8, 19.5, 20.1, 29.2, 31.2, 51.4, 61.6, 78.98, 79.02, 114.9, 115.9, 116.0, 132.5, 140.2, 140.5, 156.1, 156.5, 167.9, 172.0, 173.1. MS (FAB): 523 [(M+H)$^+$, 7%]. HRMS (FAB): calcd for $C_{25}H_{38}N_4O_8+H^+$, 523.2762; found, 523.2752.

4.5.3. G1-FV-CO_2H (2, aa^1aa^2=FV). Starting from G1-FV-CO_2Et (0.66 g, 1.1 mmol) in MeOH (10 mL) and aqueous KOH solution (2.1 mL, 1.0 M), the target product was obtained as a white solid (0.63 g, 100%). T_m 167 °C. $[\alpha]_D^{20}$ −7.3 (*c* 0.39). ^{1}H NMR: 0.90 (6H, d, *J*=6.5 Hz, CH(C*H*$_3$)$_2$), 1.32 (9H, s, C(C*H*$_3$)$_3$), 1.39 (9H, s, C(C*H*$_3$)$_3$), 1.85–2.08 (1H, m, C*H*Me$_2$), 2.72–2.91 (1H, m, C*H*HPh), 2.91–3.05 (1H, m, CH*H*Ph), 3.91 (1H, t, *J*=7.8 Hz, NC*H*CH), 4.23–4.36 (1H, m, NC*H*Bn), 6.92 (1H, d, *J*=

8.3 Hz, OCONH), 7.09–7.43 (6H, m, ArH and OCONH), 7.93 (2H, s, ArH), 8.20 (1H, s, ArH), 10.18 (1H, s, CONHAr), 10.25 (1H, s, CONHAr), 12.98 (1H, br s, COOH). ^{13}C NMR: 19.5, 20.1, 29.1, 31.2, 38.3, 57.7, 61.7, 79.1, 114.9, 116.1, 127.3, 129.0, 130.2, 132.6, 138.9, 140.2, 140.4, 156.4, 156.5, 168.0, 172.0. MS (FAB): 599 [$(M+H)^+$, 100%]. HRMS (FAB): calcd for $C_{31}H_{42}N_4O_8+H^+$, 599.3075; found, 599.3079.

4.6. General procedure for the synthesis of G1-(aa^1)$_2$-CONH-(aa^1)-CO_2Me 10

DCC (1 equiv) was added to a stirred mixture of $Cl^-H_3N^+$-(aa^1)-CO_2Me (1 equiv), G1-(aa^1)$_2$-CO_2H[8] **9** (1 equiv), 4-methylmorpholine (1 equiv) and HOBt (1 equiv) in dry THF at −10 °C. After stirring at −10 °C for 2 h and then at 25 °C for 20 h, the insoluble DCU was removed by filtration and the solvent evaporated in vacuo. The residue was dissolved in EtOAc, and washed successively with saturated $NaHCO_3$ solution, 10% citric acid solution, saturated $NaHCO_3$ solution, and water. The organic layer was dried ($MgSO_4$), filtered and evaporated in vacuo to give the target compound **10** that was purified by flash chromatography on silica gel.

4.6.1. G1-A_2-CONH-A-CO_2Me (10, aa^1=A). Starting from L-alanine methyl ester hydrochloride (1.40 g, 10.0 mmol), G1-A_2-CO_2H[8] (4.94 g, 10.0 mmol), 4-methylmorpholine (1.01 g, 10.0 mmol), DCC (2.06 g, 10.0 mmol) and HOBt (1.35 g, 10.0 mmol), the target compound was obtained as a white solid (3.60 g, 62%) after flash chromatography (eluent: EtOAc/hexane 1:1 gradient to 2:1). R_f 0.20 (EtOAc/hexane 2:1). T_m 182 °C. $[\alpha]_D^{20}$ −50.8 (*c* 1.10). ^{1}H NMR: 1.25 (6H, d, *J*=7.1 Hz, CH_3CH), 1.33–1.40 (21H, m, C(CH_3)$_3$ and CH_3CH), 3.64 (3H, s, CO_2CH_3), 3.92–4.21 (2H, m, NCHCON), 4.35–4.51 (1H, m, NCHCO_2Me), 7.09 (2H, d, *J*=7.1 Hz, OCONH), 7.68 (2H, s, ArH), 8.13 (1H, s, ArH), 8.81 (1H, d, *J*=6.8 Hz, ArCONH), 10.07 (2H, s, CONHAr). ^{13}C NMR: 17.7, 18.9, 29.2, 49.2, 51.4, 52.8, 79.0, 113.9, 114.4, 136.3, 140.2, 156.1, 167.7, 173.0, 174.0. MS (FAB): 579 (M^+, 27%). HRMS (FAB): calcd for $C_{27}H_{41}N_5O_9^+$, 579.2899; found, 579.2890.

