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Nonpeptide Integrin Antagonists: RGD Mimetics Incorporating Substituted Azabicycloalkanes as Amino Acid Replacements

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Azabicyclo[4.3.0]alkanes appropriately substituted on both cycles have been synthesized as potential scaffold mimics of the RGD signaling motif of integrin. Two sets of functionalized azabicycloalkanes have been examined. In vitro assays established that **21** has a good affinity specifically for $\alpha_v\beta_3$.

Introduction

The integrins are a class of cell surface adhesion proteins that play important roles in cell–cell and cell–matrix interactions,[1] being involved in diverse physiological processes. In particular, the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ subgroups of integrins are expressed in various cell types, such as endothelial cells, melanoma, platelets, osteoclasts, and smooth muscle cells. Furthermore, they also play important roles in angiogenesis and in tumor cell migration by interacting with vitronectin on the extracellular matrix, mainly through the recognition of the tripeptide sequence RGD (Arg-Gly-Asp).[2]

This sequence has been used as a lead for the development of different integrin antagonists. The RGD sequence was first incorporated into various linear and cyclic peptides.[3] During our studies on integrin inhibitors, we reported active and selective small-molecule antagonists of the $\alpha_v\beta_3$ receptor in the forms of the cyclic pentapeptides c(-RGDTemplate-), where "Template" represents azabicyclo[*x*.3.0]alkane amino acids.[4]

Recently, research has been focused on the development of selective nonpeptide $\alpha_v\beta_3$ integrin antagonists,[5] because of the enhanced metabolic stability, bioavailability, and biological absorption of peptidomimetic compounds.

In this paper we report the synthesis of new substituted bicyclic lactams (Figure 1) as nonpeptide scaffolds mimicking the RGD sequence.

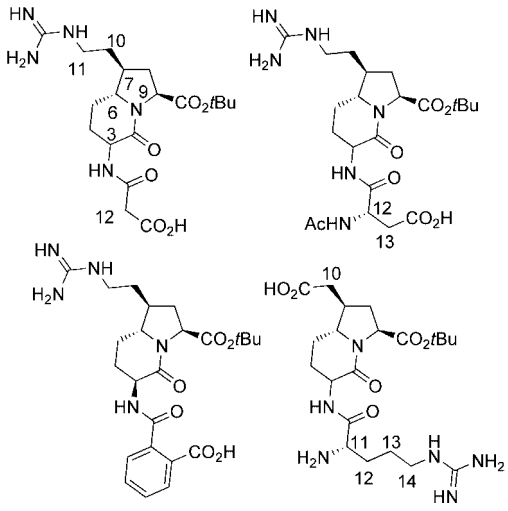

Figure 1. Library of potential functionalized bicyclic lactam mimics of the RGD sequence.

These lactams are substituted on both cyclic components, one bearing an analogue of the Arg side chain and the other bearing an analogue of the Asp side chain. The occurrence of both guanidinium and carboxylate groups in these nonpeptide molecules is an essential element for mimicking of the Arg and Asp side chains, respectively, of RGD.

Although many reports on the synthesis of bicyclic lactams can be found in the literature,[6] only a few describe the preparation of azabicyclo[*x*.*y*.0]alkanes bearing functionalized side chains.[7] In the course of our studies of peptide secondary structure mimics we have already synthesized functionalized bicyclic lactams by different strategies,[8] and in particular we have synthesized functionalized

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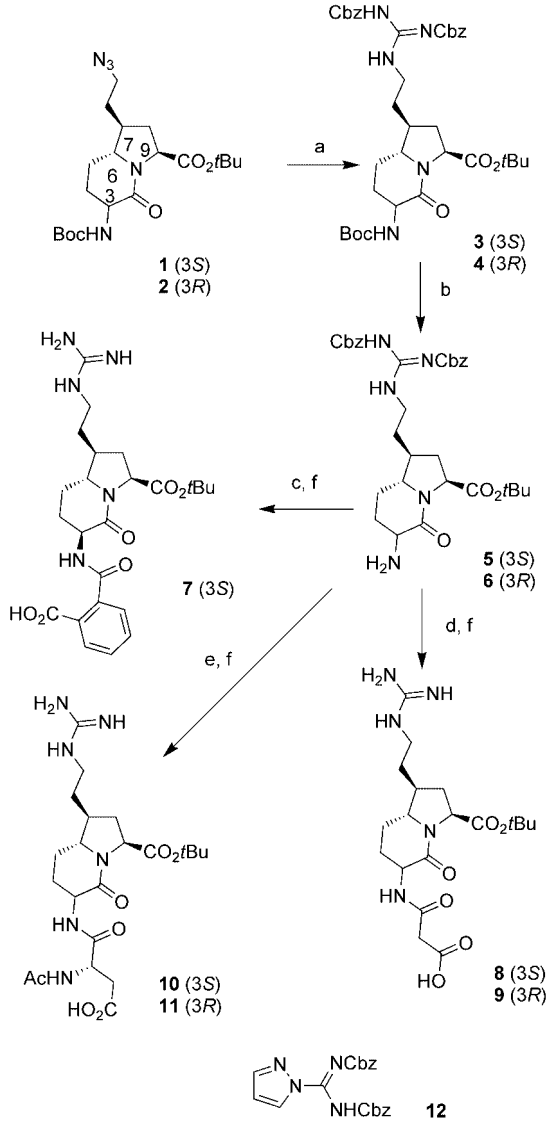
6,5-fused bicyclic lactams by a Horner–Emmons-based strategy.[9]

Here we report the use of these bicyclic lactam derivatives for the preparation of integrin inhibitors. All the non-peptide RGD mimics synthesized have been tested by an in vitro integrin-binding assay. The results showed **21** to be a good-affinity specific ligand for $\alpha_v\beta_3$ integrin.

Results and Discussion

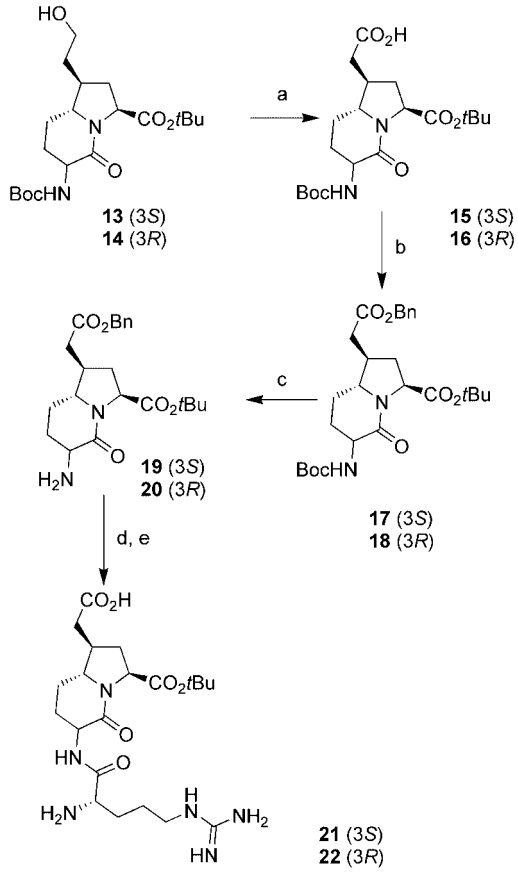
The synthetic approach is illustrated in Scheme 1. The known bicyclic lactams **1**[9] (3*S*) or **2** (3*R*) were each subjected to a one-step reduction/guanidinylation reaction sequence to give compounds **3** and **4**. The bis-Cbz carboxamidine **12** was chosen as the electrophilic guanidation reagent for this transformation.[10] The newly introduced guanidyl group mimics the arginine side chain.



