Articles

Novel Asp[32]-Replacement Tetrapeptide Analogues as Potent and Selective CCK-A Agonists

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Received December 27, 1993®

A series of novel CCK tetrapeptide analogues of the general formula Boc-Trp-Lys(Tac)-N(R)-$(CH_2)_n$CON(R')Phe-NH_2 (Tac = *o*-tolylaminocarbonyl), where R, R' = H or Me and n = 1–5, have been synthesized and tested. These analogues, which lack an acidic residue at the penultimate position, demonstrated surprisingly high CCK-A receptor affinity and selectivity. The effect of *N*-methylation pattern on CCK-A receptor affinity showed consistent trends for analogues in which n = 1, 2, or 3, with the di-*N*-methylated analogues having the highest affinity in each case. However, none of these analogues had full agonist activity, as measured by percent maximal PI hydrolysis. Two conformationally constrained analogues also demonstrated high CCK-A receptor affinity and selectivity, as well as nearly maximal agonist activity. In addition, one of these conformationally-constrained analogues demonstrated anorectic activity in rats.

The brain–gut peptide cholecystokinin (CCK) has received considerable attention for its neuromodulatory role in the regulation of food intake in animals.[1-4] Its potent anorectic actions makes it an attractive target as a novel therapeutic treatment for obesity, a major health problem in the United States and other developed nations. However, CCK's peptidic nature, high molecular weight, and *in vivo* instability limits its therapeutic potential. In an attempt to overcome these difficulties, numerous CCK analogues have been synthesized and evaluated as potential anorectic agents. However, a vast number of these analogues are *O*-sulfated heptapeptides, which still possess many of the undesirable attributes of CCK itself. Early attempts to develop smaller molecular weight analogues afforded agents which were antagonists rather than agonists.[5-9] However, several years ago we reported on a novel series of CCK tetrapeptide agonists (e.g., A-71623, Boc-Trp-Lys(Tac)-Asp-N(Me)PheNH_2, Tac = *o*-tolylaminocarbonyl, see Figure 1) which lacked the unstable *O*-sulfated moiety and had a much lower molecular weight than CCK-8 itself.[10] These analogues were shown to be potent and selective anorectic agents in rodents and primates. However, these compounds had a very low oral bioavailability (~1%) which precluded their use via po administration.

For a number of years it had been thought that the C-terminal tetrapeptide amide was the smallest fragment of CCK which would retain biological activity, and that the penultimate Asp[32] residue [CCK-33 numbering] was essential for this activity. Subsequently, it was shown that this residue could be replaced with other acidic residues, including hydroxy amino acid sulfate esters in both tetrapeptide[11] and heptapeptide analogues.[12] However, replacement of this Asp[32] residue with Glu[13] or with nonacidic residues, such as either Ala ([Ala[32]] CCK-26–33)[14] or β-Ala, caused a large decrease in biological activity. Likewise, a Z-β-Asp CCK-27–33 analogue[15] had relatively weak potency in stimulating amylase release from pancreatic acinar cells.

Recently, potent CCK-heptapeptide analogues which contain nonacidic constrained Asp[32] replacements (e.g., Aib, R-Dtc) have been reported.[16] This paper also reported on an analogue in which an R-Dtc residue had been incorporated into Abbott's A-71623 to obtain a fairly potent analogue (IC_{50} = 59 nM). We report here on a series of novel tetrapeptide analogues in which the penultimate Asp[32] residue of A-71623 has been systematically replaced with ω-aminoalkyl carboxylic acid residues of varying length and *N*-methylation pattern, as well as several conformationally-constrained β-Ala residues.

Results and Discussion

As shown in Table 3, all of the tetrapeptides synthesized had much greater affinity for the CCK-A receptor (pancreatic) than for the CCK-B receptor (cortex). Regarding the ω-aminoalkylcarboxyl series, the analogues in which n = 2 (compounds **5–8**) and n = 3 (compounds **9–12**) demonstrated the greatest potency for the CCK-A receptor, with IC_{50} values ranging from 7 to 750 nM, with compound **8** (IC_{50} = 7 nM) being the most potent analogue. The series in which n = 1 (compounds **1–4**) and n = 4 or 5 (compounds **13–14** or **15–16**, respectively) demonstrated much poorer affinity for the pancreatic receptor. Examination of the effect of *N*-methylation on pancreatic receptor affinity reveals a consistent trend for the first three series (n = 1–3) in which the di-*N*-methylated analogues (i.e., compounds **4**, **8**, and **12**) are the most potent,

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® Abstract published in *Advance ACS Abstracts*, April 15, 1994.

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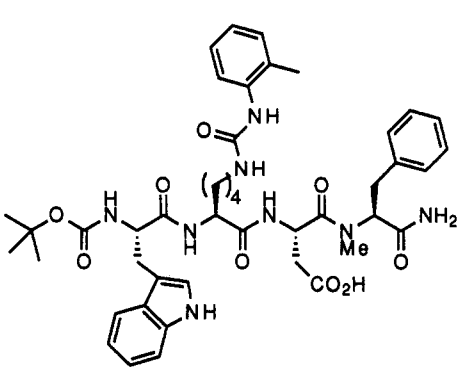
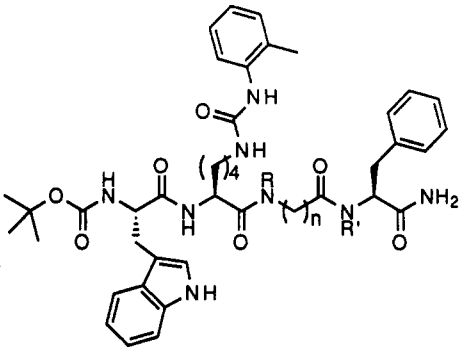


A-71623



ω-aminocarboxylic acid analogues
(R, R' = H, Me; n = 1-5)

Figure 1. Structure of A-71623 and ω-amino carboxylic acid analogues.

