N-Desmethyl Derivatives of Deoxybouvardin and RA-VII: Synthesis and Evaluation

Dale L. Boger* and Jiacheng Zhou

Contribution from the Department of Chemistry, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, California 92037

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Abstract: The synthesis of the complete set of seven *N*-desmethyl derivatives of RA-VII (**8**) are described. Thus, the synthesis of the four 14-membered cycloisodityrosine derivatives **21**−**24** and their coupling with the two tetrapeptides **32** and **33** followed by formation of the 18-membered ring with macrocyclization provided the full set of seven desmethyl derivatives **14**−**20** of RA-VII (**8**). The solution phase conformational properties of **8** and **14**−**20** were examined by 1D and 2D ^{1}H NMR to reveal the role of N-methylation on the key conformational aspects of the natural agents. In contrast to each of the simple cycloisodityrosine derivatives **21**−**24** which adopt a single, rigid solution conformation possessing a secondary or tertiary trans amide central to the 14-membered ring, the natural agents including **8** adopt a single predominant solution conformation (83−88%) that corresponds closely to the X-ray structure conformation which possesses an inherently disfavored cis C^{30}−N^{29} tertiary amide central to the 14-membered cycloisodityrosine subunit. Moreover, this cis amide is the predominant conformation (85−95%) observed with N^{29}-desmethyl RA-VII (**14**) indicating that even a secondary C^{30}−N^{29} amide adopts this inherently disfavored cis amide stereochemistry. The minor conformation of **8** observed in solution (12−17%) is shown to be derived from a minor cis C^8−N^9 tertiary amide which was not observed with its conversion to a secondary amide. Both N^9-desmethyl RA-VII (**15**) and N^9,N^{29}-desmethyl RA-VII (**18**) adopt exclusively a single solution conformation that corresponds to the major solution conformations of **8** and **14**. This conformation contains a characteristic cis C^{30}−N^{29} amide central to a type VI β-turn and the cycloisodityrosine subunit, a trans C^8−N^9 amide central to a typical type II β-turn capped with a tight Ala^4-NH-O=C-Ala^1 hydrogen bond, and a trans C^{14}−N^{15} *N*-methyl amide. In sharp contrast, removal of the N^{15} methyl group within **16**, **17**, **19**, and **20** results in the adoption of solution conformations possessing the inherently favored trans C^{30}−N^{29} amide central to the cycloisodityrosine 14-membered subunit. Thus, the N^{15}-methyl group within **8** is responsible for the agents adoption of the disfavored cis C^{30}−N^{29} amide central to the cycloisodityrosine subunit. Importantly, preceding studies have defined the cycloisodityrosine subunit of **8** as the pharmacophore and, in a reversal of the initially assigned roles, revealed that it is the tetrapeptide housed in the 18-membered ring that induces and maintains the rigid, normally inaccessible cis C^{30}−N^{29} amide conformation within the 14-membered cycloisodityrosine subunit. The studies detailed herein reveal that it is the N^{15}-methyl group that induces this conformational preference for the disfavored cis C^{30}−N^{29} amide and that its removal results in a major conformational change with adoption of the trans C^{30}−N^{29} amide and a loss of biological activity. Thus, the N^{15}-methyl group is essential for maintenance of the conformational and biological properties of **8**; the N^9-methyl group is not essential, and its removal leads to exclusive population of a single biologically active conformation; and the N^{29}-methyl group once thought essential to the adoption of the C^{30}−N^{29} cis amide is not essential, and its removal does not alter the conformational or biological properties of **8**.

Bouvardin (**1**, NSC 259968) and deoxybouvardin (**2**), bicyclic hexapeptides isolated from *Bouvardia ternifolia* (Rubiaceae) and identified by X-ray structure analysis (bouvardin) and chemical correlation (deoxybouvardin),[1] constitute the initial members of a growing class of potent antitumor antibiotics now including *O*-methyl bouvardin (**3**)[1] and RA I-XIV.[2−14] Studies of the antitumor properties of RA-VII (**8**) have revealed efficacious antitumor activity including a demonstration of cures in a solid-tumor, colon adenocarcinoma 38.[15] Both bouvardin and RA-

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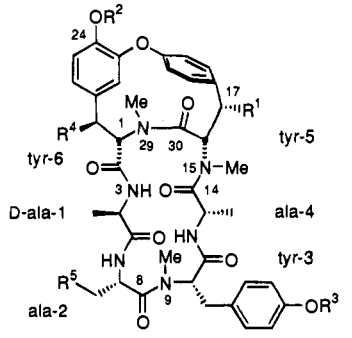
VII have been shown to inhibit protein synthesis[15-17] through eukaryotic 80S ribosomal binding[18,19] with inhibition of both amino acyl *t*RNA binding and peptidyl *t*RNA translocation, and this is presently thought to be the site of action for the agent antitumor activity.



	R^1	R^2	R^3	R^4	R^5	
1	OH	H	Me	H	H	bouvardin
2	H	H	Me	H	H	RA-V, deoxybouvardin
3	OH	Me	Me	H	H	6-*O*-methylbouvardin
4	H	H	Me	H	OH	RA-I
5	H	Me	H	H	H	RA-II
6	H	Me	Me	H	OH	RA-III
7	H	Me	Me	OH	H	RA-IV
8	H	Me	Me	H	H	RA-VII
9	H	Me	Me	H	(OH)Me	RA-VIII
10	H	Me	Me	H	CH_2CO_2H	RA-X
11	H	H	Me	H	CH_2CO_2H	RA-XI
12	H	Ac	Me	H	H	RA-V *O*-acetate

Although the examination of the structures **1**−**3** led to the initial proposal that the cycloisodityrosine-derived 14-membered ring serves the functional role of inducing and maintaining a rigid, normally inaccessible conformation within a biologically active tetrapeptide housed in the 18-membered cyclic hexapeptide,[1,20] more recent studies have shown that it is the cycloisodityrosine subunit that constitutes the agent pharmacophore.[21-27] Until recently, efforts to systematically examine the role of the cycloisodityrosine subunit have been hampered by their synthetic inaccessibility. Conventional macrolactamization techniques including transannular lactamizations,[23] Ullmann macrocyclizations with C^3-O^2 bond closure,[23,28-30] and intramolecular oxidative phenol couplings[20] have failed to date to provide the 14-membered cycloisodityrosine subunit.[31] We recently disclosed the implementation of a general C^1-O^2 Ullmann macrocyclization reaction for the preparation of such 14-membered biaryl ethers (45−60%)[32] and have reported the successful extension of the methodology to the total syntheses of RA-VII (**8**) and deoxybouvardin (**2**),[23,33] *N*-methyl cycloisodityrosine,[23,33] piperazinomycin,[34] bouvardin (**1**) and *O*-methyl bouvardin (**3**),[35] and related agents.[36-38] In these studies, the direct Ullmann macrocyclization reaction with C^1-O^2 ring closure has proven successful even with functionalized, base-sensitive substrates (30−55% yields)[33-37] and more effective than an indirect, two-step thallium trinitrate-promoted phenol coupling reaction introduced by Yamamura and co-workers.[39-43] This latter process, which requires the use of dichloro- and dibromophenol coupling partners, was employed by Inoue and co-workers[39] in the first total synthesis of RA-VII (**8**) and deoxybouvardin (**2**) albeit with the key steps proceeding in low yields (*ca.* 2−5%).

In preceding studies of the structure and solution conformation of **1**,[1] **2**, **3**, and **8** as well as N^{29}-desmethyl RA-VII (**14**)[23] a single predominant solution conformation was observed which possesses a characteristic $N^{29}-C^{30}$ cis amide and corresponds closely to the X-ray structure found for **1**.[1] Moreover, this conformation was observed even with N^{29}-desmethyl RA-VII (**14**) which was shown to possess an unusual secondary cis $N^{29}-$

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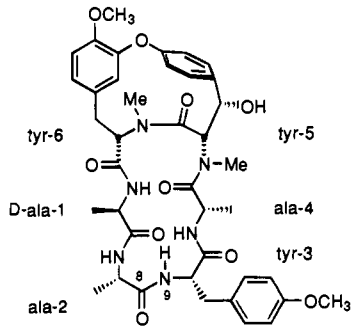
C^{30} amide.[22,23] In contrast, the *N*-methyl or N−H cycloisodityrosine derivatives **21**−**24** adopt a single, rigid conformation possessing an expected trans amide.[23,35] These studies clearly demonstrated that the bicyclic natural products adopt a conformation possessing the inherently disfavored $N^{29}-C^{30}$ cis amide. Nonetheless, one additional minor conformation of **1**−**3** may be detected in the ^1H NMR (5−20%) in nonpolar solvents including $CDCl_3$ and THF-d_8. Exhaustive conformational searches conducted on deoxybouvardin (**2**) suggested that minor conformations were not expected to be derived from a trans $N^{29}-C^{30}$ *N*-methyl amide and that of the two remaining *N*-methyl amides; it was the N^9-C^8 amide that appeared most likely to adopt an accessible cis amide conformation. Careful ^1H NMR studies of the agents including diagnostic differences in the readily assignable *N*-methyl chemical shifts and NOEs observed in the 2D $^1H-^1H$ NMR spectrum with the major and minor conformation supported this expectation.[44] The recent synthesis and evaluation of N^9-desmethyl *O*-methylbouvardin (**13**), which was found to adopt a single solution phase conformation possessing a cis $N^{29}-C^{30}$ amide and a secondary N^9-C^8 trans amide corresponding to the major conformation of **1**, confirmed that the minor conformations of **1**−**3** arise from a cis N^9-C^8 *N*-methyl amide conformation.[35]