Scheme 1. Reagents and conditions: a) Ph_3P, H_2O, THF, **12**, (**3**, 76%; **4**, 89%). b) $HClO_4$, *t*BuOAc. c) Monobenzyl phthalate, EDC·HCl, DMAP, HOBT, collidine, THF, 65%. d) Monobenzyl malonate, EDC·HCl, DMAP, HOBT, collidine, THF. e) Monobenzyl *N*-acetyl-aspartate, EDC·HCl, DMAP, HOBT, THF. f) H_2, Pd/C, MeOH, (**7**, 41% over two steps; **8**; 63% over three steps; **9**, 33% over three steps; **10**, 66% over three steps; **11**, 67% over three steps).

Removal of the nitrogen protecting group was achieved by treatment with $HClO_4$ in *t*BuOAc as solvent; these conditions enabled us to remove the Boc moiety, leaving the *tert*-butyl ester as the carboxylic protecting group. The resulting free amines **5** and **6** were coupled with the appropriate carboxyl derivative to give compounds **7**–**11** after benzyl and Cbz removal by hydrogenolysis on Pd/C.

With the aim of expanding the library of compounds and studying the influence of the positions of the pharmacophoric groups (guanidyl and carboxyl groups) in the recognition site of the receptor, we decided to synthesize a second series of RGD mimics, and so we changed the pharmacophoric group on the proline moiety, introducing a carboxyl instead of a guanidyl group in position 7 of the scaffold. The presence of the other pharmacophoric group, necessary for the binding with the receptor, was ensured by condensation with an arginine (Scheme 2).



Scheme 2. Reagents and conditions: a) PDC, DMF, (**15**, 76%; **16**, 74%). b) 1. $CsCO_3$, MeOH, H_2O, 2. BnBr, DMF (**17**, 92%; **18**, 94%). c) $HClO_4$, *t*BuOAc. d) Z-Arg(Z)$_2$-OH, DIC, HOBT, THF. e) H_2, Pd/C, MeOH, (**21**, 43% over three steps; **22**; 46% over three steps).

Compounds **15** and **16** were prepared from the known alcohols **13** and **14** by oxidation with PDC. The newly introduced carboxylic group mimics the aspartyl side chain.

Protection of the carboxyl groups of **15** and **16** as benzyl derivatives was achieved by treatment of their preformed cesium salts with benzyl bromide, and the nitrogen protecting groups were then removed by treatment with $HClO_4$ to give compounds **19** and **20**, which were coupled with suitably protected arginine components with the aid of DIC and HOBT as condensing agents to give, after hydrogenolysis, compounds **21** and **22**.

Biological Results

The RGD mimics **7**–**11** and **21**–**22** were examined in vitro for their abilities to compete with [^{125}I]-echistatin for binding to the purified $\alpha_v\beta_3$ and $\alpha_v\beta_5$ receptors (Table 1). It had previously been demonstrated that both purified and membrane-bound integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$, which had very high affinity for echistatin, could be inhibited efficiently by linear and cyclic RGD-containing peptides. Affinities of compounds c(RGDfV), EMD 121974, and ST1646 for the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins were also determined, for reference, in the same assays. Of the seven mimics tested, compound **21** showed the highest affinity towards $\alpha_v\beta_3$ integrin.

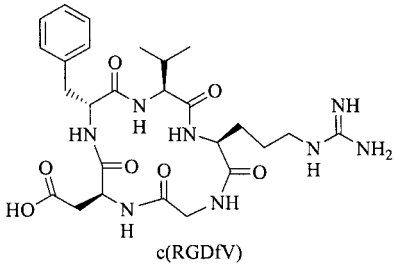
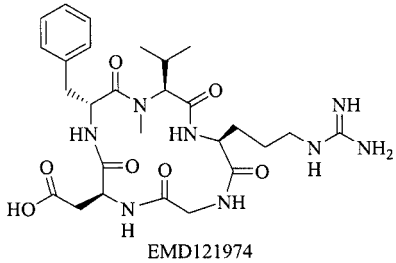
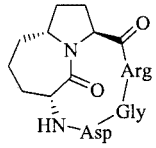
Computational Studies

To interpret the experimental results on a structural basis, molecular docking studies were performed for the compounds **7**–**11** and **21**–**22**.

The protein binding site was derived from the X-ray crystal structure of the extracellular segment of integrin $\alpha_v\beta_3$ in complexation with the cyclic pentapeptide ligand EMD 121974.[11] For each compound the global minimum energy conformer derived from an MC/EM[12] conformational search was selected as the representative starting conformation for docking studies.

The crystal structure of the peptide/integrin complex provides the actual conformation of EMD 121974 bound to the $\alpha_v\beta_3$ integrin active site and can serve as a basis for interpretation of the general mode of interaction of integrins with other RGD mimics. Examination of the three-dimensional structure of the cyclic pentapeptide ligand EMD 121974 bound to the $\alpha_v\beta_3$ integrin receptor (Protein Data Bank entry code = 1L5G[11]) reveals a conformation characterized by an inverse γ-turn with Asp at position ($i+1$) and by a distorted βII′-turn with Gly and Asp at the ($i+1$)- and ($i+2$)-positions, respectively. A distance of 8.9 Å

Table 1. Inhibition of ^{125}I-Echistatin binding to $\alpha_v\beta_3$ and $\alpha_v\beta_5$ receptors.[a]

Entry	Compounds	IC_{50} (nM) ± SD for $\alpha_v\beta_3$	IC_{50} (nM) ± SD for $\alpha_v\beta_5$
1	**7**	> 1 μM	> 1 μM
2	**8**	> 1 μM	> 1 μM
3	**9**	> 1 μM	> 1 μM
4	**10**	> 1 μM	> 1 μM
5	**11**	> 1 μM	> 1 μM
6	**21**	304.5 ± 11.4	1054 ± 127.4
7	**22**	2602 ± 71.1	1481 ± 123.0
8	echistatin	0.28 ± 0.08	0.29 ± 0.02
9	 c(RGDfV)	195.9 ± 16.8	0.11 ± 0.03
10	 EMD121974	18.9 ± 3.1	0.13 ± 0.01
11	 ST1646	3.7 ± 0.6	1.4 ± 0.2

[a] IC_{50} values were calculated as the concentration of compound required for 50% inhibition of echistatin binding as estimated by the Allfit program. All values are the means (± standard deviation) of triplicate determinations.

