Synthesis and Evaluation of Boronated Lysine and Bis(carboranylated) γ-Amino Acids as Monomers for Peptide Assembly

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We have synthesized *N*-Fmoc amino acid derivatives bearing boronated and carboranylated substituents. Thus, a 1,3,2-dioxaborolane subunit has been introduced on the side chain of N^{α}-Fmoc-L-lysine, and a bis(carboranylated) γ-amino acid building block has been prepared. As shown by the preparation of a model tetrapeptide, only the latter monomer can be successfully used in peptide synthesis.

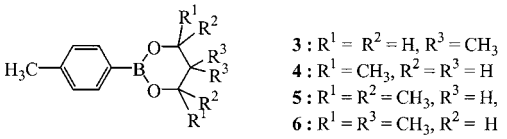
Introduction

A current aspect of Boron Neutron Capture Therapy (BNCT) research relates to the preparation of agents capable of delivering ^{10}B to malignant cells. Borocaptate (BSH)[1,2] and L-borophenylalanine (L-BPA)[3] are low-molecular weight compounds capable of concentrating boron in glioblastoma and melanoma, respectively, and are used clinically. A second approach to BNCT involves the boronation of antibodies,[4−12] with the aim of retaining antigen specificity. A third line of research involves the preparation of boron-rich entities that might be grafted to delivery vectors;[13−16] such materials have been obtained by perborylation of dendrimers[17,18] or by stepwise assembly of boronated monomers.[19−21] This last approach offers flexibility in control of size and amino acid composition, which could be advantageous in the preparation of boron-rich tails for grafting to transfecting peptides; this work presents the preparation of boronated and carboranylated *N*-Fmoc amino acid monomers with this goal in mind.

Results and Discussion

A) Synthesis of Building Blocks

An efficient synthesis of (4-formylphenyl)boronic acid (**1**), allowing high-yielding preparations of various phenylboronic acid derivatives, has been described.[22] Among these, (4-carboxyphenyl)boronic acid[23] was selected here, as its coupling to the side-chain ε-amino group of N^{α}-Fmoc-L-lysine appeared to be a straightforward procedure through which to obtain a boronated amino acid. Before doing so, however, it is necessary to esterify the boronic acid to provide a boronate that will be stable to the coupling/deprotection steps commonly encountered during peptide synthesis. Model compounds **3**−**7**[24] were obtained from *p*-tolylboronic acid with 2,2-dimethylpropane-1,3-diol, pentane-2,4-diol,[25], 2,4-dimethylpentane-2,4-diol,[26] 3,3-dimethylpentane-2,4-diol,[27] and pinacol.



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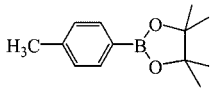
6 : $R^1 = R^3 = CH_3$, $R^2 = H$



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Each of these boronates was stable to piperidine, used to remove Fmoc groups during peptide synthesis, but only **6** and **7** were stable to 95% trifluoroacetic acid. Commercially available pinacol thus appeared to be a suitable esterification agent and so was selected for the subsequent preparation of boronated monomers; this resulted in **2**,[23] which was activated as its pentafluorophenyl ester **8** prior to coupling with N^{α}-Fmoc-L-lysine (Scheme 1). NMR examination of the crude mixture showed a triplet at δ = 6.67 ppm for the NH group of the newly formed amide bond. Although the yield of the crude product was ca. 90% (as estimated by ^1H NMR), we were unable to purify **9** either by pH-dependent extractions or by chromatography on various supports (deactivated silica gel, basic or neutral alumina, Florisil). However, the crude material could be benzylated and subjected to hydrogenolysis after purification. Cleavage of the benzyl group of **10** to give **9** pleasingly occurred without concomitant reductive cleavage of the Fmoc group.[28]

The preparation of an α,α-disubstituted glycine derivative was next considered, as glycine offers the advantage of be-