Table 1. Analytical Data for Dipeptide Intermediates

dipeptide	coupling method[a,b]	coupling yield (%)	deprotection method (R = H)[b]	deprotection yield (%)
$RNHCH_2COPheNH_2$	commercially available[c]			
$RNHCH_2CO(NMe)PheNH_2$	B (R = Boc)	64	HCl/AcOH	quantitative
$RNMeCH_2COPheNH_2$	B (R = Boc)	95	HCl/AcOH	76
$RNMeCH_2CO(NMe)PheNH_2$	B (R = Boc)	48	HCl/AcOH	quantitative
$RNH(CH_2)_2COPheNH_2$	B (R = Boc)	94	TFA/CH_2Cl_2	quantitative
$RNH(CH_2)_2CO(NMe)PheNH_2$	A (R = Boc)	39	TFA/CH_2Cl_2	quantitative
$RNMe(CH_2)_2COPheNH_2$	B (R = Boc)	70	HCl/AcOH	quantitative
$RNMe(CH_2)_2CO(NMe)PheNH_2$	B (R = Boc)	74	TFA/CH_2Cl_2	quantitative
$RNH(CH_2)_3COPheNH_2$	B(R = Cbz)	82	H_2/Pd–C/MeOH	quantitative
$RNH(CH_2)_3CO(NMe)PheNH_2$	B (R = Cbz)	83	H_2/Pd–C/MeOH	90
$RNMe(CH_2)_3COPheNH_2$	B (R = Cbz)	42	H_2/Pd–C/EtOH	99
$RNMe(CH_2)_3CO(NMe)PheNH_2$	B (R = Cbz)	42	H_2/Pd–C/EtOH	quantitative
$RNH(CH_2)_4COPheNH_2$	B (R = Cbz)	96	H_2/Pd–C/MeOH	quantitative
$RNH(CH_2)_4CO(NMe)PheNH_2$	B (R = Cbz)	88	H_2/Pd–C/MeOH	quantitative
$RNH(CH_2)_5COPheNH_2$	B (R = Boc)	85	HCl/AcOH	quantitative
$RNH(CH_2)_5CO(NMe)PheNH_2$	B (R = Boc)	91	HCl/AcOH	83

[a] All dipeptides were characterized by NMR and MS. [b] See the Experimental Section for details. [c] Commercially available from Bader, Serva, K&K, Bachem, and Sigma.

Table 2. Analytical Data for Tetrapeptides Boc-Trp-Lys(Tac)-R

compd	R	formula	anal.[a]	coupling method[b]	purification method[c]	yield (%)
1	$NHCH_2COPheNH_2$	$C_{41}H_{52}N_8O_7 \cdot 1.5H_2O \cdot 0.6AcOH$	C,H,N	A	1	54
2	$NHCH_2CON(Me)PheNH_2$	$C_{42}H_{54}N_8O_7 \cdot 0.7H_2O$	C,H,N	B	1	23
3	$N(Me)CH_2COPheNH_2$	$C_{42}H_{54}N_8O_7 \cdot H_2O$	C,H,N	A	1	9
4	$N(Me)CH_2CON(Me)PheNH_2$	$C_{43}H_{56}N_8O_7 \cdot 0.5H_2O \cdot AcOH$	C,H,N	B	1	29
5	$NH(CH_2)_2COPheNH_2$	$C_{42}H_{54}N_8O_7 \cdot 2.5H_2O$	C,H,N[d]	B	2	62
6	$NH(CH_2)_2CON(Me)PheNH_2$	$C_{43}H_{56}N_8O_7 \cdot 1.5H_2O$	C,H,N	B	3	27
7	$N(Me)(CH_2)_2COPheNH_2$	$C_{43}H_{56}N_8O_7 \cdot 0.55H_2O$	C,H,N	B	1	18
8	$N(Me)(CH_2)_2CON(Me)PheNH_2$	$C_{44}H_{58}O_7 \cdot 1.25H_2O$	C,H,N	B	3	39
9	$NH(CH_2)_3COPheNH_2$	$C_{43}H_{56}N_8O_7 \cdot 0.5H_2O$	C,H,N	B	4	29
10	$NH(CH_2)_3CON(Me)PheNH_2$	$C_{44}H_{58}N_8O_7 \cdot 1.25H_2O$	C,H,N	B	3	50
11	$N(Me)(CH_2)_3COPHeNH_2$	$C_{44}H_{58}N_8O_7 \cdot 0.25H_2O$	C,H,N	B	1	23
12	$N(Me)(CH_2)_3CON(Me)PheNH_2$	$C_{45}H_{60}N_8O_7 \cdot H_2O$	C,H,N	B	1	18
13	$NH(CH_2)_4COPheNH_2$	$C_{44}H_{58}N_8O_7 \cdot 1.5H_2O$	C,H,N	B	5	45
14	$NH(CH_2)_4CON(Me)PheNH_2$	$C_{45}H_{60}N_8O_7 \cdot 1.8H_2O \cdot 1.2AcOH$	C,H,N	B	1	32
15	$NH(CH_2)_5COPheNH_2$	$C_{45}H_{60}N_8O_7 \cdot 0.8AcOH$	C,H,N	B	1	78
16	$NH(CH_2)_5CON(Me)PheNH_2$	$C_{46}H_{62}N_8O_7 \cdot 0.8AcOH$	C,H,N	B	1	33
17	β-Ala[$NHCH_2CH_2$-N]$PheNH_2$	$C_{44}H_{56}N_8O_7 \cdot 1.5H_2O$	C,H,N	see Experimental Section		
18	β-HProN(Me)$PheNH_2$	$C_{46}H_{60}N_8O_7 \cdot 1.5H_2O$	C,H,N	see Experimental Section		
19	4(*R*)-amino-1-((*S*)-benzylcarbamoylmethyl)pyrrolidin-2-one	$C_{43}H_{54}N_8O_7 \cdot 0.5H_2O$	C,H,N	see Experimental Section		

[a] Elemental analyses were within ±0.4% of theoretical values, unless stated otherwise. [b] See the Experimental Section general methods. Coupling method A: IBCF. Coupling method B: EDCI/HOBt. [c] Purification methods (1) flash chromatography; EtOAc–Bodanszky; (2) trituration with EtOAc, Et_2O; (3) flash chromatography, EtOH–EtOAc; (4) trituration with EtOH/EtOAc, hexane; (5) trituration with Et_2O. [d] Calcd for $C_{42}H_{54}N_8O_7 \cdot 2.5H_2O$: C, 60.92; H, 7.18; N, 13.53. Found: C, 60.74; H, 6.74; N, 13.12. HRMS: calculated mass 783.4194; exact mass measured 783.4203.

with receptor affinity tapering off in the order of the N(Me)Phe analogues, N(Me)-ω-aminoalkylcarboxyl analogues, and lastly the analogues lacking *N*-methylation. These results contrast with the effect of *N*-methylations in the sulfated heptapeptide analogues of CCK [(des-NH_2)-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-Asp-Phe-NH_2] and tetrapeptide CCK-A analogues [Boc-Trp-Lys(Tac)-Asp-Phe-NH_2][17] in which *N*-methylation of the Asp[32] residue, the Phe[33] residue, or both the Asp[32] and Phe[33] residues had little effect on IC_{50} values for the pancreatic receptor. However, both of these series were full agonists (as measured by percent PI hydrolysis) whereas the ω-ami-