4.6.2. G1-F_2-CONH-F-CO_2Me (10, aa^1=F). Starting from L-phenylalanine methyl ester hydrochloride (2.16 g, 10.0 mmol), G1-F_2-CO_2H[8] (6.46 g, 10.0 mmol), 4-methylmorpholine (1.01 g, 10.0 mmol), DCC (2.06 g, 10.0 mmol) and HOBt (1.35 g, 10.0 mmol), the product was obtained as a white solid (5.12 g, 63%) after flash chromatography (eluent: EtOAc/hexane 2:3). R_f 0.46 (EtOAc/hexane 1:1). T_m 195 °C. $[\alpha]_D^{20}$ +46.6 (*c* 0.85). ^{1}H NMR: 1.33 (18H, s, C(CH_3)$_3$), 2.72–2.91 (2H, m, PhCHH), 2.91–3.08 (2H, m, PhCHH), 3.08–3.25 (2H, m, PhCH_2CHCO_2Me), 3.65 (3H, s, CO_2CH_3), 4.18–4.42 (2H, m, NCHCON), 4.58–4.72 (1H, m, NCHCO_2Me), 7.06–7.42 (17H, m, ArH and OCONH), 7.68 (2H, s, ArH), 8.13 (1H, s, ArH), 8.90 (1H, d, *J*=7.2 Hz, ArCONH), 10.23 (2H, s, CONHAr). ^{13}C NMR: 29.1, 37.1, 38.3, 52.9, 55.1, 57.6, 79.1, 114.1, 114.5, 127.3, 127.4, 129.0, 129.2, 130.0, 130.2, 136.2, 138.6, 138.9, 140.1, 156.4, 167.7, 172.0, 173.0. MS (FAB): 808 [$(M+H)^+$, 7%]. HRMS (FAB): calcd for $C_{45}H_{53}N_5O_9+H^+$, 808.3916; found, 808.3940.

4.6.3. G1-V_2-CONH-V-CO_2Me (10, aa^1=V). Starting from L-valine methyl ester hydrochloride (1.68 g, 10.0 mmol), G1-V_2-CO_2H[8] (5.50 g, 10.0 mmol), 4-methylmorpholine (1.01 g, 10.0 mmol), DCC (2.06 g, 10.0 mmol) and HOBt (1.35 g, 10.0 mmol), the titled compound was obtained as a white solid (5.34 g, 80%) after flash chromatography (eluent: EtOAc/hexane 3:8 gradient to 2:5). R_f 0.46 (EtOAc/hexane 1:1). T_m 196 °C. $[\alpha]_D^{20}$ −15.3 (*c* 0.79). ^{1}H NMR: 0.87–0.99 (18H, m, (CH_3)$_2$CH), 1.38 (18H, s, C(CH_3)$_3$), 1.85–2.08 (2H, m, CHMe$_2$), 2.08–2.28 (1H, m, CHMe$_2$), 3.65 (3H, s, CO_2CH_3), 3.93 (2H, t, *J*=7.9 Hz, NCHCON), 4.26 (1H, t, *J*=7.5 Hz, NCHCO_2Me), 6.91 (2H, d, *J*=8.5 Hz, OCONH), 7.66 (2H, s, ArH), 8.19 (1H, s, ArH), 8.63 (1H, d, *J*=7.5 Hz, ArCONH), 10.12 (2H, s, CONHAr). ^{13}C NMR: 19.5, 19.9, 20.1, 20.2, 29.2, 30.4, 31.2, 52.6, 59.5, 61.6, 79.0, 113.7, 114.6, 136.6, 139.9, 156.5, 168.4, 171.9, 173.1. MS (FAB): 663 (M^+, 11%). HRMS (FAB): calcd for $C_{33}H_{53}N_5O_9^+$, 663.3838; found, 663.3832.

4.7. General procedure for the synthesis of G1-(aa^1)$_2$-CONH-(aa^1)-CO_2H 11

A mixture of aqueous KOH solution (1.0 M) and G1-(aa^1)$_2$-CONH-(aa^1)-CO_2Me **10** in MeOH was stirred at 25 °C until completion of the reaction as monitored by TLC analysis. The solvent was evaporated in vacuo and the residue poured into large amount of water. The precipitate formed was collected by filtration, washed with water and dried in vacuo.

4.7.1. G1-A_2-CONH-A-CO_2H (11, aa^1=A). Starting from G1-A_2-CONH-A-CO_2Me (1.30 g, 2.3 mmol) in MeOH (20 mL) and aqueous KOH solution (5.0 mL, 1.0 M), the titled compound was obtained as a white solid (1.16 g, 91%). T_m 182 °C. $[\alpha]_D^{20}$ −43.9 (*c* 0.12). ^{1}H NMR: 1.26 (6H, d, *J*=7.0 Hz, CH_3CHCON), 1.29–1.49 (21H, m, C(CH_3)$_3$ and CH_3CHCO_2H), 3.92–4.21 (2H, m, NCHCON), 4.30–4.45 (1H, m, NCHCO_2H), 7.09 (2H, d, *J*=7.1 Hz, OCONH), 7.67 (2H, s, ArH), 8.14 (1H, s, ArH), 8.64 (1H, d, *J*=6.8 Hz, ArCONH), 10.07 (2H, s, CONHAr), 12.52 (1H, br s, CO_2H). ^{13}C NMR: 17.8, 18.9, 29.2, 49.1, 51.4, 79.0, 113.8, 114.4, 136.5, 140.1, 156.1, 167.5, 173.0, 175.0. MS (FAB): 565 (M^+, 8%). HRMS (FAB): calcd for $C_{26}H_{39}N_5O_9+H^+$, 566.2821; found, 566.2809.

