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Synthesis of Achiral α,α-Disubstituted β-Alanines, and Their Use in Construction of Libraries of β-Peptide Conjugates of *N*-2-alkyl-1,2,3,4-Tetrahydroisoquinolines on a Solid Support

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Abstract: Six achiral *N*-phthaloyl protected α,α-disubstituted β-amino acids were synthesized, and used to construct a 96 compound library of 1,2,3,4-tetrahydroisoquinoline conjugates of β-peptides on a solid support. The library synthesis consisted of attachment of an appropriately 5-*O*-tethered 1,2,3,4-tetrahydroisoquinoline *via* the *N*-2 atom to a vinyl sulfonyl support, elongation of the deprotected 5-*O*-tether by repeated peptide coupling, quaternarization of *N*-2 with alkylamines and release of the β-peptide conjugate into solution with triethylamine.

INTRODUCTION

Construction of libraries of small organic molecules by the methods of combinatorial and parallel solid phase synthesis has, during the past decade, gained increasing popularity as a tool for drug development.[1] Considerable attention has been paid to libraries consisting of short peptide mimetics or their conjugates bearing a non-peptidyl core structure that is known to exhibit a reasonably high affinity for a given protein target.[2] We have previously shown that some derivatives of 1,2,3,4-tetrahydroisoquinoline bind rather tightly to α_2-adrenergic receptors,[3] and a novel solid phase procedure based on a vinyl sulfone linker has been introduced for the derivatization of this core compound.[4] In the present study, the same linker chemistry is applied to the preparation of a library of tetrahydroisoquinoline conjugates of β-peptides. Novel α,α-disubstituted β-alanines were synthesized in *N*-phthaloyl protected form (**1a-f**) and used to assemble a peptide chain on a solid support bearing the appropriately derivatized tetrahydroisoquinoline core (for the general structure see Fig.1). By applying a parallel synthesis strategy, a library of 96 peptide conjugates was prepared. The unnatural β-amino acids synthesized exhibit two useful features. Firstly, they are achiral, and hence problems arising from racemization upon peptide coupling are entirely avoided. Secondly, owing to bulky α-substituents, a sterically crowded β-peptide structure is obtained. Accordingly, molecular modeling may be expected to predict rather reliably the solution conformation of the conjugates. Their binding behavior is, in turn, supposed to elucidate

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the protein ligand interactions in the close proximity of the actual binding pocket: the tetrahydroisoquinoline moiety serves as the actual binding probe, and the heavily substituted peptide moiety pinpoints the immediate molecular environment. The side chains of the amino acid constituents were selected to exhibit various steric and electronic properties. The unsubstituted hydroxy functions of **1a** may serve as hydrogen bond donors, while the ether oxygens of the methoxy groups of **1b** and those of 1,3-dioxane ring of **1e** may participate in hydrogen bonding as acceptors. Compared to **1a** and **1b**, **1e** brings aditional rigidity to the peptide chain, since the carbonyl and aminomethyl groups adopt *ax/eq* positions. The benzyl groups make **1c** bulky and hydrophobic compared to **1a,b**, and their π-electron system may participate in stacking-interactions. To the best of our knowledge, construction of peptides or peptide conjugates from this type of building bocks has not been previously described. A closely related α-amino acid, α-hydroxymethylserine, has, however, been recently used in solution phase peptide synthesis.[5]

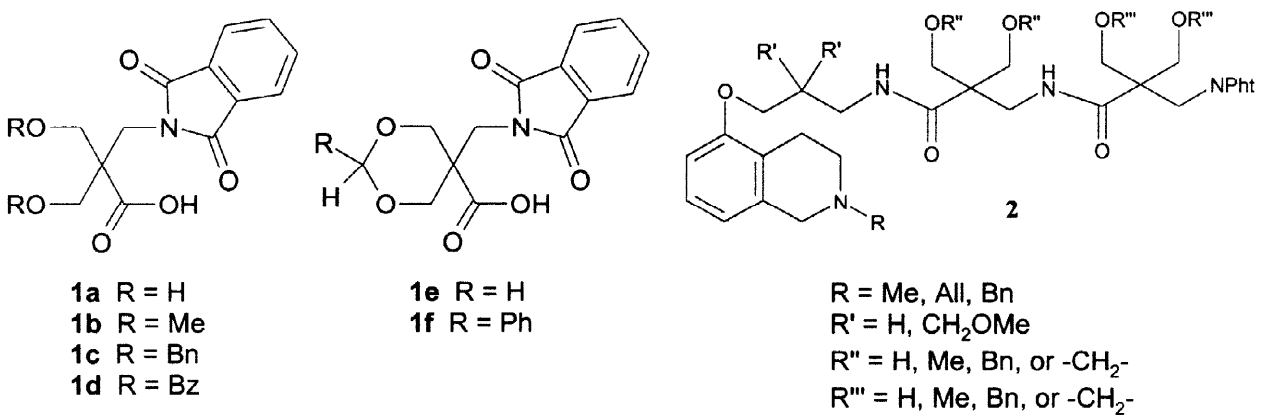
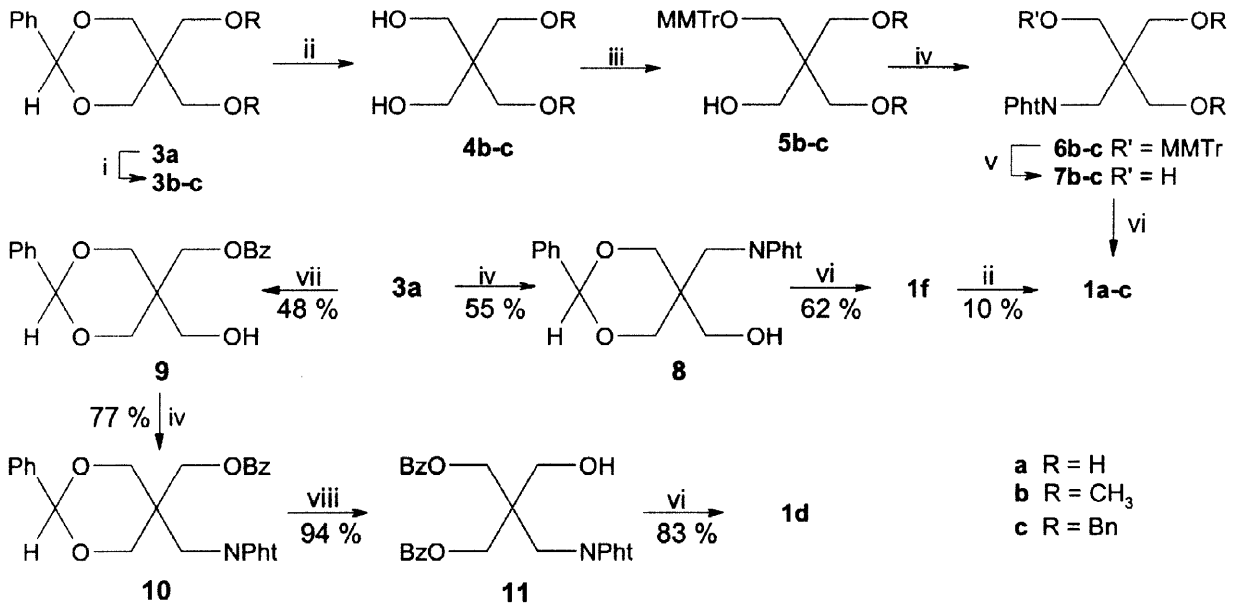


Figure 1. α,α-disubstituted β-alanine building blocks **1a-f**, and general structure of β-peptide conjugate library **2**.

