Discovery of SCH446211 (SCH6): A New Ketoamide Inhibitor of the HCV NS3 Serine Protease and HCV Subgenomic RNA Replication

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Introduction of various modified prolines at P_2 and optimization of the P_1 side chain led to the discovery of SCH6 (**24**, Table 2), a potent ketoamide inhibitor of the HCV NS3 serine protease. In addition to excellent enzyme potency (K_i*= 3.8 nM), **24** was also found to be a potent inhibitor of HCV subgenomic RNA replication with IC_{50} and IC_{90} of 40 and 100 nM, respectively. Recently, antiviral activity of **24** was demonstrated with inhibition of the full-length genotype 2a HCV genome. In addition, **24** was found to restore the responsiveness of the interferon regulatory factor 3 (IRF-3) in cells containing HCV RNA replicons.

Introduction

Hepatitis C virus (HCV), a small (+)-RNA virus belonging to the *Flaviiridae* family, infects chronically an estimated 3% of the population worldwide. Untreated HCV infections can progress to cirrhosis, hepatocellular carcinoma, and liver failure.[1] Currently, the immune system booster, alpha-interferon, alone or in combination with antiviral drug ribavirin are the only available treatment. Although combination therapy is reasonably successful with genotypes 2 and 3, its efficacy against the predominant genotype 1 is moderate at best, where 50% of patients fail to show a sustained response. Therefore, several research groups have been working toward the development of a more effective, convenient, and tolerable treatment.[2] Upon entering a suitable host cell, the HCV genome serves as a template for cap-independent translation through an internal ribosome entry (IRES) located in the 5′ untranslated region of the HCV genome. The resulting polyprotein undergoes both co- and post-translational proteolytic maturation by host and virally encoded proteases. The virally encoded NS3 serine protease is involved in the cis processing of the HCV polyprotein at the NS3−NS4A junction. Because of its central role in viral replication,[3] HCV NS3 serine protease has been actively pursued as a target for antiviral therapy.[4] Recently, the Boehringer Ingelheim group reported the antiviral activity of BILN 2061 after phase Ib clinical trial.[5] Another protease inhibitor, VX-950, was also shown to inhibit HCV replication in preclinical studies with a similar efficacy to that of BILN 2061.[6]

Oligopeptide derivatives containing α-ketoamide electrophilic trap have been reported by our group[7] and others[8] to be potent inhibitors of HCV NS3 serine protease. More recently, we reported that depeptidization of our earlier P_3-capped inhibitor **1**[9] led to the identification of **2** as a potent inhibitor of the HCV NS3 serine protease with good activity against HCV replicon (Figure 1).[10] We demonstrated that N-methylation at P_2 and replacement of the charged residue at P_2′ with a dimethyl amide

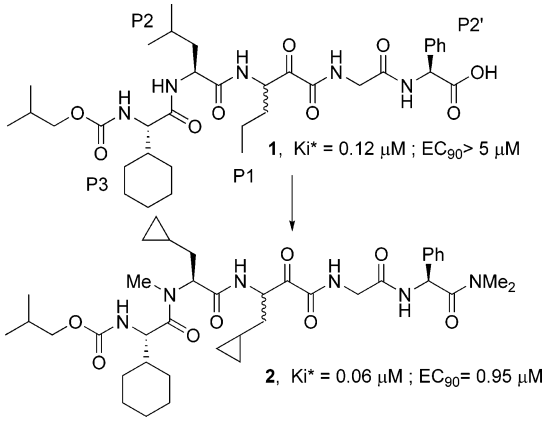

Figure 1.

cap were essential for inhibition of the HCV replicon system. N-Methylation at P_2 seemed to overcome an apparent "defect at P_2" conferred by the absence of a proline residue. Consequently, further work aimed at optimization of proline moieties at P_2 was undertaken. Herein, we report our finding that led to the discovery of SCH446211 (SCH6).

Synthesis

Preparation of the α-hydroxyl amide core of our inhibitors is depicted in Scheme 1. The P_2′ dimethylcarboxamide cap was incorporated, at low temperature, to commercially available Boc-L-phenylglycine using HATU and dimethylamine hydrochloride. Subsequent acidic removal of the Boc protecting group generated the amine hydrochloride salt, which was reacted with Boc-glycine following the same coupling protocol. After Boc deprotection, the resulting dipeptide **3** was then reacted with various Boc-protected α-hydroxyacids **4a**−**d**[11] using the aforementioned coupling and deprotection conditions to deliver the HCl salts **5**. Preparation of the modified prolines **6** was accomplished following reported procedures from our group and others.[12] Coupling with the appropriate P_3-capped amino acids **7** followed by hydrolysis of the methyl ester functionality delivered the dipeptide **8** for final assembly. The target

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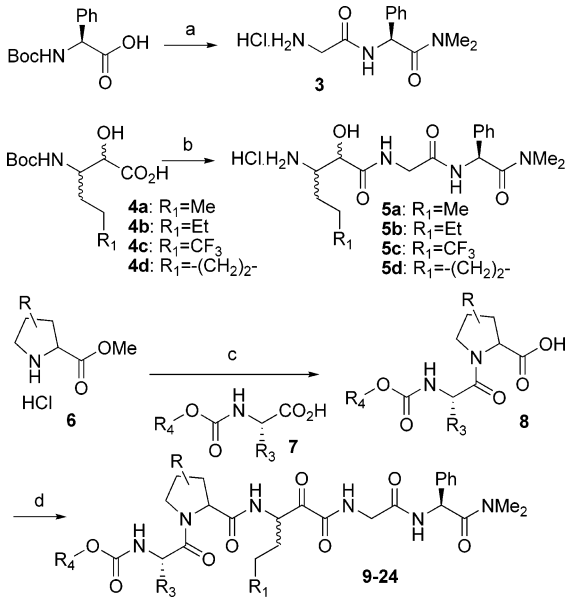
Scheme 1[a]



[a] Reagents and conditions: (a) (i) HATU, HCl·HNMe$_2$, DIPEA, CH_2Cl_2, −20 °C; (ii) 4.0 N HCl in dioxane; (iii) Boc glycine, HATU, DIPEA, CH_2Cl_2; (iiii) 4.0 N HCl in dioxane; (b) (i) HATU, **4a**−**d**, DIPEA, CH_2Cl_2, −20 °C; (ii) 4 M HCl in dioxane; (c) (i) HATU, DIPEA, CH_2Cl_2, −20 °C; (ii) aq. 1 N LiOH, THF/H_2O; (d) (i) **5a**−**d**, HATU, DIPEA, CH_2Cl_2, −20 °C; (ii) targets 9−12 and 17−24 Dess−Martin's periodinane, CH_2Cl_2; targets 13−16: DCC, DMSO, Cl_2CHCO_2H, CH_2Cl_2.

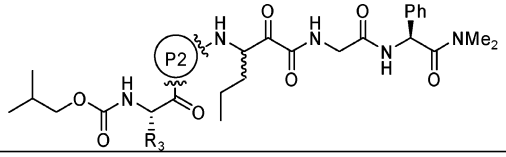
compounds were assembled by coupling together acids of type **8** and α-hydroxyl amides of type **5** according to the coupling procedures used above. Dess−Martin's periodinane[13] or Moffatt[14] oxidation of the resulting α-hydroxyl amide intermediates provided the desired α-ketoamides **9**−**24** as a mixture of diastereomers at P_1.

Results and Discussion

Inhibitors **9**−**24** (Tables 1 and 2) were tested in an HCV continuous assay using the NS4A-tethered single chain NS3 serine protease.[15] HCV replicon inhibitory activity for the targets synthesized were obtained using previously reported assay.[16]

We evaluated our initial set of inhibitors (**9**−**19**) by varying the P_2 moieties on a P_3 *i*-Boc-capped scaffold. The P_1 was kept constant as a *n*-valine residue and cyclohexyl glycine or *tert*-leucine were used as P_3 surrogates and provided similar binding potencies. Compound **9** (Table 1) was selected as the starting point for further optimization. Our initial finding that N-methylation at P_2 seemed to overcome an apparent defect conferred by the absence of a proline residue prompted us to evaluate the use of 4-alkyl-substituted prolines as conformationally restricted analogues of the acyclic moieties used in our earlier inhibitors (Figure 1).[9] We reasoned that cyclization of L-leucine to the adjacent P_2 nitrogen would be equivalent to a 4-methyl-L-proline surrogate. After examination of various X-ray structures of inhibitors bound to the active site of the NS3 protease,[9,17] we envisioned that the trans substitution would be optimal in providing enhanced hydrophobic binding with the enzyme side chain of Arg 155. Remarkably, incorporation of the *trans*-4-methyl-proline as in compound **10** provided more than 60-fold improvement in potency (K_i* = 0.14 μM) compared to the nonsubstituted proline, inhibitor **9** (K_i* = 10 μM). Additional substitution with a 4,4-dimethyl group further enhanced the binding potency of inhibitor **11** (K_i* = 0.036 μM). Because of the close proximity of the P_2 side chain to arginine 155, we decided to evaluate the possibility of larger hydrophobic

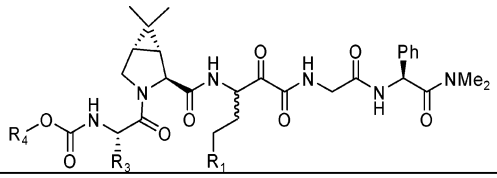
Table 1. Effect of P2 Modification on Enzyme Activity



Cmpd #	P2	R3	Ki* (μM)	Rep IC_{90} (μM)
9	R=R'= H	Chx	10.0	nt[b]
10	R= Me R'= H	*t*-Bu	0.140	nt
11[a]	R=R'= Me	*t*-Bu	0.036	nt
12	X=C, n=1	Chx	0.026	nt
13	X=S, n=1	Chx	0.026	nt
14	X=S, n=2	Chx	0.028	nt
15	X=S	Chx	0.120	nt
16	X=O	*t*-Bu	0.019	2.0
17	X=O	*t*-Bu	0.010	1.8
18	X=C	*t*-Bu	0.015	0.9
19		Chx	0.010	0.2

[a] Tested as pure (*S*) diastereomer at P1. [b] Compounds with K_i* > 0.020 μM were not tested (nt) for replicon activity.