4.7.2. G1-F_2-CONH-F-CO_2H (11, aa^1=F). Starting from G1-F_2-CONH-F-CO_2Me (2.05 g, 2.5 mmol) in MeOH (40 mL) and aqueous KOH solution (6.0 mL, 1.0 M), the target compound was obtained as a white solid (1.95 g, 97%). T_m 159 °C. $[\alpha]_D^{20}$ +42.0 (*c* 1.30). ^{1}H NMR: 1.32 (18H, m, C(CH_3)$_3$), 2.72–2.91 (2H, m, PhCHH), 2.91–3.08 (2H, m, PhCHH), 3.08–3.25 (2H, m, PhCH_2CHCO_2H), 4.18–4.42 (2H, m, NCHCON), 4.52–4.65 (1H, m, NCHCO_2H), 7.06–7.42 (17H, m, ArH and OCONH), 7.65 (2H, s, ArH), 8.13 (1H, s, ArH), 8.65 (1H, d, *J*=7.4 Hz, ArCONH), 10.21 (2H, s, CONHAr), 12.80 (1H, br s, CO_2H). ^{13}C NMR: 29.1, 37.2, 38.3, 39.6, 55.0, 57.6, 79.0, 114.0, 114.5, 127.3, 129.0, 129.1, 130.0, 130.2, 136.5, 138.9, 139.1, 140.0, 156.3, 167.4, 172.0, 174.0. MS (FAB): 794 [$(M+H)^+$, 7%]. HRMS (FAB): calcd for $C_{44}H_{51}N_5O_9+H^+$, 794.3760; found, 794.3772.

4.7.3. G1-V_2-CONH-V-CO_2H (11, aa^1=V). Starting from G1-V_2-CONH-V-CO_2Me (0.39 g, 0.6 mmol) in MeOH (10 mL) and aqueous KOH solution (1.2 mL, 1.0 M), the titled compound was obtained as a white solid (0.36 g, 94%). T_m 194 °C. $[\alpha]_D^{20}$ −8.9 (*c* 0.61). ^{1}H NMR: 0.90 (12H, d, *J*=6.0 Hz, $(CH_3)_2CH$), 0.96 (6H, d, *J*=4.7 Hz, $(CH_3)_2CH$), 1.38 (18H, s, $C(CH_3)_3$), 1.85–2.08 (2H, m, $Me_2CHCHCONH$), 2.08–2.28 (1H, m, $Me_2CHCHCO_2H$), 3.93 (2H, t, *J*=7.5 Hz, NC*H*CONH), 4.25 (1H, t, *J*=7.1 Hz, NC*H*CO_2H), 6.90 (2H, d, *J*=8.2 Hz, OCON*H*), 7.66 (2H, s, Ar*H*), 8.20 (1H, s, Ar*H*), 8.38 (1H, d, *J*=7.3 Hz, ArCON*H*), 10.12 (2H, s, CON*H*Ar), 12.64 (1H, br s, CO_2H). ^{13}C NMR: 19.4, 19.6, 20.17, 20.20, 29.1, 30.4, 31.2, 59.2, 61.6, 79.0, 113.6, 114.5, 136.8, 139.9, 156.5, 168.2, 171.9, 173.9. MS (ESI): 672 [(M+ Na)$^+$, 100%]. HRMS (ESI): calcd for $C_{32}H_{51}N_5O_9$+ Na^+, 672.3579; found, 672.3579.

4.8. General procedure for the synthesis of G2-(aa^1)$_3$(CbzNH)-CO_2Et 12

DCC or EDCI (1 equiv) was added to a stirred mixture of G1-(aa^1)$_2$-CONH-(aa^1)-CO_2H (1 equiv), ethyl 3-amino-5-(*N*-benzyloxycarbonylamino)benzoate **5** (1 equiv) and HOBt (1 equiv) in dry THF at −10 °C. The mixture was kept at −10 °C for 2 h and then at 25 °C for 20 h. When DCC was used as coupling reagent, the insoluble DCU produced was first removed by filtration. The filtrate or the reaction solvent (in the case of EDCI as the coupling agent) was then evaporated in vacuo. The residue was redissolved in EtOAc, and washed successively with saturated $NaHCO_3$ solution, 10% citric acid solution, saturated $NaHCO_3$ solution, and water. The organic layer was dried ($MgSO_4$), filtered, and evaporated in vacuo to give the target compound that was purified by precipitation or flash chromatography on silica gel.

4.8.1. G2-A_3(CbzNH)-CO_2Et (12, aa^1=A). Starting from G1-A_2-CONH-A-CO_2H (1.57 g, 2.8 mmol), ethyl 3-amino-5-(*N*-benzyloxycarbonylamino)benzoate (0.87 g, 2.8 mmol), EDCI (0.85 g, 2.8 mmol) and HOBt (0.38 g, 2.8 mmol), the desired compound was obtained as a white solid (1.66 g, 69%) after flash chromatography (eluent: EtOAc/hexane 2:1). R_f 0.24 (EtOAc/hexane 2:1). T_m 202 °C. $[\alpha]_D^{20}$ −21.3 (*c* 1.01). ^{1}H NMR: 1.26 (6H, d, *J*=7.1 Hz, surface CHCH_3), 1.30 (3H, t, *J*=7.1 Hz, CH_2CH_3), 1.32–1.48 (21H, m, $C(CH_3)_3$ and interior CHCH_3), 4.02–4.18 (2H, m, surface NC*H*), 4.30 (2H, q, *J*=7.1 Hz, CH_2Me), 4.48–4.64 (1H, m, interior NC*H*), 5.17 (2H, s, PhCH_2), 7.09 (2H, d, *J*=7.1 Hz, OCON*H*CH), 7.28–7.49 (5H, m, Ar*H*), 7.70 (2H, s, surface Ar*H*), 7.81 (1H, interior Ar*H*), 7.97 (1H, s, interior Ar*H*), 8.08 (1H, s, interior Ar*H*), 8.16 (1H, s, surface Ar*H*), 8.58 (1H, br s, ArCON*H*), 10.02 (1H, s, CON*H*Ar), 10.07 (2H, s, CON*H*Ar) 10.29 (1H, s, CON*H*Ar). ^{13}C NMR: 15.1, 18.7, 18.9, 29.2, 50.8, 51.4, 61.8, 66.8, 79.0, 113.8, 114.1, 114.5, 114.7, 115.0, 129.0, 129.4, 131.6, 136.4, 137.5, 140.1, 140.7, 154.3, 156.1, 166.4, 167.6, 172.5, 173.0. MS (FAB): 862 [(M+H)$^+$, 100%]. HRMS (FAB): calcd for $C_{43}H_{55}N_7O_{12}$+H$^+$, 862.3981; found, 862.3991.