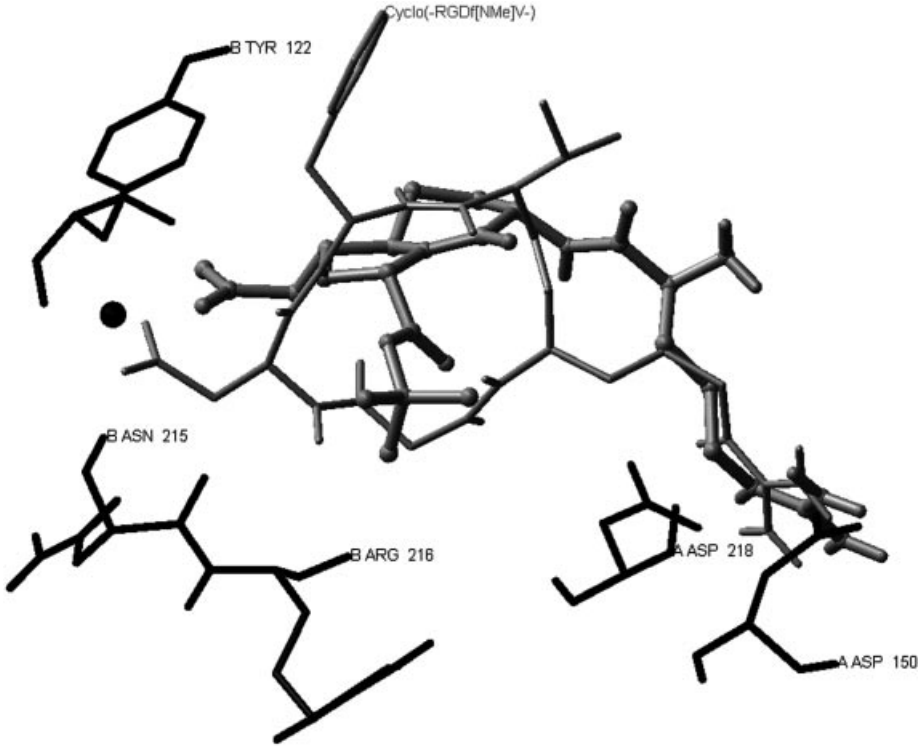


Figure 2. Top-ranking binding modes of compound **21** (gray ball and stick representation) with the crystal structure of the extracellular $\alpha_V\beta_3$ integrin domain overlaid on the bound conformation of EMD 121974 (gray tube representation). Selected integrin residues involved in the interactions with EMD 121974 are shown in black (tube representation). The Mn^{2+} ion at MIDAS is shown as a black CPK sphere. Non-polar hydrogens were removed for clarity.

between the Asp and Arg C^{β} atoms and an almost extended conformation of the RGD sequence are observed in this pentapeptide bound conformation (Figure 2). The most important interactions involve the positively charged Arg guanidinium group of the ligand and the negatively charged side chains of Asp150 and Asp218 in the α subunit, together with one of the Asp carboxylate oxygens of the ligand and the metal cation in the metal-ion-dependent adhesion site (MIDAS) region of the β subunit. Further stabilization could occur through hydrogen bonds between the backbone NH of the Asp residue and the backbone carbonyl oxygen of Arg216 in the β subunit, as well as between the other Asp carboxylate oxygen and the backbone amide hydrogen of Asn215 in the β subunit. Moreover, the central Gly residue is in close contact with the integrin surface.

Starting from this X-ray complex, structural models for the interactions of the selected compounds with the ligand-binding site of the $\alpha_v\beta_3$ integrin receptor were generated by automated computational docking by use of the Glide program,[13] after removal of the peptide ligand. Automated docking calculations of the compounds **7**–**11** produced top-ranked poses conserving only one of the important ionic interactions due to the short chain lengths.

As the guanidine and carboxy groups of the ligands are essential for binding to the integrin subunits α and β, respectively, acting like an electrostatic clamp in attaching to charged regions of the protein,[14,15] this could explain the activity data for ligands **7**–**11**.

Docking calculations for compound **21** produced top-ranked poses in which the important ionic interactions were conserved but the stabilizing hydrogen bonds with Arg216 chain β were loosened. Moreover, as the cleft in which the ligands bind is rather shallow, compound **21** shows an alternative binding mode, differing in the orientation of the carboxy group, so that it maintains the ionic interaction with Ca^{2+} (average distance 2.4 Å) but not the stabilizing hydrogen bond with Asn215 chain β (Figure 2).

For compound **22**, analogously to **21**, docking calculations showed that the inversion of stereochemistry at C-3 again causes the loss of the electrostatic clamp-like function.

In summary, if it is assumed that the X-ray pose describes the best interaction mode with the $\alpha_v\beta_3$ integrin receptor, the models constructed by docking studies for the interaction of ligands **7**–**11** and **21**–**22** with $\alpha_v\beta_3$ integrin confirmed that the bicyclic conformations of these ligands enable them to fit properly in the shallow cleft of the receptor, but that the short chain lengths (for compounds **7**–**11** and **22**) or different orientations of the carboxy groups of the ligands in the binding site (for compound **21**) may partially abolish or modify the polar interactions and the H-bonding network governing the recognition process, resulting in a reduction in activity.

The binding affinity of the best peptide mimetic – **21** – for the $\alpha_V\beta_3$ integrin is about two orders of magnitude lower than the affinity of the best cyclic RGD ligand

(ST1646). The binding properties of the nonpeptide RGD mimics based on azabicycloalkane scaffolds and described here thus need to be improved in order to develop nanomolar ligands useful for biomedical applications.

Molecular docking studies may assist this purpose through exploitation of their ability to explain differences in the biological results for closely related RGD mimetics on a structural basis. Efforts to explore new functionalized bicyclic scaffolds and substitution patterns and to develop higher affinity integrin ligands with the aid of the docking protocol as a virtual screening tool continue in our laboratories.

Experimental Section

Docking Protocol: The automated molecular docking calculations were carried out by use of the Glide (grid-based ligand docking with energetics) module implemented in Schrödinger's FirstDiscovery Suite, version 2.7.[13] Although the protein is required to be rigid, the program allows torsional flexibility in the ligand. Conformational flexibility is handled in Glide by means of an extensive conformational search, augmented by a heuristic screen that rapidly eliminates conformations deemed unsuitable for binding to a receptor. During the docking process, the bicyclic backbone conformation of the ligands was held fixed, whereas the side chain dihedral angles were free to rotate. The refined poses are scored with Schrödinger's proprietary GlideScore scoring function. GlideScore is based on Chem-Score[16] but includes a steric-clash term and adds buried polar terms devised by Schrödinger to penalize electrostatic mismatches.

For each ligand, the global minimum energy conformer derived from an MC/EM[12] (AMBER*,[17] water GB/SA[18]) conformational search was selected as the representative starting conformation.

The recently solved crystal structure of the extracellular domain of the $\alpha_v\beta_3$ integrin receptor in complexation with EMD 121974 in the presence of the proadhesive Mn^{2+} ion (PDB entry code = 1L5G[11]) was used for docking studies. Because the headgroup of integrin $\alpha_v\beta_3$ has been identified as the ligand-binding region in the X-ray structure, the docking was performed only on the globular head. The protein structure was prepared and optimized by use of the Schrödinger pprep and impref scripts[13] with replacement of Mn^{2+} ions with Ca^{2+} ions.

The grid-generation step requires mae input files of both ligand and active site, including hydrogen atoms. The center of the grid-enclosing box was defined by the centroid of the bound ligand, as described in the original PDB entry. The enclosing box dimensions, which are automatically deduced from the ligand size, fit the entire active site. For the docking step, the size of the bounding box for placing the ligand center was set to 12 Å. No further modifications were applied to the default settings. The GlideScore scoring function was used to select 30 poses for each ligand.[19]

The Glide program was initially tested for its ability to reproduce the crystallized binding geometry of EMD 121974. The program was successful in reproducing the experimentally found binding mode of this compound, as it corresponds to the best-scored pose.

Solid-Phase Receptor-Binding Assay: The receptor-binding assays were performed as described previously.[20,21] Purified receptors $\alpha_v\beta_3$ and $\alpha_v\beta_5$ (Chemicon International Inc., Temecula, CA) were diluted to 500 ng mL^{-1} and 1000 ng per well, respectively, in coating buffer [20 mM Tris-HCl (pH 7.4), 150 mM NaCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 1 mM $MnCl_2$]. Aliquots of the diluted receptors (100 μL per well) were added to 96-well microtiter plates, which were incubated overnight at 4 °C. The coating solution was removed by aspiration, and blocking solution [coating buffer containing 1% bovine serum albumin (BSA), 200 μL] was added to the wells, which were incubated for an additional 2 h at room temperature. After incubation, the plates were rinsed with blocking solution (200 μL, 3×) and incubated with the appropriate radiolabeled ligand for 3 h at room temperature. [^{125}I]Echistatin (Amersham Pharmacia, 0.05 nM and 0.1 nM, respectively) was used for $\alpha_v\beta_3$ and $\alpha_v\beta_5$. After incubation, the plates were sealed and counted in the γ-counter (Packard). Each data point is the result of the average of triplicate wells, and was analyzed by nonlinear regression analysis with the Allfit program.