Table 2. Effect of P3 and P1 Substitutions on HNE/HCV Selectivity and Potency



Cmpd #	R4	R3	R1	Ki* (nM)	HNE/HCV	Rep IC_{90} (nM)
19	*i*-Bu	Chx	Me	10	4	200
20	*i*-Bu	*t*-Bu	Me	16	81	350
21	*t*-Bu	*t*-Bu	Me	14	147	250
22	*t*-Bu	*t*-Bu	Et	10	964	400
23	*t*-Bu	*t*-Bu	CF_3	2	4600	400
24 (SCH6)	*t*-Bu	*t*-Bu	△	3.8	1000	100

interactions with the side chain of that residue and hydrophobic groups off the 4 position of the proline moiety. Thus, a spirocyclopentyl group was incorporated in compound **12** and resulted in a moderate improvement in potency (K_i* = 0.026 μM). Previous structural studies from our group indicated that introduction of a hydrophobic sulfide moiety at P_2 provided a slight improvement in binding potency.[17] Thus, to assess the effect of replacement of spiro-cyclopentyl at the 4 position of proline, we evaluated thioketals at that position. Preparation of inhibitor **13**, bearing a five-membered ring thioketal, resulted in a similar potency to **12**. Attempts to bring additional hydrophobic interactions with the larger six-membered thioketal **14** failed to provide improvement in binding potency (K_i* = 0.028 μM). The open form analogue of the thioketal was also examined; thus, *tert*-butyl thioether **15** resulted in 5-fold loss in activity (K_i* = 0.12 μM) compared to thioketals **13** and **14**. Presumably the bulky *tert*-butyl group had some detrimental steric interactions with the protein surface. In contrast, with a shorter chain length the *tert*-butyl group of compound **16** was well accommodated at that position with K_i* = 0.019 μM. The

cellular activity of inhibitor **16** was determined in the replicon assay, and its EC_{90} was found to be 2.0 μM. In an attempt to improve the potency of this type of inhibitor, we synthesized a more constrained analogue of the 4-*tert*-butoxy proline. Thus, the 3,4-fused tetrahydropyran scaffold was incorporated at P_2 in inhibitor **17** and provided about 2-fold improvement in enzyme potency but similar replicon activity ($K_i^* = 0.010$ μM, $EC_{90} = 1.8$ μM). The oxygen in cyclic ether **17** did not significantly contribute to the potency since the carbon analogue **18** was almost equipotent (Table 1). Moreover, the replicon activity of compound **18** was improved with an $EC_{90} = 0.9$ μM. Consequently, we focused on the 3,4-fused carbocycle series and decided to evaluate more conformationally restricted analogues. A recent publication from Madalengoitia identified the 2,2-dimethylcyclopropyl proline as a constrained analogue of L-leucine.[18] This new finding prompted us to replace the 2,2-dimethylcyclopentyl proline of **18** with 2,2-dimethylcyclopropyl proline as we had previously identified L-leucine as an excellent P_2 surrogate in our earlier series (Figure 1). Thus, inhibitor **19** was prepared and exhibited an excellent enzyme inhibitory activity of $K_i^* = 0.010$ μM and replicon activity as well ($EC_{90} = 0.2$ μM). On the basis of the encouraging activities demonstrated in the enzyme and replicon assays, **19** was an excellent candidate for optimization. Thus, inhibitor **19** was further evaluated in an enzyme selectivity assay against a related enzyme. The selectivity was measured against human neutrophil elastase (HNE), which is the closest serine protease to human hepatitis C virus (HCV) NS3 protease. The ratio of K_i^* values (HNE/HCV) are reported in Table 2.

Comparing inhibitors **19** and **20**, it was evident that *tert*-butyl glycine at P_3 provided better selectivity over HNE. Modification of the carbamate capping was also investigated. Use of a Boc carbamate in combination with *tert*-butyl glycine at P_3 provided an additional boost in selectivity over HNE while retaining excellent potency against the HCV protease (inhibitor **21**). We then carried out modifications aimed at optimizing the P_1 residue, and we discovered that incorporation larger moieties at P_1 provided an improvement in elastase selectivity (inhibitors **22**, **23**, and **24**). In addition to excellent selectivity over HNE, modification of the P_1 substitution revealed that incorporation of a cyclopropyl alanine at P_1 also provided a real boost in activity. Thus, inhibitor **24** exhibited an excellent enzyme potency ($K_i^* = 3.8 \pm 0.4$ nM, $n = 18$) and was also a potent inhibitor of HCV subgenomic RNA replication in vitro with an averaged IC_{90} of 100 nM. Prolonged treatment of replicon cells with **24** reduced HCV RNA by 4 logs.[19] More recently, **24** was also reported as a potent inhibitor of the full-length genotype 2a HCV genome with EC_{50} in the submicromolar range, confirming its potential antiviral properties.[20] In addition, recent studies suggested a 'dual efficacy' for **24**. HCV virus deactivates the production of interferon regulatory factor 3 or IRF-3 (a key cellular antiviral signaling molecule) that is produced by cells to defend against infection. This new protease inhibitor could actually prevent the HCV virus from blocking the immune response. Therefore, blocking viral replication by interfering with processing of the polyprotein may also restore the responsiveness of the IRF-3 in cells.[21]

The rat and monkey pharmacokinetic properties of inhibitor **24** were also investigated, and the data is summarized in Table 3. Although **24** had low oral bioavailability in rats and monkeys, its subcutaneous pharmacokinetic profile was remarkable, with high AUC and 100% bioavailability in both species.

The X-ray crystal structure of **24** bound to HCV NS3/NS4A protease was obtained (Figure 2). As anticipated, the P_1 (*S*)-diastereomer was the active component as observed in the crystal structure. A reversible covalent bond was formed between the enzyme active site serine (Ser139) hydroxyl and the ketone carbonyl of the inhibitor. The resulting oxygen anion was stabilized by hydrogen bonding with His57. The core of **24** binds to the protease through a series of hydrogen-bonding interactions. The P_3 *tert*-butyl glycine makes hydrophobic contact with the S3 pocket. The NH of the P_3 carbamate and the carbonyl at P_3 make H bonds with Ala-157. The P_2 dimethyl-cyclopropyl proline adopts a bent conformation, placing the two methyl groups in close proximity to Arg-155. The cyclopropyl alanine residue at P_1 fits well in the shallow hydrophobic S_1 pocket. The P_1' glycine moiety does not H bond with the enzyme backbone but allows the P_1-P_2' residues to form a "C-clamp" that wraps around the side chain of lysine 136 for improved overall binding.

Table 3. Full Rat and Monkey Pharmacokinetic Properties of Inhibitor **24**[a]

	rat	monkey
AUC (PO)	0.35	0.03
C_{max} (PO)	0.12	0.03
AUC (IV)	4.7	3.4
$t_{1/2}$ (IV)	5.2	5.3
Cl	24	6.9
F (PO)	4	1
AUC (SC)	19.5	5.6
C_{max}(SC)	4.37	1.6
F (SC)	100	100

[a] AUC, μM h; C_{max}, μM; $t_{1/2}$, h; Cl, mL/min/kg; F, %; SC, subcutaneous; rat SC and PO were dosed at 10 mpk, IV was dosed at 5 mpk. Monkey SC and IV were dosed at 1 mpk and PO at 3 mpk. Vehicle: IV (40% HPBCD); SC (20% HPBCD + 0.3% NaCl); PO (0.4% MC).

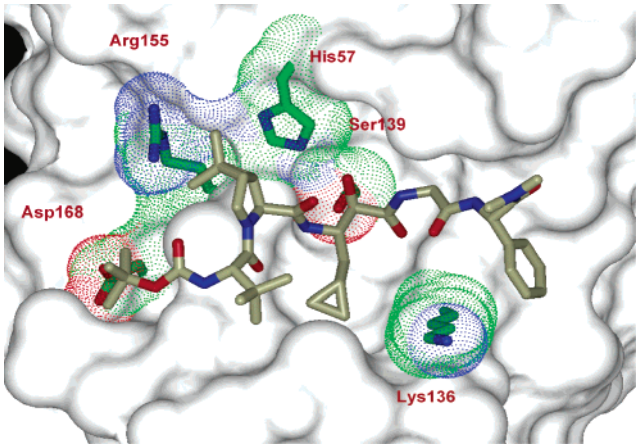


Figure 2. X-ray structure of **24** bound to the protease.