4.8.2. G2-F_3(CbzNH)-CO_2Et (12, aa^1=F). Starting from G1-F_2-CONH-F-CO_2H (1.40 g, 1.8 mmol), ethyl 3-amino-5-(*N*-benzyloxycarbonylamino)benzoate (0.55 g, 1.8 mmol), DCC (0.36 g, 1.8 mmol), and HOBt (0.24 g, 1.8 mmol), the target product was obtained as a white solid (1.23 g, 64%) after precipitation from H_2O/MeOH. T_m 195 °C. $[\alpha]_D^{20}$+ 54.9 (*c* 1.01). ^{1}H NMR: 1.18–1.42 (21H, m, CH_2CH_3 and $C(CH_3)_3$), 2.72–2.91 (2H, m, PhC*H*H), 2.91–3.08 (2H, m, PhC*H*H), 3.08–3.25 (2H, m, interior PhCH_2C), 4.20–4.42 (4H, m, surface NC*H* and CH_2Me), 4.78–4.92 (1H, m, interior NC*H*), 5.18 (2H, s, PhCH_2O), 7.06–7.42 (22H, m, Ar*H* and OCON*H*CH), 7.69 (2H, s, surface Ar*H*), 7.84 (1H, s, interior Ar*H*), 7.98 (1H, s, interior Ar*H*), 8.11 (1H, s, interior Ar*H*), 8.14 (1H, s, surface ArH), 8.71 (1H, br s, ArCON*H*), 10.05 (1H, s, CON*H*Ar), 10.22 (2H, s, surface CON*H*Ar), 10.43 (1H, s, interior CON*H*Ar). ^{13}C NMR: 15.1, 29.1, 38.1, 38.3, 56.7, 57.6, 61.8, 66.8, 79.0, 114.0, 114.2, 114.6, 114.8, 115.1, 127.3, 129.0, 129.1, 129.4, 130.2, 131.6, 136.5, 137.5, 138.9, 140.0, 140.6, 140.8, 154.3, 156.4, 166.4, 167.7, 171.4, 172.0. MS (FAB): 1090 [(M+H)$^+$, 100%]. HRMS (FAB): calcd for $C_{61}H_{67}N_7O_{12}$+H$^+$, 1090.4920; found, 1090.4942.

4.9. General procedure for the synthesis of G2-(aa^1)$_3$(NH_2)-CO_2Et 13

A suspension of G2-(aa^1)$_3$(CbzNH)-CO_2Et **12** and 10% palladium on charcoal in MeOH was stirred under H_2 for 1 h. After the reaction, the catalyst was removed by filtration and the solvent evaporated in vacuo to give the target compound.

4.9.1. G2-A_3(NH_2)-CO_2Et (13, aa^1=A). Starting from G2-A_3(CbzNH)-CO_2Et (1.30 g, 1.5 mmol), the titled compound was obtained as a white solid (1.08 g, 98%). T_m 189 °C. $[\alpha]_D^{20}$ −27.9 (*c* 0.69). ^{1}H NMR: 1.26 (6H, d, *J*=6.2 Hz, surface NCHCH_3), 1.29 (3H, t, *J*=7.1 Hz, CH_2CH_3), 1.32–1.42 (21H, m, $C(CH_3)_3$ and interior NCHCH_3), 4.02–4.18 (2H, m, surface NC*H*), 4.25 (2H, q, *J*=7.1 Hz, CH_2Me), 4.42–4.60 (1H, m, interior NC*H*), 5.42 (2H, s, NH_2), 6.90 (1H, s, interior Ar*H*), 7.09 (2H, d, *J*=7.2 Hz, OCON*H*), 7.20 (1H, s, interior Ar*H*), 7.35 (1H, s, interior Ar*H*), 7.70 (2H, s, surface Ar*H*), 8.15 (1H, s, surface Ar*H*), 8.52 (1H, br s, ArCON*H*), 9.95 (1H, s, CON*H*Ar), 10.07 (2H, s, CON*H*Ar). ^{13}C NMR: 15.2, 18.8, 18.9, 29.2, 50.8, 51.4, 61.3, 79.0, 108.7, 109.7, 110.6, 113.8, 114.4, 131.6, 136.5, 140.2, 140.8, 150.3, 156.1, 167.1, 167.5, 172.2, 173.0. MS (FAB): 727 (M^+, 100%). HRMS (FAB): calcd for $C_{35}H_{49}N_7O_{10}^+$, 727.3535; found, 727.3542.