RESULTS AND DISCUSSION

Synthesis of the building blocks (**1a-f**). The acyclic building blocks, **1a-d**, were synthesized from benzylidene protected pentaerythritol[6] (**3a**) as depicted in *Scheme 1*. To obtain **1a**, one of the unprotected hydroxy groups of **3a** was displaced by Mitsunobu reaction with a phthalimido group (**8**), and the remaining alcohol function was oxidized to the carboxylic acid group (**1f**) by Jones' oxidation. Removal of the benzylidene protection by acid-catalyzed hydrolysis yielded **1a**. To obtain **1b** and **1c**, the hydroxy functions of **3a** were first alkylated with methyl iodide (**3b**) or benzyl bromide (**3c**). The benzylidene protection was then removed (**4b,c**), and one of the released hydroxy functions was temporarily protected with a 4-monomethoxytrityl group (**5b,c**). The remaining hydroxy group was displaced with a phthalimido group, the trityl protection was removed, and the released hydroxy group was oxidized to the carboxylic acid group. The cyclic building block **1e** was synthesized analogously, using commercially available 5,5-bis(hydroxymethyl)-1,3-dioxane as starting material.

The benzoyl protected building block **1d** was prepared from **3a**, which was first converted to monobenzoate (**9**). The phthalimido group was then introduced (**10**), and the benzylidene protection was converted to benzoyl protection (**11**) by Pd^{2+}-catalyzed oxidation with *t*-butylhydroperoxide. Oxidation to the carboxylic acid with Jones' reagent accomplished the synthesis

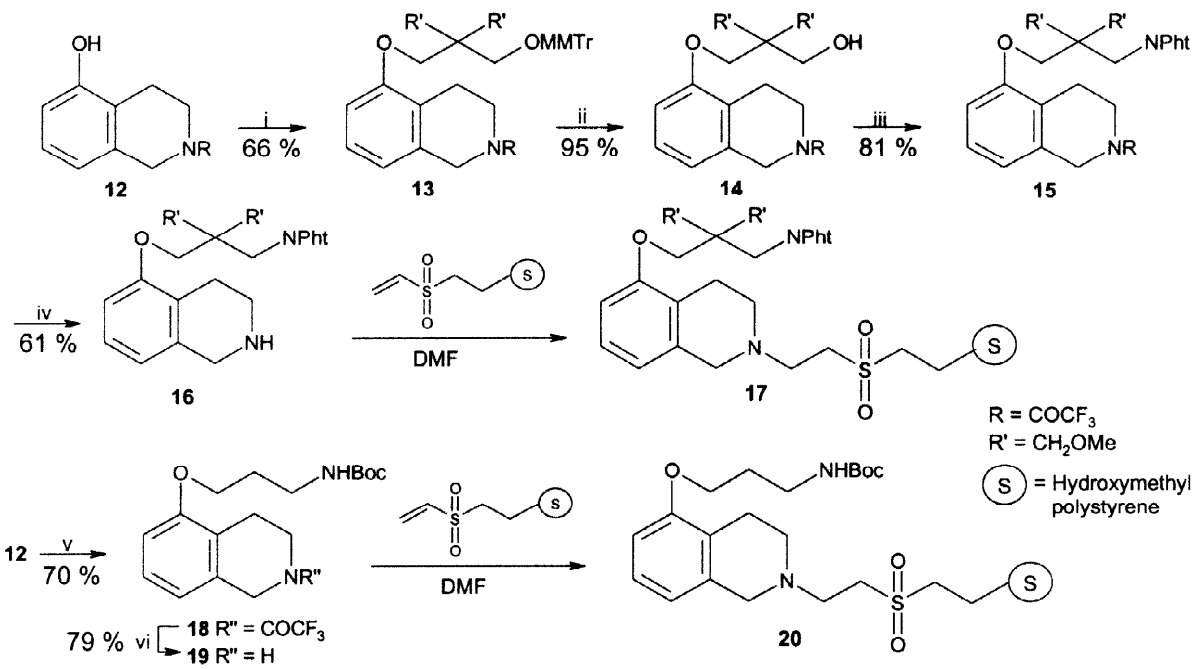


Scheme 1. Synthesis of buildings blocks. MMTr = 4-monomethoxytrityl, Pht = phthaloyl, i) NaH, RX (X=Br or I), THF, 91 % for **3b**, 72 % for **3c**, ii) H^+/H_2O, 88 % for **4b**, 51 % for **4c**, iii) MMTrCl, Py, 51 % for **5b**, 67 % for **5c**, iv) phthalimide, PPh_3, DEAD, THF, 59 % for **6b**, 88 % for **6c**, v) dichloroacetic acid, dichloromethane, 69 % for **7b**, 82 % for **7c**, vi) CrO_3, H_2SO_4, acetone, 60 % for **1b**, 60 % for **1c**, vii) BzCl, pyridine, viii) Bu'OOH, $Pd(OAc)_2$, benzene.

Synthesis of the solid supports (**17,20**). The solid supports for synthesis of the β-peptide conjugates of 1,2,3,4-tetrahydroisoquinoline were prepared as outlined in *Scheme 2*. To provide the tetrahydroisoquinoline core with a handle that later allows peptide chain assembly on a solid support, *N*-2-trifluoroacetylated 5-hydroxy-1,2,3,4-tetrahydroisoquinoline (**12**) was first subjected to Mitsunobu reaction with **5b** in solution (**13**). Detritylation (**14**), displacement of the released hydroxy function with a phthalimido group (**15**), and removal of the trifluoroacetyl protection then gave the appropriately derivatized core structure (**16**), which was attached by Michael addition to the vinyl sulfone support prepared as described previously (**17**).[4] The loading obtained was approximately 250 μmol / g, as determined by RP HPLC. The Mitsunobu reaction of **12** with **5b** was slow, and elevated temperature had to be used. Somewhat unexpectedly, the attempted reaction of **12**, or its *N*-2-*t*-Boc counterpart, with **7b** (the phthalimido analog of **5b**) was even slower, and hence this more straightforward synthesis of **15** had to be rejected. The trifluoroacetyl group was removed from **15** with methanolic sodium methoxide. Potassium carbonate turned out to be too weakly basic for the purpose, and aqueous sodium hydroxide yielded significant amount of side products, probably due to partial hydrolysis of the phthalimido

group.