Conclusion

Introduction of various modified prolines at P_2 resulted in the identification of 2,3-dimethylcyclopropyl proline, which dramatically improved the potency of our inhibitors. Optimization of the P_1 side chain led to the discovery of **24** with 80-fold improvement in potency over our earlier P_3-capped inhibitors. In addition to excellent enzyme potency, **24** was also a potent inhibitor of HCV subgenomic RNA replication in vitro and the full-length genotype 2a HCV genome. Its subcutaneous pharmacokinetic profile was remarkably high in rats and monkeys with 100% bioavailability in both species. Modifications that aimed at improving the oral pharmacokinetic properties of Sch 446211 culminated in the identification of our clinical candidate Sch 503034 and will be reported shortly.

Experimental Section

General Methods. Reagents and solvents, including anhydrous THF, dichloromethane, and DMF, were purchased from Aldrich or other commercial sources and used without further purification. Reactions that were moisture sensitive or using anhydrous solvents were performed under either a nitrogen or an argon atmosphere. Analytical thin-layer chromatography (TLC) was performed on precoated silica gel plates obtained from Analtech. Visualization was accomplished with UV light or by staining with basic $KMnO_4$ solution, ethanolic H_2SO_4, or Vaughn's reagent. Compounds were purified by flash chromatography either on a glass column using Merck silica gel 60 (230−400 mesh) or on a Biotage disposable silica gel column. NMR spectra were recorded at 300, 400, or 500 MHz for ^{1}H and at 75, 100, or 125 MHz for ^{13}C on a Bruker or Varian spectrometer with $CDCl_3$ or DMSO-d_6 as solvent. The chemical shifts are given in ppm, referenced to the deuterated solvent signal.

2-(2-Aminoacetylamino)-*N*,*N*-dimethyl-2-(*S*)-phenylacetamide Hydrochloride (3). To a −20 °C solution of (*S*)-*N*-Boc-phenylglycine (4.50 g, 17.9 mmol,), HATU (20 mmol, 7.6 g), and dimethylamine hydrochloride (1.61 g, 19.7 mmol) in anhydrous CH_2Cl_2 (300 mL) was added DIPEA (9.35 mL, 53.7 mmol). After being stirred at this temperature for 18 h, the reaction mixture was then allowed to warm to room temperature, and EtOAc (500 mL), brine (100 mL), and 5% H_3PO_4 (100 mL) were added. After the layers were separated, the organic layer was washed with 5% H_3PO_4 (100 mL), saturated aqueous sodium bicarbonate solution (2 × 150 mL), water (150 mL), and brine (150 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo to afford 4.86 g of a white solid. 4 N HCl in dioxane (60 mL, 240 mmol) was added, and the resulting solution was stirred at room temperature. The progress of the reaction was monitored by TLC. After 4 h, the solution was concentrated in vacuo to yield 4.95 g (99%) of 2-amino-*N*,*N*-dimethyl-2-(*S*)-phenylacetamide hydrochloride as a white solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.54−7.49 (m, 5 H), 5.50 (s, 1 H), 3.00 (s, 3 H), 2.86 (s, 3 H).^{13}C NMR (DMSO-d_6, 125 MHz) δ 167.4, 132.4, 130.3, 129.8, 128.7, 55.4, 36.2, 35.3. HRMS Calcd for $C_{10}H_{15}N_2O$: 179.1184 $(M + H)^+$. Found: 179.1189. To a −20 °C solution of *N*-Boc-glycine, (1.4 g, 8 mmol), HATU (8.8 mmol, 3.37 g), and 2-amino-*N*,*N*-dimethyl-2-(*S*)-phenylacetamide hydrochloride (1.72 g, 8 mmol) in anhydrous CH_2Cl_2 (100 mL) was added DIPEA (4.18 mL, 24 mmol). After 18 h, the reaction was worked up as described above and the dipeptide was subsequently deprotected using 4 M HCl in dioxane (30 mL, 120 mmol) to provide 2.1 g (100%) of 2-(2-aminoacetylamino)-*N*,*N*-dimethyl-2-(*S*)-phenylacetamide hydrochloride (**3**) as a white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.44−7.37 (m, 5 H), 5.91 (s, 1 H), 3.78−3.69 (ABq, J_{AB} = 16 Hz, 2H), 2.99 (s, 3 H), 2.98 (s, 3 H). ^{13}C NMR (CD_3OD, 125 MHz) δ 170.5, 165.6, 136.2, 129.3, 128.8, 128.3, 67.1, 55.2, 40.5, 36.3, 35.3. HRMS Calcd for $C_{12}H_{18}N_3O_2$: 236.1399 $(M + H)^+$. Found: 236.1398.

3-*tert*-Butoxycarbonylamino-2-hydroxyhexanoic Acid (4a). To a stirred solution of 1-nitrobutane (16.5 g, 0.16 mol) and glyoxylic acid in H_2O (28.1 g, 0.305 mol) and MeOH (122 mL) at 0−5 °C was added dropwise triethylamine (93 mL, 0.667 mol) over 2 h. The solution was warmed to room temperature, stirred overnight, and concentrated to dryness to give a colorless oil. The oil was then dissolved in H_2O and acidified to pH 1 with 10% HCl, followed by extraction with EtOAc. The combined organic solution was washed with brine, dried over Na_2SO_4, filtered, and concentrated to dryness to give 2-hydroxy-3-nitrohexanoic acid (28.1 g, 99% yield). To a stirred solution of 2-hydroxy-3-nitrohexanoic acid (240 g, 1.35 mol) in acetic acid (1.25 L) was added 10% Pd/C (37 g). The resulting solution was hydrogenated at 59 psi for 3 h and then at 60 psi overnight. The acetic acid was then evaporated and azeotroped three times with toluene, then triturated with MeOH and ether. 3-Amino-2-hydroxy-hexanoic acid was separated by filtration and azeotroped twice with toluene to give an off-white solid (131 g, 0.891 mol, 66%). To a stirred solution of the amino acid (2.0 g, 13.6 mmol) in dioxane (10 mL) and H_2O (5 mL) at 0 °C was added 1 N NaOH solution (4.3 mL, 14.0 mmol). The resulting solution was stirred for 10 min, followed by addition of di-*tert*-butyl dicarbonate (3.10 g, 14.0 mmol), and stirred at 0 °C for 15 min. The solution was then warmed to room temperature, stirred for 45 min, kept in the refrigerator overnight, and concentrated to dryness to give a crude material. To the solution of this crude material in EtOAc (100 mL) and ice were added $KHSO_4$ (3.36 g) and H_2O (32 mL), and this was stirred for 4−6 min. The organic layer was then separated, and the aqueous layer was extracted twice with EtOAc, and the combined organic layers were washed with water and brine, dried (Na_2SO_4), filtered, and concentrated to dryness to yield the product **4a** as a clear gum (3.0 g, 89% yield). ^{1}H NMR (CD_3OD, 500 MHz, mixture of two diastereomers) δ 4.19 and 4.15 (d, J = 4 and 2.5 Hz, 1H), 3.98−3.95 and 3.91−3.90 (m, 1 H), 1.59−1.35 (m, 4 H), 1.46 and 1.43 (s, 9 H), 0.98 and 0.94 (t, J = 7.0 Hz, 3H). ^{13}C NMR (CD_3OD, 125 MHz, mixture of two diastereomers) δ 175.2, 174.8, 157.1, 156.9, 79.1, 73.3, 72.1, 53.2, 42.9, 34.1, 31.1, 27.8, 27.7, 19.4, 19.3, 13.2, 13.1. HRMS Calcd for $C_{11}H_{22}NO_5$: 248.1498 $(M + H)^+$. Found: 248.1510.

3-*tert*-Butoxycarbonylamino-2-hydroxyheptanoic Acid (4b). Procedure to prepare **4b** is identical to the procedure used for the preparation of **4a**, replacing in step1 1-nitrobutane with 1-nitropentane. ^{1}H NMR (DMSO-d_6, 500 MHz, mixture of two diastereomers) δ 6.51 and 6.22 (d, J = 9.1 and 9.3 Hz, 1H), 3.93 and 3.91 (d, J = 3.2 and 4.7 Hz, 1H), 3.77−3.72 and 3.71−3.63 (m, 1H), 1.51−1.39 (m, 2H), 1.38 and 1.36 (s, 9H), 1.33−1.13 (m, 4H), 0.90−0.82 (m, 3H). ^{13}C NMR (DMSO-d_6, 125 MHz, mixture of two diastereomers) δ 175.1, 175.0, 156.1, 156.0, 78.5, 78.4, 73.5, 72.5, 53.6, 53.5, 32.0, 29.2, 29.1, 29.0, 28.7, 28.6, 22.8, 22.7, 14.8, 14.7. HRMS Calcd for $C_{12}H_{24}NO_5$: 262.1654 $(M + H)^+$. Found: 262.1666.