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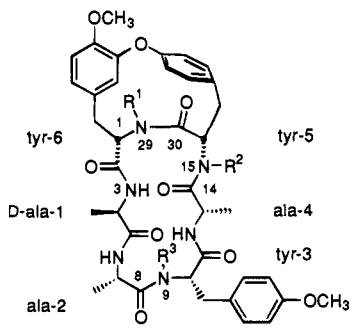
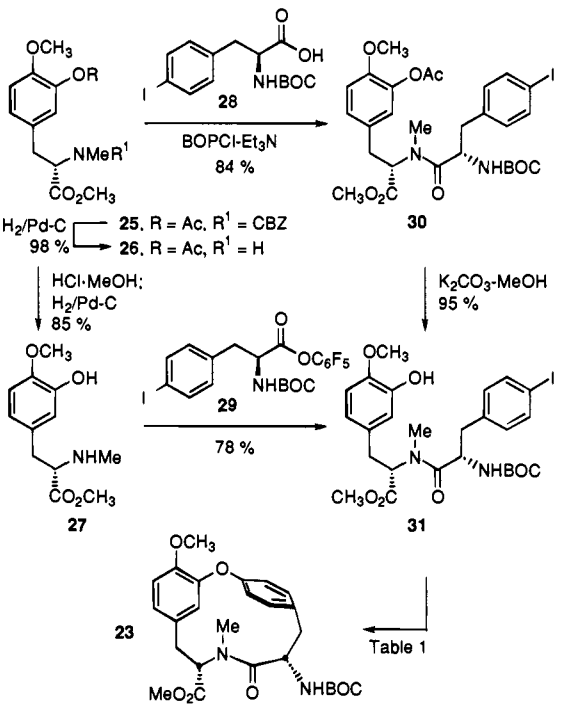
Herein, we detail studies on the preparation and evaluation of **15**−**20**, the complete series of *N*-desmethyl derivatives of RA-VII (**8**) and deoxybouvardin (**2**), which unambiguously establish the site and stereochemistry of the minor amide conformations of **1**−**12** and through their comparative evaluation serve to establish the surprising role of the three N-methylation sites within the natural products.



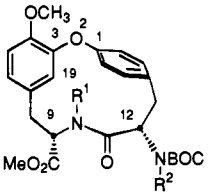
	R^1	R^2	R^3	
14	H	Me	Me	N^{29}-desmethyl RA-VII
15	Me	Me	H	N^9-desmethyl RA-VII
16	Me	H	Me	N^{15}-desmethyl RA-VII
17	Me	H	H	N^9,N^{15}-desmethyl RA-VII
18	H	Me	H	N^9,N^{29}-desmethyl RA-VII
19	H	H	Me	N^{15},N^{29}-desmethyl RA-VII
20	H	H	H	N^9,N^{15},N^{29}-desmethyl RA-VII

(44) Boger, D. L.; Patane, M. A.; Zhou, J. accompanying article.

Scheme 1



The 14-Membered Cycloisodityrosine Subunits. The cycloisodityrosine subunits **21**−**24** incorporating the four possible variations in the extent of N-methylation were required for preparation of the full series of agents **14**−**20**. Both **21** and **22** were available from our studies[22,23,33] that led to the total synthesis of deoxybouvardin, RA-VII, and N^{29}-desmethyl RA-VII (**14**), respectively. The agent **24**, which lacks both the N^{10} and C12 N^{α}-methylation, was prepared in efforts that led to the total synthesis of piperazinomycin.[34] Only the agent **23**, which lacks the C12 N^{α}-methylation had not yet been prepared and was required to complete the series.



	R^1	R^2
21	Me	Me
22	H	Me
23	Me	H
24	H	H

Direct coupling of methyl O^3-acetyl-O^4-methyl-L-DOPA (**26**)[45] with *N*-BOC-L-4-iodophenylalanine (**28**) provided **30** in excellent yield (84%). The use of the O^3-acetate **26** prevented competitive O-acylation generally observed with the free phenol **27** under standard amide coupling procedures which, in the case of **26**, may be attributed to the diminished rate of tertiary amide formation. Mild methanolysis of **30** provided **31** and our substrate for the required Ullmann closure to **23**. In the examination of methods for the preparation of **31**, the competitive O-acylation of **27** was found to be minimized if the coupling of methyl O^4-methyl-L-DOPA (**27**)[44] was conducted with the pentafluorophenyl ester of *N*-BOC-L-4-iodophenylalanine (**29**, DMF, 25 °C, 24 h, 78%) and this also provided **31** in excellent conversions (Scheme 1). Subjection of **31** to the set of

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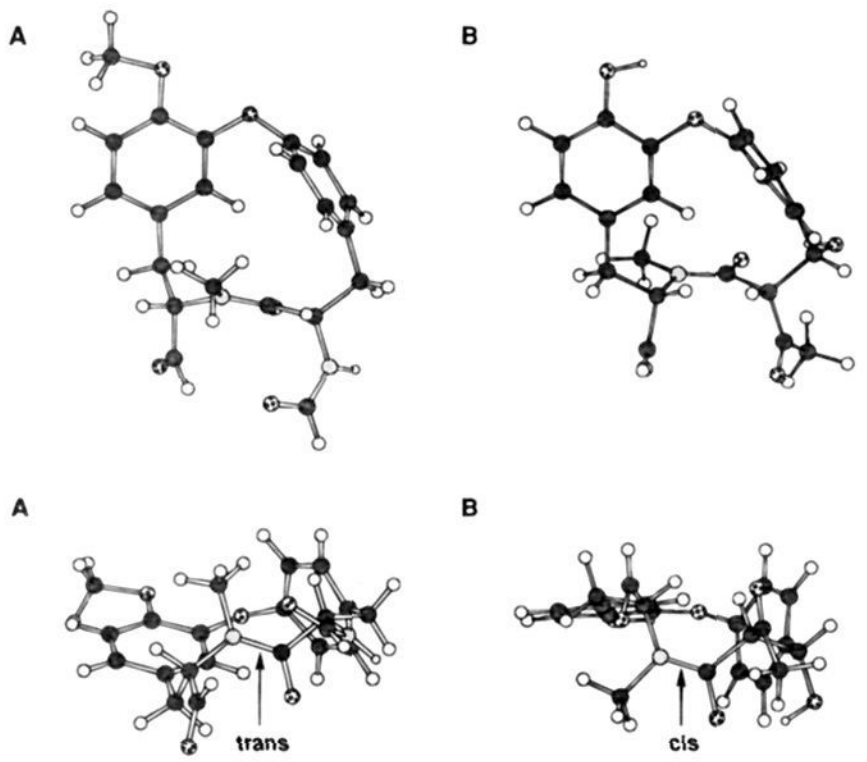

Figure 1. A: OPLSA low energy conformation of **23**. B: Fourteen-membered ring conformation taken from X-ray crystal structure of bouvardin (**1**).

conditions established for the Ullmann macrocyclization[32−37] afforded the cycloisodityrosine derivative **23**.

The generation of the 14-membered ring in the cyclization of **31** was confirmed upon observation of the diagnostic, strongly shielded C19-H (d, J = 2.2 Hz) at 4.73 ppm ($CDCl_3$). Like **21**, **22**, and **24**, the cycloisodityrosine derivative **23** adopts a rigid solution conformation possessing a trans $N^{10}-C^{11}$ amide. Consistent with expectations based on a conformational analysis,[46−48] the global and low lying conformations (≤12 kcal/mol) of **23** each possess a trans $N^{10}-C^{11}$ *N*-methyl amide (Figures 1 and 2). The conformational search of **23**, like that of **21**,[23] revealed a single, low energy conformation that was 4.6 kcal/mol lower in energy than any other located conformation and 6.2 kcal/mol lower in energy than the lowest energy conformation possessing a cis amide bond. The calculated coupling constants for the C9 and C12 hydrogens in the lowest energy conformation of **23** are 3.1, 11.8 Hz (dd) and 5.4, 9.0, 11.1 Hz (ddd), respectively, and match the experimentally measured values of 2.8, 12.0 Hz (dd, 4.58 ppm) and 5.4, 9.8, 12.6 Hz (ddd, 4.92 ppm). Confirmation that **23** adopts a solution conformation that possesses a trans *N*-methyl $N^{10}-C^{11}$ amide was derived from 2D $^1H-^1H$ NOESY NMR. Strong NOE crosspeaks were observed for C9-H/N10-CH_3 and C12-H/N10-CH_3 and are uniquely diagnostic of the trans amide stereochemistry. Similarly, a C9-H/C12-H NOE crosspeak was not detected and would be both intense and diagnostic of a cis amide stereochemistry. Consequently, **23** adopts a single rigid solution conformation possessing a trans $N^{10}-C^{11}$ amide like the preceding cycloisodityrosine derivatives. Table 1 summarizes the diagnostic comparison properties of **21**−**24** and Tables 4 and 5 in the Experimental Section provide a detailed comparison of their ^1H and ^{13}C NMR properties.