Table 3. Biological Data for Tetrapeptides Boc-Trp-Lys(Tac)-R

compd	R	guinea pig binding: pancreas[a,b] IC_{50} (nM)	guinea pig binding: cortex[c] % inhibition	PI[d] (% max)	FI (ED_{50}),[e,f] nmol/kg; rat
1	$NHCH_2COPheNH_2$	921 ± 360 (3)	14 ± 3 (3)	17 ± 3 (4)	NT
2	$NHCH_2CON(Me)PheNH_2$	141 ± 46 (3)	0 (4)	28 ± 4 (4)	NT
3	$N(Me)CH_2COPheNH_2$	265 ± 91 (3)	5 ± 2.6 (3)	24 ± 2 (4)	NT
4	$N(Me)CH_2CON(Me)PheNH_2$	84 ± 9.6 (3)	0 (4)	33 ± 4 (4)	NT
5	$NH(CH_2)_2COPheNH_2$	126 ± 12 (3)	6 ± 3.8 (3)	16 ± 2 (3)	NT
6	$NH(CH_2)_2CON(Me)PheNH_2$	27 ± 8.6 (3)	9 ± 2 (3)	47 (1)	NT
7	$N(Me)(CH_2)_2COPheNH_2$	62 ± 11 (3)	14 ± 1 (4)	61 (2)	NT
8	$N(Me)(CH_2)_2CON(Me)PheNH_2$	7.0 ± 2.1 (3)	10 ± 1 (4)	36 ± 6 (4)	NT
9	$NH(CH_2)_3COPheNH_2$	749 ± 303 (3)	11 ± 2 (4)	9 ± 4 (4)	NT
10	$NH(CH_2)_3CON(Me)PheNH_2$	27 ± 3.8 (3)	11 ± 2 (4)	67 ± 5 (4)	NT
11	$N(Me)(CH_2)_3COPheNH_2$	393 ± 92 (3)	11 ± 3 (3)	8 ● 4 (3)	NT
12	$N(Me)(CH_2)_3CON(Me)PheNH_2$	19 ± 6.8 (3)	4 ± 2 (4)	64 ± 4 (3)	NT
13	$NH(CH_2)_4COPheNH_2$	1803 ± 381 (3)	10 ± 4 (3)	2 ± 2 (3)	NT
14	$NH(CH_2)_4CON(Me)PheNH_2$	1179 ± 151 (3)	7 ± 2 (4)	7 ± 2 (3)	NT
15	$NH(CH_2)_5COPheNH_2$	1195 ± 224 (3)	6 ± 2 (3)	5 (2)	NT
16	$NH(CH_2)_5CON(Me)PheNH_2$	1333 ± 215 (3)	10 ± 2 (4)	4 (2)	NT
17	β-Ala[NCH_2CH_2-N]$PheNH_2$	750 ± 355 (3)	8 ± 2 (3)	1 ± 1 (4)	NT
18	β-HProN(Me)$PheNH_2$	17 ± 3.4 (3)	13 ± 2 (3)	92 ± 1 (4)	no effect
19	4(*R*)-amino-1-((*S*)-benzylcarbamoylmethyl)pyrrolidin-2-one	16 ± 2.2 (4)	39 ± 11 (4)	89 ± 2 (4)	30

[a] Concentration of the peptide that inhibited 50% of the specific binding of [^{125}I]Bolton–Hunter CCK-8 binding. [b] Number of determinations is in parentheses. [c] Percent inhibition of specific [^{125}I]Bolton–Hunter CCK-8 binding at 10^{-5} M. [d] Percent response of peptide at 10^{-4} M in PI hydrolysis (guinea pig) relative to maximal response elicited by CCK-8 (10^{-6} M). [e] Dose of peptide (ip) required to reduce food intakes by 50%. [f] NT = not tested.

noalkylcarbonyl analogues are not; thus it is not too surprising to see nonparallel SAR between these series.

Functionally, these ω-aminoalkylcarboxyl analogues ranged from being poor to moderately good agonists, with the percent maximal PI hydrolysis ranging from 1 to 67%. Unlike binding affinity, there were not any obvious trends for the effect of *N*-methylation on agonist activity within each series (n = 1, 2, or 3), nor does there seem to be any correlation between receptor affinity and agonist activity. Interestingly, the series in which n = 3 (compounds **9–12**) are much more sensitive to changes in *N*-methylation pattern, in terms of percent PI hydrolysis, than the other series. The analogues with longer alkyl chains (n = 4 or 5) had essentially no agonist activity. Because of the low *in vitro* agonist activity, these analogues were not evaluated for their anorectic activity. On the basis of the SAR of these ω-aminoalkylcarboxyl analogues, it is tempting to speculate that an acidic residue at the Asp^{32} position is not absolutely necessary for good pancreatic receptor affinity, but is required for good agonist efficacy. However, the SAR of several constrained analogues (*vida infra*) belies this conclusion.

Compounds **17–19** can all be considered as conformationally-constrained analogues of the β-Ala analogue **5** (see Figure 2). Compound **17** had poor affinity (IC_{50} = 750 nM) for the receptor, whereas analogues **18** and **19** demonstrated high affinity for the pancreatic receptor (IC_{50} values of 17 and 16 nM, respectively). Although compounds **18** and **19** are similar to their acyclic counterparts in terms of receptor affinity, they are much better agonists, exhibiting near maximal *in vitro* agonist activity (92% and 89%, respectively).

Compounds **18** and **19** were tested *in vivo* to assess their anorectic activity. No dose (1–300 nmol/kg, ip) (n = 8/group) of **18** significantly reduced intakes below that of controls. In contrast, compound **19**, at a dose of 100 nmol/kg (ip, n = 7–8/group per experiment) was found to reduce intakes to 36% of controls values, with the dose required to reduce food intakes by 50% calculated to be 30 (21.2–43.6) nmol/kg. Interestingly, comparison of this anorectic effect with that of the prototypic lead, A-71623, reveals

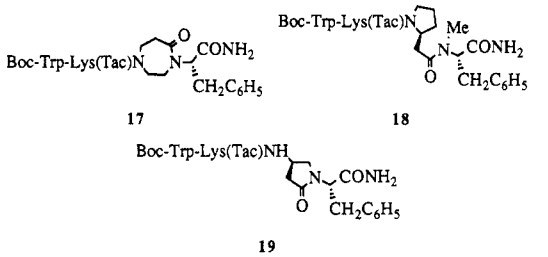
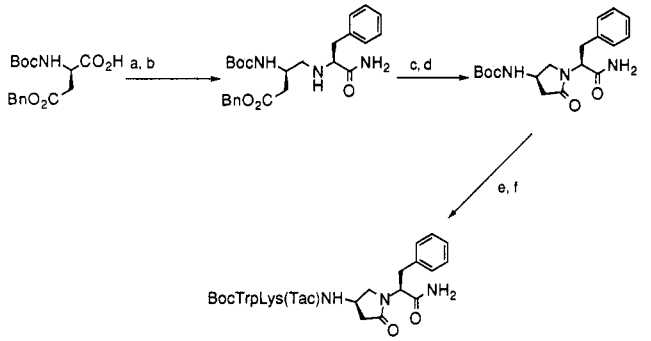


Figure 2. Structures of conformationally constrained CCK-A tetrapeptides.

that the IC_{50} ratio for A-71623 and **19** (3 vs 16 nM, respectively) is roughly equal to the ED_{50} ratio for food intake (4 vs 30 nmol/kg, respectively). However, the differences between **18** and **19** in feeding behavior, despite their very similar CCK-A binding affinity and *in vitro* agonist activity, is difficult to explain. Further investigation would be needed to delineate the factor(s) involved in these discrepancies.