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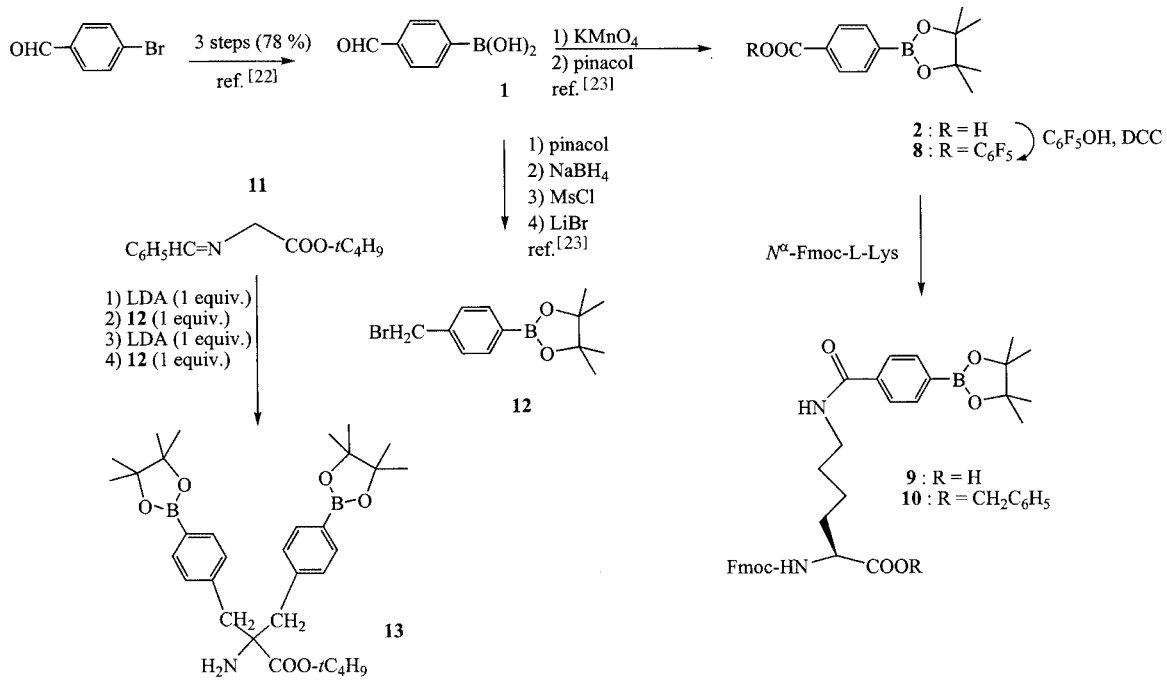


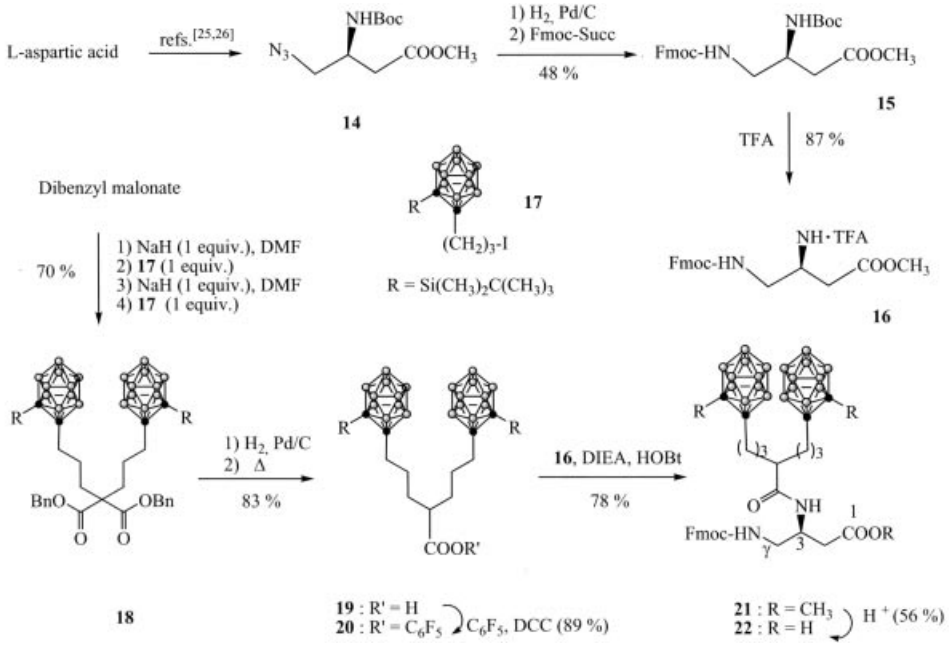
Scheme 1. Preparation of α-amino acid boronated building blocks