(46) Global and close low-lying minima (≤12 kcal/mol for **23** and ≤5 kcal/mol for **20**) were located in conformational searches with use directed Monte Carlo sampling and subsequent minimization of conformations generated by random variations (0−180°) in the available torsional angles[47] excluding those originating in the phenyl rings (MacroModel,[48] version 3.5, OPLSA force field, MCMM = 5000, MCSS = 2, 12, or 5 kcal/mol window). The global minima for **23** and **20** were located ≥25 times.

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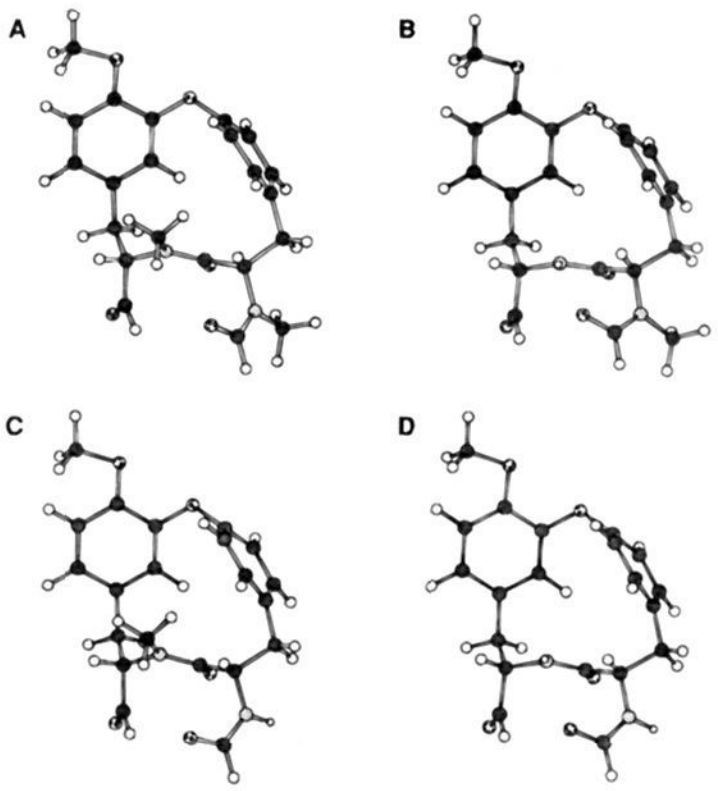
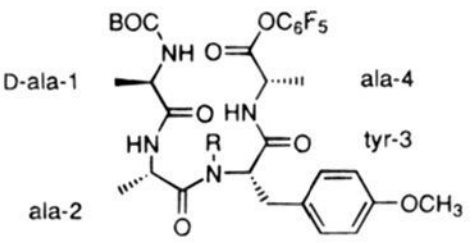


Figure 2. A: OPLSA low energy conformation of **21**. B: OPLSA low energy conformation of **22**. C: OPLSA low energy conformation of **23**. D: OPLSA low energy conformation of **24**.

The Tetrapeptide Subunits: BOCNH-D-Ala-Ala-NMe-Tyr(OCH_3)-Ala-OC_6F_5 (32) and BOCNH-D-Ala-Ala-Tyr-(OCH_3)-Ala-OC_6F_5 (33). Completion of the preparation of the full range of agents **14**−**20** required the two tetrapeptides **32** and **33**. The tetrapeptide **32**, which incorporates the remaining N^9-C^8 *N*-methyl amide characteristic of the natural products **1**−**12**, was prepared through esterification of the corresponding carboxylic acid[25] (1.2 equiv of C_6F_5OH, 1.2 equiv of EDCI, CH_2Cl_2, 25 °C, 4 h, 75%) available from studies on the total synthesis of **1**−**3** and **8**. The tetrapeptide **33** which lacks the remaining *N*-methyl amide that is key to the definition of the role and stereochemistry of natural product N^9-C^8 amide was prepared by a similar route (Scheme 2).



32, R = Me
33, R = H

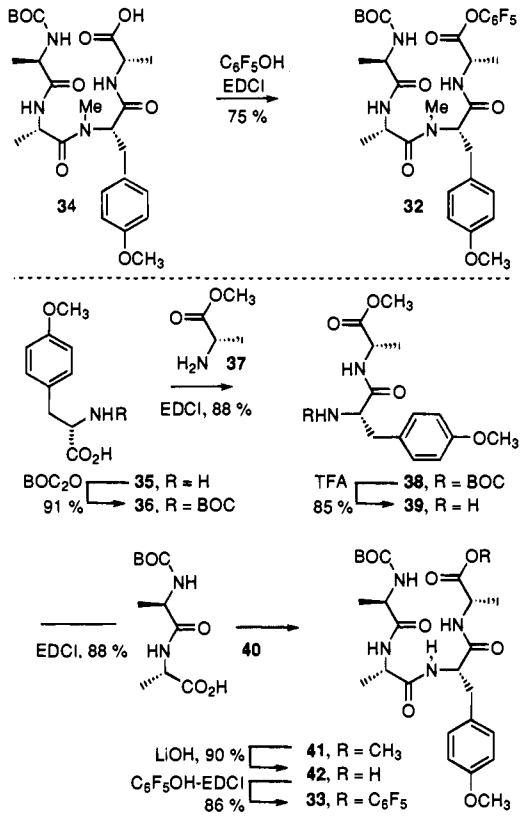
Coupling of BOCNH-L-Tyr(OCH_3)-OH (**36**)[25] with L-alanine methyl ester (**37**, 1.1 equiv of EDCI, 1.1 equiv of HOBt, DMF, 25 °C, 10 h, 88%) provided **38**. Acid-catalyzed BOC deprotection (TFA, CH_2Cl_2, 25 °C, 1 h, 85%) followed by coupling of the free base **39** with BOCNH-D-Ala-L-Ala-OH (**40**,[25] 1.1 equiv of EDCI, 1.1 equiv of HOBt, DMF, 25 °C, 12 h, 88%) provided the tetrapeptide **41**. Conversion of **41** to the activated pentafluorophenyl ester **33** was accomplished upon saponification (2.0 equiv of LiOH, THF−CH_3OH-H_2O 3:1:1, 25 °C, 6 h, 90−96%) and esterification of the intermediate carboxylic acid **42** (1.2 equiv of C_6F_5OH, 1.2 equiv of EDCI, CH_2Cl_2, 25 °C, 4 h, 75−86%).

Synthesis of 15−20: *N*-Desmethyl Derivatives of RA-VII. Acid-catalyzed deprotection of **21**−**24** (4 M HCl−EtOAc, 25 °C, 30 min) followed by coupling of the liberated free amine with **32** or **33** (THF, 25−50 °C, 2−12 h, 81−88%) provided **47**−**52** (Scheme 3). The higher reaction temperatures (50 °C) and longer reaction times (12 h) were required only for the couplings of the secondary amines **43** and **44** while the primary

Table 1. Comparison of the Chemical and Biological Properties of **21**–**24**

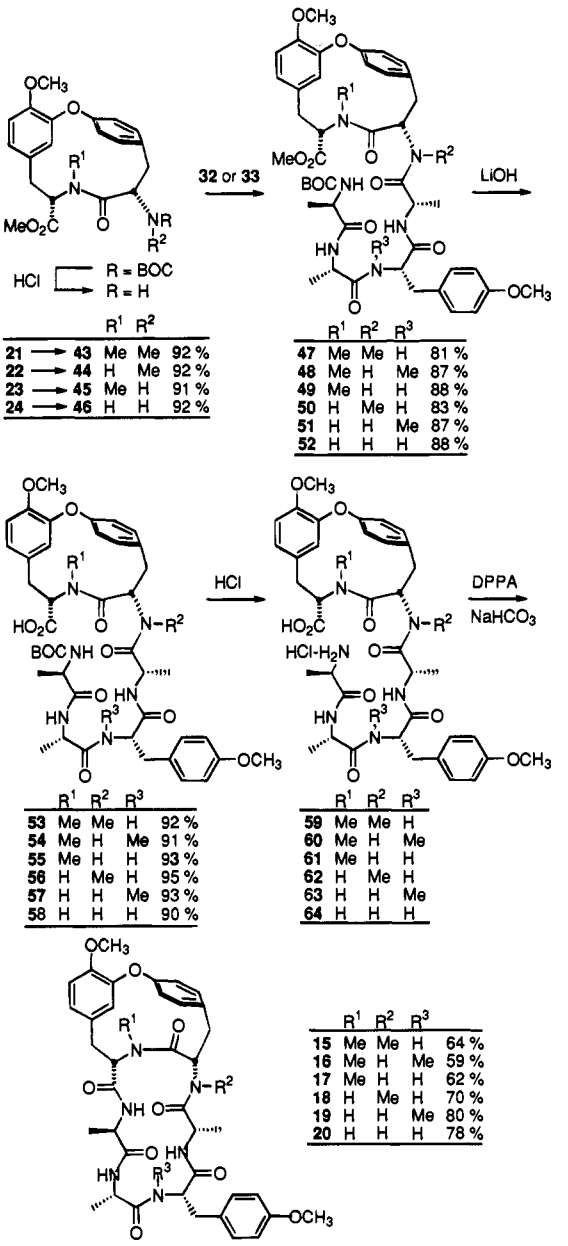
	% yield of Ullmann reaction		^{1}H NMR C-19H (d)		
	NaH/CuBrMe$_2$S	CH$_3$Cu	δ and J (Hz), CDCl$_3$	$[\alpha]^{22}_D$	rel IC$_{50}$ (L1210)
21[23]	22		4.75, 2.2	−23 (c 0.25, CH$_3$OH)	1.0
22[23]	30	36	5.14, 1.8	−6.7 (c 0.2, CH$_3$OH)	0.5
23	34	31	4.73, 2.2	−49 (c 0.2, CHCl$_3$)	1.1
24[34]	25		5.05, 2.0	−32 (c 0.25, CHCl$_3$)	2