The synthesis of support **20**, bearing a *t*-Boc protected 3-aminopropoxy tether at *C*5 of the tetrahydroisoquinoline core, was more straightforward. Compound **12** was coupled with *t*-Boc protected 3-aminopropanol in solution (**18**), the trifluoroacetyl protection was removed (**19**), and the product was attached to the vinyl sulfone support to give a loading of ca. 275 μmol / g.



Scheme 2. Synthesis of solid supports. i) **5b**, PPh_3. DEAD, THF, ii) DCA, DCM, iii) phthalimide, PPh_3, DEAD, THF, iv) NaOMe, MeOH, v) $HOCH_2$-CH_2-CH_2NHBoc, PPh_3, DEAD, THF, vi) NaOH aq, dioxane, methanol.

Deprotection of amine functions on solid supports. With support **20**, quantitative removal of the *t*-Boc group was achieved by mild TFA treatment. In contrast, removal of the phthaloyl protection from **17** had to be optimized rather carefully. This protection is rather rarely used,[7,8] although according to our experience, it is an attractive alternative for amino group protection in solid phase synthesis. On removal of phthaloyl group by hydrazinolysis, alkaline hydrolysis tends to compete,[9,10] and hence the correct choice of solvent is essential. The alkaline hydrolysis first yields a phthalamidic acid derivative, which is relatively stable under alkaline conditions.[9,10] This intermediate may, during acetylation and peptide bond formation, partly convert back to a phthalimido group. After several trials, we came to the conclusion that with polystyrene solid supports, 2 M hydrazine hydrate in 1:2 DMF:dioxane solution (v/v) afforded complete deprotection in 3 h at 55 °C without marked appearance of side products. If either DMF or dioxane were used as solvent, a significant amount of hydrolysis by-products was obtained. On using TentaGel type supports, a higher concentration of hydrazine hydrate (4 M) and higher reaction temperature (3 h at 80 °C) were needed to avoid interference of hydrolysis.

Peptide coupling on solid support. To elucidate the coupling efficiency of our α,α-disubstituted β-amino acid building blocks on solid support, four successive couplings of building block **1b** were carried out using **17** as the solid support. Standard HATU coupling protocol was applied.[11] As seen from Fig 2., the crude reaction product obtained after four couplings is free from any considerable amounts of side products. For our library synthesis, shorter β-peptide sequences were sufficient. Usually only two couplings were carried out on the support.

Introduction of hydroxy functions in the peptide chain. To introduce unprotected hydroxy functions in the peptide chain, the compatibility of three alternative building blocks, *viz.* **1a**, **1d**, and **1f**, with the coupling and deprotection steps was studied. During hydrazinolysis of the phthaloyl protection, the benzoyl protections of the hydroxy functions (cf. **1d**) were also lost. We also failed to remove the benzoyl protection selectively in the presence of the phthalimido moiety. Because all three building blocks employed were coupled cleanly, the use of **1d** did not offer any significant advantage over **1a** and **1f**. In fact, the only advantage is the more convenient building block synthesis when compared to the latter two. The benzylidene protection survived the hydrazinolysis, as expected, but it could not be easily removed on a solid support. The removal was conveniently achieved by mild aqueous acid treatment only after release from the support.

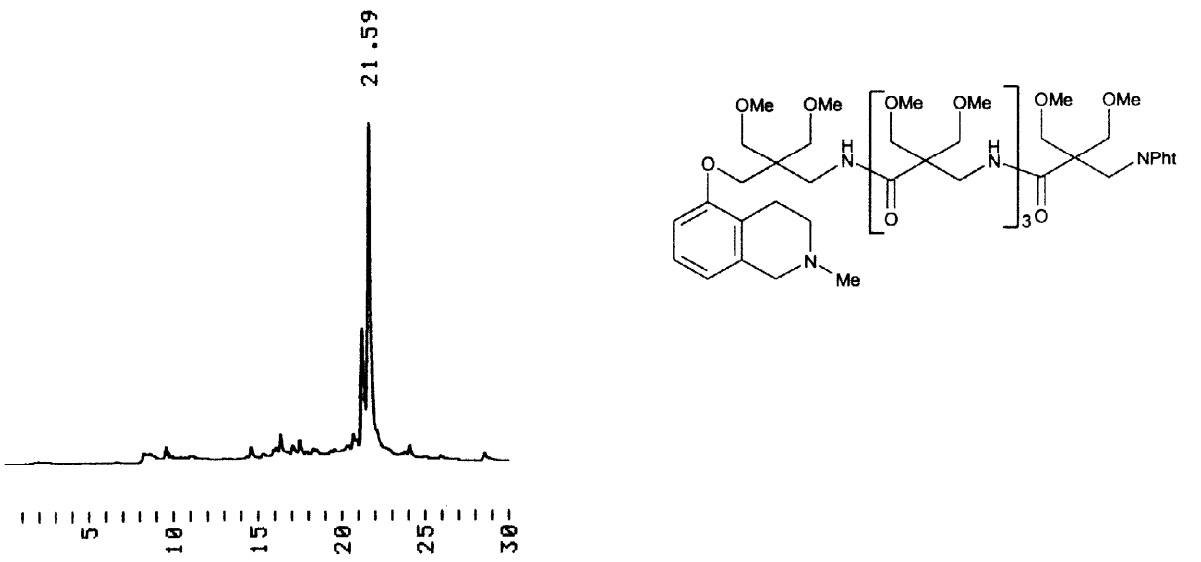
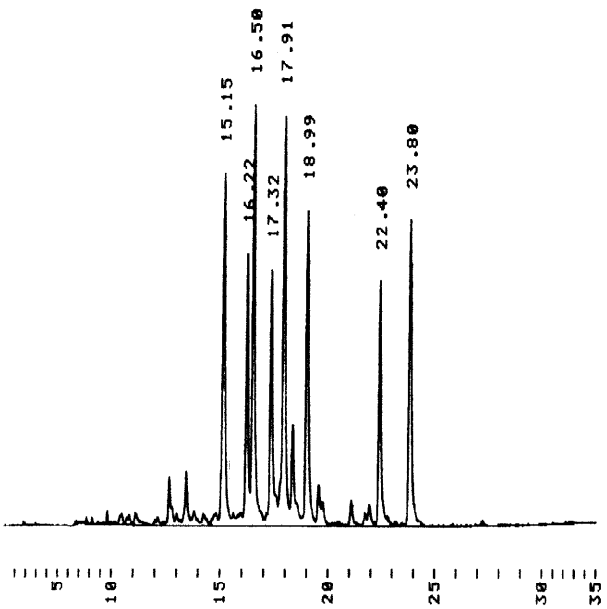


Figure 2. HPLC chromatogram of crude synthesis product after four coupling reactions. Hypersil Hypurity C18, gradient from aqueous 0.1 % TFA to acetonitrile in 30 min, flow 1.0 mL/ min, detection at 276 nm.