The conformationally-constrained analogues **18** and **19** provide valuable insight into the conformational requirements for agonist activity. The β-Ala moiety, a common element shared by both analogues, appears to be an important feature for good agonist activity in these series; e.g., an analogue of **18** (without the *N*-methyl group) containing L-Proline instead of L-β-HPro was found to have much poorer binding affinity and agonist activity (IC_{50} = 239 nM, PI hydrolysis (% max.) = 68%; MDT unpublished results). The conformationally-constrained γ-lactam (*S*-isomer) of **19** has been successfully incorporated into other bioactive peptides[21] and may, along with the β-HPro moiety of **18**, have utility as constraining elements in other bioactive peptides. However, despite their common elements, it is not possible for **18** and **19** to adapt exactly the same conformation; complete overlay of **18** onto **19** requires that the *N*-methyl group and the C-3 pyrrolidine carbon of **18** occupy the same space. This would suggest that, in terms of agonist efficacy, more than one conformation can fulfill the requirements for good agonist activity. Conversely it may be that, despite their conformational differences, the key elements in **18** and **19**

Scheme 1. Synthesis of 4(*R*)-(Boc-Trp-Lys(Tac)-amino)-1-((*S*)-benzylcarbamoylmethyl)pyrrolidin-2-one[a]



[a] (a) 3,5-dimethylpyrazole/DCC (98%); (b) LAH/THF; then $PheNH_2$/EtOH/$NaCNBH_3$/AcOH (31%); (c) H_2/10% Pd-C/AcOH (100%); (d) EDCI/HOBt/DMF/DIEA (73%); (e) 1.5 N HCl in AcOH; then BocLys(Tac)OSu/DMF/DIEA (100%); (f) 1.5 N HCl in AcOH; then BocTrpOSu/DMF/DIEA (42%).

needed for agonist activity are cospacial in the receptor-bound conformation. The finding that the Asp^{32} carboxylic acid is not absolutely necessary for good binding and agonist activity should be of great aid in designing further potent CCK-A agonists with improved physicochemical and pharmacokinetic properties.

Experimental Section

Methods. The ω-aminoalkylcarboxyl-substituted tetrapeptide analogues were made by standard solution-phase peptide coupling of the *N*-protected ω-amino carboxylic acid to $PheNH_2$ or N(Me)-$PheNH_2$[17] to afford the dipeptide (see Table 1), followed by *N*-deprotection and subsequent EDCI-mediated coupling with Boc-Trp-Lys(Tac)-OH[17] to afford the tetrapeptide. The synthetic route to the conformationally constrained β-Ala analogue **19** is outlined in Scheme 1. All final compounds were fully characterized by MS, NMR, and elemental analysis.

The target compounds were tested at both the CCK-A receptor (guinea pig pancreas) and CCK-B receptor (guinea pig cortex) as previously described[18] using [^{125}I]Bolton–Hunter CCK-8 as the radioligand. Functional activity of these analogues in stimulating PI hydrolysis were performed as described previously.[19] The activity of analogues in feeding behavior was measured in rats as previously described.[20]

All solvents and reagents were reagent grade unless otherwise noted. Amino acids were purchased from Aldrich Chemical Co. or Sigma Chemical Co., and all are L-amino acids, unless otherwise noted. ^{1}H-NMR spectra were generally taken in DMSO-d_6 or $CDCl_3$ and are recorded at 300 MHz, and are reported in ppm downfield from tetramethylsilane. Elemental analyses were obtained from Abbott Laboratories Analytical Department, North Chicago, IL; Midwest Laboratories, Indianapolis, IN; or Robertson Laboratories, Madison, NJ. Chromatographic purifications were carried out using flash chromatography (60 mesh; 0.04–0.063 mm, E. Merck). All final compounds, unless otherwise noted, were analyzed by ^{1}H-NMR, MS, and elemental analysis and in general had a chemical purity >95%. Abbreviations: HOBt = *N*-hydroxybenzotriazole hydrate, TFA = trifluoroacetic acid, DMF = *N*,*N*-dimethylformamide, DIEA = diisopropylethylamine, Bodanszky solution = H_2O/pyridine/acetic acid, 55:100:30, v:v:v, DCC = 1,3-dicyclohexylcarbodiimide, EDCI = 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride. Boc-β-Ala-*p*-nitrophenyl ester, *N*-Boc-β-OBn-D-aspartic acid, Boc-β-Ala-OH, and Cbz-γ-aminobutyric acid were purchase from Sigma. Boc-Trp-*N*-hydroxysuccinimide ester can be purchased from Fluka. Boc-Lysine was purchased from Bachem.

Boc-N(Me)-$(CH_2)_2$COOH. To sodium hydride (660 mg, 22.0 mmol; washed with 2 × hexane) in THF (6 mL) was added Boc-$NH(CH_2)_2COOH$ (1.89 g, 10.0 mmol) in THF (6 mL) at 0 °C over a 3-min period. After several minutes THF (25 mL) was added, and after an additional 10 min iodomethane (684 μL, 11.0 mmol) was added. After being stirred for 16 h at room temperature the reaction mixture was quenched with H_2O (50 mL) and extracted with Et_2O (2 × 50 mL); then the aqueous layer was acidified with 10% HCl to pH ~1–2 and extracted with EtOAc (2 × 70 mL). The combined EtOAc extracts were dried ($MgSO_4$) and concentrated to afford the product as a yellow oil (1.95 g, 96%): MS (DCI/NH_3) *m/z* 204 $(M + H)^+$, 221 $(M + NH_4)^+$; ^{1}H-NMR ($CDCl_3$) δ 1.46 (s, 9H), 2.60 (m, 2H), 2.89 (s, 3H), 3.51 (m, 2H).

Cbz-N(Me)-$(CH_2)_3$COOH. To sodium hydride (0.78 g, 32.5 mmol; washed with 2 × hexane) in THF (10 mL) was added Cbz-$NH(CH_2)_3COOH$ (3.5 g, 14.8 mmol) at 0 °C and the reaction mixture stirred at room temperature. Iodomethane (1.4 mL, 16.25 mmol) was then added and reaction mixture was stirred overnight at ambient temperature. The reaction mixture was then quenched with H_2O and extracted with Et_2O (2 × 50 mL); then the aqueous layer acidified with 10% HCl to pH ~2 and extracted with EtOAc (3 × 100 mL). The combined EtOAc layers were washed with H_2O and brine, dried ($MgSO_4$), and concentrated *in vacuo* to afford the product as a white solid (3.5 g, 95%): MS (DCI/NH_3) *m/z* 252 $(M + H)^+$, 269 $(M + NH_4)^+$; ^{1}H-NMR (DMSO-d_6) δ 1.78 (m, 2H), 2.12 (m, 2H), 2.85 (br d, 3H), 3.35 (br m, 2H), 5.08 (s, 2H), 7.36 (m, 5H), 12.09 (br s, 1H).