Scheme 2



amines **45** and **46** reacted at room temperature (2 h). The free amines **43**–**46** were isolated and fully characterized in the course of these efforts, and the spectroscopic properties of **43** differ significantly from that reported by Inoue and co-workers and employed in the initial low yielding total synthesis of **2**.[39] Sequential hydrolysis of the methyl esters **47**–**52** (2.5–3.0 equiv of LiOH, THF–CH$_3$OH–H$_2$O, 25 °C, 6–10 h, 90–95%), acid-catalyzed N-BOC deprotection of **53**–**58** (3–4 M HCl–EtOAc, 0–25 °C, 1 h), and subsequent macrocyclization of **54**–**64** upon treatment with diphenyl phosphorazidate[49] (DPPA, 2.0 equiv, 8–10 equiv of NaHCO$_3$, 0.003 M DMF, 4 °C, 48 h, 59–80%) provided **15**–**20** in excellent conversions. Macrocyclization with C^2–N^3 amide bond formation and closure of the 18-membered ring was conducted strategically at the one common secondary amine site that possesses a D-amino acid amine terminus[50,51] under the improved DPPA reaction conditions.[52]

Scheme 3



Comparisons of 8 with 14–20: Role of N-Methylation on the Conformational Properties. Each of the agents **14**–**20** were subjected to extensive spectroscopic comparison alongside **8** including complete ^{1}H NMR, ^{13}C NMR, 2D ^{1}H–^{1}H NOESY, and ROESY NMR in efforts to establish their conformational properties. The details of these comparisons and the resulting spectral assignments are summarized in the Experimental Section. However, the comparisons revealed a simple paradigm that controls the conformational properties of the natural products including **8** which ultimately has a pronounced influence on their biological properties. The X-ray crystal structure of bouvardin (**1**)[1] and deoxybouvardin (**2**)[12] revealed that the three secondary amides and the C^8–N^9 and C^{14}–N^{15}

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N-methyl amides possess the trans stereochemistry, while the $C^{30}-N^{29}$ *N*-methyl amide central to the cycloisodityrosine 14-membered ring adopts a cis stereochemistry. In addition, the X-ray structure conformation has been shown to correspond to the major predominant solution conformation ($CDCl_3$, THF-d_8) for **1**, **2**, and RA-VII (**8**).[1] Moreover, RA-VII has been shown to adopt this near exclusive solution conformation upon complexation with LiCl (THF-d_8/LiCl)[44] indicating that this represents the preferred conformation even under conditions that may reflect its behavior in an aqueous media. Under such conditions, the solution conformation of the agent even more closely matches the X-ray structure conformation.[44] This conformation contains a characteristic cis $C^{30}-N^{29}$ *N*-methyl amide central to a type VI β-turn and the cycloisodityrosine subunit, a trans C^8-N^9 *N*-methyl amide central to a typical type II β-turn capped with a tight Ala^4-NH-O=C-Ala^1 hydrogen bond and a trans $C^{14}-N^{15}$ *N*-methyl amide. Diagnostic of this conformation, the Ala^2-NH is fully accessible to solvent and exhibits fast exchange rates ($t_{1/2}$ < 30 min, DMSO), the Ala^4-NH is inaccessible to solvent due to the tight hydrogen bond and exhibits both a very slow exchange rate ($t_{1/2}$ > 2 day) and little solvent dependent chemical shifts changes, and the Ala^1-NH which participates in a weak hydrogen bond in aprotic solvents ($CDCl_3$, THF-d_8, DMSO-d_6) exhibits an intermediate exchange rate ($t_{1/2} \leq 10$ h). This weak hydrogen bond of Ala^1-NH is not observed in the X-ray and is disrupted upon complexation with LiCl (LiCl/THF-d_8).[44]

A second spectroscopically detected conformation for **1**, **2**, or **8** is observed in $CDCl_3$ or 15% $CD_3OD-CDCl_3$ and may be attributed to an additional conformation within the flexible portion of the 18-membered ring possessing a cis C^8-N^9 *N*-methyl amide. N^{29}-Desmethyl RA-VII (**14**) behaves essentially identical to **8** and possesses a single predominant solution conformation in $CDCl_3$ (85−95%, Table 2). Diagnostic of this major conformation is a strong and characteristic NOE observed between C^1-H and $C^{16}-H$. Within the X-ray structure conformation of **1** and **2**, the $C^1-H/C^{16}-H$ proton−proton distance is only 1.7−1.8 Å, and accordingly the $C^1-H/C^{16}-H$ NOE crosspeak in the 2D $^1H-^1H$ NOESY NMR spectrum of **1**, **2**, and **8** constitutes the strongest observed NOE. Expectedly absent are NOE crosspeaks between C^1-H or $C^{16}-H$ and $N^{29}-CH_3$ that would be present if **1**, **2**, or **8** adopts a trans $C^{30}-N^{29}$ amide bond. In the trans $C^{30}-N^{29}$ conformation, the $C^1-H/C^{16}-H$ proton−proton distance is 4.9 Å, and the methyl group of $N^{29}-CH_3$ lies directly between the C^1-H and $C^{16}-H$ with proton−proton distances of 1.8−1.95 Å. Thus, the presence of a strong $C^1-H/C^{16}-H$ NOE crosspeak in the 2D $^1H-^1H$ NMR spectrum is uniquely diagnostic of a cis $C^{30}-N^{29}$ amide while the presence of strong $C^1-H/N^{29}-R$ and $C^{16}-H/N^{29}-R$ NOE crosspeaks may be considered uniquely diagnostic of a trans $C^{30}-N^{29}$ amide (Figure 2). As detailed in the accompanying paper, the major conformation of **8** also exhibited strong C^7-H/N^9-CH_3 and $C^{10}-H/N^9-CH_3$ NOEs but no $C^7-H/C^{10}-H$ NOE diagnostic of a trans C^8-N^9 amide as well as $C^{13}-H$ and $C^{13}-CH_3/N^{15}-CH_3$ NOEs characteristic of a trans $C^{14}-N^{15}$ amide and its backbone orientation. For **14**, not only were the spectral characteristics of the agent essentially identical to those of **8** (δ and $^1H-^1H$ NOE) and the ratio of major and minor conformational isomers relatively unperturbed, but it exhibited the strong characteristic $C^1-H/C^{16}-H$ NOE crosspeak in the $^1H-^1H$ NOESY NMR diagnostic of a cis $C^{30}-N^{29}$ amide. Thus, even **14** which possesses a secondary $C^{30}-N^{29}$ amide adopts the characteristic conformation of the natural products in which the amide central to the cycloisodityrosine 14-membered ring possesses the inherently disfavored cis stereochemistry. This result was initially surprising since it was anticipated that this N^{29} methylation would be critical to the adoption of the $C^{30}-N^{29}$ cis amide stereochemistry. In contrast to such expectations, the removal of the N^{29} methyl group had no perceptible effect on the conformational equilibria of the agents. In addition, this preferential adoption of the cis $C^{30}-N^{29}$ amide is in marked contrast to the simple 14-membered cycloisodityrosines **21**−**24**, each of which adopts a single solution conformation possessing a trans amide. Thus, these initial results suggested that it is not the rigid 14-membered cycloisodityrosine that serves the scaffolding role of inducing and maintaining a rigid, normally inaccessible conformation within the tetrapeptide[1] but rather that it is the tetrapeptide that induces a rigid, normally inaccessible conformation within the 14-membered cycloisodityrosine ring.[22,23]

Table 2. Conformational Composition of **8** and **14**−**20**[a]

	Solvent = 15% $CD_3OD-CDCl_3$			
	% CTT[b]	CCT	TTT	TCT
8	83	17		
14	83	17		
15	100			
16			56	44
17			100	
18	>98	<2		
19			56	44
20			100	
	Solvent = $CDCl_3$			
	% CTT	CCT (%)	TTT	TCT
8	88	12		
14[c]	85−95	15−5		
15[d]				
16			66	34
17			100	
18	84	16		
19			62	38
20[d]				
	Solvent = DMSO-d_6			
	% CTT	CCT	TTT	TCT
8[e]	64	32		
14				
15	100			
16			38	62
17			100	
18	100			
19			18	82
20			100	

[a] All data were obtained by ^1H NMR (400 MHz, 295 K). [b] The C or T refer to cis or trans amide and are listed in the order of $C^{30}-N^{29}$, C^8-N^9, and $C^{14}-N^{15}$. [c] Reference 23. [d] Compounds **15** and **20** were not soluble in $CDCl_3$. [e] An additional CCC conformation (4%) was detected.