Model library synthesis. A library of 96 tri-β-peptide conjugates (see Fig. 1) was prepared as mixtures of 8 compounds. Diversity of the library was generated by: (i) using different combinations of building blocks **1a-c** and **1e**, (ii) carrying out the synthesis on supports **17** and **20**, and (iii) using three different alkylating agents (MeI, BnBr, AllylBr) to quaternize the support bound tetrahydroisoquinoline before cleavage. The identity of all the compounds obtained was verified by RP HPLC using both UV and mass spectroscopic detection. The

HPLC analysis also showed that all 96 compounds were produced in high purity. In addition, two of the



rt/min	M⁺+1	R'	R''
15.15	667	H	H
16.22	679	H	$-CH_2-$
16.50	755	CH_2OMe	H
17.32	695	H	Me
17.91	767	CH_2OMe	$-CH_2-$
18.99	783	CH_2OMe	Me
22.40	847	H	Bn
23.80	935	CH_2OMe	Bn

Figure 3. HPLC chromatogram of a library of eight compounds **2** (R = All, R''' = Me). Hypersil Hypurity C18, gradient from aqueous 0.1 % TFA to acetonitrile in 30 min, flow 1.0 mL/ min, detection at 276 nm.

compounds were prepared on larger scale and analyzed by ^1H NMR spectroscopy. Satisfactory spectra were obtained.

Concluding remarks. The β-amino acids employed should be considered as illustrative examples of symmetrical α,α-dialkoxymethyl substituted β-alanines. The protocol developed, however, allows construction of more extensive libraries with various substitution patterns. The free hydroxy functions can also be substituted after the peptide chain synthesis on the solid support. The model library synthesis verifies that coupling and deprotection proceed without problems with all combinations of phthalimido protected amino acids. The methodology can be used to prepare either peptide conjugates, as in the present study, or libraries of non-conjugated β-peptides.

EXPERIMENTAL

General methods. The NMR spectra were recorded on JEOL JNM-GX 400, JEOL JNM-A 500 or Bruker 200 NMR spectrometers. The chemical shifts are given in ppm from internal TMS. The mass spectra of small molecular compounds were recorded on a 7070E VG mass spectrometer. RP HPLC analysis were performed using Hypersil 150x4.6 mm, 5μm HyPurity™ Elite C18 column, gradient from aqueous 0.1 % TFA to

acetonitrile in 30 min, flow 1.0 mL/min, detection at 276 nm. LC/ESI-MS analysis were performed on Perkin-Elmer Sciex API 365 LC/MS/MS triple quadrupole mass spectrometer.

5,5-Bis(methoxymethyl)-2-phenyl-1,3-dioxane (**3b**). Sodium hydride (0.47 g, 19.6 mmol) was added portionwise to the solution of 5,5-bis(hydroxymethyl)-2-phenyl-1,3-dioxane[6] (2.0 g, 8.9 mmol) in THF (20 mL). When NaH was entirely consumed, methyl iodide (5.1 g, 35.7 mmol) in THF (10 mL) was added from a dropping funnel, and the reaction mixture was agitated overnight at ambient temperature. The reaction mixture was diluted with dichloromethane, and washed with brine. The organic solution was dried over Na_2SO_4, and evaporated to dryness. The yield after silica gel purification (1:99, MeOH:CH_2Cl_2) was 2.0 g (91 %) as a white solid. ^{1}H NMR($CDCl_3$, 400 MHz): 7.5-7.3 (5H, m), 5.40 (1H, s), 4.10 (2H, d, J = 11.7 Hz), 3.85 (2H, d, J = 11.7 Hz), 3.69 (2H, s), 3.38 (3H, s), 3.28 (3H, s), 3.18 (2H, s); MS(EI$^+$): 252 (30, M^+), 251 (43, M^+-1), 237 (10, $(M - CH_3)^+$).

5,5-Bis(benzyloxymethyl)-2-phenyl-1,3-dioxane (**3c**). Sodium hydride (2.67 g, 110 mmol) was added portionwise to the solution of *O*-monobenzylidenepentaerythritol (11.2 g, 50 mmol), and the mixture was stirred until no liberation of hydrogen was observed. Benzyl bromide (25.9 g , 150 mmol) was added, and the reaction mixture was stirred for 48 h. The solvent was removed by evaporation, and the residue was dissolved in dichloromethane, washed with water, dried over Na_2SO_4, and evaporated. Crystallization from ethyl acetate / petroleum ether yielded 14.55 g (72 %) of **3c** as white crystals. ^{1}H NMR($CDCl_3$, 400 MHz): 7.5-7.2 (15 H, m), 5.45 (1H, s), 4.57 (2H, s), 4.46 (2H, s), 4.16 (2H, d, J = 11.7 Hz), 3.90 (2H, d, J = 11.8 Hz), 3.85 (2H, s), 3.34 (2H, s); ^{13}C NMR($CDCl_3$, 125 MHz): 138.7, 138.4, 138.3, 129.0, 128.4, 128.31, 128.29, 127.6, 127.5, 127.46, 127.44, 126.2, 101.8, 73.5, 73.4, 70.3, 70.2, 67.0, 39.0; MS(EI$^+$): 404 (2, M^+), 313 (10), 297 (12), 207 (22), 105 (15), 91 (100).

2,2-Bis(methoxymethyl)-1,3-propanediol (**4b**). 6.0 g (23.8 mmol) of **3b** was stirred for 8h in solution of 1 M aqueous hydrochloric acid and 1,4-dioxane, and evaporated. Benzaldehyde was removed by repeated evaporations with water. Yield 3.4 g (88 %) as a colourless oil. ^{1}H NMR($CDCl_3$, 400 MHz): 3.68 (4H, s), 3.45 (4H, s), 3.36 (6H, s), 2.95 (2H, bs); ^{13}C NMR($CDCl_3$, 100 MHz): 74.6, 64.8, 59.6, 44.8; IR(film): ν = 3410, 2928, 1460, 1107, 1042 cm^{-1}.

2,2-Bis(benzyloxymethyl)-1,3-propanediol (**4c**). **4c** was prepared from **3c** as previously described for **4b**. Recrystallization from hexane yielded 51 % of the product as white needles. ^{1}H NMR($CDCl_3$, 400 MHz): 7.4-7.2 (10 H, m), 4.49 (2H, s), 3.68 (2H, s), 3.56 (2H, s), 2.66 (2H, bs); ^{13}C NMR($CDCl_3$, 125Hz): 137.9, 128.5, 127.8, 127.6, 73.7, 72.0, 65.0, 45.0.