ing able to introduce *two* boronated substituents by double alkylation (Scheme 1). Starting with the readily available glycinate **11**,[29,30] two successive in situ monoalkylations with bromide **12**[23] afforded **13**, with the imine being cleaved during workup. However, protection of the amino group of **13** could not be achieved either with Fmoc chloroformate[31] or with fluorenyl succinimidyl carbonate.[32] Noting that harsh conditions had been found necessary to protect an amino group in a similar case[33] it was anticipated that, even if the desired Fmoc derivative of **13** were to be obtained, its use in peptide synthesis could result in low coupling efficiencies, especially since both the amino and carboxylic acid groups are of neopentylic character.[34] Derivatives in which the amino group and the carboxylic group would be placed further apart − namely γ-amino acids − were therefore considered.

The neurotransmitter GABA (γ-aminobutyric acid) is a well-known member of this family. Additionally, the introduction of γ-amino acid units into peptides has also been known for quite some time[35−37] and such peptides have recently been shown to produce stable helical secondary structures[38−41] or to display resistance to certain proteolytic enzymes.[42] For this work, we considered a γ-amino acid core, which would be substituted at position 3 (see Scheme 2); in this respect azido derivative **14**, which can be obtained from L-aspartic acid,[43,44] attracted our attention. Functional group manipulations of **14** involved reduction of the azido group and introduction of an Fmoc protecting group to yield **15**, followed by unmasking of the 3-amino group to afford **16**. To load this amino acid with large amounts of boron, introduction of carboranes was considered.[45] Thus, dibenzyl malonate was bis(alkylated) with (iodopropyl)carborane **17**,[19] which gave the bis(carboranyl) derivative **18**; after hydrogenolysis and thermal decarboxylation, **19** could be obtained in ca. 50% yield from dibenzyl malonate. The carboxylic acid group was then activated as its pentafluorophenyl ester **20** prior to coupling with **16** (which must be freshly prepared in order to achieve good yields). The bis(carboranylated) γ-amino ester **21** thus obtained was finally hydrolyzed to **22**, a bis(carboranylated) amino acid building block.

B) Evaluation in Solid-Phase Peptide Synthesis

To evaluate the compatibility of monomers **9** and **22** with peptide synthesis, the preparation of tetrapeptides incorporating these units was carried out. This was accomplished by standard Fmoc-based solid-phase methodology: (*p*-chlorotrityl)polystyrene resin[46] was treated with Fmoc-glycine, followed by removal of the Fmoc protecting group. The amino group thus liberated was treated with boronated N^{α}-Fmoc-L-lysine **9** [(benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBop), diisopropylethylamine (DIEA)/DMF]. This deprotection/coupling sequence was reiterated, allowing the successive introduction of L-phenylalanine and L-lysine residues. The tetrapeptide was then liberated from the resin by acidic treatment and analyzed. To confirm the sequence, TOCSY-1D NMR experiments were carried out to allow the assignments of NH groups; NOESY experiments then allowed observation of NH−C(=O)−CH cross correlations, which confirmed the Lys−Phe−**9**−Gly sequence. HPLC purification, however, resulted in unacceptable losses of material, a problem reminiscent of the difficulties encountered with chromatographic purifications of the boronated lysine monomer **9** (see above). It is apparent that **9** is not a suitable monomer for peptide synthesis.



Scheme 2. Preparation of a *N*-Fmoc bis(carboranylated) γ-amino acid

The use of **22** gave more promising results. By the same solid-phase methodology, **22**−Gly−Arg−Gly, incorporating a bis(carboranyl) γ-amino acid unit, could be obtained (coupling yield > 95%). After acidic treatment (to cleave the peptide from the resin), the Fmoc group was removed by treatment with tris(2-aminoethyl)amine.[19,47] Mass spectrometric analysis of the peptide isolated after HPLC purification confirmed its structure with the presence of two carborane groups (i.e., 20 boron atoms; m/z = 1275−1287 with the highest peak at m/z = 1281, with relative intensities matching those of the theoretical spectrum). Compound **22** is thus a bis(carboranylated) building block suitable for peptide synthesis, and the preparation of polycarboranylated peptides is a logical extension of this work. It is also noted that intermediates such as the orthogonally protected amino acid **15** could be useful in the development of derivatives of GABA and analogues and other γ-amino acids.