The minor conformations of **8** and **14** each exhibited a strong C7−H/C10−H NOE and lacked the C^7-H and $C^{10}-H/N^9-CH_3$ NOEs diagnostic of a cis C^8-N^9 amide, and the remaining elements of the spectra were the same indicating that the differences were due to cis-trans isomerization about the C^8-N^9 amide. The subsequent examination of N^9-desmethyl RA-VII (**15**) served to confirm this origin of the minor solution conformation observed with **1**, **2**, **8**, and **14**. The agent **15** could be expected to adopt a conformation possessing a secondary trans C^8-N^9 amide. The ^1H NMR spectrum of **15** revealed a single solution conformation in any solvent that corresponds to the major solution conformation of **1**, **2**, **8**, and **14** and which lacked the diagnostic signals observed for their minor conformations. Since **15** incorporates a secondary C^8-N^9 amide capable of adopting only a trans amide stereochemistry and no longer

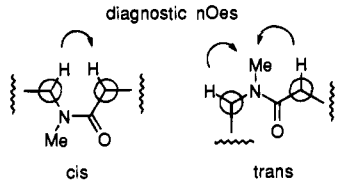


Figure 3.

adopts the minor conformations of **1**, **2**, and **8**, their minor conformation can be localized to the C^8-N^9 amide and assigned a cis stereochemistry. Diagnostic of this conformation, **15** exhibited an intense $C^1-H/C^{16}-H$ NOE (cis $C^{30}-N^{29}$ amide), C^7-H and $C^{10}-H/N^9-H$ NOEs (trans C^8-N^9 amide), and $C^{13}-H$ and $C^{13}-CH_3/N^{15}-CH_3$ NOEs (trans $C^{14}-N^{15}$ amide and backbone orientation).

The examination of N^{15}-desmethyl RA-VII (**16**) was just as revealing. This agent which can be expected to adopt a conformation possessing a trans $C^{14}-N^{15}$ amide exhibited a nearly equal mixture of two solution conformations in any solvent (Table 2). Thus, the minor conformations of **1**, **2**, **8**, and **14** are not due simply to minor amounts of the corresponding cis $C^{14}-N^{15}$ amide. More revealing, both conformations of **16** were found to lack the diagnostic $C^1-H/C^{16}-H$ NOE crosspeak in the $^1H-^1H$ NOESY NMR spectrum and to exhibit a set of characteristic $C^1-H/N^{29}-CH_3$ and $C^{16}-H/N^{29}-CH_3$ NOE crosspeaks diagnostic of a trans $C^{30}-N^{29}$ amide stereochemistry. One conformation exhibited C^7-H and $C^{10}-H/N^9-CH_3$ NOEs diagnostic of a trans C^8-N^9 amide, while the other exhibited a strong $C^7-H/C^{10}-H$ NOE diagnostic of a cis C^8-N^9 amide. Thus, the agent adopts two new conformations each possessing the trans $C^{30}-N^{29}$ amide stereochemistry central to the isodityrosine subunit, a trans $C^{14}-N^{15}$ amide, and a corresponding trans or cis C^8-N^9 *N*-methyl amide stereochemistry. Importantly, this observation defines the N^{15} methylation as the structural feature of **1**, **2**, and **8** that is responsible for the unusual adoption of the inherently disfavored $C^{30}-N^{29}$ cis amide.

Consistent with this interpretation, the further removal of N^{15} methyl group from **16** with N^9,N^{15}-desmethyl RA-VII (**17**) provided an agent that adopts a single solution conformation in any solvent which assuredly possesses both a trans C^8-N^9 and $C^{14}-N^{15}$ amide. Again, the 2D $^1H-^1H$ NOESY NMR spectrum of **17** exhibited a set of C^1-H and $C^{16}-H/N^{29}-CH_3$ NOE crosspeaks diagnostic of a trans $C^{30}-N^{29}$ amide and lacked the corresponding $C^1-H/C^{16}-H$ NOE crosspeak that would indicate the presence of a cis amide. Characteristic of the trans C^8-N^9 and $C^{14}-N^{15}$ trans amides, C^7-H and $C^{10}-H/N^9-H$ NOEs and $C^{13}-H$ and $C^{13}-CH_3/N^{15}-H$ NOEs were observed.

Similarly, the further removal of the N^{29}-methyl group from **17** with N^9,N^{15},N^{29}-desmethyl RA-VII (**20**) provided an agent that also adopts a single solution conformation in any solvent which corresponds to the all-trans amide conformation. Diagnostic of the trans $C^{30}-N^{29}$ trans amide, strong C^1-H and $C^{16}-H/N^{29}-H$ NOE crosspeaks were observed in the 2D $^1H-^1H$ NOESY NMR, and no evidence for the cis amide $C^1-H/C^{16}-H$ NOE was detected. Characteristic of the trans C^8-N^9 and $C^{14}-N^{15}$ trans amide, C^7-H and $C^{10}-H/N^9-H$ NOEs and $C^{13}-H$ and $C^{13}-CH_3/N^{15}-H$ NOEs were observed. Thus, the removal of the *N*-methyl group from the $C^{14}-N^{15}$ amide in **16**, **17**, **19**, and **20** results in the adoption of the inherently preferred trans $C^{30}-N^{29}$ amide central to the cycloisodityrosine 14-membered ring.

Two further observations confirmed these conclusions. N^9,N^{29}-Desmethyl RA-VII (**18**), in which the N^{29} methyl group of **15** has been further removed or in which the N^9 methyl group of **14** has been further removed, provided an agent that adopts a single conformation containing a trans C^8-N^9 amide and maintains the cis $C^{30}-N^{29}$ amide induced by the presence of the N^{15} methyl group. For **18**, the diagnostic $C^1-H/C^{16}-H$ intense NOE was observed (cis $C^{30}-N^{29}$ amide) as well as C^7-H and $C^{10}-H/N^9-H$ NOEs (trans C^8-N^9 amide) and $C^{13}-H$ and $C^{13}-CH_3/N^{15}-CH_3$ NOEs (trans $C^{14}-N^{15}$ amide).

In addition, N^{15},N^{29}-desmethyl RA-VII (**19**), in which the N^{29}-methyl group of **16** has been further removed, was found to behave essentially identical to **16**. Two major conformations were detected each of which possesses trans $C^{30}-N^{29}$ and $C^{14}-N^{15}$ amides and constitute a mixture of cis and trans C^8-N^9 *N*-methyl amides. Again, the $C^{30}-N^{29}$ amide central to the 14-membered cycloisodityrosine subunit adopts the inherently preferred trans amide stereochemistry in the absence of the N^{15}-methyl group.

Although a number of diagnostic ^1H NMR signals could be utilized to distinguish the cis and trans $C^{30}-N^{29}$ amides, the easiest and most reliable proved to be the Ala^4-CH_3 signal. For **8**, **14** and **15**, and **18**, its chemical shift was δ 1.10−1.18, whereas it was δ 1.53−1.78 for **16**, **17**, **19**, and **20**. Similarly, the agents possessing the trans $C^{30}-N^{29}$ amide exhibited a diagnostic and weak $C^{16}-H/N^{15}-H$ NOE, while the agents possessing a cis $C^{30}-N^{29}$ amide lacked a comparable $C^{16}-H/N^{15}-CH_3$ NOE. These differences may be attributed to the inward rotation of the Ala^4-Tyr^5 amide (Figure 4). Illustrated in Figure 4 are models of the X-ray conformation of **1**,[12] the major solution conformation of **8** (CTT)[44] which corresponds to the major or exclusive solution conformations of **14**,[22,23] **15**, and **18**, and the solution conformation of **20** (TTT) which corresponds to the exclusive solution conformation of **17** as well. The definition of the former have been described elsewhere, and the latter was derived from an exhaustive conformational search of **20**[46] to locate all accessible TTT conformations followed by further minimization with imposition of NOE distance constraints (±15%) derived from the $^1H-^1H$ NOESY NMR (100 KJ/Å^2) and fixed amide torsional angles (180 ± 10°, 1000 KJ/mol). With the exception of variations in the Tyr^3 side chain, only one located conformation fit the imposed NOE distance constraints and satisfied unrestrained hydrogen bonding constraints. The hydrogen bonding constraints were derived from amide NH exchange rates and solvent dependent chemical shift perturbations. These latter studies revealed that only N^3-H and $N^{12}-H$ were engaged in H-bonding to a comparable extent (δ = 7.40 and 7.42, $t_{1/2}$ exchange = 10 h, DMSO-d_6), while N^6-H, N^9-H, $N^{15}-H$, and $N^{29}-H$ were fully solvent accessible and not engaged in H-bonding (δ = 8.14−8.38, 8.61, $t_{1/2}$ exchange = ≤10 min, DMSO-d_6). This conformation was found to match not only the NOE distance constraints exceptionally well but also all other unrestrained experimental results surprisingly well. First, the unrestrained transannular hydrogen bond distances for the Ala^1-NH-O=C-Ala^4 and Ala^4-NH-O=C-Ala^1 are 2.68 and 2.52 Å, respectively, in this conformation and cap two typical type II β-turns. In addition, the calculated coupling constants for the six amide protons and the six α-protons matched the experimental values extraordinarily well without imposing deliberate restraints (Table 3). Only the orientation of the Tyr^3 side chain varied in a number of the located conformations and that which most closely matched the experimental coupling constants ($Tyr^{3\alpha}$-H/$Tyr^{3\beta}$-H_β J = 4.2 Hz, calcd 3.8 Hz; $Tyr^{3\alpha}$-H/$Tyr^{3\beta}$-H_α J = 11.3 Hz, calcd 11.7 Hz) is represented in Figure 4.