General Procedure for the Coupling of XN(R)$(CH2)_n$-COOH with HN(R′)$PheNH_2$ (X = Boc or Cbz). To a stirred solution of the N-protected ω-amino carboxylic acid (1.0 equiv), HN(R)$PheNH_2$ (TFA or HCl salt, 1.0 equiv; R = H or Me), NMM (1.2 equiv), and HOBt (1.1 equiv) in DMF (~0.1 to 0.5 M) at room temperature was added EDCI (1.2 equiv). The reaction was stirred at room temperature until complete (typically the reactions were run for 12–48 h), diluted with EtOAc, washed successively with 10% aqueous citric acid, saturated aqueous $NaHCO_3$, and brine, dried ($MgSO_4$), and concentrated to obtain the crude product (see Table 1 for yields). The dipeptides were used subsequently without further purification.

General Procedure for the Deprotection of Boc-Protected Amines. To a cooled solution (0 °C) of the Boc-protected amine in CH_2Cl_2 was added an equal volume of TFA, and then the reaction mixture was allowed to warm to room temperature. Upon completion of the reaction (typically 0.5–1 h) the solvents were removed *in vacuo* to afford the amine (TFA salt) as a hydroscopic solid (see Table 1 for yields). The dipeptides were used subsequently without further purification.

General Procedure for the Deprotection of Cbz-Protected Amines. A solution of the Cbz-protected amine in solvent (usually MeOH or EtOH) containing 5–10 mol % Pd–C (10 mol %) was placed under an atmosphere of H_2 (1 atm) and stirred at room temperature. Upon completion of the reaction, the mixture was filtered through Celite, the Celite pad was washed with solvent (2×), and the combined solvents were removed *in vacuo* to afford the free amine (see Table 1 for yields), which was used subsequently without further purification.

General Procedure for IBCF Coupling of Boc-Trp-Lys-(Tac)-OH with HN(R)$(CH_2)_n$CON(R′)$PheNH_2$ (Coupling Method A). To a solution of Boc-Trp-Lys(Tac)-OH (1.0 equiv) in THF (~0.2 M) cooled to −20 °C was added NMM (1.0 equiv), followed by isobutyl chloroformate (1.0 equiv). After 10 min a solution of the amine (TFA salt, 1.0 equiv) and NMM (1.0 equiv) in DMF (~0.2 M) was added, and then the reaction mixture was allowed to gradually warm to room temperature. Upon completion of the reaction the solvents were removed *in vacuo*, and the residue was partitioned between aqueous 1 M H_3PO_4 and EtOAc. The organic phase was washed with saturated aqueous $NaHCO_3$, water, and brine, dried ($MgSO_4$), and concentrated to afford the crude product. The purification method and yield for each analogue is listed in Table 2.

General Procedure for EDCI Coupling Boc-Trp-Lys-(Tac)-OH with HN(R)$(CH2)_n$CON(R′)$PheNH_2$ (Coupling Method B). To a stirred solution of the amine (1.0 equiv, TFA salt), Boc-Trp-Lys(Tac)-OH (1.0 equiv), NMM (1.2 equiv), and HOBt (1.1 equiv) in DMF (~0.1 to 0.5 M) at room temperature was added EDCI (1.2 equiv). The reaction was stirred at room temperature until complete (typically the reactions were run for 12–48 h), diluted with EtOAc, washed with 10% aqueous citric acid, saturated aqueous $NaHCO_3$, and brine, dried ($MgSO_4$), and concentrated to obtain the crude product. The purification method and yield for each analogue are listed in Table 2.

Boc-Trp-Lys(Tac)-β-Ala[$NHCH_2CH_2$-N]$PheNH_2$(17). (A) *N*-Cbz-*N*-(hydroxyethyl)-β-alanine Methyl Ester. Methyl

acrylate (9.0 mL, 100 mmol) was added dropwise to a solution of 2-aminoethanol (6.2 g, 100 mmol) in toluene (70 mL) at room temperature. The mixture was refluxed for 4 h, the volatiles were evaporated, and the residue was taken up in THF (150 mL) and saturated aqueous $NaHCO_3$ (80 mL). Benzyl chloroformate (17 mL, 119 mmoL) was added dropwise to the above solution at 0 °C while the reaction medium was maintained at pH ~8–9 with 1 N NaOH (100 mL). After stirring overnight at room temperature, the THF was removed *in vacuo* and the residue diluted with water and extracted with CH_2Cl_2 (3 × 60 mL), dried (Na_2SO_4), and concentrated. Removal of impurities (e.g., benzyl alcohol) by Kugelrohr distillation followed by chromatography afforded the title compound (6.0 g, 23%): MS (DCI/NH_3) *m/z* 282 $(M + H)^+$, 299 $(M + NH_4)^+$; ^{1}H-NMR ($CDCl_3$) δ 2.52–2.78 (br m, 2H), 3.47 (br m, 2H), 3.62 (s, 3H), 3.65–3.81 (br m, 4H), 5.13 (s, 2H), 7.28–7.40 (m, 5H).

(B) *N*-Cbz-*N*-(1-oxoethyl)-β-alanine Methyl Ester. A solution of DMSO (1.5 mL) in CH_2Cl_2 (3 mL) was added to a solution of oxalyl chloride (0.88 mL, 10 mmol) in CH_2Cl_2 (5 mL) at −78 °C. The reaction mixture was stirred for 5 min, and then *N*-Cbz-*N*-(hydroxyethyl)-β-alanine methyl ester (2.0 g, 7 mmol) in CH_2Cl_2 (10 mL) was added over 5 min. After an additional 30 min of stirring, triethylamine (7.0 mL, 50 mmol) was added, and after 10 min at −78 °C the reaction mixture was allowed to warm to −20 °C. Ice-cold aqueous 10% citric acid solution was then added, the layers were separated, and the aqueous layer was reextracted with CH_2Cl_2 (2 × 15 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated to afford the title compound (1.6 g, 80%): MS (DCI/NH_3) *m/z* 280 $(M + H)^+$, 279 $(M + NH_4)^+$; ^{1}H-NMR ($CDCl_3$) δ 2.60 and 2.69 (2 t, 2H), 3.60 (q, 2H), 3.64, 3.68 (2 s, 3H), 4.15 and 4.18 (2 s, 2H), 5.10 and 5.16 (2 s, 2H), 7.25–7.40 (5H), 9.51 and 9.57 (2 s, 1H).