2,2-Bis(methoxymethyl)-3-(4-monomethoxytrityloxy)propanol (**5b**). Compound **4b** (4.0 g, 24.4 mmol) was coevaporated twice with dry pyridine, and dissolved in dry pyridine. To this solution, 4-monomethoxytrityl chloride (7.5 g, 24.3 mmol) was added, and the reaction mixture was stirred overnight. Dichloromethane was added, the solution was washed with aq. $NaHCO_3$ and brine, dried over Na_2SO_4, and evaporated. Purification by silica gel chromatography (MeOH gradient in CH_2Cl_2) yielded 5.4 g (51 %) of **5b** as a white solid foam. ^{1}H NMR($CDCl_3$, 400 MHz): 7.5-6.8 (14H, m), 3.82 (3H, s), 3.68 (2H, d, J = 6.1 Hz), 3.47 (4H, q_{AB}, J = 9.1 Hz), 3.29 (6H, s), 3.11 (2H, s), 2.75 (1H, t, J = 6.1 Hz); ^{13}C NMR($CDCl_3$, 100 MHz): 158.5, 149.7, 144.5, 143.7, 135.5, 130,4, 128.4, 127.7, 126.8, 113.0, 86.2, 73.7, 66.2, 62.6, 59.3, 55.2, 45.0.

2,2-Bis(benzyloxymethyl)-3-(4-monomethoxytrityloxy)propanol (**5c**). **5c** was prepared from **4c** as previously described for **5b**. Yield 67 % as a white solid foam. ^{1}H NMR($CDCl_3$, 400 MHz): 7.5-6.6 (24H, m), 4.46 (4H, s),

3.73 (3H, s), 3.60 (4H, q_{AB}, J = 9.0 Hz), 3.56 (2H, s), 3.20 (2H, s), 2.72 (1H, bs); MS(EI$^+$): 588 (2, M^+), 511 (1), 315 (6), 273 (100), 91 (75).

5-Hydroxymethyl-5-(4-monomethoxytrityloxymethyl)-1,3-dioxane (**5d**). **5d** was prepared from 5,5-bis(hydroxymethyl)-1,3-dioxane as previously described for **5b**. Yield 49.3 % as a white solid foam. ^{1}H NMR($CDCl_3$, 400 MHz): 7.5-7.2 (12H, m), 6.8 (2H, m), 4.82 (1H, d, J = 6.1 Hz), 4.76 (1H, d, J = 6.1 Hz), 3.85-3.65 (9H, m), 3.20 (2H, s), 2.01 (1H, bs); MS(EI$^+$): 420 (M^+, 7), 343 (5), 273 (100), 213 (11), 165 (8).

2,2-Bis(methoxymethyl)-1-(4-monomethoxytrityloxymethyl)-3-phthalimidopropanol (**6b**). Diethyl azodicarboxylate (4.2 g , 24 mmol) was added dropwise to the solution of **5b** (8.7 g, 20 mmol), phthalimide (3.5 g, 24 mmol) and triphenylphosphine (6.3 g, 24 mmol) in THF. The reaction mixture was stirred overnight and evaporated. Silica gel chromatography (CH_2Cl_2: MeOH, 99:1) yielded 11.3 g (59 %) of **6b** as a white solid foam. ^{1}H NMR($CDCl_3$, 400 MHz): 7.86-7.65 (4H, m), 7.44-7.17 (12H, m), 6.84-6.78 (2H, m), 3.84 (2H, s), 3.78 (3H, s), 3.43 (4H, s), 3.19 (6H, s), 3.12 (2H, s); ^{13}C NMR($CDCl_3$, 100 MHz): 168.8, 158.5, 144.5, 135.6, 133.6, 132.4, 130.5, 128.6, 127.7, 126.7, 123.0, 113.0, 86.2, 73.2, 63.6, 59.0, 55.2, 45.8, 40.3.

2,2-Bis(benzyloxymethyl)-1-(4-monomethoxytrityloxymethyl)-3-phthalimidopropanol (**6c**). **6c** was prepared from **5c** as previously described for **6b**. Yield 88 % as a white solid foam. ^{1}H NMR($CDCl_3$, 400 MHz): 7.6 (2H, m), 7.5 (2H, m), 7.45-7.0 (22H, m), 6.65 (2H, m), 4.40 (4H, s), 3.89 (2H, s), 3.73 (3H, s), 3.67 (2H, s), 3.23 (2H, s); MS(EI$^+$): 718 (1, M^+), 641 (1), 273 (100), 91 (72).

5-(4-Monomethoxytrityloxymethyl)-5-phthalimidomethyl-1,3-dioxane (**6d**). **6d** was prepared from **5d** as previously described for **6b**. Yield quantitative. White solid foam. ^{1}H NMR($CDCl_3$, 400 MHz): 7.9-7.6 (4H, m), 7.5-7.2 (12H, m), 6.8 (2H, m), 4.77 (1H, d, J = 6.1 Hz), 4.63 (1H, d, J = 6.1 Hz), 3. 98 (2H, d, J = 11.7 Hz), 3.75 (7H, m), 3.25 (2H, s); ^{13}C NMR($CDCl_3$, 100 MHz): 168.6, 158.4, 144.1, 136.2, 133.9, 132.0, 130.4, 128.4, 127.7, 126.8, 123.3, 113.0, 93.8, 86.4, 70.8, 63.8, 55.1, 40.7, 38.9; MS(EI$^+$): 550 (M^+, 3), 473 (2), 273 (100), 260 (8), 229 (6), 160 (27).

2,2-Bis(methoxymethyl)-3-phthalimidopropanol (**7b**). Compound **6b** (6.6g, 9.2 mmol) was dissolved in 10 % (v/v) dichloroacetic acid solution in dichloromethane. After 1 h, the reaction mixture was diluted with dichloromethane and washed with aq. $NaHCO_3$ and dried over Na_2SO_4. Silica gel chromatography (CH_2Cl_2: MeOH, 90:10) yielded 2.4 g (69 %) of **7b** as a colourless oil. ^{1}H NMR($CDCl_3$, 400 MHz): 7.9-7.7 (4H, m), 3.81 (2H, s), 3.53 (2H, d, J = 7.1 Hz), 3.4 (4H, q_{AB}, J = 9.3 Hz), 3.28 (6H, s), 1.84 (1H, bs); ^{13}C NMR($CDCl_3$, 100 MHz): 169.3, 134.1, 132.0, 123.3, 74.7, 73.7, 64.9, 63.1, 59.3.