Conclusions. Thus, the N^{15}-methyl group is essential for the induction and maintenance of the conformational properties of the agents and is responsible for their adoption of the inherently disfavored $C^{30}-N^{15}$ cis amide; the N^9-methyl group is not

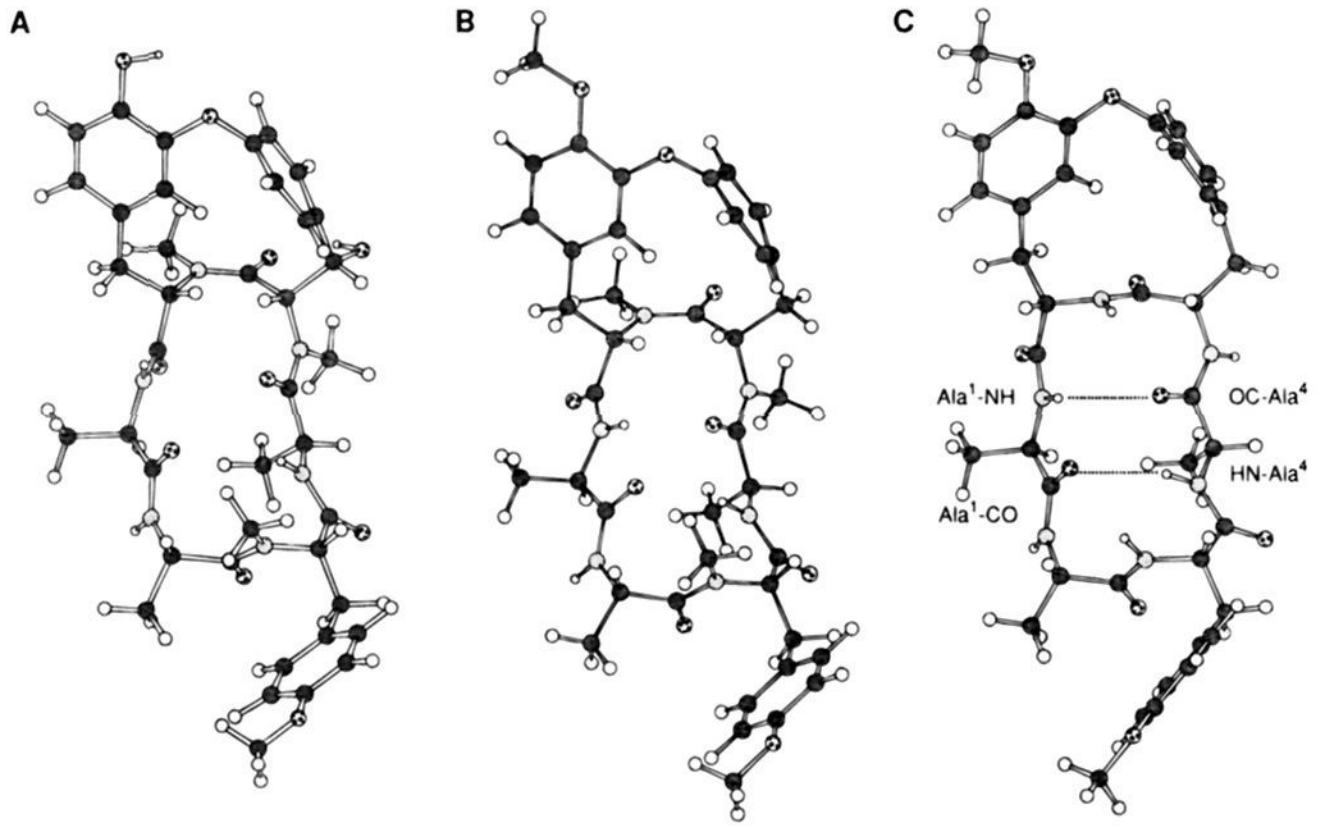


Figure 4. A: X-ray crystal structure of bouvardin (**1**). B: Major solution phase ctt conformation of RA-VII (**8**) in $CDCl_3$ or THF-d_8 which also corresponds to the major or exclusive solution conformation of **14**, **15**, and **18**. C: ttt solution conformation of **20** which also corresponds to the exclusive conformation of **17**.

Table 3. Comparison of the Calculated[a] and Observed[b] ^{1}H NMR Coupling Constants of **20**

	coupling constant (J, Hz)	
	calcd	obsd
Ala^1-NH/Ala$^{1\alpha}$-H	4.3	4.2
Ala$^{1\alpha}$-H/Ala$^{1\beta}$-CH$_3$	6.3[c]	7.0
Ala^2-NH/Ala$^{2\alpha}$-H	6.2	6.0
Ala$^{2\alpha}$-H/Ala$^{2\beta}$-CH$_3$	6.3[c]	7.2
Tyr^3-NH/Tyr$^{3\alpha}$-H	7.4	7.6
Tyr$^{3\alpha}$-H/Tyr$^{3\beta}$-H$_\beta$	3.8	4.2
Tyr$^{3\alpha}$-H/Tyr$^{3\beta}$-H$_\alpha$	11.7	11.3
Ala^4-NH/Ala$^{4\alpha}$-H	6.9	6.8
Ala$^{4\alpha}$-H/Ala$^{4\beta}$-CH$_3$	6.3[c]	7.5
Tyr^5-NH/Tyr$^{5\alpha}$-H	4.5	4.5
Tyr$^{5\alpha}$-H/Tyr$^{5\beta}$-H$_\beta$	5.5	5.0
Tyr$^{5\alpha}$-H/Tyr$^{5\beta}$-H$_\alpha$	11.6	12.2
Tyr^6-NH/Tyr$^{6\alpha}$-H	6.3	6.4
Tyr$^{6\alpha}$-H/Tyr$^{6\beta}$-H$_\beta$	2.4	2.2
Tyr$^{6\alpha}$-H/Tyr$^{6\beta}$-H$_\alpha$	11.8	10.5

[a] Taken from the computer generated model (Figure 4). [b] DMSO-d_6. [c] Average value given.

essential, and its removal leads to the exclusive adoption of a single biologically active conformation;[35] and the N^{29}-methyl group once thought to be key to the adoption of the $C^{30}-N^{29}$ cis amide is not essential, and its removal does not alter the conformational or biological properties[23] of the agents. Consistent with these findings, the agents lacking the essential N^{15}-methyl group (**16**, **17**, **19**, and **20**) were found to be biologically inactive (IC_{50}, L1210, >10 μg/mL), while **14** and **15** were essentially equipotent with **8** (IC_{50}, L1210, 0.0007−0.002 μg/mL).[23]

Experimental Section

3-Acetoxy-*N*,*O*-dimethyl-L-tyrosine Methyl Ester (26). A solution of **25**[45] (2.075 g, 5.0 mmol) in anhydrous CH_3OH (25 mL) was treated with 10% Pd−C (210 mg, 10% wt equiv) and stirred under an atmosphere of H_2 (1 atm) at 25 °C for 3 h. The reaction mixture was filtered through Celite (CH_3OH wash), concentrated in vacuo, and dried thoroughly under vacuum to afford **26** (1.377 g, 1.405 g theoretical, 98%) as a pale-yellow oil: $[\alpha]^{25}_D$ +27 (c 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.99 (dd, 1H, J = 2.2, 8.4 Hz, C6-H), 6.87 (d, 1H, J = 8.4 Hz, C5-H), 6.84 (d, 1H, J = 2.2 Hz, C2-H), 3.79 (s, 3H, $ArOCH_3$), 3.65 (s, 3H, CO_2CH_3), 3.38 (t, 1H, J = 6.8 Hz, C*H*CH_2), 2.86 (d, 2H, J = 6.8 Hz, CHCH_2), 2.34 (s, 3H, $NHCH_3$), 2.29 (s, 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.8, 169.0, 149.9, 139.5, 129.7, 127.6, 123.7, 112.2, 64.6, 55.9, 52.0, 38.7, 34.7, 20.7; IR (neat) ν_{max} 3360, 2951, 2844, 2800, 1769, 1732, 1619, 1514, 1444, 1370, 1267, 1203, 1125, 1023, 900, 815, 777 cm^{-1}; FABHRMS (NBA-NaI) *m/e* 282.1350 (M^+ + H, $C_{14}H_{19}NO_5$ requires 282.1341). Anal. Calcd for $C_{14}H_{19}NO_5$: C, 59.79; H, 6.76; N, 4.98. Found: C, 59.64; H, 7.01; N, 4.79.