3-*tert*-Butoxycarbonylamino-2-hydroxy-5-trifluoromethylheptanoic Acid (4c). Procedure to prepare **4c** is identical to the procedure used for the preparation of **4a**, replacing in step1 1-nitrobutane with 3-trifluoro-1-nitrobutane. ^{1}H NMR (DMSO-d_6, 500 MHz, mixture of two diastereomers) δ 6.78 and 6.46 (d, J = 8.9 and 9.1 Hz, 1H), 4.01 and 3.95 (d, J = 3.1 and 5.3 Hz, 1H), 3.86−3.81 and 3.78−3.73 (m, 1 H), 2.35−2.11 (m, 2H), 1.80−1.52 (m, 2H), 1.38 and 1.37 (s, 9H). ^{13}C NMR (DMSO-d_6, 125 MHz, mixture of two diastereomers) δ 174.7, 174.1, 156.1, 156.0, 128.5 and 128.4 (q, J = 276.4 and 275.7 Hz), 78.8, 78.7, 72.9, 72.3, 52.8, 52.6, 30.5 and 30.4 (q, J = 27.6 and 26.6 Hz), 29.0, 28.9, 22.4, 21.9. HRMS Calcd for $C_{11}H_{19}F_3NO_5$: 302.1215 $(M + H)^+$. Found: 302.1228.

3-*tert*-Butoxycarbonylamino-4-cyclopropyl-2-hydroxybutyric Acid (4d). To a −20 °C solution of Boc-(D,L)-cyclopropyl alanine (114.6 g, 0.5 mol) ((Boc-(D,L)-cyclopropyl alanine was prepared according to the procedure described ref 11b by replacing, in the alkylation step, (bromomethyl)cyclobutane with (bromomethyl)-cyclopropane)) in DCM (1 L) was added *N*,*O*-dimethylhydroxylamine hydrochloride (1.05 equiv, 51.2 g), NMM (1.05 equiv, 53.1 g) followed by EDCI (1.05 equiv, 100.6 g). The reaction was stirred at −20 °C for 2 h, and HCl (0.5 N, 500 mL) was added. The organic layer was separated and washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness under vacuo to yield 126.2 g (83%) of 1-cyclopropylmethyl-2-hydroxy-2-(methoxymethylcarbamoyl)ethyl]-carbamic acid *tert*-butyl ester. To a 0 °C solution of LAH in Et_2O (1.0 M, 2 L) was added, over 1 h, the Weinreb amide prepared above (126.2 g, 0.463 mol) in Et_2O (1 L). After 2 h of stirring at 0 °C, $NaHSO_4$ (50 g in 300 mL of water) was slowly added. The reaction was stirred for 30 min, and the solid was filtered off. The Et_2O filtrate was washed with HCl (1.0 N, 600 mL) followed by brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness under reduced pressure to yield 126 g (100%) of (2-cyclopropyl-1-formylethyl)carbamic acid *tert*-butyl ester. To a room temperature solution of aldehyde (126 g, 0.463 mol) in DCM (1 L) was added acetonecyanohydrine (0.926 mmol, 2 equiv, 79 g) and Et_3N (1.2 equiv, 56.1 g). The reaction was stirred at room temperature for 18 h; then volatiles were removed under

vacuo to yield 103 g (92%) of (2-cyano-1-cyclopropylmethyl-2-hydroxyethyl)carbamic acid *tert*-butyl ester. To a −10 °C solution of the above compound (103 g, 0.428 mol) in MeOH (1 L) was added dropwise AcCl (250 mL). After the addition the temperature was brought to 50 °C and the mixture was stirred for 18 h. The volatiles were removed under vacuo to yield 90 g (100%) of 3-amino-4-cyclopropyl-2-hydroxybutyric acid methyl ester hydrochloride. To a 0 °C solution of the above ester (90 g, 0.429 mol) in CH_3CN (1.1 L) was added Boc_2O (100 g, 0.45 mol) and DIPEA (84 g, 0.643 mol). The reaction was stirred at room temperature for 18 h. EtOAc (1 L) and saturated aqueous citric acid (500 mL) were added, and the two layers were separated. The EtOAc layer was washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness under vacuo to yield 106 g (90%) of 3-*tert*-butoxycarbonylamino-4-cyclopropyl-2-hydroxybutyric acid methyl ester that solidified upon standing. To a room temperature solution of the above methyl ester (106 g, 0.388 mol) in THF/MeOH (400 mL/400 mL) was added LiOH (33 g, 2.0 equiv in 400 mL of water). After 2 h, Et_2O (2 L) was added and the layers were separated. The aqueous layer was acidified with HCl (3 N) to pH 2 and extracted with EtOAc twice (2 × 1.5 L). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated to dryness under vacuo to yield 82.4 g (81%) of 3-*tert*-butoxycarbonylamino-4-cyclopropyl-2-hydroxybutyric acid (**4d**). ^{1}H NMR (DMSO-d_6, 500 MHz, mixture of two diastereomers) δ 6.55 and 6.22 (d, J = 8.8 and 9.8 Hz, 1H), 4.06 and 3.93 (d, J = 2.9 and 4.7 Hz, 1H), 3.88−3.83 and 3.82−3.76 (m, 1H), 1.51−1.42 (m, 2H), 1.38 and 1.36 (s, 9H), 1.33−1.23 (m, 1H), 1.10−1.05 (m, 1H), 0.67−0.31 (m, 2H), 0.45−0.27 (m, 4H), 0.10−0.05 (m, 3H), −0.08 to −0.13 (m, 1H). ^{13}C NMR (DMSO-d_6, 125 MHz, mixture of two diastereomers) δ 175.2, 175.0, 156.1, 155.8, 78.5, 78.4, 73.3, 72.0, 54.5, 54.4, 37.2, 34.8, 29.2, 29.1, 8.9, 8.8, 5.6, 5.1, 5.0, 4.8. HRMS Calcd for $C_{12}H_{22}NO_5$: 260.1498 $(M + H)^+$. Found: 260.1493.

3-Amino-4-cyclopropyl-*N*-{[(dimethylcarbamoylphenylmethyl)carbamoyl]methyl}-2-hydroxybutyramide Hydrochloride (5d). To a −20 °C solution of 3-*tert*-butoxycarbonylamino-4-cyclopropyl-2-hydroxybutyric acid (**4d**) (4.74 g, 18.3 mmol) and 2-(2-aminoacetylamino)-*N*,*N*-dimethyl-2-(*S*)-phenylacetamide hydrochloride (**3**) (4.97 g, 18.3 mmol) in DCM (100 mL) was added HATU (6.96 g, 18.3 mmol) followed by DIPEA (54.9 mmol, 9.56 mL). The reaction was gradually brought to room temperature and stirred for 18 h. EtOAc (250 mL), brine (100 mL), and 5% H_3PO_4 (100 mL) were added. After the layers were separated, the organic layer was washed with 5% H_3PO_4 (100 mL), saturated aqueous $NaHCO_3$ solution (2 × 150 mL), water (150 mL), and brine (150 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo to afford 6.81 g (78%) of 3-amino-4-cyclopropyl-*N*-{[(dimethylcarbamoylphenylmethyl)-carbamoyl]methyl}-2-hydroxybutyramide as a white solid. The above compound (5.57 g, 11.7 mmol) was stirred at room temperature in 55 mL of 4.0 N HCl in dioxane for 1 h. Et_2O (100 mL) was added, and the mixture was concentrated to deliver 5d as an off-white solid (4.82 g, 100%). ^{1}H NMR (CD_3OD, 500 MHz, mixture of four diastereomers) δ 7.39−7.34 (m, 10H), 5.86−5.84 (m, 2H), 4.37−4.34 (m, 2H), 4.19−4.14 (m, 1H), 4.03−3.84 (m, 3H), 3.64−3.58 (m, 2H), 2.96−2.94 (m, 12H), 1.77−1.70 (m, 1H), 1.60−1.55 (m, 3H), 0.84−073 (m, 2H), 0.64−0.48 (m, 4H), 0.27−0.09 (m, 4H). ^{13}C NMR (CD_3OD, 125 MHz, mixture of diastereomers) δ 173.9, 173.7, 172.7, 170.6, 169.9, 169.7, 169.3, 169.2, 136.5, 136.4, 129.2, 128.8, 128.7, 128.3, 128.2, 71.0, 70.0, 69.9, 67.1, 55.2, 55.0, 54.9, 42.0, 41.8, 36.3, 35.3, 34.4, 7.0, 6.9, 4.8, 4.1, 3.9, 3.6. HRMS Calcd for $C_{19}H_{29}N_4O_4$: 377.2189 $(M + H)^+$. Found: 377.2185.

3-Amino-2-hydroxyhexanoic Acid {[(Dimethyl Carbamoylphenylmethyl)carbamoyl]methyl}amide (5a). Synthesis of intermediate **5a** was identical to the synthesis of **5d** where 3-*tert*-butoxycarbonylamino-4-cyclopropyl-2-hydroxybutyric acid (**4d**) was replaced with 3-*tert*-butoxycarbonylamino-2-hydroxyhexanoic acid (**4a**). It was subsequently deprotected using 4 M HCl in dioxane. ^{1}H NMR (CD_3OD, 500 MHz, mixture of four diastereomers) δ 7.44−7.34 (m, 10H), 5.90−5.80 (m, 2H), 4.33−4.32 (m, 1H), 4.28−4.26 (m, 1H), 4.20−4.16 (m, 2H), 4.06−3.87 (m, 3H), 3.58−3.50 (m, 2H), 2.99−2.96 (m, 12H), 1.86−1.79 (m, 1H), 1.72−1.64 (m, 3H), 1.55−1.48 (m, 3H), 1.46−1.36 (m, 1H), 1.41 (t, J = 7.2 Hz, 3H), 1.00−0.97 (m, 3H). ^{13}C NMR (CD_3OD, 125 MHz, mixture of four diastereomers) δ 173.7, 176.5, 172.7, 170.5, 169.7, 169.3, 136.5, 136.4, 129.2, 128.8, 128.7, 128.3, 128.2, 71.1, 69.8, 55.1, 55.0, 54.9, 54.1, 41.9, 41.8, 36.3, 35.3, 31.8, 18.8, 18.7, 18.6, 13.1, 13.0. HRMS Calcd for $C_{18}H_{29}N_4O_4$: 365.2189 $(M + H)^+$. Found: 365.2185.