4.9.2. G2-F_3(NH_2)-CO_2Et (13, aa^1=F). Starting from G2-F_3(CbzNH)-CO_2Et (1.22 g, 1.1 mmol), the titled compound was obtained as a white solid (1.02 g, 95%). T_m 191 °C. $[\alpha]_D^{20}$+53.0 (*c* 0.70). ^{1}H NMR: 1.18–1.42 (21H, m, CH_2CH_3 and $C(CH_3)_3$), 2.72–2.91 (2H, m, PhC*H*H), 2.91–3.08 (2H, m, PhC*H*H), 3.08–3.25 (2H, m, PhCH_2), 4.26 (2H, q, *J*=7.1 Hz, CH_2Me), 4.20–4.42 (2H, m, surface NC*H*), 4.78–4.92 (1H, m, interior NC*H*), 5.45 (2H, s, NH_2), 6.92 (s, 1H, interior Ar*H*), 7.06–7.42 (19H, m, Ar*H*, OCON*H* and interior Ar*H*), 7.69 (2H, s, surface Ar*H*), 8.13 (1H, s, surface Ar*H*), 8.67 (1H, br s, ArCON*H*), 10.10 (1H, s, interior CON*H*Ar), 10.23 (2H, s, surface CON*H*Ar). ^{13}C NMR: 15.2, 29.1, 38.2, 38.3, 56.8, 57.6, 61.4, 79.0, 108.8, 109.8, 110.7, 113.9, 114.6, 127.3, 129.0, 129.1, 130.2, 131.6, 136.5, 138.9, 140.0, 140.6, 150.3, 156.4, 167.1, 167.6,

171.1, 172.0. MS (FAB): 955 (M^+, 100%). HRMS (FAB): calcd for $C_{53}H_{61}N_7O_{10}^+$, 955.4474; found, 955.4492.

4.10. General procedure for the preparation of G2-(aa^1)$_3$(aa^2)$_3$-CO$_2$Et 3

DCC or EDCI (1 equiv) was added to a stirred mixture of G2-(aa^1)$_3$(NH$_2$)-CO$_2$Et **13** (1 equiv), G1-(aa^2)$_2$-CONH-(aa^2)-CO$_2$H (1 equiv) and HOBt (1 equiv) in dry THF at −10 °C. The mixture was kept at −10 °C for 2 h and then at 25 °C for 48 h. When DCC was used as the coupling agent, the insoluble DCU produced was removed by filtration. The filtrate or the reaction solvent (in the case of EDCI as the coupling agent) was evaporated in vacuo. The residue was redissolved in $CHCl_3$, and washed successively with saturated $NaHCO_3$ solution, 10% citric acid solution, saturated $NaHCO_3$ solution and water. The organic layer was dried ($MgSO_4$), filtered and evaporated in vacuo to give the target compound that was purified by flash chromatography on silica gel.

4.10.1. G2-A$_3$F$_3$-CO$_2$Et (3, aa^{1}aa^2=AF). Starting from G2-F$_3$(NH$_2$)-CO$_2$Et (0.75 g, 0.78 mmol), G1-A$_2$-CONH-A-CO$_2$H (0.44 g, 0.78 mmol), DCC (0.16 g, 0.78 mmol) and HOBt (0.11 g, 0.81 mmol), the target compound was obtained as a white solid (0.51 g, 43%) after flash chromatography (eluent: $CHCl_3$/MeOH 60:1). R_f 0.14 ($CHCl_3$/MeOH 50:1). T_m 191 °C. $[\alpha]_D^{20}$ +25.8 (*c* 0.89). ^{1}H NMR: 1.26 (6H, d, *J*=7.1 Hz, surface CHC*H*$_3$), 1.27–1.51 (42H, m, C(C*H*$_3$)$_3$, interior CHC*H*$_3$ and CH$_2$C*H*$_3$), 2.70–2.91 (2H, m, surface PhC*H*H), 2.91–3.08 (2H, m, surface PhC*H*H), 3.08–3.25 (2H, m, interior PhC*H*$_2$), 3.98–4.19 (2H, m, surface NC*H*Me), 4.19–4.44 (4H, m, surface NC*H*Bn and C*H*$_2$Me), 4.48–4.64 (1H, m, interior NC*H*Me), 4.75–4.92 (1H, m, interior NC*H*Bn), 7.08 (2H, d, *J*=7.1 Hz, OCON*H*Me), 7.11–7.45 (17H, m, Ph*H* and OCON*H*Bn), 7.69 (2H, s, surface Ar*H*), 7.71 (2H, s, surface Ar*H*), 7.99 (2H, s, interior Ar*H*), 8.14 (1H, s, surface Ar*H*), 8.16 (1H, s, surface Ar*H*), 8.31 (1H, s, interior Ar*H*), 8.60 (1H, br s, ArCON*H*CHMe), 8.73 (1H, br s, ArCON*H*CHBn), 10.08 (2H, s, surface CON*H*Ar), 10.22 (2H, s, surface CON*H*Ar), 10.34 (1H, s, interior CON*H*Ar), 10.44 (1H, s, interior CON*H*Ar). ^{13}C NMR: 15.1, 18.6, 18.9, 29.1, 38.1, 38.3, 50.9, 51.4, 56.8, 57.6, 61.8, 78.96, 79.04, 113.8, 114.5, 114.6, 115.3, 115.8, 127.3, 129.0, 129.1, 130.2, 131.5, 136.5, 138.9, 140.0, 140.1, 140.5, 140.6, 156.1, 156.4, 166.4, 167.7, 171.4, 172.0, 172.6, 173.0. MS (FAB): 1526 [(M+ Na)$^+$, 100%]. HRMS (ESI): calcd for $C_{79}H_{98}N_{12}O_{18}$+ Na$^+$, 1525.7014; found, 1525.7036.