Chemistry

General: ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solution as indicated, at 400 and 100.6 MHz, respectively. The chemical shift values are given in ppm and the coupling constants in Hz. Thin-layer chromatography (TLC) was carried out with Merck precoated silica gel F-254 plates. Flash chromatography was carried out with Macherey–Nagel silica gel 60, 230–400 mesh. Solvents were dried by standard procedures, and reactions requiring anhydrous conditions were performed under nitrogen or argon. Elemental analyses were performed by the staff of the microanalytical laboratory in our department.

General Procedure A. Reduction/Guanidinylation Reaction: Bis-Cbz carboxamidine **12** (1.1 equiv.), water (6 equiv.), and triphenylphosphane (1.1 equiv.) were added to a solution of azide (1 equiv.) in THF (1.2 mL). The reaction mixture was allowed to stir at room temperature for 48 h, the solvent was removed under reduced pressure, and the crude product was purified by flash chromatography (EtOAc/petroleum ether 6:4).

Compound 3 (3*S*,6*R*,7*S*,9*S*): The compound was obtained as a yellow oil by General Procedure A (76%). $[\alpha]_D^{25\,°C}$ = –8.16 (*c* = 1.4, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.44 (s, 10 H, *Boc*, *H*^{8}), 1.46 [s, 10 H, *C*(*CH*$_3$)$_3$, *H*^{5}], 1.72 (m, 2 H, *H*$_4$, *H*$^{5'}$), 1.9 (m, 1 H, *H*$^{10'}$), 2.15 (m, 2 H, *H*^{4}, *H*$^{8'}$), 2.2 (m, 2 H, *H*^{7}, *H*^{10}) 3.37 (m, 1 H, *H*^{6}), 3.55 (s, 2 H, *H*^{11}), 4.03 (m, 1 H, *H*^{3}), 4.31 (m, 1 H, *H*^{9}), 5.15 (m, 5 H, *NHBoc*, *OCH*$_2$*Ph*), 7.36 (m, 11 H, *Ph*, *NHCbz*), 8.5 [s, 1 H, *NH*(=*NCbz*)*NHCbz*] ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 26.5, 28.1, 28.5, 28.7, 30.8, 34.6, 39.8, 52.4, 58.2, 64.6, 67.6, 68.6, 79.7, 81.8, 128.2, 128.3, 128.6, 128.9, 129.1, 167.7, 171.3 ppm. MS (FAB): *m*/*z* found: 709 [M + H]$^+$ (calcd. for $C_{37}H_{49}N_5O_9$: 707.35). $C_{37}H_{49}N_5O_9$ (707.35): calcd. C 62.78, H 6.98, N 9.89; found: C 62.76, H 6.97, N 9.88.

Compound 4 (3*R*,6*R*,7*S*,9*S*): The compound was obtained as a white solid by General Procedure A (89%). M.p. 58–59 °C. $[\alpha]_D^{25\,°C}$ = –41.4 (*c* = 0.86, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.42 (s, 9 H, *Boc*), 1.43 [s, 9 H, *C*(*CH*$_3$)$_3$], 1.52 (m, 3 H, *H*$^{10'}$, *H*$^{8'}$, *H*$^{5'}$), 1.8 (m, 3 H, *H*$^{4'}$, *H*^{5}, *H*^{10}), 2.08 (m, 1 H, *H*^{7}), 2.35 (m, 1 H, *H*^{4}) 2.55 (m, 1 H, *H*^{8}), 3.38 (m, 1 H, *H*^{6}), 3.55 (s, 2 H, *H*^{11}), 4.12 (m, 1 H, *H*^{3}), 4.40 (m, 1 H, *H*^{9}), 5.16 (2s, 4 H, *OCH*$_2$*Ph*), 5.50 (br s, 1 H, N*H*Boc), 7.35 (m, 10 H, *Ph*), 8.35 [s, 1 H, *NH*(=*NCbz*)-*NHCbz*] ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 24.9, 27.0, 28.1, 28.2, 31.3, 34.5, 39.4, 44.2, 50.5, 58.9, 61.8, 67.3, 68.4, 79.7, 81.9, 128.1, 128.2, 128.6, 128.7, 128.9, 129.0, 134.6, 136.8, 154.1, 156.2, 163.2, 163.8, 168.7, 170.9 ppm. MS (FAB): *m*/*z* found: 709 [M + H]$^+$ (calcd. for $C_{37}H_{49}N_5O_9$: 707.35). $C_{37}H_{49}N_5O_9$ (707.35): calcd. C 62.78, H 6.98, N 9.89; found: C 62.75, H 6.96, N 9.89.

General Procedure B. Cleavage of the Boc Protecting Group: $HClO_4$ (70% in water, 3 equiv.) was added at 0 °C to a solution of compound **3** or **4** (1 equiv.) in *t*BuOAc (0.03 M). The reaction mixture was then allowed to warm to room temperature. When the reaction was complete (TLC), triethylamine was added and after 30 min the aqueous phase was extracted with EtOAc. The collected organic phases were dried with Na_2SO_4 and filtered, and the solvent was evaporated under reduced pressure. The crude product was carried over to the next reaction without further purification.

Compound 5 (3*S*,6*R*,7*S*,9*S*): The compound was obtained as a colorless oil by General Procedure B.

Compound 6 (3*R*,6*R*,7*S*,9*S*): The compound was obtained as a white solid by General Procedure B.

Compound 7 (3*S*,6*R*,7*S*,9*S*): EDC (0.075 g, 0.376 mmol), HOBT (0.0376 mmol), and collidine (22.7 μL, 0.171 mmol) were added under nitrogen to a solution of monobenzyl phthalate (0.963 g, 0.376 mmol) in dry THF (3.5 mL). The solution was stirred for 10 min, and a solution of **5** (0.104 g, 0.171 mmol) in dry THF (2 mL) was added by cannula, followed by a catalytic quantity of DMAP. After 12 h the solvent was evaporated under reduced pressure and the crude product was purified by flash chromatography (EtOAc/petroleum ether, 7:3) to give the protected compound as a yellow oil. $[\alpha]_D^{25\,°C} = -9.75$ (c = 0.83, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ = 1.40 [s, 9 H, *C(CH$_3$)$_3$*], 1.60, 1.80 (m, 5 H, H^8, $H^{4'}$, $H^{10'}$, H^5), 2.05 (m, 1 H, H^{10}), 2.15 (m, 1 H, H^7), 2.65 (m, 1 H, H^8) 2.75 (m, 1 H, H^4), 3.40 (m, 1 H, H^6), 3.52 (m, 2 H, H^{11}), 4.25 (m, 1 H, H^3), 4.38 (m, 1 H, H^9), 5.15 (m, 6 H, *OCH$_2$Ph*), 6.52 (s, 1 H, N*H*C=O), 7.20–7.35 (m, 19 H, *Ph*), 8.35 [s, 1 H, *NH(=NCbz)-NHCbz*] ppm. HETCOR NMR ($CDCl_3$, 400 MHz): δ = 24.0, 25.5, 27.0, 31.0, 34.0, 40.0, 44.0, 58.5, 64.5, 68.0, 130.0 ppm. MS (FAB): *m*/*z* found: 846 $[M + H]^+$ (calcd. for $C_{47}H_{51}N_5O_{10}$: 845.36). $C_{47}H_{51}N_5O_{10}$ (845.36): calcd. C 66.73, H 6.08, N 8.28; found: C 66.70, H 6.05, N 8.25.