2,2-Bis(benzyloxy)-3-phthalimidopropanol (**7c**). **7c** was prepared from **6c** as previously described for **7b**. Yield 82 % as a white solid. ^{1}H NMR($CDCl_3$, 400 MHz): 7.75 (2H, m), 7.65 (2H, m), 7.3-7.1 (10H, m), 4.43 (4H, s), 3.87 (2H, s), 3.6 (7H, m); ^{13}C NMR($CDCl_3$, 100 MHz): 169.4, 138.2, 133.9, 131.8, 128.2, 127.3, 127.3, 123.3, 73.4, 71.6, 63.0, 46.1, 39.0; MS(EI$^+$): 445 (2, M^+), 354 (18), 248 (42), 160 (91).

5-Hydroxymethyl-5-phthalimidomethyl-1,3-dioxane (**7d**). **7d** was prepared from **6d** as previously described for **7b**. Yield 52 % as a white solid. ^{1}H NMR($CDCl_3$, 400 MHz): 7.9-7.4 (4H, m), 4.86 (2H, s), 3.79 (2H, s), 3.75 (4H, s), 3.52 (2H, bs), 1.76 (1H, bs); ^{13}C NMR($CDCl_3$, 100 MHz): 169.5, 134.5, 131.6, 123.7, 94.2, 70.2, 61.4, 40.6, 38.1; MS(EI$^+$): 277 (M^+, 3), 247 (10), 229 (11), 217 (23), 160 (100), 148 (25).

5-Hydroxymethyl-2-phenyl-5-phthalimidomethyl-1,3-dioxane (**8**). **8** was prepared from **3a** as previously

described for **6b**. Yield 55 % as a white solid foam. ^{1}H NMR(DMSO-d_6, 200 MHz): 7.95-7.80 (4H, m), 7.36 (5H, m), 5.42 (1H, s), 4.86 (1H, t, J = 5.3 Hz), 4.0-3.7 (6H, m), 3.47 (2H, s). MS(EI$^+$): 353 (M^+, 57), 304 (9), 276 (10), 231 (14), 217 (23), 200 (27), 188 (23), 160 (100), 148 (25).

2,2-Bis(methoxymethyl)-3-phthalimidopropanoic acid (**1b**). A solution of CrO_3 (0.81 g, 8.1 mmol) and H_2SO_4 (0.8 mL) in 3 mL of water was added dropwise to the solution of **7b** (2.36 g, 8.05 mmol) in acetone. After 1h mixing, 25 mL of methanol and 25 mL of water were added, and the mixture was evaporated to half of its initial volume. The mixture was extracted three times with chloroform, the combined extracts were dried over Na_2SO_4, and evaporated to dryness. Crystallization from chloroform yielded 1.49 g (60 %) of **1b** as a white solid. ^{1}H NMR($CDCl_3$, 400 MHz): 9.75 (1H, s), 8.9-8.6 (4H, m), 4.09 (2H, s), 3.67 (4H, s), 3.34 (6H, s); ^{13}C NMR($CDCl_3$, 100 MHz): 175.1, 168.3, 134.0, 131.9, 123.4, 71.4, 59.3, 52.3, 39.0, IR(KBr): ν = 3200-2800 (br), 1725, 1614, 1431, 1392, 1356, 1196, 1109, 717 cm^{-1}, HRMS(EI): M^+ found 307.1060. $C_{15}H_{17}NO_6$ requires 307.1056.

2,2-Bis(benzyloxymethyl)-3-phthalimidopropanoic acid (**1c**). **1c** was prepared from **7c** as previously described for **1b**. Yield 60 % as a white solid. ^{1}H NMR($CDCl_3$, 400 MHz): 7.75 (2H, m), 7.65 (2H, m), 7.23 (10H, m), 4.45 (4H, q_{AB}, J = 11.9 Hz), 4.09 (2H, s), 3.84 (4H, q_{AB}, J = 9.2 Hz); ^{13}C NMR($CDCl_3$, 100 MHz): 175.5, 168.4, 137.8, 133.9, 131.9, 128.2, 127.4, 123.3, 73.5, 69.3, 52.5, 39.3. IR(KBr): ν = 3200-2800 (br), 1713, 1615, 1394, 1207, 1101, 725 cm^{-1}, HRMS(EI): M^+ found 459.1680. $C_{27}H_{25}NO_6$ requires 459.1682.

5-Phthalimidomethyl-1,3-dioxane-5-carboxylic acid (**1e**). **1e** was prepared from **7d** as previously described for **1b**. Yield 74 % as a white solid. ^{1}H NMR(DMSO-d_6, 400 MHz): 12.9 (1H, bs), 7.9-7.8 (4H, m), 4.71 (2H, q_{AB}, J = 5.9 Hz), 4.10 (2H, d, J = 11.2 Hz), 3.75 (2H, d, J = 11.4 Hz), 3.71 (2H, s); ^{13}C NMR(DMSO-d_6, 100 MHz): 172.5, 167.9, 134.6, 131.4, 123.2, 92.8, 69.5, 46.3; MS(EI$^+$): 291 (M^+,15), 214 (6), 185 (10), 160 (100); IR(KBr): ν = 3200-2800 (br), 1724, 1633, 1397, 1361, 1163, 1062, 1035, 927, 723 cm^{-1}, HRMS(EI): M^+ found 291.0746. $C_{14}H_{13}NO_6$ requires 291.0743.

2-Phenyl-5-phthalimidomethyl-1,3-dioxane-5-carboxylic acid (**1f**). **1f** was prepared from **8** as previously described for **1b**. Yield 62 % as a white solid. ^{1}H NMR(DMSO-d_6, 200 MHz): 7.9 (4H, bs), 7.3 (5H, bs), 5.55 (1H, s), 4.43 (2H, d, J = 11.0 Hz), 3.95 (2H, d, J = 11.5 Hz), 3.61 (2H, s); MS(EI$^+$): 367 (M^+, 37), 214 (24), 160 (54), 105 (100), IR(KBr): ν = 3200-2800 (br), 1723, 1632, 1396, 1226, 1122, 1074, 993, 906, 727 cm^{-1}, HRMS(EI): M^+ found 367.1062. $C_{20}H_{17}NO_6$ requires 367.1056.

2,2-Bis(hydroxymethyl)-3-phthalimidopropanoic acid (**1a**). **1a** was prepared from **1f** as previously described for **4b.** Yield 10 % as a white solid. ^{1}H NMR($CDCl_3$, 200 MHz): 8.7 (3H, bs), 7.8-7.6 (4H, m), 3.93 (2H, s), 3.68 (2H, d, J = 12.0 Hz), 3.45 (2H, d, J = 12.0 Hz), ^{13}C NMR($CDCl_3$, 200 MHz): 174.0, 168.6, 133.8, 131.1, 122.9, 61.2, 53.5, 36.8, MS(EI$^+$): 279 (M^+, 9), 249 (17), 231 (10), 185 (15), 160 (100), 148 (12), IR(KBr): ν = 3400 (br), 3200-2800 (br), 1711, 1631, 1396, 1226, 1032, 725 cm^{-1}, HRMS(EI): M^+ found 279.0744. $C_{13}H_{13}NO_6$ requires 279.0743.