4.10.2. G2-A$_3$V$_3$-CO$_2$Et (3, aa^{1}aa^2=AV). Starting from G2-A$_3$(NH$_2$)-CO$_2$Et (0.42 g, 0.58 mmol), G1-V$_2$-CONH-V-CO$_2$H (0.37 g, 0.58 mmol), DCC (0.12 g, 0.58 mmol) and HOBt (0.08 g, 0.58 mmol), the target product was obtained as a white solid (0.27 g, 35%) after flash chromatography (eluent: $CHCl_3$/MeOH 70:1 gradient to 30:1). R_f 0.55 ($CHCl_3$/MeOH 50:1). T_m 194 °C. $[\alpha]_D^{20}$ +38.5 (*c* 0.86). ^{1}H NMR: 0.90 (12H, d, *J*=6.5 Hz, surface CH(C*H*$_3$)$_2$), 0.97 (3H, d, *J*=6.9 Hz, interior CH(C*H*$_3$)$_2$), 1.00 (3H, d, *J*=6.9 Hz, interior CH(C*H*$_3$)$_2$), 1.26 (6H, d, *J*=7.1 Hz, surface CHC*H*$_3$), 1.32–1.55 (42H, m, C(C*H*$_3$)$_3$, CH$_2$C*H*$_3$ and interior CHC*H*$_3$), 1.85–2.08 (2H, m, surface C*H*Me$_2$), 2.10–2.29 (1H, m, interior C*H*Me$_2$), 3.92 (2H, t, *J*=7.9 Hz, surface NC*H*CHMe$_2$), 4.05–4.19 (2H, m, surface NC*H*Me), 4.31 (2H, q, *J*=7.1 Hz, C*H*$_2$Me), 4.35 (1H, t, *J*=7.0 Hz, interior NC*H*CHMe$_2$), 4.48–4.64 (1H, m, interior NC*H*Me), 6.90 (2H, d, *J*=8.4 Hz, OCON*H*), 7.08 (2H, d, *J*=7.1 Hz, OCON*H*), 7.70 (4H, s, surface Ar*H*), 7.98 (1H, s, Ar*H*), 8.00 (1H, s, Ar*H*), 8.16 (1H, s, Ar*H*), 8.20 (1H, s, Ar*H*), 8.31 (1H, s, Ar*H*), 8.43 (1H, br s, ArCON*H*), 8.59 (1H, br s, ArCON*H*), 10.07 (2H, s, surface CON*H*Ar), 10.13 (2H, s, surface CON*H*Ar), 10.32 (1H, s, CON*H*Ar), 10.41 (1H, s, CON*H*Ar). ^{13}C NMR: 15.1, 18.6, 18.9, 19.4, 20.0, 20.2, 29.1, 31.1, 31.2, 50.9, 51.4, 61.0, 61.6, 61.8, 79.0, 113.7, 114.5, 115.2, 115.7, 115.8, 131.5, 136.4, 136.7, 139.9, 140.1, 140.3, 140.6, 156.1, 156.5, 166.4, 167.6, 168.1, 171.6, 171.9, 172.6, 173.0. MS (FAB): 1382 [(M+ Na)$^+$, 12%]. HRMS (FAB): calcd for $C_{67}H_{98}N_{12}O_{18}$+ Na$^+$, 1381.7014; found, 1381.7036.