Experimental Section

General: Dry THF was obtained by distillation under Ar from sodium with benzophenone ketyl as an indicator. Amines were distilled from powdered potassium hydroxide. Dry DMF and acetonitrile were obtained by distillation from calcium hydride. NMR spectra are referenced from the residual peak of the NMR solvent;[48] a large singlet is abbreviated as "sl".

Solid-Phase Synthesis: Assembly of protected peptides were carried out manually by the Fmoc/*t*Bu strategy in a glass reaction vessel fitted with a sintered glass frit. Before use, this reaction vessel was silanized overnight with dichlorodimethylsilane, and was then washed abundantly with dichloromethane and dried; DMF was degassed for 30 min with argon. Coupling reactions were performed by use of 1.5−2 equiv. (relative to the resin loading) of N^{α}-Fmoc-protected amino acid, activated in situ with 1.5−2 equiv. PyBOP and 3−4 equiv. DIEA in DMF (10 mL/g resin) for 45 min. The coupling efficiency was controlled by the Kaiser test and/or TNBS tests. For the Kaiser test the following solutions were prepared: A) ninhydrin (500 mg) in ethanol (10 mL), B) phenol (80 g) in ethanol (20 mL), and C) 1 nM aqueous potassium cyanide (2 mL) in pyridine (98 mL). Three drops of each solution were added to a few beads of resin, and heating at 110 °C was carried out for 3 min; a blue color is indicative of free amino groups. For the TBNS test, 6 drops each of solution A (1% trinitrobenzenesulfonic acid in DMF) and of solution B (11% diisopropylethylamine in DMF) were added to a few beads of resin, a red color after 1 min being indicative of the presence of amino groups. N^{α}-Fmoc protecting groups were removed by treatment with a piperidine/DMF solution (1:4) (10 mL/g resin) for 10 min or, if a carborane was present, with tris-(aminoethyl)amine (16% in DMF). The process was repeated three times, and the degree of completeness of deprotection was verified by the UV absorption of the combined washings at 299 nm. At the end of the coupling processes the peptides were recovered from the resin after acid cleavage (1% TFA in CH_2Cl_2 for 3 min), which was repeated until the resin beads became dark purple. After filtration, the combined washings were concentrated under reduced pressure and the residue were purified by reversed-phase HPLC on C-18 columns with 0.09% TFA in water and an acetonitrile gradient.

Methyl (3*S*)-3-(*tert*-Butyloxycarbonylamino)-4-(fluorenylmethoxycarbonylamino)butanoate (15): A solution of the azide **14**[44] (158 mg, 0.61 mmol) in methanol (1 mL) containing Pd (10% on C; 16 mg) was stirred under hydrogen for 30 min. The Pd/C was filtered through Celite, and the solvent was evaporated under reduced pressure. The crude solid was dissolved in a mixture of water (0.6 mL) and THF (0.9 mL), and the pH was adjusted to 9.0 with aq. sodium hydrogencarbonate (10% w/w). A solution of *N*-Fmoc-succinimide (235 mg, 0.73 mmol, 1.1 equiv.) in THF (1 mL) was then added, while the pH was kept at 9−10, and the reaction mixture was stirred for 1 h. The solution was then acidified with diluted hydrochloric acid and extracted with dichloromethane. The organic layers were combined and washed with brine. Volatiles were removed under reduced pressure, and column chromatography (silica gel; DCM/EtOAc, 9:1) of the residue gave **15** (133 mg, 48%) as a white solid. M.p. 161−162 °C. $[\alpha]_D^{20}$ = +2.5 (c = 1, $CHCl_3$).