A catalytic amount of Pd/C was added to a solution of the protected compound (0.101 g, 0.170 mmol) in MeOH (5 mL). The reaction mixture was hydrogenated at 1 atm for 4 h, the catalyst was then removed by filtration through a Celite pad, and the solvent was removed under reduced pressure to give **7** as a white solid in 41% yield over three steps. $[\alpha]_D^{25\,°C} = -41.8$ (c = 0.32, H_2O). ^1H NMR (D_2O, 400 MHz): δ = 1.40 [s, 9 H, *C(CH$_3$)$_3$*], 1.50 (m, 3 H, H^8, $H^{10'}$, H^7), 1.85 (m, 3 H, $H^{5'}$, $H^{4'}$, H^{10}), 2.25 (m, 2 H, H^5, H^4), 2.55 (m, 1 H, H^8), 3.20 (m, 2 H, H^{11}), 3.45 (m, 1 H, H^6), 4.34 (dd, J = 8.9, J = 8.9 Hz, 1 H, H^9), 4.55 (m, 1 H, H^3), 7.5 (m, 4 H, *Ph*) ppm. HETCOR NMR (D_2O, 400 MHz): δ = 26.5, 27.5, 29.5, 34.0, 40.0, 43.0, 51.0, 60.0, 66.0, 130.0 ppm. MS (FAB): *m*/*z* found: 488 $[M + H]^+$ (calcd. for $C_{24}H_{33}N_5O_6$: 487.24). $C_{24}H_{33}N_5O_6$ (487.24): calcd. C 59.12, H 6.82, N 14.36; found: C 59.10, H 6.80, N 14.37.

General Procedure C. Coupling with the Monobenzyl Malonate and Hydrogenolysis: EDC (2 equiv.), HOBT (1 equiv.), and collidine (1 equiv.) were added under nitrogen to a solution of monobenzyl malonate (2.2 equiv.) in dry THF (0.1 M). The solution was stirred for 10 min, and a solution of **5** or **6** (1 equiv.) in dry THF (0.1 M) was then added by cannula, followed by a catalytic quantity of DMAP. After 12 h the solvent was evaporated under reduced pressure, and the crude product was purified by flash chromatography.

A catalytic amount of Pd/C was added to a solution of the protected compound (1 equiv.) in MeOH (0.1 M). The reaction mixture was hydrogenated at 1 atm for 4 h, the catalyst was then removed by filtration through Celite pad, and the solvent was removed under reduced pressure to give the desired product.

Compound 8 (3*S*,6*R*,7*S*,9*S*): The compound was obtained as a white solid by General Procedure C (63% over two steps).

Protected Product: Yellow oil, flash chromatography: EtOAc/petroleum ether, 7:3. $[\alpha]_D^{25\,°C} = -4.4$ (c = 0.34, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ = 1.30 (m, 2 H, $H_{5'}$, $H_{10'}$), 1.48 [s, 9 H, *C(CH$_3$)$_3$*], 1.60 (m, 4 H, $H^{8'}$, H^{10}, $H^{4'}$, H^7), 2.20 (m, 1 H, H^5), 2.60 (m, 2 H, H^4, H^8), 3.40 (m, 3 H, H^{12}, H^6), 3.55 (s, 2 H, H^{11}), 4.40 (m, 2 H, H^9, H^3), 5.18 (m, 6 H, *OCH$_2$Ph*), 7.30–7.45 (m, 16 H, *Ph*, *NHC=O*), 8.35 [m, 1 H, *NH(=NCbz)NHCbz*] ppm. HETCOR NMR ($CDCl_3$, 400 MHz): δ = 28.5, 30.0, 40.0, 43.0, 44.0, 52.0, 57.5, 65.0, 68.0, 129.0 ppm. MS (FAB): *m*/*z* found: 784 $[M + H]^+$ (calcd. for $C_{42}H_{49}N_5O_{10}$: 783.35). $C_{42}H_{49}N_5O_{10}$ (783.35): calcd. C 64.35, H 6.30, N 8.93; found: C 64.30, H 6.28, N 8.91.

Deprotected Product: $[\alpha]_D^{25\,°C} = -44.4$ (c = 1.57, MeOH). ^1H NMR (D_2O, 400 MHz): δ = 1.39 [s, 9 H, *C(CH$_3$)$_3$*], 1.50 (m, 3 H, $H^{8'}$, $H^{10'}$, H^5), 1.85 (m, 3 H, $H^{4'}$, H^7, H^{10}), 2.20 (m, 2 H, H^4, H^5), 2.55 (m, 1 H, H^8), 3.20 (m, 4 H, H^{12}, H^{11}), 3.41 (m, 1 H, H^6), 4.30 (dd, J = 8.9, J = 8.9 Hz, 1 H, H^9),4.40 (m, 1 H, H^3) ppm. HETCOR NMR (D_2O, 400 MHz): δ = 26.0, 27.5, 29.5, 34.0, 40.0, 43.0, 45.5, 50.5, 60.0, 65.0 ppm. MS (FAB): *m*/*z* found: 426 $[M + H]^+$ (calcd. for $C_{19}H_{31}N_5O_6$: 425.23). $C_{19}H_{31}N_5O_6$ (425.23): calcd. C 53.63, H 7.34, N 16.46; found: C 53.60, H 7.32, N 16.42.

Compound 9 (3*R*,6*R*,7*S*,9*S*): The compound was obtained as a white solid by General Procedure C (33% over two steps).

Protected Product: Flash chromatography: toluene/acetone, 85:5. $[\alpha]_D^{25\,°C} = -10.5$ (c = 1, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ = 1.49 [s, 13 H, $H^{4'}$, $H^{8'}$, $H^{5'}$, $H^{10'}$, *C(CH$_3$)$_3$*], 1.70 (m, 2 H, H^{10}, H^7), 2.15 (m, 1 H, H^5), 2.60 (m, 2 H, H^4, H^8), 3.40 (m, 3 H, H^{12}, H^6), 3.50 (m, 2 H, H^{11}), 4.35 (m, 2 H, H^9, H^3), 5.15 (m, 6 H, *OCH$_2$Ph*), 7.35 (m, 16 H, *Ph*, *NHC=O*), 8.35 [m, 1 H, *NH(=NCbz)NHCbz*] ppm. HETCOR NMR ($CDCl_3$, 400 MHz): δ = 27.0, 28.0, 40.0, 42.0, 51.5, 58.5, 68.0, 129.0 ppm. MS (FAB): *m*/*z* found: 784 $[M + H]^+$ (calcd. for $C_{42}H_{49}N_5O_{10}$: 783.35). $C_{42}H_{49}N_5O_{10}$ (783.35): calcd. C 64.35, H 6.30, N 8.93; found: C 64.37, H 6.30, N 8.94.

Deprotected Product: $[\alpha]_D^{25\,°C} = -50.8$ (c = 0.92, MeOH). ^1H NMR (D_2O, 400 MHz): δ = 1.48 [s, 9 H, *C(CH$_3$)$_3$*], 1.57 (m, 3 H, $H^{8'}$, $H^{10'}$, $H^{5'}$), 1.90 (m, 3 H, $H^{4'}$, H^5, H^{10}), 2.22 (m, 2 H, H^4, H^7), 2.60 (m, 1 H, H^8), 3.25 (m, 2 H, H^{11}), 3.41 (m, 1 H, H^{12}, H^6), 4.32 (dd, J = 8.9, J = 8.9 Hz, 1 H, H^9),4.42 (m, 1 H, H^3) ppm. HETCOR NMR (D_2O, 400 MHz): δ = 26.0, 27.0, 33.5, 38.5, 42.0, 42.5, 50.9, 59.9, 64.5 ppm. MS (FAB): *m*/*z* found: 426 $[M + H]^+$ (calcd. for $C_{19}H_{31}N_5O_6$: 425.23). $C_{19}H_{31}N_5O_6$ (425.23): calcd. C 53.63, H 7.34, N 16.46; found: C 53.65, H 7.33, N 16.43.