4.10.3. G2-F$_3$V$_3$-CO$_2$Et (3, aa^{1}aa^2=FV). Starting from G2-F$_3$(NH$_2$)-CO$_2$Et (0.41 g, 0.43 mmol), G1-V$_2$-CONH-V-CO$_2$H (0.28 g, 0.43 mmol), EDCI (0.13 g, 0.43 mmol) and HOBt (0.06 g, 0.43 mmol), the target compound was obtained as a white solid (0.31 g, 46%) after flash chromatography (eluent: $CHCl_3$/MeOH 80:1 gradient to 60:1). R_f 0.18 ($CHCl_3$/MeOH 50:1). T_m 192 °C. $[\alpha]_D^{20}$ +78.3 (*c* 0.79). ^{1}H NMR: 0.90 (12H, d, *J*=6.5 Hz, surface CH(C*H*$_3$)$_2$), 0.99 (3H, d, *J*=6.8 Hz, interior CH(C*H*$_3$)$_2$), 1.01 (3H, d, *J*=6.8 Hz, interior CH(C*H*$_3$)$_2$), 1.14–1.35 (21H, m, CH$_2$C*H*$_3$ and C(C*H*$_3$)$_3$), 1.38 (18H, s, C(C*H*$_3$)$_3$), 1.85–2.08 (2H, m, surface C*H*Me$_2$), 2.10–2.29 (1H, m, interior C*H*Me$_2$), 2.70–2.91 (2H, m, surface PhC*H*H), 2.91–3.08 (2H, m, surface PhC*H*H), 3.08–3.25 (2H, m, interior PhC*H*$_2$), 3.93 (2H, t, *J*=8.0 Hz, surface NC*H*CHMe$_2$), 4.17–4.49 (5H, C*H*$_2$Me, interior NC*H*CHMe$_2$ and surface C*H*Bn), 4.70–4.92 (1H, m, interior C*H*Bn), 6.90 (2H, d, *J*=8.6 Hz, OCON*H*), 7.03–7.45 (17H, m, Ar*H* and OCON*H*), 7.69 (2H, s, surface Ar*H*), 7.71 (2H, s, surface Ar*H*), 8.00 (2H, s, Ar*H*), 8.13 (1H, s, Ar*H*), 8.19 (1H, s, Ar*H*), 8.31 (1H, s, Ar*H*), 8.42 (1H, br s, ArCON*H*), 8.73 (1H, br s, ArCON*H*), 10.13 (2H, s, surface CON*H*Ar), 10.21 (2H, s, surface CON*H*Ar), 10.43 (1H, s, interior CON*H*Ar), 10.44 (1H, s, interior CON*H*Ar). ^{13}C NMR: 15.2, 19.5, 20.0, 20.2, 29.1, 31.1, 31.2, 38.0, 38.3, 56.9, 57.6, 61.1, 61.6, 61.9, 79.0, 113.7, 114.0, 114.6, 115.3, 116.0, 127.3, 129.0, 129.1, 130.2, 131.6, 136.5, 136.7, 138.9, 140.0, 140.3, 140.5, 156.4, 156.5, 166.4, 167.8, 168.1, 171.5, 171.6, 171.9, 172.0. MS (ESI): 1610 [(M+Na)$^+$, 100%]. HRMS (ESI): calcd for $C_{85}H_{110}N_{12}O_{18}$+ Na$^+$, 1609.7953; found, 1609.7946.

4.11. General procedure for the synthesis of G2-(aa^1)$_3$(aa^2)$_3$-CO$_2$H 4

A mixture of aqueous KOH solution (1.0 M) and G2-(aa^1)$_3$(aa^2)$_3$-CO$_2$Et **3** in MeOH was stirred at 25 °C until completion of the reaction as monitored by TLC. The solvent was evaporated in vacuo and the residue was poured into large amount of water. The precipitate formed was collected by filtration, washed with water, and dried in vacuo.

4.11.1. G2-A$_3$F$_3$-CO$_2$H (4, aa^{1}aa^2=AF). Starting from G2-A$_3$F$_3$-CO$_2$Et (0.32 g, 0.21 mmol) in MeOH (10 mL) and aqueous KOH solution (0.5 mL, 1.0 M), the target

compound was obtained as a white solid (0.30 g, 95%). T_m 169 °C. $[\alpha]_D^{20}+22.9$ (*c* 0.52). ^{1}H NMR: 1.26 (6H, d, $J=$ 7.2 Hz, surface CHCH_3), 1.27–1.51 (39H, m, C(CH_3)$_3$, interior CHCH_3), 2.70–2.91 (2H, m, surface PhC*H*H), 2.91–3.08 (2H, m, surface PhC*H*H), 3.08–3.25 (2H, m, interior PhCH_2), 3.98–4.19 (2H, m, surface NC*H*Me), 4.19–4.44 (2H, m, surface NC*H*Bn), 4.48–4.64 (1H, m, interior NC*H*Me), 4.75–4.92 (1H, m, interior NC*H*Bn), 7.08 (2H, d, $J=7.1$ Hz, OCON*H*), 7.11–7.45 (17H, m, Ar*H* and OCON*H*), 7.69 (2H, s, surface Ar*H*), 7.71 (2H, s, surface Ar*H*), 7.96 (1H, s, Ar*H*), 7.98 (1H, s, Ar*H*), 8.14 (1H, s, Ar*H*), 8.16 (1H, s, Ar*H*), 8.26 (1H, s, Ar*H*), 8.60 (1H, br s, ArCON*H*), 8.72 (1H, br s, ArCON*H*), 10.08 (2H, s, surface CON*H*Ar), 10.22 (2H, s, surface CON*H*Ar), 10.29 (1H, s, interior CON*H*Ar), 10.40 (1H, s, interior CON*H*Ar), 12.93 (1H, br s, COO*H*). ^{13}C NMR: 18.7, 18.9, 29.2, 38.1, 38.3, 50.9, 51.4, 56.8, 57.6, 79.0, 79.1, 113.8, 114.5, 114.6, 115.1, 116.1, 127.3, 129.0, 129.1, 130.2, 132.5, 136.5, 138.9, 140.0, 140.2, 140.3, 140.5, 156.1, 156.4, 167.7, 168.0, 171.4, 172.0, 172.6, 173.0. MS (FAB): 1476 [$(M+H)^+$, 10%]. HRMS (FAB): calcd for $C_{77}H_{94}N_{12}O_{18}+H^+$, 1475.6882; found, 1475.6873.