$C_{25}H_{30}N_2O_6$ (454.52): calcd. C 66.06, H 6.65; found C 65.97, H 6.70. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.80−7.25 (m, 8 H, H_{ar}), 5.44−5.35 (m, 2 H, NH), 4.37 (d, *J* = 6.5 Hz, 2 H, $CH_{2,Fmoc}$), 4.21−4.06 (m, 2 H, CH_{Fmoc} + 3-H), 3.66 (s, 3 H, $CH_{3,ester}$), 3.36 (sl, 2 H, 4-H), 2.55 (sl, 2 H, 2-H), 1.42 (s, 9 H, $CH_{3,Boc}$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.8 (C-1), 157.1 ($C{=}O_{Fmoc}$), 155.8 ($C{=}O_{Boc}$), 144.0−141.4 (C^{IV}_{ar}), 127.7−127.1−125.1−120.0 (CH_{ar}), 79.8 (C^{IV}_{Boc}), 66.9 ($CH_{2,Fmoc}$), 51.9 ($CH_{3,ester}$), 48.3, 47.3, 44.6 (CH_2), 36.6 (CH_2), 28.4 ($CH_{3,Boc}$).

Methyl (3*S*)-3-Amino-4-[(fluorenylmethoxycarbonyl)amino]butanoate Trifluoroacetate Salt (16): Trifluoroacetic acid (3 mL) was added dropwise at 0 °C to a stirred solution of **15** (597 mg, 1.31 mmol) in dichloromethane (8 mL). After the mixture had been stirred at room temperature for 45 min, toluene (20 mL) was added and volatiles were removed under reduced pressure. Column chromatography (silica gel; EtOAc/MeOH, 9:1) of the residue gave **16** (503 mg, 87%) as a white foam; it is advisable to prepare it freshly before use. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.70−7.20 (m, 8 H, H_{ar}), 6.33 (sl, 1 H, NH), 5.63 (s), 4.31−4.28 (m, 2 H, $CH_{2,Fmoc}$), 4.13−4.09 (m, 1 H, CH_{Fmoc}), 3.59 (s, 4 H, 3-H + $CH_{3,ester}$), 3.38 (s, 2 H, 4-H), 2.65−2.62 (m, 2 H, 2-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.8 (C-1), 162.7−162.2, 157.5 ($C{=}O_{Fmoc}$), 143.9−141.3 (C^{IV}_{ar}), 127.8−127.2−125.2−120.0 (CH_{ar}), 67.2 ($CH_{2,Fmoc}$), 52.3 ($CH_{3,ester}$), 48.9, 47.1, 43.5 (CH_2), 35.2 (CH_2) ppm. ^{19}F NMR (282 MHz, $CDCl_3$): δ = −75.8 (CF_3) ppm.

Dibenzyl Bis[3-(2-*tert*-butyldimethylsilyl-*o*-carboranyl)propyl]malonate (18): Dibenzyl malonate (1.17 mL, 4.7 mmol, 1 equiv.) in DMF (10 mL) was added to a suspension of sodium hydride (188 mg, 4.7 mmol, 1 equiv.) in DMF (10 mL). After stirring at 40 °C for 45 min, the solution was cooled to room temp., and iodide **17**[19] (2 g, 4.7 mmol, 1 equiv.) in DMF (10 mL) was added. The solution was stirred at 40 °C for 2 h, after which the whole above procedure was repeated to effect the second alkylation. The mixture was then hydrolyzed with brine and extracted with diethyl ether/pentane (1:1). Solvents were removed under reduced pressure, and pure product was obtained after column chromatography (silica gel; pentane/DCM, 6:4) as a white solid (yield 70%). M.p = 126−127 °C. MS (ESI): *m*/*z* = 916.9 $[M + Cl]^-$. IR: $\tilde{\nu}$ = 2576 cm^{-1} (B−H), 1730 cm^{-1} (C=O). $C_{39}H_{76}B_{20}O_4Si_2$ (881.41): calcd. C 53.14, H 8.69, B 24.53; found C 52.05, H 8.57, B 24.09. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.35−7.23 (m, 10 H, H_{ar}), 5.10 (s, 4 H, $CH_{2,Bn}$), 2.01 (m, 4 H), 1.76 (m, 4 H), 1.27 (m, 4 H), 1.03 (s, 18 H, CCH_3), 0.28 (m, 12 H, $SiCH_3$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.5 (C=O), 135.2 (C^{IV}_{ar}), 128.9−128.8−128.6 (CH), 80.8 (CB), 76.5 (CB), 67.6 ($CH_{2,Bn}$), 57.2 (OC*C*CO), 37.8−32.2 (CH_2), 27.6 (CH_3), 25.2 (CH_2), 20.4 (SiC), −2.4 (CH_3) ppm.