5-Benzoyloxymethyl-5-hydroxymethyl-2-phenyl-1,3-dioxane (**9**). Benzoylchloride (2.8 g, 20 mmol) was added from a dropping funnel to the solution of **3a** (5g, 22.3 mmol) in pyridine at 0 °C during 2 h. After 2 h stirring in room temperature, the reaction mixture was evaporated to dryness, and the residue dissolved in dichloromethane. The solution was washed with aq. $NaHCO_3$, dried over Na_2SO_4, and evaporated. Purification by silica gel chromatography (95:5 dichloromethane: methanol) yielded 3.18 g (48 %) of **9** as a mixture of two isomers as a white solid foam. ^{1}H NMR($CDCl_3$, 200 MHz): 8.1-8.0 (2H, m), 7.7-7.3 (8H, m), 5.58 (1H, s), 4.87 (2H, s), 4.27 (2H, d, J = 12.1 Hz), 3.85 (2H, d, J = 12.1 Hz), 3.35 (2H, d, J = 6.8 Hz), 2.78 (1H, t, J = 6.9 Hz);

MS(EI$^+$): 327 (M$^+$-1, 11), 206 (14), 123 (15), 105 (100).

5-Benzoyloxymethyl-2-phenyl-5-phthalimidomethyl-1,3-dioxane (**10**). **10** was prepared from **9** as described for **6b**. Yield 77 % as a white solid foam. ^{1}H NMR($CDCl_3$, 200 MHz): 8.0-7.2 (14H, m), 5.46 (1H, s), 4.72 (2H, s), 4.30 (2H, d, J = 12.0 Hz), 4.00 (2H, d, J = 11.9 Hz), 3.71 (2H, s); MS(EI$^+$): 457 (M$^+$, 18), 216 (10), 201 (9), 160 (21), 105 (100).

1-O-Benzoyl-2-benzoyloxymethyl-2-phthalimidomethyl-1,3-propanediol (**11**). A solution of *tert*-butyl hydroperoxide in decane (3.1 mL, 6 M, 28 mmol) and a catalytic amount of palladium acetate was added to a solution of **10** (2.56 g, 5.6 mmol) in benzene. The reaction mixture was stirred for 2 weeks at room temperature, after which it was diluted with dichloromethane, and washed with aq Na_2SO_3 until no peroxide was present. The organic solution was dried over Na_2SO_4, and evaporated. Purification by silica gel chromatography (97:3 CH_2Cl_2: MeOH) yielded 2.5 g (94 %) of the product as a white foam. ^{1}H NMR ($CDCl_3$, 200 MHz): 8.0-7.3 (14H, m), 4.49 (4H, s), 4.03 (2H, s), 3.67 (2H, s); ^{13}C NMR($CDCl_3$, 50 MHz): 169.3, 166.2, 134.4, 133.1, 131.7, 129.7, 128.6, 128.4, 123.7, 64.3, 61.2, 45.4, 38.2.

2,2-Bis(benzoyloxymethyl)-3-phthalimidopropanoic acid (**1d**). **1d** was prepared from **11** as previously described for **1b**. Yied 83% as a white solid. ^{1}H NMR($CDCl_3$, 200 MHz): 8.0-7.2 (14H, m), 4.70 (4H, q_{AB}, J = 11.4 Hz), 4.27 (2H, s); ^{13}C NMR($CDCl_3$, 50 MHz): 175.6, 168.4, 165.9, 134.2, 133.0, 131.6, 129.6, 128.3, 123.6, 63.4, 51.1, 39.2, MS(EI$^+$): 487 (M$^+$, 1), 216 (7), 160 (33), 105 (100), IR(KBr): ν = 3200-2800 (br), 1723, 1631, 1392, 1277, 1117, 713, HRMS(EI): M$^+$ found 487.1264. $C_{27}H_{21}NO_8$ requires 487.1267.

5-[2,2-Bis(methoxymethyl)-3-(4-monomethoxytrityloxy)propoxy]-2-trifluoroacetyl-1,2,3,4-tetrahydro-isoquinoline (**13**). Diethyl azodicarboxylate (2.62 mL, 16.6 mmol) was added dropwise to a solution of 2-trifluoroacetyl-1,2,3,4-tetrahydro-5-isoquinolinol (2.77 g, 11.4 mmol), **5b** (5.6 g, 12.8 mmol) and triphenylphosphine (4.35 g, 16.6 mmol) in THF. The reaction mixture was stirred overnight, and evaporated. Purification by silica gel chromatography (dichloromethane) yielded 4.96 g (66 %) of **13** as a colourless oil. ^{1}H NMR($CDCl_3$, 400 MHz): 7.4-6.6 (17H, m), 4.77 (2H, ds), 3.93 (2H, s), 3.85-3.70 (5H, m), 3.52 (4H, s), 3.31 (6H, s), 3.24 (2H, s), 2.50 (2H, m).

5-[2,2-Bis(methoxymethyl)-3-hydroxypropoxy]-2-trifluoroacetyl-1,2,3,4-tetrahydroisoquinoline (**14**). 4.9 g of **13** was dissolved in dichloromethane and 10 % (v/v) dichloroacetic acid and methanol were added. After the reaction was completed (TLC: CH_2Cl_2: MeOH, 97:3), dichloromethane was added, and the solution was washed with aqueous $NaHCO_3$ solution. Purification by silica gel chromatography (CH_2Cl_2: MeOH, 97:3) yielded 2.8 g (95 %) of **14** as a colourless oil. ^{1}H NMR($CDCl_3$, 400 MHz): 7.18 (1H, t, J = 8.1 Hz), 6.7 (2H, m), 4.75 (2H, ds), 4.03 (2H, s), 3.8 (4H, m), 3.54 (4H, s), 3.35 (6H, s), 2.8 (2H, m), 2.69 (1H, bs); MS(EI$^+$): 391 (35, M$^+$-1), 245 (15), 147 (23).