4.11.2. G2-A$_3$V$_3$-CO$_2$H (4, aa^{1}aa^2=AV). Starting from G2-A$_3$V$_3$-CO$_2$Et (0.34 g, 0.25 mmol) in MeOH (7.0 mL) and aqueous KOH solution (1.0 mL, 1.0 M), the titled compound was obtained as a white solid (0.33 g, 99%). T_m 181 °C. $[\alpha]_D^{20}+27.9$ (*c* 0.89). ^{1}H NMR: 0.90 (12H, d, $J=$ 6.4 Hz, surface CH(CH_3)$_2$), 0.97 (3H, d, $J=7.0$ Hz, interior CH(CH_3)$_2$), 1.00 (3H, d, $J=7.0$ Hz, interior CH(CH_3)$_2$), 1.26 (6H, d, $J=6.9$ Hz, surface CHCH_3), 1.31–1.55 (39H, m, C(CH_3)$_3$ and interior CHCH_3), 1.95–2.10 (2H, m, surface C*H*Me$_2$), 2.10–2.29 (1H, m, interior C*H*Me$_2$), 3.93 (2H, t, $J=7.7$ Hz, surface NC*H*CMe$_2$), 4.07–4.21 (2H, m, surface NC*H*Me), 4.39 (1H, t, $J=7.0$ Hz, interior NC*H*CMe$_2$), 4.48–4.64 (1H, m, interior NC*H*Me), 6.90 (2H, d, $J=7.5$ Hz, OCON*H*), 7.08 (2H, d, $J=7.1$ Hz, OCON*H*), 7.70 (4H, s, surface Ar*H*), 7.96 (2H, s, Ar*H*), 8.17 (1H, s, Ar*H*), 8.20 (2H, s, Ar*H*), 8.25 (1H, s, Ar*H*), 8.41 (1H, br s, ArCON*H*), 8.59 (1H, br s, ArCON*H*), 10.07 (2H, s, surface CON*H*Ar), 10.13 (2H, s, surface CON*H*Ar), 10.27 (1H, s, interior CON*H*Ar), 10.36 (1H, s, interior CON*H*Ar). ^{13}C NMR: 18.7, 18.9, 19.5, 20.0, 20.2, 29.2, 31.3, 50.9, 51.4, 61.0, 61.6, 78.97, 79.01, 113.7, 113.8, 114.5, 115.0, 116.1, 116.2, 132.7, 136.4, 136.7, 140.0, 140.1, 140.5, 156.1, 156.5, 167.7, 168.0, 168.1, 171.5, 171.9, 172.5, 173.0. MS (FAB): 1353 [$(M+Na)^+$, 15%]. HRMS (FAB): calcd for $C_{65}H_{94}N_{12}O_{18}+Na^+$, 1353.6701; found, 1353.6727.

4.11.3. G2-F$_3$V$_3$-CO$_2$H (4, aa^{1}aa^2=FV). Starting from G2-F$_3$V$_3$-CO$_2$Et (0.26 g, 0.16 mmol) in MeOH (7 mL) and aqueous KOH solution (0.6 mL, 1.0 M), the titled compound was obtained as a white solid (0.24 g, 94%). T_m 175 °C. $[\alpha]_D^{20}+63.8$ (*c* 0.90). ^{1}H NMR: 0.90 (12H, d, $J=$ 6.5 Hz, surface CH(CH_3)$_2$), 0.98 (3H, d, $J=6.8$ Hz, interior CH(CH_3)$_2$), 1.00 (3H, d, $J=6.8$ Hz, interior CH(CH_3)$_2$), 1.32 (18H, s, C(CH_3)$_3$), 1.38 (18H, s, C(CH_3)$_3$), 1.85–2.08 (2H, m, surface C*H*Me$_2$), 2.10–2.29 (1H, m, interior C*H*Me$_2$), 2.70–2.91 (2H, m, surface PhC*H*H), 2.91–3.08 (2H, m, surface PhC*H*H), 3.08–3.25 (2H, m, interior PhCH_2), 3.93 (2H, t, $J=8.0$ Hz, surface NC*H*CHMe$_2$), 4.17–4.49 (3H, interior NC*H*CHMe$_2$ and surface NC*H*Bn), 4.70–4.92 (1H, m, interior NC*H*Bn), 6.90 (2H, d, $J=8.3$ Hz, OCON*H*), 7.03–7.45 (17H, m, Ar*H* and OCON*H*), 7.68 (2H, s, surface Ar*H*), 7.71 (2H, s, surface Ar*H*), 7.98 (2H, s, Ar*H*), 8.14 (1H, s, Ar*H*), 8.20 (1H, s, Ar*H*), 8.25 (1H, s, Ar*H*), 8.42 (1H, br s, ArCON*H*), 8.73 (1H, br s, ArCON*H*), 10.13 (2H, s, surface CON*H*Ar), 10.22 (2H, s, surface CON*H*Ar), 10.39 (1H, s, interior CON*H*Ar), 10.40 (1H, s, interior CON*H*Ar), 12.91 (1H, br s, COO*H*). ^{13}C NMR: 19.5, 20.0, 20.2, 29.1, 31.2, 38.0, 38.3, 56.9, 57.6, 61.0, 61.6, 78.99, 79.04, 113.6, 113.9, 114.5, 115.1, 116.2, 127.3, 129.0, 129.1, 130.2, 132.5, 136.5, 136.7, 138.9, 139.96, 140.01, 140.2, 140.3, 156.4, 156.5, 167.8, 168.0, 168.1, 171.4, 171.5, 171.9, 172.0, 172.2. MS (FAB): 1582 [$(M+Na)^+$, 100%]. HRMS (ESI): calcd for $C_{83}H_{106}N_{12}O_{18}+Na^+$, 1581.7640; found, 1581.7656.

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