Bis[3-(2-*tert*-Butyldimethylsilyl-*o*-carboranyl)propyl]acetic Acid (19): A solution of **18** (2.54 g, 2.88 mmol) in ethyl acetate (250 mL) and in the presence of Pd/C (10%; 254 mg) was stirred under hydrogen for 2 h. The mixture was then filtered through Celite, and the solvent was evaporated under reduced pressure. The resulting solid was then heated at reflux in toluene (200 mL) and DMF (20 mL) for 3 h. Toluene and DMF were evaporated, and **19** was obtained after column chromatography (silica gel; DCM/MeOH, 9.8:0.2) as a white solid (1.555 g, 83%). M.p. 188−189 °C. MS (ESI): *m*/*z* = 656.6 $[M - H]^+$. IR: $\tilde{\nu}$ = 2575 cm^{-1} (B−H). $C_{24}H_{64}B_{20}O_2Si_2$ (657.15): calcd. C 43.86, H 9.82, B 32.90; found C 44.07, H 9.99, B 30.78. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.22 (m, 1 H, CH), 2.22−2.16 (m, 4 H), 1.57−1.39 (m), 1.06 (s, 18 H, CCH_3), 0.32 (m, 12 H, $SiCH_3$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 180.6 (C=O), 81.0 (CB), 76.4 (CB), 44.5 (CH), 37.7−31.1−28.0 (CH_2), 27.7 (CH_3), 20.5 ($SiCH_3$), −2.3 (CH_3) ppm.

Pentafluorophenyl Bis[3-(2-*tert*-butyldimethylsilyl-*o*-carboranyl)propyl]acetate (20): Pentafluorophenol (331 mg, 1.64 mmol, 1.1 equiv.) and DCC (371 mg, 1.8 mmol, 1.1 equiv.) were added to a solution of **19** (1.076 g; 1.64 mmol) in dichloromethane (10 mL) and DMF (0.5 mL). The reaction mixture was then stirred overnight, and, after filtration, solvents were evaporated under reduced pressure. Column chromatography (silica gel; pentane/DCM, 7:3) gave ester **20** (1.393 g, 89%) as a white foam. IR: $\tilde{\nu}$ = 2576 cm^{-1} (B−H), 1780 cm^{-1} (C=O). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.71−2.66 (m, 1 H, CH), 2.26−2.20 (m, 4 H), 1.74−1.50 (m), 1.06 (s, 18 H, CCH_3), 0.32 (s, 12 H, $SiCH_3$) ppm.^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.2 (C=O), 80.9 (CB), 76.5 (*C*B), 44.9 (CH), 37.7−31.7−27.8 (CH_2), 27.7 (CH_3), 20.5 ($SiCH_3$), −2.3 (CH_3) ppm. ^{19}F NMR (282 MHz, $CDCl_3$): δ = −152.8 to −153.0 (m, 2 F), −157.5 (t, *J* = 21.3 Hz, 1 F), −161.8 to −162.0 (m, 2 F) ppm.