General Procedure D. Coupling with Monobenzyl *N*-Acetyl-aspartate and Hydrogenolysis: DIC (1.1 equiv.) and HOBT (0.1 equiv.) were added under nitrogen to a solution of monobenzyl *N*-acetyl-aspartate (1 equiv.) in dry THF (0.1 M). The solution was stirred for 10 min, and a solution of either **5** or **6** (1 equiv.) in dry THF (0.5 M) was then added by cannula. After 4 h the solvent was evaporated under reduced pressure and the crude product was purified by flash chromatography.

A catalytic amount of Pd/C was added to a solution of the protected compound (1 equiv.) in MeOH (0.1 M). The reaction mixture was hydrogenated at 1 atm for 4 h, the catalyst was then removed by filtration through a Celite pad, and the solvent was removed under reduced pressure to give the desired product.

Compound 10 (3*S*,6*R*,7*S*,9*S*): The compound was obtained as a white solid by General Procedure C (66% over two steps).

Protected Product: Yellow oil, flash chromatography: toluene/acetone, 6:4. $[\alpha]_D^{25\,°C} = -15.6$ (c = 0.93, $CHCl_3$). ^1H NMR ($CDCl_3$,

400 MHz): δ = 1.42 [s, 9 H, $C(CH_3)_3$], 1.51 (m, 3 H, H^{10}, $H^{4'}$, H^5), 1.78 (m, 3 H, H^{10}, H^5, $H^{8'}$), 2.0 (s, 3 H, CH_3C=O), 2.18 (m, 1 H, H^7), 2.47 (m, 1 H, H^4), 2.61 (m, 1 H, H^8), 2.73 (ddd, J = 6.63, J = 6.63, J = 16.9 Hz, 1 H, $H^{13'}$), 3.02 (ddd, J = 4.82, J = 4.82, J = 16.9 Hz, 1 H, H^{13}), 3.40 (m, 1 H, H^6), 3.55 (s, 2 H, H^{11}), 4.32 (m, 2 H, H^9, H^3), 4.86 (m, 1 H, H^{12}), 5.21, 5.86 (2× s, 7 H, OCH_2Ph, N*H*), 6.67 (d, J = 8.55 Hz, 1 H, N*H*Ac), 6.96 (d, J = 6.36 Hz, 1 H, *NHC*=*O*), 7.38 (m, 15 H, *Ph*), 8.46 [s, 1 H, *NH(=NCbz)-NHCbz*] ppm. HETCOR NMR ($CDCl_3$, 400 MHz): δ = 27.0, 28.8, 31.8, 33.0, 35.0, 37.0, 40.5, 44.0, 50.0, 58.5, 65.2 ppm. MS (FAB): *m*/*z* found: 855 $[M + H]^+$ (calcd. for $C_{45}H_{54}N_6O_{11}$: 854.39). $C_{45}H_{54}N_6O_{11}$ (854.39): calcd. C 63.22, H 6.37, N 9.83; found: C 63.18, H 6.35, N 9.82.

Deprotected Product: $[\alpha]_D^{25\,°C}$ = –34.9 (*c* = 1.01, MeOH). ^{1}H NMR (D_2O, 400 MHz): δ = 1.38 [s, 9 H, $C(CH_3)_3$], 1.40 (m, 3 H, H^8, H^{10}, H^5), 1.78 (m, 3 H, H^4, $H^{5'}$, $H^{10'}$), 1.90 (s, 3 H, CH_3CO), 2.08 (m, 2 H, $H^{4'}$, H^7), 2.45 (m, 2 H, H^{13}, $H^{8'}$), 2.58 (m, 1 H, H^{13}), 3.15 (m, 2 H, H^{11}), 3.35 (m, 1 H, H^6), 4.22 (m, 2 H, H^3, H^9), 4.48 (m, 1 H, H^{12}) ppm. HETCOR NMR (D_2O, 400 MHz): δ = 22.2, 26.0, 27.0, 29.0, 33.8, 34.0, 39.0, 40.0, 43.0, 49.2, 52.5, 59.8, 65.0 ppm. MS (FAB): *m*/*z* found: 497 $[M + H]^+$ (calcd. for $C_{22}H_{36}N_6O_7$: 496.26). $C_{22}H_{36}N_6O_7$ (496.26): calcd. C 53.21, H 7.31, N 16.92; found: C 53.2, H 7.30, N 16.91.

Compound 11 (3*R*,6*R*,7*S*,9*S*): This compound was obtained as a white solid by General Procedure C (67% over two steps).

Protected Product: White solid, flash chromatography: toluene/acetone, 1:1). $[\alpha]_D^{25\,°C}$ = –16.3 (*c* = 2.37, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.40 [s, 9 H, $C(CH_3)_3$], 1.50 (m, 4 H, $H^{8'}$, $H^{10'}$, $H^{4'}$, H^5), 1.85 (m, 1 H, H^{10}), 2.05 (s, 3 H, CH_3C=O), 2.10 (m, 1 H, H^5), 2.30 (m, 2 H, H^7, H^4), 2.55 (m, 1 H, H^8), 2.74 (ddd, J = 5.57, J = 5.57, J = 16.8 Hz, 1 H, $H^{13'}$), 3.03 (ddd, J = 4.66, J = 4.66, J = 17.1 Hz, 1 H, H^{13}), 3.38 (m, 1 H, H^6), 3.50 (s, 2 H, H^{11}), 4.25 (m, 1 H, H^3), 4.41 (dd, J = 8.57, J = 8.57 Hz, 1 H, H^9), 4.89 (m, 1 H, H^{12}), 5.15, (ms, 6 H, OCH_2Ph), 6.80 (m, 1 H, N*H*Ac), 7.30 (m, 17 H, *NH*Asp, *Ph*), 8.35 [s, 1 H, *NH(=NCbz)-NHCbz*] ppm. HETCOR NMR ($CDCl_3$, 400 MHz): δ = 23.6, 23.8, 28.4, 30.1, 31.5, 34.6, 36.4, 39.6, 44.1, 49.8, 59.1, 62.4, 67.2, 67.6, 69.7, 82.2, 128.4, 128.5, 128.7, 128.8, 128.9, 129.1, 129.5, 134.9, 135.8, 137.1, 154.3, 156.4, 157.4, 156.4, 157.4, 168.3, 170.5, 170.8, 171.1, 171.9 ppm. MS (FAB): *m*/*z* found: 855 $[M + H]^+$ (calcd. for $C_{45}H_{54}N_6O_{11}$: 854.39). $C_{45}H_{54}N_6O_{11}$ (854.39): calcd. C 63.22, H 6.37, N 9.83; found: C 63.20, H 6.35, N 9.84.

Deprotected Product: $[\alpha]_D^{25\,°C}$ = –21.8 (*c* = 0.22, MeOH). ^{1}H NMR (D_2O, 400 MHz): δ = 1.42 [s, 9 H, $C(CH_3)_3$], 1.51 (m, 3 H, $H^{8'}$, $H^{10'}$, $H^{5'}$), 1.85 (m, 3 H, H^4, H^7, H^{10}), 1.98 (s, 3 H, CH_3CO), 2.06 (m, 2 H, H^4, H^5), 2.55 (m, 1 H, H^8), 2.58 (m, 1 H, $H^{13'}$), 2.66 (m, 1 H, H^{13}), 3.21 (m, 1 H, $H^{4'}$), 3.34 (m, 1 H, H^6), 4.32 (m, 2 H, H^3, H^9), 4.50 (m, 1 H, H^{12}) ppm. HETCOR NMR (D_2O, 400 MHz): δ = 22.1, 23.8, 25.0, 28.8, 29.0, 34.5, 39.0, 40.0, 42.5, 49.0, 51.8, 60.0, 65.0 ppm. MS (FAB): *m*/*z* found: 497 $[M + H]^+$ (calcd. for $C_{22}H_{36}N_6O_7$: 496.26). $C_{22}H_{36}N_6O_7$ (496.26): calcd. C 53.21, H 7.31, N 16.92; found: C 53.19, H 7.32, N 16.90.