(C) β-Ala[NCH_2CH_2-N]PheNH$_2$. A mixture of the *N*-Cbz-*N*-(1-oxoethyl)-β-alanine methyl ester (1.4 g, 5.1 mmol), H-$PheNH_2$ (1.0 g, 6.1 mmol), and AcOH (240 μL) in CH_3CN (10 mL) was stirred at room temperature for 20 min. Sodium cyanoborohydride (850 mg, 13.5 mmoL) was added to the above mixture in 4 portions at 0 °C over 30 min. The mixture was stirred at room temperature overnight, quenched by the addition of 10% aqueous K_2CO_3 (10 mL), stirred for 10 min, and then extracted with CH_2Cl_2 (3 × 25 mL). The combined extracts were washed with brine, dried (Na_2SO_4), and concentrated *in vacuo*. The residue was filtered through silica (CH_2Cl_2/EtOH, 98:2) to afford the crude amino ester (2.05 g). This material was dissolved in 10% aqueous MeOH (20 mL), K_2CO_3 (1.0 g) was added, and the reaction mixture was refluxed for 3 h, after which the solvents were removed *in vacuo*. The resulting residue was dissolved in H_2O (15 mL), the pH of the medium was adjusted to ~7 with AcOH, the water was decanted, and the residue was rinsed with H_2O and dried *in vacuo* to afford the crude carboxylic acid. To a solution of this material in CH_2Cl_2 (20 mL) was added EDCI (700 mg, 3.65 mmol), and after stirring at room temperature overnight the reaction was quenched with 15% aqueous citric acid (15 mL), the aqueous layer was extracted with CH_2Cl_2 (3 × 15 mL), and the combined organic extracts were washed with brine, dried (Na_2SO_4), and concentrated *in vacuo*. Chromatographic purification (flash chromatography, silica, EtOAc) afforded *N*-Cbz-β-Ala[NCH_2CH_2-N]$PheNH_2$ (0.25 g, 13%): MS (DCI/NH_3) *m/z* 396 $(M + H)^+$, 413 $(M + NH_4)^+$; ^{1}H-NMR ($CDCl_3$, 55°C) δ 2.64 (br m, 2H), 3.03 (dd, *J* = 5.4, 9 Hz, 1H), 3.30 (dd, *J* = 4.2, 9 Hz, 2H), 3.40–3.60 (m, 6H), 5.15 (s, 2H), 5.36 (dd, 1H), 7.22–7.40 (m, 10H).

Hydrogenolysis of this material (240 mg, 0. 6 mmol) under H_2 (1 atm) using 10% Pd/C (10 mg) in EtOH (10 mL) for 16 h, followed by removal of the catalyst and concentration, afforded the title compound (110 mg, 70%): MS (DCI/NH_3) *m/z* 262 $(M + H)^+$; ^{1}H-NMR ($CDCl_3$) δ 2.58–2.66 (m, 2H), 2.73–2.78 (m, 2H), 2.82–2.90 (m, 2H), 2.98 (dd, 1H), 3.32 (dd, 1H), 3.40–3.50 (m, 1H), 5.23 (br m, 1H), 5.32 (dd, 1H), 6.28 (br, 1H), 7.20–7.33 (m, 5H).

(D) Boc-Trp-Lys(Tac)-β-Ala[NCH_2CH_2-N]PheNH$_2$ (17). A solution of Boc-Lys(Tac)-OH (90 mg, 0.24 mmol), β-Ala[NCH_2CH_2-N]$PheNH_2$ (56 mg, 0.21 mmol), and EDCI (60 mg, 0.3 mmol) in CH_2Cl_2 (2 mL) was stirred at room temperature overnight. The mixture was then partitioned between 10% aqueous citric acid and CH_2Cl_2, and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined CH_2Cl_2 extracts were washed with brine, dried (Na_2SO_4), and concentrated *in vacuo* to afford the crude product. Chromatographic purification (flash chromatography, silica, CH_2Cl_2/EtOH, 95:5) afforded 85 mg (65%) of Boc-Lys(Tac)-β-Ala[NCH_2CH_2-N]$PheNH_2$.

A solution of this material (85 mg, 0.136 mmol) in CH_2Cl_2 (2 mL) and TFA (2 mL) was stirred at room temperature for 1 h. The reaction mixture was then evaporated to dryness, the residue was dissolved in CH_2Cl_2 (2 mL), and DIEA (80 μL, 0.46 mmol) and Boc-Trp-OSu (100 mg, 0.25 mmol) were added. After the mixture was stirred overnight at room temperature, the reaction was quenched with 15% aqueous citric acid (15 mL), and the aqueous layer was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4), and concentrated *in vacuo* to afford the crude product. Chromatographic purification (flash chromatography, silica, CH_2Cl_2/EtOH, 95:5) afforded 50 mg (50%) of the title compound: MS (DCI/NH_3) *m/z* 809 $(M + H)^+$; ^{1}H-NMR (DMSO-d_6, 130 °C) δ 1.33 (s, 9H), 1.43–1.58 (m, 3H), 1.66–1.73 (m, 1H), 2.20 (s, 3H), 2.47–2.57 (m, 2H), 2.92 (dd, 1H), 2.97 (dd, 1H), 3.06–3.12 (m, 2H), 3.17 (dd, 1H), 3.26 (dd, 1H), 3.40–3.56 (m, 6H), 4.24–4.31 (m, 1H), 4.70–4.78 (m, 1H), 5.21 (dd, 1H), 6.10–6.15 (m, 1H), 6.17–6.22 (m, 1H), 6.73 (br m, 2H), 6.94 (t, 1H), 6.96 (t, 1H), 7.03–7.18 (m, 5H), 7.22–7.33 (m, 6H), 7.52 (d, 1H), 7.55 (d, 1H), 7.69 (d, 1H), 10.40 (s, 1H). Anal. Cald for $C_{44}H_{56}N_8O_7 \cdot 1.5H_2O$: C, 63.22; H, 7.11; N, 13.40. Found: C, 63.42; H, 6.90; N, 13.03.

Boc-Trp-Lys(Tac)-β-HProPheNH$_2$ (18). To a solution of Cbz-β-HomoPro[22] (209 mg, 0.794 mmol), HN(Me)$PheNH_2$ hydrochloride salt (170 mg, 0.794 mmol), NMM (105 μL, 0.952 mmol), and HOBt (118 mg, 0.873 mmol) in DMF (2 mL) was added EDCI (167 mg, 0.873 mmol), and the reaction mixture was stirred at room temperature. After 5 days the reaction mixture was diluted with Et_2O (60 mL) and washed with 15-mL portions of 10% aqueous citric acid (1×), saturated aqueous $NaHCO_3$ (1×), and brine (1×), dried ($MgSO_4$), and concentrated to obtain *N*-Cbz-β-HProN(Me)$PheNH_2$ as a white solid (167 mg, 49% yield): MS (DCI/NH_3) *m/z* 424 $(M + H)^+$, 441 $(M + NH_4)^+$; ^{1}H-NMR (DMSO-d_6, two conformers ca. 1:1) δ 1.49–1.83 (br m, 3H), 2.01–2.45 (br m, 2H), 2.63–2.93 (br m, 1H), 2.75, 2.79 (2 s, 3H), 3.14–3.37 (br m, 4H), 3.70, 4.00 (2 br m, 1H), 5.04, 5.06 (2 s, 2H), 5.14–5.28 (br m, 1H), 7.06–7.27 (br m, 6H), 7.27–7.44 (br m, 6H).