Methyl (3*S*)-3-{Bis[5-(2-*tert*-butyldimethylsilyl-*o*-carboranyl)pentan-2-oylamino]}-4-[(fluorenylmethoxycarbonyl)amino]butanoate (21): Diisopropylethylamine (397 μL, 2.28 mmol, 2 equiv.), *N*-hydroxybenzotriazole (208 mg, 1.37 mmol, 1.2 equiv.), and then diisopropylethylamine (199 μL, 1.14 mmol, 1 equiv.) were added to a stirred solution of freshly prepared **16** (503 mg, 1.14 mmol) and **21** (1.088 g, 1.14 mmol, 1 equiv.) in dichloromethane (17 mL). After stirring overnight, the reaction mixture was diluted with dichloromethane (20 mL) and washed twice with diluted hydrochloric acid and saturated sodium chloride solutions. The solvent was then evaporated under reduced pressure, and column chromatography (silica gel; pentane/EtOAc, 7:3) of the residue afforded a colorless oil, which was precipitated in pentane to **21** as a white solid (887 mg, 78%). M.p. 174−175 °C. MS (ESI): *m*/*z* = 1016.7 $[M + Na]^+$, 1031.7 $[M + K]^+$. IR: $\tilde{\nu}$ = 2573 cm^{-1} (B−H). $[\alpha]^{20}_D$ = +4.1 (*c* = 1, $CHCl_3$). $C_{44}H_{84}B_{20}N_2O_5Si_2$ (993.54): calcd. C 53.19, H 8.52, B 21.76; found C 53.20, H 8.60, B 21.76. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.78−7.27 (m, 8 H, H_{ar}), 6.92 (d, *J* = 7.4 Hz, 1 H, NH_{amide}), 5.63 (sl, 1 H, NH), 4.46−4.18 (m, 3 H, $CH_{2,Fmoc}$, CH_{Fmoc}, 3-H), 3.69 (s, 3 H, $CH_{3,ester}$), 3.40 (sl, 2 H, 4-H), 2.73−2.46 (m, 2-H), 2.16−2.07 (m), 1.45 (sl), 1.28−1.24 (m), 1.03−1.01 (2 s, 18 H, CH_3), 0.29−0.26 (2 s, 12 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.4 ($C{=}O_{amide}$), 171.8 (C-1), 157.8 ($C{=}O_{carbamate}$), 143.8−143.7−141.4 (C^{IV}_{ar}), 127.9−127.2−125.1−120.1 (CH_{ar}), 81.2 (CB), 76.3* (CB), 67.4 ($CH_{2,Fmoc}$), 52.0 ($CH_{3,ester}$), 47.7−47.2* (CH), 37.8−35.5−32.0−28.2 (CH_2), 27.6 (CH_3), 20.4 ($C^{IV}Si$), −2.4 (CH_3Si) ppm.

(3*S*)-3-{Bis[5-(2-*tert*-butyldimethylsilyl-*o*-carboranyl)pentan-2-oylamino]}-4-[(fluorenylmethoxycarbonyl)amino]butanoic Acid (22): Hydrochloric acid (2 N, 1 mL) was added to a solution of **21** (230 mg, 0.23 mmol) in dioxane (1 mL). This solution was heated at reflux for 2 d before being extracted with dichloromethane. The organic layer was dried and volatiles were removed under reduced pressure. Column chromatography (silica gel; EtOAc/DCM, 3:7) of the residue gave **22** (127 mg, 56%) as a white foam. MS (ESI): *m*/*z* = 1002.7 $[M + Na]^+$. IR: $\tilde{\nu}$ = 2572 cm^{-1} (B−H). $[\alpha]^{20}_D$ = +2.9 (*c* = 1, $CHCl_3$). $C_{43}H_{82}B_{20}N_2O_5Si_2$ (979.51): calcd. C 52.73, H 8.44, B 22.07; found C 52.61, H 8.44, B 20.48. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.77−7.22 (m, 9 H, H_{ar}, NH), 5.51 (t, *J* = 5.5 Hz, 1 H, NH_{Fmoc}), 4.45−4.15 (m, 4 H, $CH_{2,Fmoc}$, CH_{Fmoc}, 3-H), 3.44 (sl, 2 H, $CH_{2,next\ to\ Fmoc}$), 2.75−2.50 (m, CH_2), 2.05 (sl, CH_2), 1.03−1.00 (2 s, 18 H, CH_3), 0.28−0.25 (2 s, 12 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 175.1 ($C{=}O_{acid}$), 174.7 ($C{=}O_{amide}$), 158.4 ($C{=}O_{Fmoc}$), 143.7−141.4 (C^{IV}_{ar}), 128.0−127.3−125.2−120.2 (CH_{ar}), 81.3 (CB), 76.4* (CB), 67.7 ($CH_{2,Fmoc}$), 47.9−47.1 (CH), 44.0−37.8−35.7−31.9−28.2−28.1 (CH_2), 27.6 (CH_3), 20.4 ($C^{IV}Si$), −2.4 (CH_3) ppm.

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