General Procedure E. Oxidation: A solution of PDC in DMF (1 M, 4.8 equiv.) was added to a solution of either **13** or **14** (1 equiv.) in DMF (0.1 M). The reaction mixture was stirred for 12 h, a saturated solution of NaCl was then added, and the aqueous phase was extracted with EtOAc. The collected organic layers were evaporated under reduced pressure, the residue was dissolved in EtOAc and washed with a saturated solution of $NaHCO_3$, and the aqueous phase was acidified with a solution of HCl (0.5 M) and then extracted with EtOAc. The organic phase was dried with Na_2SO_4 and filtered, and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography.

Compound 15 (3*S*,6*R*,7*S*,9*S*): The compound was obtained as a yellow oil by General Procedure E (flash chromatography: petroleum ether/EtOAc, 8:2, 76%). $[\alpha]_D^{25\,°C}$ = –39.7 (*c* = 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.45 [s, 9 H, $C(CH_3)_3$], 1.48 [s, 9 H, $C(CH_3)_3$], 1.54 (m, 2 H, $H^{8'}$, $H^{5'}$), 1.73 (m, 1 H, $H^{4'}$), 2.15 (m, 2 H, H^5, H^7), 2.36 (m, 1 H, H^{10}), 2.54 (m, 2 H, H^{10}, H^4), 2.68 (m, 1 H, H^8), 3.41 (m, 1 H, H^6), 4.12 (m, 1 H, H^3), 4.38 (m, 1 H, H^9), 5.43 (br d, 1 H, *NHBoc*) ppm. HETCOR NMR ($CDCl_3$, 100.6 MHz): δ = 27.0, 29.0, 35.1, 36.0, 42.0, 52.5, 58.5, 64.5 ppm. MS (FAB): *m*/*z* found: 413 $[M + H]^+$ (calcd. for $C_{20}H_{32}N_2O_7$: 412.32). $C_{20}H_{32}N_2O_7$ (412.32): calcd. C 58.24, H 7.82, N 6.79; found: C 58.27, H 7.80, N 6.78.

Compound 16 (3*R*,6*R*,7*S*,9*S*): The compound was obtained as a yellow oil by General Procedure E (flash chromatography: petroleum ether/EtOAc, 8:2, 74%). $[\alpha]_D^{25\,°C}$ = –44.7 (*c* = 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.46 [s, 9 H, $C(CH_3)_3$], 1.48 [s, 9 H, $C(CH_3)_3$], 1.62 (m, 3 H, $H^{4'}$, $H^{8'}$, $H^{5'}$), 2.05 (m, 1 H, $H^{10'}$), 2.20 (m, 1 H, H^5), 2.37 (m, 2 H, H^4, H^7), 2.61 (m, 2 H, H^{10}, H^8), 3.44 (m, 1 H, H^6), 4.18 (m, 1 H, H^3), 4.43 (m, 1 H, H^9), 5.65 (br s, 1 H, *NHBoc*) ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 21.0, 28.1, 28.4, 29.8, 34.5, 36.0, 42.2, 50.4, 58.7, 78.0, 79.0, 80.0, 82.1, 170.9, 175.9, 176.6 ppm. MS (FAB): *m*/*z* found: 413 $[M + H]^+$ (calcd. for $C_{20}H_{32}N_2O_7$: 412.32). $C_{20}H_{32}N_2O_7$ (412.32): calcd. C 58.24, H 7.82, N 6.79; found: C 58.22, H 7.79, N 6.80.

General Procedure F. Esterification with Benzyl Bromide: An aqueous solution of $CsCO_3$ (20%) was added to a solution of either **15** or **16** (1 equiv.) in a mixture of MeOH/H_2O (10:1, 0.1 M) until pH 7 was reached. The solvent was evaporated under reduced pressure, the residue was dissolved in DMF and evaporated again, and a solution of benzyl bromide in DMF (0.4 M, 1.1 equiv.) was added to the remaining cesium salt. After 1 h the solvent was evaporated under reduced pressure, and the residue was then dissolved in EtOAc and washed with water. The organic phase was dried with Na_2SO_4, filtered, and evaporated under reduced pressure, and the crude product was purified by flash chromatography.

Compound 17 (3*S*,6*R*,7*S*,9*S*): The compound was obtained as a white solid by General Procedure F (flash chromatography: petroleum ether/EtOAc, 6:4, 92%). $[\alpha]_D^{25\,°C}$ = –23.5 (*c* = 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.46 [s, 9 H, $C(CH_3)_3$], 1.48 [s, 9 H, $C(CH_3)_3$], 1.48–1.52 (m, 3 H, $H^{4'}$, $H^{8'}$, $H^{5'}$), 2.05 (m, 1 H, H^5), 2.17 (m, 1 H, H^7), 2.35 (m, 1 H, H^{10}), 2.56 (m, 2 H, $H^{10'}$, H^4), 2.63 (m, 1 H, H^8), 3.38 (m, 1 H, H^6), 4.05 (m, 1 H, H^3), 4.34 (m, 1 H, H^9), 5.15 (s, 2 H, OCH_2Ph), 5.28 (br s, 1 H, *NHBoc*), 7.40 (m, 5 H, Ph) ppm. HETCOR NMR ($CDCl_3$, 400 MHz): δ = 26.5, 28.7, 29.0, 35.5, 36.7, 42.5, 52.8, 58.7, 64.6, 67.5 ppm. MS (FAB): *m*/*z* found: 503 $[M + H]^+$ (calcd. for $C_{27}H_{38}N_2O_7$: 502.27). $C_{27}H_{38}N_2O_7$ (502.27): calcd. C 64.52, H 7.62, N 5.57; found: C 64.49, H 7.60, N 5.57.

Compound 17 (3*R*,6*R*,7*S*,9*S*): The compound was obtained as a yellow oil by General Procedure F (flash chromatography: petroleum ether/EtOAc, 6:4, 94%). $[\alpha]_D^{25\,°C}$ = –38.1 (*c* = 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.44 [s, 9 H, $C(CH_3)_3$], 1.48 [s, 9 H, $C(CH_3)_3$], 1.50–1.72 (m, 3 H, $H^{4'}$, $H^{8'}$, $H^{5'}$), 2.05 (m, 1 H, H^5), 2.22 (m, 1 H, H^7), 2.36 (m, 2 H, H^4, H^{10}), 2.60 (m, 2 H, $H^{10'}$, H^8), 3.41 (m, 1 H, H^6), 4.10 (m, 1 H, H^3), 4.41 (m, 1 H, H^9), 5.15 (s, 2 H, OCH_2Ph), 5.50 (br s, 1 H, *NHBoc*), 7.40 (m, 5 H, Ph) ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 24.7, 26.9, 28.1, 28.5, 34.5, 36.4, 42.5, 50.4, 58.7, 61.3, 65.9, 79.8, 82.0, 128.6, 128.7, 128.9, 135.6, 155.8, 170.8, 171.4 ppm. MS (FAB): *m*/*z* found: 503 $[M + H]^+$

(calcd. for $C_{27}H_{38}N_2O_7$: 502.27). $C_{27}H_{38}N_2O_7$ (502.27): calcd. C 64.52, H 7.62, N 5.57; found: C 64.53, H 7.63, N 5.57.

Compound 19 (3*S*,6*R*,7*S*,9*S*): The compound was obtained as a yellow oil by General Procedure B.

Compound 20 (3*R*,6*R*,7*S*,9*S*): The compound was obtained as a white solid by General Procedure B.