A solution of this material (100 mg, 0.236 mmoL) in EtOH (6 mL) was hydrogenated (10% Pd–C, 1 atm) for 30 h, the reaction mixture was filtered through Celite, and the celite pad was washed with EtOH (4 × 2 mL) and water (15 mL). Lyophilization of these filtrates afford β-$HProPheNH_2$ as a white powder (63 mg, 92%): MS (DCI/NH_3) *m/z* 290 $(M + H)^+$; ^{1}H-NMR (DMSO-d_6, two conformers ca. 2:1) δ 1.46–1.71 (m, 3H), 2.11–2.44 (m, 2H), 2.56–2.92 (m, 3H), 2.74, 2.80 (2 s, 3H), 2.99–3.49 (br m, 6H), 4.60, 5.24 (2 dd, 1H), 7.10–7.32 (m, 5H).

To a solution of β-$HProPheNH_2$ (29 mg, 0.10 mmol), Boc-Trp-Lys(Tac)-OH (56.5 mg, 0.10 mmol), and HOBt (15 mg, 0.11 mmol) in DMF (1 mL) was added EDCI (21 mg, 0.11 mmol), and the reaction was stirred at room temperature. After 31 h the reaction was quenched with EtOH (2 mL) and water (15 mL), and the solution was lyophilized. Chromatographic purification (EtOAc/EtOH, 9:1) afforded the title compound as a white powder (59 mg, 70%): MS (FAB$^+$) *m/z* 837 $(M + H)^+$, 820 $(M - NH_2)^+$; ^{1}H-NMR (DMSO-d_6, 135 °C) δ 1.35 (s, 9H), 1.41–1.54 (m, 4H), 1.63–1.85 (m, 4H), 2.20 (s, 3H), 2.74–2.94 (m, 7H), 2.96–3.19 (m, 6H), 3.26 (dd, 1H), 3.37–3.54 (br m, 3H), 4.23 (br m, 1H), 4.30 (m, 1H), 4.47–4.56 (m, 1H), 5.18 (br m, 1H), 6.06–6.18 (m, 2H), 6.69 (br m, 2H), 6.90 (t, 1H), 6.96 (dd, 1H), 7.04–7.13 (m, 4H), 7.14–7.27 (m, 3H), 7.28 (s, 1H), 7.33 (dd, 1H), 7.38–7.44 (m, 1H), 7.55 (dd, 1H), 7.68 (d, 1H), 10.39 (br s, 1H). Anal. Calcd for $C_{46}H_{60}N_8O_7 \cdot 1.5H_2O$: C, 63.94; H, 7.35; N, 12.97. Found: C, 63.83; H, 7.16; N, 12.95.

4(*R*)-(Boc-Trp-Lys(Tac)-amino)-1-((*S*)-benzylcarbamoylmethyl)pyrrolidin-2-one (19). Synthesis of Boc-Lys(Tac)-OSu. To a suspension of Boc-lysine (1.0 g, 4.06 mmol) in DMF (10 mL) at 0–5 °C was added DIEA (0.74 mL, 4.26 mmol) followed by the dropwise addition of *o*-tolyl isocyanate (0.5 mL, 4.06 mmol) in EtOAc (10 mL). After being stirred for 4 h at room temperature, the solution was partitioned between dilute aqute HCl and EtOAc (100 mL), and the organic phase was dried

($MgSO_4$) and concentrated *in vacuo* to give Boc-Lys(Tac)-OH in quantitative yield as a viscous amber oil: MS (DCI/NH_3) *m/z* 380 (M + H)$^+$; ^{1}H-NMR ($CDCl_3$) δ 1.41 (s, 9H), 1.30–1.55 (m, 3H), 1.70–1.90 (m, 3H), 2.28 (s, 3H), 3.22 (m, 2H), 4.29 (m, 1H), 4.90 (br m, 2H), 5.23 (br d, J = 7.5 Hz, 1H), 7.10–7.31 (m, 4H).

A portion of this material (0.65 g, 1.71 mmol) was combined with *N*-hydroxysuccinimide (0.20 g, 1.71 mmol) and DCC (0.35 g, 1.71 mmol) in EtOAc (15 mL). After being stirred at room temperature for 5 h the reaction mixture was filtered and the filtrate concentrated *in vacuo* to give the crude product. Flash chromatography (silica, EtOAc–hexane/EtOAc) afforded the title compound as a white foam (0.57 g, 70%): MS (DCI/NH_3) *m/z* 477 (M + H)$^+$, 494 (M + NH_4)$^+$; ^{1}H-NMR ($CDCl_3$) δ 1.46 (s, 9H), 2.84 (br s, 4H), 3.18–3.41 (m, 2H), 4.70 (m, 1H), 5.11 (t, 2H), 6.29 (br s, 1H), 7.02–7.10 (m, 1H), 7.16–7.23 (m, 3H), 7.58 (d, 1H).

(A) 4(*R*)-(Boc-amino)-1-((*S*)-benzylcarbamoylmethyl)-pyrrolidin-2-one. A solution of *N*-Boc-β-OBn-D-aspartic acid (1.0 g, 3.09 mmoL) and 3,5-dimethylpyrazole (0.3 g, 3.12 mmoL) in EtOAc (75 mL) was cooled to 0 °C, and DCC (0.64 g, 3.10 mmol) was added. After being stirred at room temperature overnight the mixture was filtered and the filtrate concentrated *in vacuo* to afford 1.36 g (98%) of the desired *N*-Boc-β-benzyl-D-aspartyl-3,5-dimethylpyrazolide. This material was used without further purification: ^{1}H-NMR ($CDCl_3$) δ 1.23 (s, 9H), 2.20 (s, 3H), 2.47 (s, 3H), 3.04–3.24 (br m, 2H), 5.10 (s, 2H), 5.67 (br m, 2H), 5.94 (s, 1H), 7.33–7.38 (m, 5H).