3-Acetoxy-*N*,*O*-dimethyl-*N*-[[(*tert*-butyloxy)carbonyl]-L-4′-iodophenylalanyl]-L-tyrosine Methyl Ester (30). A solution of **26** (334 mg, 1.19 mmol) and **28**[34,53] (465 mg, 1.19 mmol, 1.0 equiv) in anhydrous CH_2Cl_2 (10 mL) was treated with bis(2-oxo-3-oxazolidinyl)-phosphinic chloride (BOP-Cl, 303 mg, 1.19 mmol, 1.0 equiv) and Et_3N (240 mg, 0.33 mL, 2.38 mmol, 2.0 equiv) at 0 °C under Ar. The resulting reaction mixture was stirred at 4 °C for 12 h before H_2O (5 mL) was added. The two layers were separated, and the aqueous phase was extracted with additional CH_2Cl_2 (3 × 5 mL). The combined organic phases were washed with H_2O (5 mL) and saturated aqueous NaCl (5 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 3 × 8 cm, 10−40% EtOAc−hexane gradient elution) afforded **30** (654 mg, 778 mg theoretical, 84%) as a white foam: mp 74−76 °C (white foam); $[\alpha]^{25}_D$ −40 (c 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) mixture of two rotamers, δ 7.56 and 7.50 (two d, 2H, J = 8.2 Hz, C3′-H and C5′-H), 6.93 and 6.91 (two d, 2H, J = 8.2 Hz, C2′-H and C6′-H), 6.89 and 6.69 (two dd, 1H, J = 2.2, 8.4 Hz, C6-H), 6.84 and 6.82 (two d, 1H, J = 2.2 Hz, C2-H), 6.83 and 6.79 (two d, 1H, J = 8.4 Hz, C5-H), 5.16 and 5.08 (two d, 1H, J = 9.6 Hz, NHBOC), 5.13 and 4.96 (two dd, 1H, J = 5.8, 9.6 Hz, C*H*NCH_3), 4.67 and 4.46 (two dd, 1H, 7.0, 15.8 Hz, C*H*NHBOC), 3.79 and 3.77 (two s, 3H, $ArOCH_3$), 3.71 and 3.69 (two s, 3H, CO_2CH_3), 3.25 and 3.02 (two dd, 1H, J = 6.3, 14.6 Hz, ArC*H*H), 2.78−2.98 (m, 3H, ArCH*H*), 2.87 and 2.74 (two s, 3H, NCH_3), 2.29 and 2.26 (two s, 3H, $COCH_3$), 1.34 and 1.32 (two s, 9H, $CO_2C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) mixture of two rotamers, δ 172.1 and 171.7, 171.2 and 170.6, 169.1 and 168.8, 155.0 and 154.8, 150.1 and 149.9, 139.5 and 139.3, 138.1 and 137.6, 137.3 and 137.1, 131.7 and 131.6, 131.5 and 131.2, 129.1 and 127.2, 124.1 and 123.3, 112.7 and 112.3, 92.5 and 92.1, 79.8 and 79.7, 58.6 and 58.4, 56.0 and 55.9, 53.1 and 52.4, 51.3 and 50.2, 38.3 and 36.9, 34.2 and 33.8, 32.8 and 31.4, 28.2 and 28.0, 20.8 and 20.7; IR (neat) ν_{max} 3354, 2976, 2930, 2837, 1765, 1743, 1706, 1647, 1514, 1482, 1367, 1267, 1205, 1164, 1125, 1008, 898, 811 cm^{-1}; FABHRMS (NBA) *m/e* 655.1534 (M^+ + H, $C_{28}H_{35}IN_2O_8$ requires

(53) Schwabacher, A. W.; Lee, J.; Lei, H. *J. Am. Chem. Soc.* **1992**, *114*, 7597.

655.1516). Anal. Calcd for $C_{28}H_{35}IN_2O_8$: C, 51.38; H, 5.35; N, 4.28. Found: C, 50.92; H, 5.40; N, 4.04.

3-Hydroxy-*N*,*O*-dimethyl-*N*-[[(*tert*-butyloxy)carbonyl]-L-4′-iodophenylalanyl]-L-tyrosine Methyl Ester (31). Method A. A solution of **27**[23] (382 mg, 1.6 mmol) and **29**[34] (890 mg, 1.6 mmol, 1.0 equiv) in DMF (10 mL) was stirred at 25 °C under Ar for 24 h before H_2O (10 mL) and EtOAc (15 mL) were added. After separation of two layers, the aqueous phase was extracted with EtOAc (2 × 15 mL). The combined EtOAc extracts were washed with 10% aqueous HCl (5 mL), H_2O (10 mL), saturated aqueous $NaHCO_3$ (5 mL), H_2O (10 mL), and saturated aqueous NaCl (10 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 3 × 10 cm, 15−35% EtOAc−hexane gradient elution) afforded **31** (765 mg, 979 mg theoretical, 78%) as a colorless oil which solidified upon standing: mp 72−74 °C (white foam); $[\alpha]^{25}_D$ −22 (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) mixture of two rotamers, δ 7.55 and 7.48 (two d, 2H, J = 8.2 Hz, C3′-H and C5′-H), 6.92 and 6.90 (two d, 2H, J = 8.2 Hz, C2′-H and C6′-H), 6.70 and 6.63 (two d, 1H, J = 8.2 Hz, C5-H), 6.68 and 6.57 (two d, 1H, J = 2.1 Hz, C2-H), 6.51 and 6.45 (two dd, 1H, J = 2.1, 8.2 Hz, C6-H), 5.90 (br s, 1H, OH), 5.17 and 5.02 (two d, 1H, J = 8.9 Hz, NHBOC), 5.11 and 4.94 (two dd, 1H, J = 5.8, 9.8 Hz, C*H*NCH_3), 4.69 and 4.32 (two dd, 1H, J = 6.9, 15.3 Hz, C*H*NHBOC), 3.82 and 3.81 (two s, 3H, $ArOCH_3$), 3.69 and 3.68 (two s, 3H, CO_2CH_3), 3.22 and 2.94 (two dd, 1H, J = 5.8, 14.4 Hz, ArC*H*H), 2.76−2.92 (m, 3H, ArCH*H*), 2.87 and 2.75 (two s, 3H, NCH_3), 1.36 and 1.30 (two s, 9H, $CO_2C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) mixture of two rotamers, δ 171.8 and 171.4, 171.1 and 170.6, 155.0 and 154.8, 145.9 and 145.8, 145.6 and 145.5, 137.6 and 137.3, 136.2 and 136.0, 131.7 and 131.4, 129.7 and 128.4, 120.6 and 120.2, 115.6 and 115.1, 111.0 and 110.8, 92.4 and 92.2, 80.4 and 79.8, 61.4 and 58.9, 55.9 and 55.4, 53.3 and 52.4, 51.3 and 50.1, 38.4 and 37.9, 37.8 and 37.0, 33.8 and 32.9, 28.2 and 28.1; IR (KBr) ν_{max} 3419, 2977, 2937, 1735, 1700, 1645, 1584, 1509, 1438, 1364, 1268, 1168, 1022, 866, 801, 756 cm^{-1}; FABHRMS (NBA-CsI) *m/e* 745.0387 (M^+ + Cs, $C_{26}H_{33}IN_2O_7$ requires 745.0387). Anal. Calcd for $C_{26}H_{33}IN_2O_7$: C, 50.98; H, 5.39; N, 4.56. Found: C, 50.64; H, 5.59; N, 4.27.

Method B. A solution of **30** (524 mg, 0.80 mmol) in THF−CH_3OH−H_2O (3:1:1, 10 mL) was treated with K_2CO_3 (552 mg, 4.0 mmol, 5.0 equiv) at 25 °C under Ar. The resulting reaction mixture was stirred at 25 °C for 12 h before the organic solvents were removed in vacuo. H_2O (10 mL) and EtOAc (20 mL) were added, and the resulting mixture was treated with 10% aqueous HCl (pH 3.0). Two layers were separated, and the aqueous phase was extracted with EtOAc (3 × 10 mL). The combined EtOAc extracts were washed with H_2O (10 mL) and saturated aqueous NaCl (10 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 3 × 8 cm, 10% EtOAc−hexane gradient elution) afforded **31** (463 mg, 490 mg theoretical, 95%) which was identical in all respects with the product obtained by method A.