3-Amino-2-hydroxyheptanoic acid {[(Dimethyl Carbamoylphenylmethyl)carbamoyl]methyl}amide Hydrochloride (5b). Synthesis of intermediate 5b was identical to the synthesis of 5d where 3-*tert*-butoxycarbonylamino-4-cyclopropyl-2-hydroxybutyric acid (**4d**) was replaced with 3-*tert*-butoxycarbonylamino-2-hydroxyheptanoic acid (**4b**). It was subsequently deprotected using 4 M HCl in dioxane. ^{1}H NMR (CD_3OD, 400 MHz, mixture of four diastereomers) δ 7.42−7.37 (m, 10H), 5.89−5.87 (m, 2H), 4.29−4.24 (m, 2H), 4.17−4.13 (m, 1H), 4.03−3.83 (m, 4H), 3.75−3.68 (m, 1H), 3.54−3.48 (m, 2H), 3.25−3.15 (m, 1H), 2.96 and 2.94 (s, 6H), 1.83−1.77 (m, 1H), 1.69−1.60 (m, 3H), 1.43−1.36 (m, 8H), 0.99−0.93 (m, 6H). ^{13}C NMR (CD_3OD, 125 MHz, mixture of four diastereomers) δ 172.7, 169.2, 136.4, 129.1, 128.8, 128.7, 128.2, 71.1, 69.7, 54.9, 54.8, 54.5, 54.3, 41.9, 41.8, 36.3, 35.3, 29.4, 27.6, 27.5, 22.4, 17.7, 16.3, 13.1. HRMS Calcd for $C_{19}H_{31}N_4O_4$: 379.2345 $(M + H)^+$. Found: 379.2330.

3-Amino-6,6,6-trifluoro-2-hydroxyhexanoic Acid {[(Dimethylcarbamoylphenylmethyl)carbamoyl]methyl}amide Hydrochloride (5c). Synthesis of intermediate 5c was identical to the synthesis of 5d where 3-*tert*-butoxycarbonylamino-4-cyclopropyl-2-hydroxybutyric acid (**4d**) was replaced with 3-*tert*-butoxycarbonylamino-2-hydroxy-5-trifluoromethylheptanoic acid (**4c**). It was subsequently deprotected using 4 M HCl in dioxane. ^{1}H NMR (DMSO-d_6, 500 MHz, mixture of four diastereomers) δ 8.72 and 8.58 (dd, J = 13.9 and 7.8 Hz and J = 7.6 and 5.9 Hz, 1H), 8.29−8.24 and 8.11−8.04 (m, 3 H), 7.40−7.30 (m, 5H), 6.80 and 6.70 (t, J = 5.9 and 5.0 Hz, 1H), 5.84−5.81 (m, 1H), 4.32 and 4.21 (bs, 1H), 3.88−3.67 (m, 2H), 3.52−3.47 (m, 1H), 2.93 (bs, 3H), 2.85 (bs, 3H), 2.48−2.37 (m, 2H), 1.85−1.74 (m, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz, mixture of four diastereomers) δ 172.0, 171.6, 171.5, 170.2, 170.1, 170.0, 168.9, 168.5, 138.5, 138.3, 129.5, 128.8, 128.7, 128.6, 71.3, 54.0, 53.9, 53.8, 52.8, 42.6, 42.4, 37.5, 36.2, 30.1, (q, J = 29.0 Hz), 22.6, 20.9. HRMS Calcd for $C_{18}H_{26}F_3N_4O_4$: 419.1906 $(M + H)^+$. Found: 419.1910.

1,1-Dimethylethyl [1(*S*)-[[(1*R*,5*S*)-2(*S*)-[[[1-(Cyclopropylmethyl)-3-[[2-[[2-(dimethylamino)-2-oxo-1(*S*)-phenylethyl]-amino]-2-oxoethyl]amino]-2,3-dioxopropyl]amino]carbonyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexan-3-yl]carbonyl]-2,2-dimethylpropyl]carbamate (24-SCH6). To a −20 °C solution of 3-(2-*tert*-butoxycarbonylamino-3,3-dimethylbutyryl)-6,6-dimethyl-3-aza-bicyclo[3.1.0]hexane-2-carboxylic acid (0.2 mmol, 74 mg) and 3-amino-4-cyclopropyl-*N*-{[(dimethylcarbamoylphenylmethyl)-carbamoyl]methyl}-2-hydroxybutyramide hydrochloride (0.2 mmol, 82 mg) (**5d**) in CH_2Cl_2 (5 mL) was added HATU (1.2 equiv, 0.24 mmol, 91 mg) followed by DIPEA (3 equiv, 0.6 mmol, 0.1 mL). After being stirred at this temperature for 18 h, the reaction mixture was then allowed to warm to room temperature, and EtOAc (25 mL) and water (20 mL) were added. After the layers were separated, the organic layer was washed with 5% H_3PO_4 (20 mL), saturated aqueous $NaHCO_3$ solution (2 × 20 mL), and brine (20 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The intermediate product, α-hydroxyamide, was obtained as a mixture of diastereomers (0.145 g, 0.2 mmol, 100% yield), which was used in subsequent reactions without further purification. To the solution of this product (0.145 g, 0.2 mmol) in anhydrous CH_2Cl_2 (20 mL) at room temperature was added Dess−Martin reagent (0.21 g, 0.5 mmol). The mixture was stirred for 3 h. Saturated $NaHCO_3$ and $Na_2S_2O_3$ solutions (20 mL each) were added. After stirring for 10 min, the layers were separated. The aqueous solution was extracted with EtOAc (2 × 50 mL). The organic solutions were combined, dried with magnesium sulfate, filtered, and concentrated in vacuo. Flash chromatography (20−80% EtOAc/hexane) afforded 24 (86

mg, 0.118 mmol, 86% yield) as a mixture of two diastereomers. ^{1}H NMR (DMSO-d_6, 500 MHz, mixture of two diastereomers) δ 8.77−8.73 (m, 1H), 8.56 (t, J = 7.8 Hz, 1H), 8.46 and 8.39 (d, J = 7.0 and 7.6 Hz, 1H), 7.39−7.30 (m, 5H), 6.63−6.59 (m, 1H), 5.82 (d, J = 7.8 Hz, 1H), 5.15−5.11 and 5.07−5.03 (m, 1H), 4.36 and 4.35 (bs, 1H), 4.05−4.01 (m, 1H), 3.90−3.74 (m, 4H), 2.92 (s, 3H), 2.85 (s, 3H), 1.72−1.64 and 1.61−1.58 (m, 2H), 1.47−1.39 (m, 2H), 1.36 (s, 9H), 0.98 and 0.95 (s, 3H), 0.91 (s, 9H), 0.88 and 085 (s, 3H), 0.80−0.74 (m, 1H), 0.43−0.34 (m, 2H), 0.16−0.09 (m, 1H), 0.08−0.01 (m, 1H). ^{13}C NMR (DMSO-d_6, 125 MHz, mixture of two diastereomers) δ 197.6, 196.9, 171.9, 171.6, 170.8, 170.6, 170.1, 167.8, 161.7, 161.3, 156.8, 138.4, 129.5, 128.7, 79.0, 60.3, 60.1, 59.6, 55.3, 55.1, 53.9, 53.8, 48.4, 46.5, 42.4, 37.5, 36.2, 35.8, 34.9, 31.6, 29.0, 28.9, 27.8, 27.2, 27.1, 27.0, 19.5, 13.4, 13.3, 12.4, 12.2, 8.8, 8.6, 5.9, 5.2, 4.9. HRMS Calcd for $C_{38}H_{57}N_6O_8$: 725.4238 (M + H)$^+$. Found: 725.4231.

***N*-[(2-Methylpropoxy)carbonyl]cyclohexylglucyl-*N*-[1-[2-[[2-[[2-(dimethylamino)-2-oxo-1(*S*)-phenylethyl]amino]-2-oxoethyl]-amino]-1,2-dioxoethyl]butyl]prolinamide (9).** Coupling and oxidation procedures for the preparation of **9** were carried out in a manner similar to that described above for the preparation of **24**. ^{1}H NMR (DMSO-d_6, 500 MHz, mixture of two diastereomers) δ 8.77−8.52 and 8.04−8.01 (m, 3H), 7.50−7.24 (m, 6H), 5.82 (d, J = 7.6 Hz, 1H), 5.09−4.88 (m, 1H), 4.38−4.29 (m, 1H), 4.06 and 4.00 (t, J = 7.3 Hz and J = 8.8 Hz, 1H), 3.88−3.69 (m, 4H), 3.67−3.59 (m, 1H), 3.56−3.48 (m, 1H), 2.93 (s, 3H), 2.85 (s, 3H), 2.05−1.08 (m, 20H), 0.98−0.80 (m, 9H). ^{13}C NMR (DMSO-d_6, 125 MHz, mixture of two diastereomers) δ 197.5, 197.4, 172.4, 172.1, 171.4, 171.0, 170.1, 167.8, 161.6, 157.7, 157.5, 138.4, 129.5, 128.7, 70.9, 70.8, 68.2, 60.4, 59.7, 58.2, 54.3, 53.9, 53.8, 47.8, 42.5, 40.9, 39.9, 39.8, 37.5, 36.2, 32.4, 30.4, 29.8, 29.5, 29.3, 28.5, 26.7, 26.5, 26.3, 24.8, 24.6, 23.7, 19.7, 19.4, 14.4, 14.2. HRMS Calcd for $C_{36}H_{55}N_6O_8$: 753.4163 (M + H)$^+$. Found: 753.4133.