5-[2,2-Bis(methoxymethyl)-3-phthalimidopropoxy]-2-trifluoroacetyl-1,2,3,4-tetrahydroisoquinoline (**15**). Diethyl azodicarboxylate (1.43 mL, 9.0 mmol) was added dropwise to the solution of **14** (2.8 g, 7.1 mmol), phthalimide (1.33 g, 9.0 mmol) and triphenylphosphine (2.36 g, 9.0 mmol) in tetrahydrofuran. The reaction mixture was stirred overnight, and evaporated. Purification by silica gel chromatography (CH_2Cl_2: MeOH, 99:1) yielded 3.69 g (81 %) of **15** as a white solid foam. ^{1}H NMR($CDCl_3$, 400 MHz): 7.83 (2H, m), 7.79 (2H, m), 7.16 (1H, t, J = 7.8 Hz), 6.78-6.66 (2H, m), 4.68 (2H, ds), 4.03 (2H, s), 3.93 (2H, s), 3.75 (2H, m), 3.51 (4H, s), 3.27 (6H, s), 2.71-2.64 (2H, m); MS(EI$^+$): 520 (42, M$^+$), 309 (11), 296 (12), 276 (74), 244 (39), 163 (34), 160 (78).

5-[2,2-Bis(methoxymethyl)-3-phthalimidopropoxy]-1,2,3,4-tetrahydroisoquinoline (**16**). 1.0 g (1.9 mmol) of **15** was dissolved in an ethanolic solution of sodium ethoxide (16 mL, 0.5 mol dm^{-3}). After 6 h, the reaction mixture was evaporated and purified by silica gel chromatography (CH_2Cl_2: MeOH, 92:8). Yield 0.5 g, 61 % as a white solid foam. ^{1}H NMR($CDCl_3$, 400MHz): 7.82 (2H, m), 7.70 (2H, m), 7.09 (1H, t, J = 7.8 Hz), 6.71 (1H, d, J = 8.1 Hz), 6.62 (1H, d, J = 7.6 Hz), 4.03 (2H, bs), 3.99 (2H, s), 3.98 (2H, s), 3.51 (4H, s), 3.25 (6H, s), 3.12 (2H, m), 2.67 (2H, m); MS(EI$^+$): 424 (100, M^+), 276 (48), 160 (59), 148 (25).

3-(t-Butyloxycarbonylamino)propanol. 6 mL (78 mmol) of 3-aminopropanol was dissolved in acetonitrile, and 43 mL of 2 M aq. NaOH, and 18.8 g (86 mmol) of di-*t*-butyl dicarbonate were added. The reaction mixture was stirred overnight, diluted with dichloromethane, and washed with aq. $NaHCO_3$, and brine. Yield after drying with Na_2SO_4 and evaporation was 12.8 g (94 %) as a white solid. ^{1}H NMR($CDCl_3$, 400MHz): 5.04 (1H, bs), 3.65 (2H, bs), 3.51 (1H, bs), 3.27 (2H, m), 1.67 (2H, m), 1.44 (9H, s); ^{13}C NMR($CDCl_3$, 100 MHz): 157.1, 79.5, 59.3, 37.0, 32.8, 28.4.

5-[(3-t-Butyloxycarbonylamino)propoxy]-2-trifluoroacetyl-1,2,3,4-tetrahydroisoquinoline (**18**). Diethyl azodicarboxylate (2.2 mL, 12.7 mmol) was added dropwise to the solution of 2-trifluoroacetyl-1,2,3,4-tetrahydro-5-isoquinolinol (2.5 g, 10.2 mmol), 3-*t*-butyloxycarbonylpropanol (2.7 g, 15.3 mmol) and triphenylphosphine (3.34 g, 12.7 mmol) in THF. The reaction mixture was stirred for 20 h, and evaporated. Purification by silica gel chromatography (CH_2Cl_2: MeOH, 97:3) yielded 2.9 g (70 %) of **18** as a white solid. ^{1}H NMR($CDCl_3$, 400 MHz): 7.18 (1H, t, J = 8.05 Hz), 6.76 (1H, d, J = 7.87 Hz), 6.72 (1H, d, J = 8.90 Hz), 4.81 (1H, bs), 4.78 (2H, s), 4.04 (2H, t, J = 5.86 Hz), 3.86 (2H, m), 3.35 (2H, m), 2.88 (2H, m), 2.00 (2H, m), 1.44 (9H, s).

5-[(3-t-Butyloxycarbonylamino)propoxy]-1,2,3,4-tetrahydroisoquinoline (**19**). 0.3 g (0.75 mmol) of **18** was dissolved in dioxane and methanol, and 5 mL of 2 M aq. NaOH was added. When the reaction was completed, dichloromethane was added, and solution was washed with water and brine. The dichloromethane fraction was dried over Na_2SO_4 and evaporated. Purification by silica gel chromatography (CH_2Cl_2: MeOH: TEA, 89:8:3) yielded 0.18 g (79 %) of **19** as a white solid foam. ^{1}H NMR($CDCl_3$, 400 MHz): 7.11 (1H, t, J = 8.04 Hz), 6.67 (2H, t, J = 8.29 Hz), 4.04 (4H, m), 3.34 (2H, m), 3.21 (2H, t, J = 6.10 Hz), 2.74 (2H, t, J = 8.05 Hz), 2.34 (2H, bs), 2.00 (2H, m), 1.44 (9H, s); MS (EI$^+$): 306 (25, M^+), 249 (95), 233 (29), 205 (14), 148 (52), 132 (22), 120 (35).

Solid supports 17 and 20. The solid supports **17** and **20** were prepared from **16** and **19**, respectively. The secondary amine (**16** or **19**, 10 molar equivalance) was shaken with vinyl sulfone support[4] in DMF for 20 h, and washed with dichloromethane and methanol. The solid supports were dried under reduced pressure before use.

Deprotection of phthaloyl group on solid support. 3 mL of 2 M hydrazine hydrate solution in 1:2 DMF:dioxane (v/v) was added to the reaction vessel containing 5 to 50 mg of solid support. The stoppered vessel was held at 55 °C for 3h, and the mixture was shaken occasionally. The solid support was filtered, washed with dichloromethane and methanol, and dried under reduced pressure.

Deprotection of 20. 5 to 100 mg of support **20** was suspended in 3 ml of dichloromethane, and 1 mL of trifluoroacetic acid was added. The mixture was shaken at room temperature for 1 h. The solid support was filtered, washed with dichloromethane and methanol, and dried under reduced pressure.

General method for amino acid coupling. 20 mg of solid support was suspended in 0.5 mL of DMF. To this

suspension, the appropriate amino acid (5 mol eq in 0.5 mL DMF), *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophoshate (HATU) (5 mol eq in 0.5 mL DMF), and diisopropyl ethyl amine (10 mol eq in 0.1 mL DMF) were added. The mixture was shaken for 1 h, and the solid support was filtered, washed with dichloromethane and methanol, and dried under reduced pressure.

Cleavage from solid support. The solid support was shaken with 1.5 mmol of appropriate alkyl iodide or bromide (MeI, allyl bromide, or benzyl bromide) in DMF for 1 h, and then filtered, washed with dichloromethane and methanol, and dried under reduced pressure. The solid support was suspended in solution of triethyl amine and dichloromethane (1:1, v/v). After 1 h shaking, the solution was collected by filtration, and evaporated under reduced pressure.

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