A solution of the pyrazolide (1.36 g, 3.02 mmoL) in anhydrous THF (10 mL) was added dropwise over 4 min to a cooled (−78 °C) suspension of LAH (0.13 g, 3.09 mmoL) in THF (30 mL) under nitrogen. After 40 min the reaction was quenched with EtOAc (10 mL) followed by 10% aqueous citric acid solution (10 mL), and the reaction mixture was partitioned between EtOAc (100 mL) and dilute citric acid/brine solution (~100 mL). The organic phase was dried ($MgSO_4$) and concentrated *in vacuo* to give Boc-D-Asp aldehyde in quantitative yield. The crude aldehyde (928 mg, 3.02 mmoL) was dissolved in absolute EtOH (15 mL) and treated with $PheNH_2$ (0.46 g, 2.78 mmoL). After 15 min 1 equiv (0.16 mL) of glacial AcOH was added, and after an additional 10 min of stirring, $NaCNBH_3$ (0.35 g, 5.56 mmoL) was added in portions over 2 min. The resulting heterogeneous solution was stirred for 70 min and then partitioned between EtOAc (125 mL) and dilute sodium bicarbonate/brine solution (~100 mL). The resulting aqueous phase was extracted with EtOAc (50 mL), and the combined EtOAc layers were dried ($MgSO_4$) and concentrated *in vacuo* to afford the crude product. This material was purified by flash chromatography (silica, EtOAc–hexane, then EtOAc–acetone) to afford 3(*R*)-(Boc-amino)-4-[((*S*)-benzylcarbamoylmethyl)amino]butyric acid benzyl ester (0.43 g, 31%) as a 3:1 mixture of diastereoisomers: MS (DCI/NH_3) *m/z* 456 (M + H)$^+$; ^{1}H-NMR ($CDCl_3$) δ 1.35 (s, 9H), 1.84 (m, 1H), 2.05–2.18 (br m, 3H), 2.27–2.83 (m, 2H), 3.15 (m, 1H), 3.80 (br m, 1H), 5.03 (br s, 2H), 6.66 (br d, 1H), 6.99 (br s, 1H), 7.14–7.40 (m, 12 H).

The diastereisomeric mixture of 3(*R*)-(Boc-amino)-4-[((*S*)-benzylcarbamoylmethyl)amino]butyric acid benzyl ester (0.43 g, 0.94 mmoL) was subjected to catalytic hydrogenolysis at room temperature (4 atm H_2/10% Pd–C) in glacial acetic acid (20 mL). Upon completion the reaction mixture was filtered through Celite, and the filtrate was concentrated *in vacuo* to afford a quantitative yield of the desired carboxylic acid as a white foam. This material was dissolved in DMF (5 mL) and EDCI (0.2 g, 1.03 mmoL) and HOBt monohydrate (0.14 g, 1.03 mmoL) added, and after 30 min DIEA (0.18 mL, 1.03 mmoL) was added. After 2 h the reaction mixture was partitioned between EtOAc and dilute aqueous HCl, and the organic phase was washed with water, dried ($MgSO_4$), and concentrated *in vacuo* to yield the crude product. Chromatographic purification (silica, EtOAc/acetone) afforded 4(*R*)-(Boc-amino)-1-((*S*)-benzylcarbamoylmethyl)pyrrolidin-2-one as a white foam (0.23 g, 73%): ^{1}H-NMR ($CDCl_3$) δ 1.26 (s, 9H), 2.28 (dd, 1H), 2.58 (dd, 1H), 3.00 (dd, 1H), 3.31–3.42 (m, 2H), 3.67 (dd, 1H), 4.09 (br m, 1H), 4.95 (dd, 1H), 5.26 (br s, 1H), 5.56 (br s, 1H), 6.70 (br s, 1H), 7.18–7.28 (m, 5H).

4(*R*)-(Boc-Lys(Tac)-amino)-1-((*S*)-benzylcarbamoylmethyl)pyrrolidin-2-one. The Boc-protected lactam (0.228 g, 0.66 mmoL) from the above reaction was treated with 1.5 N HCl in glacial acetic acid (7 mL) at room temperature for 1 h, and then the solvent was removed by lyophilization to afford 4(*R*)-amino-1-((*S*)-benzylcarbamoylmethyl)pyrrolidin-2-one hydrochloride as an oil. This material was dissolved in MeOH and concentrated *in vacuo* (2×) to afford the hydrochloride salt as a foam in quantitative yield. This material was combined with *N*-Boc-Lys(Tac)-OSu (0.31 g, 0.66 mmoL) in DMF (5 mL) followed by DIEA (0.126 mL, 0.726 mmoL), and the reaction mixture was stirred overnight at room temperature. The reaction mixture was then partitioned between EtOAc and dilute sodium bicarbonate/brine solution, and the organic phase was dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product as a foam (400 mg, 100%): MS (FAB) *m/z* 609 (M + H)$^+$, 631 (M + Na)$^+$; ^{1}H-NMR (DMSO-d_6) δ 1.20–1.62 (br m, 6H), 1.37 (s, 9H), 2.06–2.19 (m, 1H), 2.16 (s, 3H), 2.36 (dd, 1H), 2.79–2.91 (m, 1H), 3.05 (m, 2H), 3.18 (dd, 1H), 3.24–3.35 (m, 1H), 3.64 (dd, 1H), 3.83 (br m, 1H), 4.10 (br m, 1H), 4.82 (dd, 1H), 6.52 (br t, 1H), 6.77 (br d, 1H), 6.85 (dd, 1H), 7.03–7.12 (m, 2H), 7.16–7.31 (m, 6H), 7.48 (br s, 1H), 7.56 (br s, 1H), 7.81 (d, 1H), 8.21 (br d, 1H).

4(*R*)-(Boc-Trp-Lys(Tac)-amino)-1-((S)-benzylcarbamoylmethyl)pyrrolidin-2-one (19). The Boc-protected material from the above reaction (400 mg, 0.66 mmoL) was treated with 1.5 N HCl (7.5 mL) in glacial AcOH at room temperature, and after 1 hthe contents of the flask were frozen and lyophilized. The residue was dissolved in MeOH and concentrated *in vacuo* (2×) to afford the hydrochloride salt. Half of this material (0.33 mmoL) was combined with commercially available Boc-Trp-OSu (0.13 g, 0.33 mmoL) in DMF (5 mL) and treated with DIEA (65 μL, 0.36 mmoL). The resulting solution was stirred at room temperature under nitrogen, and after 3 h the reaction mixture was added dropwise to a large volume of dilute aqueous HCl (~150 mL). The crude product was filtered and the solid precipitate washed with water. Preparative reverse-phase HPLC purification (C_{18}) HPLC (Dynamax, MeCN–H_2O) afforded, after lyophilization, the title compound (0.112 g, 42%): MS (FAB$^+$) *m/z* 795 (M + H)$^+$, 817 (M + Na)$^+$; ^{1}H-NMR (DMSO-d_6) δ 1.09–1.69 (br m, 4H), 1.31 (s, 9H), 2.11 (dd, 1H), 2.15 (s, 3H), 2.36 (dd, 1H), 2.82–2.96 (m, 2H), 3.00–3.22 (m, 4H), 3.25–3.38 (m, 3H), 3.65 (dd, 1H), 4.06 (m, 1H), 4.21 (m, 2H), 4.82 (dd, 1H), 6.53 (t, 1H), 6.81–6.87 (m, 2H), 6.93–6.99 (m, 1H), 7.02–7.14 (m, 4H), 7.17–7.35 (m, 7H), 7.50 (br s, 1H), 7.58 (t, 2H), 7.81 (d, 1H), 7.89 (d, 1H), 8.35 (d, 1H), 10.80 (br s, 1H). Anal. Calcd for $C_{43}H_{54}N_8O_7 \cdot 0.5H_2O$: C, 64.23; H, 6.91; N, 13.94. Found: C, 63.96; H, 6.82; N, 13.81.

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