***N*-[(2-Methylpropoxy)carbonyl]-(*S*)-*tert*-leucyl-*N*-[1(S)-[2-[[2-[[2-(dimethylamino)-2-oxo-1-phenylethyl]amino]-2-oxoethyl]-amino]-1,2-dioxoethyl]butyl]-4(*R*)-methyl-(*S*)-prolinamide (10).** Coupling and oxidation procedures for the preparation of **10** were carried out in a manner similar to that described for the preparation of **24**. ^{1}H NMR (DMSO-d_6, 500 MHz, mixture of two diastereomers) δ 8.75−8.69 (m, 1H), 8.57 (t, J = 7.9 Hz, 1H), 8.21 and 8.20 (d, J = 6.9 and 7.0 Hz, 1H), 7.41−7.28 (m, 5H), 6.95 (d, J = 8.8 Hz, 1H), 5.83 (d, J = 7.6 Hz, 1H), 5.08−4.96 (m, 1H), 4.37−4.30 (m, 1H), 4.19−4.11 (m, 2H), 4.03−3.95 (m, 1H), 3.87 3.66 (m, 4H), 2.93 (s, 3H), 2.85 (s, 3H), 2.33−2.22 (m, 1H), 2.20−2.09 (m, 1H), 1.83 (sept, J = 6.6 Hz, 1H), 1.73−1.66 (m, 1H), 1.54−1.19 (m, 4H), 1.01 (d, J = 6.6 Hz, 3H), 0.94−0.86 (m, 18H). ^{13}C NMR (DMSO-d_6, 125 MHz, mixture of two diastereomers) δ 197.4, 174.1, 172.5, 172.4, 170.3, 170.1, 167.9, 167.8, 161.9, 161.5, 157.5, 138.4, 135.3, 133.3, 132.6, 132.5, 132.0, 130.9, 129.5, 129.4, 128.7, 70.8, 68.3, 60.9, 60.5, 60.1, 60.0, 56.0, 54.0, 53.9, 42.5, 42.4, 40.9, 39.9, 38.9, 38.1, 37.5, 36.2, 35.6, 34.1, 32.8, 32.6, 30.7, 29.2, 28.5, 27.3, 27.2, 24.1, 23.3, 19.8, 19.5, 19.4, 17.4, 14.8, 14.4, 14.3, 11.7. HRMS Calcd for $C_{35}H_{55}N_6O_8$: 687.4081 (M + H)$^+$. Found: 687.4061.

2-Methylpropyl [1-Cyclohexyl-2-[2-[8(*S*)-[[[1-[2-[[2-[[2-(dimethylamino)-2-oxo-1-phenylethyl]amino]-2-oxoethyl]amino]-1,2-dioxoethyl]butyl]amino]carbonyl]-1,4-dithia-7-azaspiro[4.4]-nonan-7-yl]-2-oxoethyl]carbamate (13). Coupling procedures for the preparation of **13** were carried out in a manner similar to that described for the preparation of **24**. For the oxidation of the intermediate hydroxyl amide to the corresponding ketoamide, the Moffat procedure was used (ref 14). ^{1}H NMR (DMSO-d_6, 500 MHz, mixture of two diastereomers) δ 8.77−8.72 (m, 1H), 8.57 (d, J = 7.5 Hz, 1H), 8.32 and 8.13 (d, J = 6.7 and 7.9 Hz, 1H), 7.38−7.23 (m, 6H), 5.83 (d, J = 7.8 Hz, 1H), 5.04−4.96 (m, 1H), 4.45 and 4.38 (t, 1H), 4.31−4.26 (m, 1H), 4.03−3.99 (m, 1H), 3.88−3.71 (m, 4H), 3.43−3.32 (m, 5H), 2.93 (s, 3H), 2.85 (s, 3H), 2.60−2.55 (m, 1H), 2.35−2.25 (m, 1H), 1.87−1.79 (m, 1H), 1.75−1.54 (m, 7H), 1.46−1.34 (m, 3H), 1.13−1.07 (m, 3H), 0.96−0.84 (m, 11H). ^{13}C NMR (DMSO-d_6, 125 MHz, mixture of two diastereomers) δ 196.50, 196.38, 170.14, 170.08, 169.99, 169.80, 169.15, 166.81, 160.66, 160.55, 156.39, 156.32, 137.42, 128.49, 127.75, 127.70, 127.69, 69.82, 69.77, 68.40, 66.90, 66.87, 61.24, 59.95, 59.10, 56.89, 55.73, 53.39, 52.93, 52.91, 44.09, 41.51, 41.44, 38.47, 38.44, 36.49, 35.26, 35.25, 32.00, 31.69, 31.48, 29.50, 28.48, 28.46, 27.89, 27.56, 25.75, 25.43, 25.41, 25.33, 18.79, 18.61, 18.54, 13.38. HRMS Calcd for $C_{38}H_{57}N_6O_8S_2$: 789.3679 (M + H)$^+$. Found: 789.3678.

2-Methylpropyl [1-Cyclohexyl-2-[3(*S*)-[[[1-[2-[[2-[[2-(Dimethylamino)-2-oxo-1-phenylethyl]amino]-2-oxoethyl]amino]-1,2-dioxoethyl]butyl]amino]carbonyl]-6,10-dithia-2-azaspiro[4.5]-decan-2-yl]-2-oxoethyl]carbamate (14). Coupling procedures for the preparation of **14** were carried out in a manner similar to that described for the preparation of **24**. For the oxidation of the intermediate hydroxyl amide to the corresponding ketoamide, the Moffat procedure was used. ^{1}H NMR (DMSO-d_6, 500 MHz, mixture of two diastereomers) δ 8.75 (q, J = 5.9 Hz, 1H), 8.56 and 8.55 (d, J = 7.7 and 7.5 Hz, 1H), 8.46 and 8.31 (d, J = 7.9 and 6.9 Hz, 1H), 7.39−7.26 (m, 6 H), 5.83 and 5.82 (d, J = 7.6 and 7.7 Hz, 1H), 5.02−4.95 (m, 1H), 4.71 and 4.63 (d, J = 11.0 and 11.3 Hz, 1H), 4.52−4.46 (m, 1H), 4.07 (t, J = 8.9 Hz, 1H), 3.86−3.57 (m, 5H), 3.08−2.97 (m, 2H), 2.92 (s, 3H), 2.89−2.82 (m, 1H), 2.84 (s, 3H), 2.63−2.54 (m, 1H), 2.02−1.90 (m, 2 H), 1.84−1.58 (m, 10 H), 1.47−1.23 (m, 3 H), 1.11−1.06 (m, 3 H), 0.89−0.76 (m, 11 H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 196.5, 196.4, 170.4, 170.31, 170.27, 170.2, 170.1, 169.2, 166.8, 160.7, 160.6, 156.45, 156.35, 137.4, 128.5, 128.3, 127.8, 127.71, 127.68, 127.65, 127.6, 69.9, 69.8, 69.7, 59.8, 59.7, 59.6, 58.8, 58.0, 56.9, 55.7, 53.4, 53.1, 53.0, 52.95, 52.94, 52.86, 52.1, 42.59, 42.58, 42.55, 41.55, 41.47, 38.7, 36.5, 35.27, 35.26, 31.7, 31.5, 29.5, 28.6, 28.33, 28.29, 28.2, 27.6, 26.6, 25.84, 25.80, 25.5, 25.4, 24.8, 20.7, 18.8, 18.64, 18.58, 14.0, 13.42, 13.40. HRMS Calcd for $C_{39}H_{59}N_6O_8S_2$: 803.3836 (M + H)$^+$. Found: 803.3835.