General Procedure G. Coupling with Z-Arg(Z)$_2$OH and Hydrogenolysis: DIC (1.3 equiv.) and HOBT (1.3 equiv.) were added under nitrogen to a solution of Z-Arg(Z)$_2$OH in dry THF (0.1 M, 1.3 equiv.). The solution was stirred for 10 min, and a solution of either **19** or **20** (1 equiv.) in dry THF (0.5 M) was then added by cannula. After 12 h the solvent was evaporated under reduced pressure and the crude product was purified by flash chromatography.

A catalytic amount of Pd/C was added to a solution of the protected compound (1 equiv.) in MeOH (0.1 M). The reaction mixture was hydrogenated at 1 atm for 4 h, the catalyst was then removed by filtration through a Celite pad, and the solvent was removed under reduced pressure to give the desired product.

Compound 21 (3*S*,6*R*,7*S*,9*S*): The compound was obtained as a white solid by General Procedure G (43% over three steps).

Protected Product: Yellow oil, flash chromatography: EtOAc/petroleum ether, 1:1. $[\alpha]_D^{25\,°C} = -8.13$ (c = 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.31–1.50 (m, 3 H, $H^{8'}$, $H^{4'}$, $H^{5'}$), 1.46 [s, 9 H, $C(CH_3)_3$], 1.60–1.82 (m, 4 H, H^{13}, H^{12}), 1.96 (m, 1 H, H^5), 2.14 (m, 1 H, H^7), 2.23 (m, 1 H, H^4), 2.35 (dd, J = 8.7, J = 15.7 Hz, 1 H, $H^{10'}$), 2.51 (dd, J = 5.4, J = 15.7 Hz, 1 H, H^{10}), 2.60 (m, 1 H, H^8), 3.33 (m, 1 H, H^6), 3.93 (m, 1 H, H^{14}), 4.06 (m, 1 H, $H^{14'}$), 4.17 (m, 1 H, H^3), 4.29 (dd, J = 8.9, J = 8.9 Hz, 1 H, H^9), 4.35 (m, 1 H, H^{11}), 5.0–5.30 (m, 8 H, OCH_2Ph), 5.88 (br d, 1 H, NH), 6.90 (br d, 1 H, NH), 7.23–7.43 (m, 20 H, Ph) ppm. HETCOR NMR ($CDCl_3$, 400 MHz): δ = 25.2, 27.0, 28.1, 30.3, 36.5, 42.3, 45.0, 51.5, 54.8, 58.4, 64.1, 67.5, 94.0 ppm. MS (FAB): m/z found: 961 [M + H]$^+$ (calcd. for $C_{52}H_{60}N_6O_{12}$: 960.43). $C_{52}H_{60}N_6O_{12}$ (960.43): calcd. C 64.99, H 6.29, N 8.74; found: C 64.98, H 6.30, N 8.73.

Deprotected Product: White solid. $[\alpha]_D^{25\,°C} = -87.4$ (c = 0.63, H_2O). ^{1}H NMR (D_2O, 400 MHz): δ = 1.42 [s, 9 H, $C(CH_3)_3$], 1.48–1.59 (m, 2 H, $H^{8'}$, $H^{5'}$), 1.63–1.72 (m, 2 H, $H^{13'}$, H^{13}), 1.82–1.95 (m, 3 H, H^{12}, $H^{12'}$, H^4), 2.07–2.22 (m, 4 H, H^4, H^5, H^{10}, H^7), 2.23 (m, 1 H, H^4), 2.33 (dd, J = 5.6, J = 13.6 Hz, 1 H, $H^{10'}$), 2.54 (m, 1 H, H^8), 3.18 (m, 2 H, H^{14}, $H^{14'}$), 3.40 (m, 1 H, H^6), 3.97 (m, 1 H, H^{11}), 4.36 (dd, J = 8.9, J = 8.9 Hz, 1 H, H^9), 4.39 (m, 1 H, H^3) ppm. HETCOR NMR (D_2O, 400 MHz): δ = 24.0, 26.7, 26.9, 28.5, 35.0, 39.5, 41.2, 43.0, 50.9, 53.8, 59.9, 65.3 ppm. MS (FAB): m/z found: 469 [M + H]$^+$ (calcd. for $C_{21}H_{36}N_6O_6$: 468.27). $C_{21}H_{36}N_6O_6$ (468.27): calcd. C 53.83, H 7.74, N 17.94; found: C 53.81, H 7.73, N 17.93.

Compound 22 (3*R*,6*R*,7*S*,9*S*): The compound was obtained as a white solid by General Procedure G (46% over three steps).

Protected Product: White foam, flash chromatography: EtOAc/petroleum ether, 6:4. $[\alpha]_D^{25\,°C} = -20.9$ (c = 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.46 [s, 9 H, $C(CH_3)_3$], 1.50–1.75 (m, 6 H, H^{13}, $H^{13'}$, $H^{12'}$, $H^{8'}$, $H^{4'}$, $H^{5'}$), 1.84 (m, 1 H, H^{12}), 1.95 (m, 1 H, H^5), 2.18 (m, 1 H, H^7), 2.25 (m, 1 H, H^4), 2.35 (dd, J = 8.7, J = 15.7 Hz, 1 H, $H^{10'}$), 2.56 (m, 2 H, H^8, H^{10}), 2.60 (m, 1 H, H^8), 3.38 (m, 1 H, H^6), 3.98 (m, 2 H, $H^{14'}$, H^{14}), 4.18 (m, 1 H, H^3), 4.30 (m, 1 H, H^{11}), 4.40 (dd, J = 8.4, J = 8.4 Hz, 1 H, H^9), 5.00–5.20 (m, 8 H, OCH_2Ph), 5.80 (br s, 1 H, NH), 7.05 (br s, 1 H, NH), 7.31–7.43 (m, 20 H, Ph) ppm. HETCOR NMR ($CDCl_3$, 400 MHz): δ = 25.1, 28.3, 30.0, 35.0, 37.0, 42.8, 45.0, 50.0, 55.2, 59.8, 62.2, 68.0, 70.0 ppm. MS (ESI): m/z found: 961 [M + H]$^+$ (calcd. for $C_{52}H_{60}N_6O_{12}$: 960.43). $C_{52}H_{60}N_6O_{12}$ (960.43): calcd. C 64.99, H 6.29, N 8.74; found: C 64.97, H 6.28, N 8.75.

Deprotected Product: White solid. $[\alpha]_D^{25\,°C} = -39.3$ (c = 1, H_2O). ^{1}H NMR (D_2O, 400 MHz): δ = 1.42 [s, 9 H, $C(CH_3)_3$], 1.50–1.71 (m, 4 H, $H^{13'}$, H^{13}, $H^{8'}$, $H^{5'}$), 1.85 (m, 2 H, $H^{12'}$, $H^{4'}$), 1.98–2.40 (m, 6 H, $H^{10'}$, H^{12}, H^4, H^5, H^{10}, H^7), 2.52 (m, 1 H, H^8), 3.20 (m, 2 H, H^{14}, $H^{14'}$), 3.38 (m, 1 H, H^6), 3.91 (m, 1 H, H^{11}), 4.38 (m, 1 H, H^9), 4.50 (m, 1 H, H^3) ppm. HETCOR NMR (D_2O, 400 MHz): δ = 24.0, 27.8, 29.0, 40.0, 41.5, 48.8, 53.8, 60.0, 66.0 ppm. MS (ESI): m/z found: 469 [M + H]$^+$ (calcd. for $C_{21}H_{36}N_6O_6$: 468.27). $C_{21}H_{36}N_6O_6$ (468.27): calcd. C 53.83, H 7.74, N 17.94; found: C 53.84, H 7.75, N 17.92.

Supporting Information (see footnote on the first page of this article): NMR spectra of all new synthesized compounds.

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