2-Methylpropyl [1-Cyclohexyl-2-[2-[[[1-[2-[[2-[[2-(dimethylamino)-2-oxo-1-phenylethyl]amino]-2-oxoethyl]amino]-1,2-dioxoethyl]butyl]amino]carbonyl]-4(*S*)-[(1,1-dimethylethyl)thio]-1-pyrrolidinyl]-2-oxoethyl]carbamate (15). Coupling procedures for the preparation of **15** were carried out in a manner similar to that described for the preparation of **24**. For the oxidation of the intermediate hydroxyl amide to the corresponding ketoamide, the Moffat procedure was used. ^{1}H NMR (DMSO-d_6, 500 MHz, mixture of two diastereomers) δ 8.76−8.72 (m, 1H), 8.57 (d, J = 7.5 Hz, 1H), 8.25 and 8.01 (d, J = 7.0 and 7.9 Hz, 1H), 7.38−7.29 (m, 6H), 5.83 (d, J = 7.5 Hz, 1H), 5.06−5.01 and 4.97−4.93 (m, 1H), 4.51−4.48 and 4.45−4.43 (m, 1H), 4.08−3.96 (m, 2H), 3.87−3.68 (m, 4H), 3.49−3.37 (m, 4H), 2.93 (s, 3H), 2.85 (s, 3H), 2.27−2.22 and 2.19−2.14 (m, 1H), 1.96−1.90 (m, 1H), 1.85−1.57 (m, 9H), 1.32 and 1.31 (s, 9H), 1.30−1.28 (m, 1H), 1.12−1.10 (m, 2H), 0.89−0.86 (m, 11H). ^{13}C NMR (DMSO-d_6, 125 MHz, mixture of diastereomers) δ 196.47, 196.30, 171.14, 171.00, 169.98, 169.15, 166.83, 160.64, 156.42, 156.35, 137.42, 137.41, 128.50, 127.76, 127.70, 127.69, 69.80, 69.77, 68.40, 59.15, 58.42, 56.82, 56.71, 55.73, 54.07, 53.53, 53.31, 52.93, 52.91, 42.92, 42.89, 41.50, 41.47, 38.06, 37.88, 37.67, 36.50, 35.26, 32.01, 31.53, 31.30, 31.15, 31.14, 31.08, 29.50, 28.92, 28.52, 28.22, 28.09, 27.56, 25.80, 25.60, 25.57, 25.44, 18.80, 18.67, 18.60, 13.39, 13.35. HRMS Calcd for $C_{40}H_{63}N_6O_8S$: 787.4428 (M + H)$^+$. Found: 787.4437.

***N*-[(2-Methylpropoxy)carbonyl]-(*S*)-*tert*-leucyl-*n*-[1-[2-[[2-[[2-(dimethylamino)-2-oxo-1(*S*)-phenylethyl]amino]-2-oxoethyl]amino]-1,2-dioxoethyl]butyl]-4(*R*)-(1,1-dimethylethoxy)-(*S*)-prolinamide (16).** Coupling and oxidation procedures for the preparation of **16** were carried out in a manner similar to that described for the preparation of **24**. ^{1}H NMR (DMSO-d_6, 500 MHz, mixture of two diastereomers) δ 8.73 (t, J = 6.0 Hz, 1H), 8.57 (t, J = 7.9 Hz, 1H), 8.26 and 8.15 (d, J = 7.9 and 6.9 Hz, 1H), 7.38−7.29 (m, 5H), 7.11 and 7.04 (d, J = 9.1 and 9.5 Hz, 1H), 5.82 (d, J = 7.6 Hz, 1H), 5.03−4.95 (m, 1H), 4.49−4.41 (m, 1H), 4.35−4.30 (m, 1H), 4.18−4.14 (m, 1H), 3.83−3.79 (m, 2H), 3.77−3.70 (m, 4H), 2.93 (s, 3H), 2.85 (s, 3H), 1.97−1.91 (m, 2H), 1.86−1.79 (m, 1H), 1.72−1.66 (m, 1H), 1.46−1.23 (m, 3H), 1.15−1.13 (m, 9H), 0.92−0.95 (m, 9H), 0.90−0.82 (m, 9H). ^{13}C NMR (DMSO-d_6, 125 MHz, mixture of two diastereomers) δ 197.6, 172.4, 172.2, 170.1, 167.8,

161.5, 157.5, 157.4, 138.4, 129.5, 128.7, 110.0, 90.8, 74.3, 70.8, 70.3, 70.1, 59.7, 59.0, 58.7, 55.8, 54.2, 53.9, 42.5, 42.4, 40.9, 39.9, 38.2, 38.1, 37.5, 37.1, 36.2, 35.6, 28.9, 28.5, 27.2, 27.1, 19.8, 19.5, 19.4, 14.3. HRMS Calcd for $C_{38}H_{61}N_6O_9$: 745.4495 $(M + H)^+$. Found: 745.4500.

2-Methylpropyl [1(*S*)-[[(3a*R*,6a*R*)-4(*S*)-[[[1-[2-[[2-[[2-(Dimethylamino)-2-oxo-1(*S*)-phenylethyl]amino]-2-oxoethyl]amino]-1,2-dioxoethyl]butyl]amino]carbonyl]hexahydro-2,2-dimethyl-5*H*-furo[2,3-*c*]pyrrol-5-yl]carbonyl]-2,2-dimethylpropyl]carbamate (17). Coupling and oxidation procedures for the preparation of **17** were carried out in a manner similar to that described for the preparation of **24**. ^{1}H NMR (DMSO-d_6, 500 MHz, mixture of two diastereomers) δ 8.73 and 8.70 (t, J = 6.3 and 6.3 Hz, 1H), 8.58−8.54 (m, 1H), 8.39−8.35 (m, 1H), 7.38−7.28 (m, 5H), 7.00−6.97 (m, 1H), 5.82 (d, J = 7.9 Hz, 1H), 5.06−5.01 and 4.95−4.93 (m, 1H), 4.56−4.54 (m, 1H), 4.47−4.41 (m, 1H), 4.17 (d, J = 9.1 Hz, 1H), 4.12−4.09 (m, 1H), 3.89−3.83 (m, 1H), 3.82−3.79 (m, 1H), 3.78−3.71 (m, 1H), 3.18 (d, J = 5.0 Hz, 1H), 2.97 (s, 1H), 2.93 (s, 3H), 2.85 and 2.86 (s, 3H), 2.07−1.98 (m, 1H), 1.87−1.77 (m, 1H), 1.75−1.43 (m, 3H), 1.41−1.26 (m, 3H), 1.23 (d, J = 9.1 Hz, 3H), 1.14 (d, J = 5.4 Hz, 3H), 0.92 (s, 9H), 0.89−0.89 (m, 9H). ^{13}C NMR (DMSO-d_6, 125 MHz, mixture of two diastereomers) δ 197.6, 197.2, 170.7, 170.1, 167.8, 161.9, 161.5, 157.3, 138.4, 129.5, 128.7, 83.4, 82.0, 70.8, 65.7, 59.6, 59.5, 55.3, 55.3, 54.2, 54.1, 53.9, 49.5, 48.3, 44.7, 44.6, 42.5, 42.4, 37.5, 36.2, 35.7, 32.5, 29.7, 28.6, 28.6, 27.2, 27.2, 27.1, 19.7, 19.5, 19.5, 14.4, 14.3. HRMS Calcd for $C_{38}H_{59}N_6O_9$: 743.4344 $(M + H)^+$. Found: 743.4324.

2-Methylpropyl [1(*S*)-[[(3a*R*,6a*S*)-1(*S*)-[[[1-[2-[[2-[[2-(Dimethylamino)-2-oxo-1(*S*)-phenylethyl]amino]-2-oxoethyl]amino]-1,2-dioxoethyl]butyl]amino]carbonyl]hexahydro-5,5-dimethylcyclopenta[*c*]pyrrol-2(1*H*)-yl]carbonyl]-2,2-dimethylpropyl]carbamate (18). Coupling and oxidation procedures for the preparation of **18** were carried out in a manner similar to that described for the preparation of **24**. ^{1}H NMR (DMSO-d_6, 500 MHz, mixture of two diastereomers) δ 8.78−8.69 (m, 1H), 8.61−8.54 (m, 1H), 8.24 and 8.20 (d, J = 7.6 and 6.9 Hz, 1H). 7.40−7.29 (m, 5H), 7.07 and 7.03 (d, J = 8.8 and 9.1 Hz, 1H), 5.82 (d, J = 7.6 Hz, 1H), 4.97−4.92 and 5.04−4.98 (m, 1H), 4.38 (d, J = 10.7 Hz, 1H), 4.20−4.14 (m, 1H), 3.84−3.67 (m, 6H), 2.93 (s, 3H), 2.85 (s, 3H), 2.79−2.72 (m, 1H), 2.68−2.59 (m, 1H), 1.90−1.60 (m, 4H), 1.19−1.51 (m, 5H), 1.30 (d, J = 9.5 Hz, 3H), 0.88−0.95 (m, 12H), 0.82−0.89 (m, 9H). ^{13}C NMR (DMSO-d_6, 125 MHz, mixture of two diastereomers) δ 197.6, 197.3, 172.7, 172.4, 170.1, 167.8, 161.8, 161.5, 157.5, 138.4, 129.5, 128.7, 128.6, 71.3, 70.7, 65.8, 59.7, 59.6, 54.4, 54.3, 54.1, 53.9, 53.8, 47.7, 47.5, 47.4, 47.3, 47.2, 43.0, 42.9, 42.5, 42.4, 41.6, 37.5, 36.2, 36.2, 35.3, 35.1, 32.5, 32.4, 30.2, 29.0, 28.6, 27.3, 27.2, 27.1, 25.7, 19.8, 19.7, 19.6, 19.5, 14.4, 14.3. HRMS Calcd for $C_{39}H_{60}N_6NaO_8$: 763.4370 $(M + Na)^+$. Found: 763.4394.

2-Methylpropyl [1(*S*)-Cyclohexyl-2-[2(*S*)-[[[1-[2-[[2-[[2-(dimethylamino)-2-oxo-1(*S*)-phenylethyl]amino]-2-oxoethyl]amino]-1,2-dioxoethyl]butyl]amino]carbonyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hex-3-yl]-2-oxoethyl]carbamate (19). Coupling and oxidation procedures for the preparation of **19** were carried out in a manner similar to that described for the preparation of **24**. ^{1}H NMR (DMSO-d_6, 500 MHz, mixture of two diastereomers) δ 8.78−8.71 (m, 1H), 8.60−8.57 (m, 1H), 8.39 and 8.21 (d, J = 7.0 and 7.6 Hz, 1H), 7.38−7.36 (m, 6H), 5.82 (d, J = 7.9 Hz, 1H), 5.03−4.98 (m, 1H), 4.28 and 4.25 (s, 1H), 3.94−3.65 (m, 7H), 2.93 (bs, 3H), 2.86 and 2.85 (s, 3H), 1.79−1.00 (m, 18H), 0.89−0.84 (m, 15H).^{13}C NMR (DMSO-d_6, 125 MHz, mixture of two diastereomers) δ 197.8, 197.3, 171.9, 171.1, 170.9, 170.8, 170.1, 167.8, 161.6, 161.5, 157.4, 138.4, 129.5, 128.7, 70.6, 70.6, 60.8, 60.3, 58.1, 58.0, 54.2, 54.1, 53.9, 47.8, 47.7, 42.5, 42.4, 39.6, 39.4, 37.4, 36.2, 35.1, 32.7, 32.4, 31.7, 31.6, 29.5, 29.4, 28.6, 27.8, 27.7, 27.0, 26.9, 26.8, 26.5, 26.4, 26.3, 19.7, 19.6, 19.5, 14.4, 14.3, 13.5, 13.4. HRMS Calcd for $C_{39}H_{58}N_6NaO_8$: 761.4220 $(M + Na)^+$. Found: 761.4214.

2-Methylpropyl [1(*S*)-[[(1*R*,5*S*)-2-[[[1-[2-[[2-[[[2-(Dimethylamino)-2-oxo-1(*S*)-phenylethyl]amino]-2-oxoethyl]amino]-1,2-dioxoethyl]butyl]amino]carbonyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexan-3-yl]carbonyl]-2,2-dimethylpropyl]carbamate (20). Coupling and oxidation procedures for the preparation of **20** were carried out in a manner similar to that described for the preparation of **24**. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.75 (t, J = 6.5 Hz, 1H), 8.58 (d, J = 8.0 Hz, 1H), 8.40 (d, J = 7.0 Hz, 1H), 7.37−7.31 (m, 5H), 7.03 (d, J = 8.5 Hz, 1H), 5.83 (d, J = 8.0 Hz, 1H), 5.06−5.02 (m, 1H), 4.32 (s, 1H), 4.05 (d, J = 9.0 Hz, 1H), 3.88−3.67 (m, 6H), 2.93 (s, 3H), 2.85 (s, 3H), 1.83−1.80 (m, 1H), 1.73−1.67 (m, 1H) 1.50−1.32 (m, 5H), 1.03 (s, 3H), 0.93 (s, 9H), 0.87 (s, 3H), 0.85 (d, J = 7.5 Hz, 6H), 0.86 (t, 3H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 197.8, 172.0, 170.1, 167.8, 161.5, 157.6, 138.4, 129.5, 128.7, 70.7, 60.2, 60.1, 48.4, 42.4, 37.5, 36.2, 35.2, 32.4, 31.6, 28.9, 28.6, 27.9, 27.3, 27.0, 19.7, 19.6, 19.5, 14.3, 13.4. HRMS Calcd for $C_{37}H_{57}N_6O_8$: 713.4238 $(M + H)^+$. Found: 713.4238.

1,1-Dimethylethyl [1(*S*)-[[(1*R*,5*S*)-2-[[[1-[2-[[2-[[[2-(Dimethylamino)-2-oxo-1(*S*)-phenylethyl]amino]-2-oxoethyl]amino]-1,2-dioxoethyl]butyl]amino]carbonyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexan-3-yl]carbonyl]-2,2-dimethylpropyl]carbamate (21). Coupling and oxidation procedures for the preparation of **21** were carried out in a manner similar to that described for the preparation of **24**. ^{1}H NMR (DMSO-d_6, 500 MHz, mixture of two diastereomers) δ 8.77−8.71 (m, 1H), 8.59−8.54 (m, 1H), 8.39 and 8.29 (d, J = 6.8 and 7.7 Hz, 1H), 7.39−7.36 (m, 5H), 6.63−6.58 (m, 1H), 5.82 (d, J = 7.5 Hz, 1H), 5.05−4.91 (m, 1H), 4.33 (bs, 1H), 4.07−4.00 (m, 1H), 3.89−3.76 (m, 4H), 2.93 (bs, 3H), 2.85 (bs, 3H), 1.93−1.85 (m, 1H), 1.51−1.25 (m, 14H), 1.05−0.85 (m, 18H).^{13}C NMR (DMSO-d_6, 125 MHz, mixture of two diastereomers) δ 197.8, 197.1, 172.0, 170.6, 170.1, 167.8, 161.5, 156.8, 138.4, 129.5, 128.7, 79.0, 68.2, 60.4, 60.1, 59.7, 54.3, 54.1, 53.9, 48.4, 42.5, 37.5, 36.2, 34.9, 33.2, 32.5, 31.6, 28.9, 27.9, 27.8, 27.3, 27.0, 23.7, 19.6, 19.5, 14.4, 14.3, 13.4. HRMS Calcd for $C_{37}H_{57}N_6O_8$: 713.4238 $(M + H)^+$. Found: 713.4246.

1,1-Dimethylethyl [1(*S*)-[[(1*R*,5*S*)-2-[[[1-[2-[[2-[[2-(Dimethylamino)-2-oxo-1(*S*)-phenylethyl]amino]-2-oxoethyl]amino]-1,2-dioxoethyl]pentyl]amino]carbonyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexan-3-yl]carbonyl]-2,2-dimethylpropyl]carbamate (22). Coupling and oxidation procedures for the preparation of **22** were carried out in a manner similar to that described for the preparation of **24**. ^{1}H NMR (DMSO-d_6, 500 MHz, mixture of two diastereomers) δ, 8.77−8.68 (m, 1H), 8.58−8.50 (m, 1H), 8.38 and 8.26 (d, J = 7.0 and 7.2 Hz, 1H), 7.44−7.27 (m, 5H), 6.61 and 6.57 (d, J = 9.0 and 9.5 Hz, 1H), 5.82 (d, J = 8.0 Hz, 1H), 5.04−4.90 (m, 1H), 4.33 and 4.17(d, J = 7.2 and 15.2 Hz, 1H), 4.07−4.01 (m, 1H), 3.92−3.74 (m, 4H), 2.93 (bs, 3H), 2.85 (bs, 3H), 1.93−1.85 (m, 1H), 1.51−1.25 (m, 16H), 1.03−0.81 (m, 18H).^{13}C NMR (DMSO-d_6, 125 MHz, mixture of two diastereomers) δ 197.8, 197.1, 172.0, 171.8, 170.8, 170.6, 170.1, 167.8, 161.9, 161.5, 156.7, 138.4, 129.5, 128.7, 79.0, 60.5, 60.1, 59.7, 54.4, 54.3, 53.9, 42.9, 42.5, 37.4, 36.2, 34.9, 32.1, 31.6, 30.1, 30.0, 28.9, 28.4, 28.3, 27.8, 27.3, 27.0, 22.6, 22.5, 19.5, 14.6. HRMS Calcd for $C_{38}H_{59}N_6O_8$: 727.4394 $(M + H)^+$. Found: 727.4392.

1,1-Dimethylethyl [1(*S*)-[[(1*R*,5*S*)-2(*S*)-[[[1-[2-[[2-[[2-(Dimethylamino)-2-oxo-1(*S*)-phenylethyl]amino]-2-oxoethyl]amino]-1,2-dioxoethyl]-4,4,4-trifluorobutyl]amino]carbonyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexan-3-yl]carbonyl]-2,2-dimethylpropyl]carbamate (23). Coupling and oxidation procedures for the preparation of **23** were carried out in a manner similar to that described above for the preparation of **24**. ^{1}H NMR (DMSO-d_6, 500 MHz, mixture of two diastereomers) δ 8.80−8.76 (m, 1H), 8.61−8.54 (m, 2H), 7.42−7.30 (m, 5H), 6.64 (t, J = 8.9 Hz, 1H), 5.82 and 5.80 (d, J = 7.8 and 8.0 Hz, 1H), 5.15−5.10 and 4.62−4.58 (m, 1H), 4.26 and 4.19 (s, 1H), 3.99 (t, J = 9.5 Hz, 1H), 3.90−3.77 (m, 4H), 2.93 and 2.91 (s, 3H), 2.85 and 2.84 (s, 3H), 2.43−2.23 (m, 1H), 2.10−1.89 (m, 1H), 1.70−1.43 (m, 1H), 1.36 (s, 9H), 1.32−1.23 (m, 3H), 1.02 (s, 3H), 0.92 and 0.89 (s, 9H), 0.87 (s, 3H).^{13}C NMR (DMSO-d_6, 125 MHz, mixture of two diastereomers) δ 196.4, 196.0, 172.4, 172.2, 170.7, 170.1, 167.8, 162.6, 161.5, 156.8, 138.4, 129.5, 128.7, 79.0, 60.5, 60.4, 59.6, 53.9, 53.8, 53.0, 48.3, 42.5, 42.4, 37.5, 37.4, 36.2, 34.8, 28.9, 27.2,

26.9, 26.8, 19.6, 13.3. HRMS Calcd for $C_{37}H_{54}F_3N_6O_8$: 767.3955 $(M + H)^+$. Found: 767.3932.

Supporting Information Available: Synthesis and analytical data for the preparation of modified prolines and P3−P2 intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

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