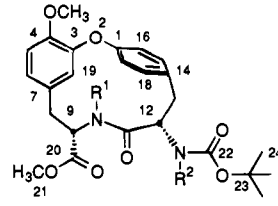
Methyl 4-Methoxy-12(*S*)-[[*N*-(*tert*-butyloxy)carbonyl]amino]-10-methyl-11-oxo-10-aza-2-oxatricyclo[12.2.2.1^{3,7}]nonadeca-3,5,7-(19),14,16,17-hexaen-9(*S*)-carboxylate (23). Method A. A solution of **31** (122 mg, 0.20 mmol) in anhydrous collidine (2 mL) was added dropwise to a suspension of NaH (60% dispersion in mineral oil, 16 mg, 0.40 mmol, 2.0 equiv) in anhydrous collidine (1 mL) at 0 °C under Ar, and the solution was allowed to stir for 15 min (0 °C) under Ar. The solution was treated with CuBr−SMe_2 (412 mg, 2.0 mmol, 10.0 equiv) and allowed to stir at 25 °C for 1 h before the mixture was diluted with anhydrous degassed collidine (47 mL) to 0.004 M and warmed at 130 °C (oil bath) for 10 h. The cooled reaction mixture was concentrated in vacuo. The residue was treated with EtOAc (30 mL) and saturated aqueous NH_4Cl (20 mL) and stirred at 25 °C for 30 min. The two phases were separated, and the aqueous phase was extracted with EtOAc (4 × 20 mL). The combined organic extracts were washed with saturated aqueous NH_4Cl (2 × 10 mL), H_2O (10 mL) and saturated aqueous NaCl (20 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatograhy (SiO_2, 2 × 15 cm, 10−40% EtOAc−hexane gradient elution) afforded **23** (32.9 mg, 96.8 mg theoretical, 34%) as a clear yellow oil which solidified upon standing and recovered **27** (19 mg, 15%). For **23**: mp 132−133 °C; $[\alpha]^{25}_D$ −49 (*c* 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.43 (dd, 1H, J = 2.2, 8.3 Hz, C18-H), 7.22 (dd, 1H, J = 2.2, 8.3 Hz, C15-H), 7.05 (dd, 1H, J = 2.2, 8.3 Hz, C17-H), 7.02 (dd, 1H, J = 2.2, 8.3 Hz, C16-H), 6.80 (d, 1H, J = 8.3 Hz, C5-H), 6.62 (dd, 1H, J = 2.2, 8.3 Hz, C6-H), 5.09 (d, 1H, J = 9.8 Hz, NHBOC), 4.92 (ddd, 1H, J = 5.4, 9.8, 12.6 Hz, C12-H), 4.73 (d, 1H, J = 2.2 Hz, C19-H), 4.58 (dd, 1H, J = 2.8, 12.0 Hz, C9-H), 3.93 (s, 3H, $ArOCH_3$), 3.66 (s, 3H, CO_2CH_3), 3.32 (dd, 1H, J = 5.4, 12.0 Hz, C13-H$_\beta$), 3.06 (dd, 1H, J = 2.8, 18.0 Hz, C8-H$_\beta$), 2.98 (dd, 1H, J = 12.0, 18.0 Hz, C8-H$_\alpha$), 2.88 (dd, 1H, J = 12.0, 12.6 Hz, C13−H$_\alpha$), 2.83 (s, 3H, NCH_3), 1.46 (s, 9H, $CO_2C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.4, 171.8, 156.6, 155.1, 152.3, 146.3, 134.6, 132.5, 130.3, 129.5, 125.3, 123.9, 121.0, 113.4, 111.9, 80.1, 57.0, 56.1, 52.7, 52.3, 39.4, 31.1, 30.8, 28.3; IR (KBr) ν_{max} 3428, 2927, 2846, 1743, 1708, 1644, 1511, 1452, 1369, 1526, 1164, 1128, 1021, 867, 805 cm^{-1}; FABHRMS (NBA-NaI) *m/e* 507.2110 (M^+ + Na, $C_{26}H_{32}N_2O_7$ requires 507.2107).

^1H NMR ($CDCl_3$, 400 MHz) with irradiation at 7.43 ppm (C18-H) led to the collapse of the signals at 7.22 ppm (dd, C15-H) and 7.05 ppm (dd, C17-H) to doublets; irradiation at 7.02 ppm (C16-H) led to the collapse of the signals at 7.22 ppm (dd, C15-H) and 7.05 ppm (dd, C17-H) to doublets; irradiation at 4.92 ppm (C12-H) led to the collapse of the signal at 5.09 ppm (d, C12−NHBOC) to a broadened singlet and to the collapse of the signals at 3.32 (dd, C13-H$_\beta$) and 2.88 (dd, C13-H$_\alpha$) to doublets; irradiation at 4.73 ppm (C19-H) led to the collapse of the signal at 6.62 ppm (dd, C6-H) to a doublet; irradiation at 4.58 ppm (C9-H) led to the collapse of the signals at 3.06 ppm (dd, C8-H$_\beta$) and 2.98 ppm (dd, C8-H$_\alpha$) to doublets.

The 2D ^1H−^1H NOESY NMR spectrum of **23** ($CDCl_3$, 400 MHz) displayed diagnostic NOE crosspeaks for C18-H/C17-H, C18-H/C12-H, C15-H/C16-H, C15-H/C13-H$_\alpha$, C17-H/C19-H, C5−H/C6-H, C5-H/C4-OCH_3, C6-H/C8-H$_\beta$, C12-NHBOC/C12-H, C12-NHBOC/C13-H$_\alpha$, C12-H/N10−CH_3, C12-H/C13-H$_\beta$, C12-H/C13-H$_\alpha$, C19-H/C9-H, C19-H/N10-CH_3, C9-H/C8-H$_\alpha$, C9-H/N10−CH_3, C9-H/C8-H$_\beta$, C13-H$_\beta$/C13-H$_\alpha$ and C8-H$_\alpha$/C8-H$_\beta$.

Method B. Methyllithium (1.4 M solution in Et_2O, 0.36 mL, 0.5 mmol, 2.5 equiv) was added dropwise to a solution of CuI−$(SBu_2)_2$ (242 mg, 0.5 mmol, 2.5 equiv) in 8 mL of anhydrous Et_2O at −78 °C under Ar. The bright-yellow slurry was stirred well before the solution was allowed to warm to 0 °C. The precipitated methylcopper was collected by removal of supernatant and washed with anhydrous Et_2O (3 × 8 mL) under Ar. After careful removal of the residual Et_2O in vacuo, pyridine (2 mL) was added to the methylcopper at −78 °C. A solution of **31** (122 mg, 0.2 mmol) in anhydrous collidine (2 mL) was then added dropwise to the mixture at −78 °C, and the resulting brown mixture was stirred at 25 °C for 1 h. The mixture was diluted further with anhydrous collidine (46 mL) and warmed at 130 °C (oil bath) for 10 h. The cooled reaction mixture was concentrated in vacuo. The residue was treated with EtOAc (30 mL) and saturated aqueous NH_4Cl (20 mL) and stirred at 25 °C for 30 min. After separation of two layers, the aqueous phase was extracted with EtOAc (3 × 30 mL). The combined organic extracts were washed with saturated aqueous NH_4Cl (2 × 10 mL), H_2O (10 mL), and saturated aqueous NaCl (20 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 2 × 10 cm, 10−40% EtOAc−hexane gradient elution) afforded **23** (30 mg, 96.8 mg theoretical, 31%) which was identical in all respects with the product from method A and recovered **31** (13 mg, 11%).

Summarized in Tables 4 and 5 are the comparison ^1H and ^{13}C NMR properties of **21**−**24**.



BOC-D-Ala-L-Ala-NMe-L-Tyr(OMe)-L-Ala-OC$_6$F$_5$ (32). A solution of BOC-D-Ala-L-Ala-NMe-L-Tyr(OMe)-L-Ala-OH[25] (**34**, 236 mg, 0.45 mmol) in CH_2Cl_2 (5 mL) was treated with C_6F_5OH (100 mg, 0.54 mmol, 1.2 equiv) and EDCI (104 mg, 0.54 mmol, 1.2 equiv) at 25 °C under Ar, and the resulting reaction mixture was stirred at 25 °C for 4 h. The solvent was removed in vacuo, and the residue was purified by flash chromatography (SiO_2, 2 × 10 cm, 30−50% EtOAc−hexane

Table 4. Comparison ^{13}C NMR of **22**–**24**[a]

assignment	**22** $R^1 = H$, $R^2 = CH_3$	**23** $R^1 = CH_3$, $R^2 = H$	**24** $R^1 = R^2 = H$
C24	28.6 (o)	28.3 (o)	28.3 (o)
N10-CH_3		30.8 (o)	
C12 N^{α}-CH_3	29.7 (o)		
C8	34.7 (e)	31.1 (e)	34.3 (e)
C13	35.6 (e)	39.4 (e)	38.9 (e)
C21	52.6 (o)	52.7 (o)	52.5 (o)
C9	53.5 (o)	52.3 (o)	54.0 (o)
C4-OCH_3	56.3 (o)	56.1 (o)	56.1 (o)
C12	61.4 (o)	57.0 (o)	58.2 (o)
C23	80.9 (e)	80.1 (e)	80.3 (e)
C5	111.7 (o)	111.9 (o)	111.5 (o)
C19	114.9 (o)	113.4 (o)	115.0 (o)
C6	121.9 (o)	121.0 (o)	121.2 (o)
C16	124.7 (o)	125.3 (o)	125.0 (o)
C17	124.7 (o)	123.9 (o)	124.7 (o)
C14	129.7 (e)	129.5 (e)	129.8 (e)
C7	130.5 (e)	130.3 (e)	130.5 (e)
C18	131.5 (o)	132.5 (o)	132.5 (o)
C15	133.7 (o)	134.6 (o)	134.5 (o)
C4	147.0 (e)	146.3 (e)	146.0 (e)
C3	152.6 (e)	152.3 (e)	152.3 (e)
C1	155.2 (e)	155.1 (e)	155.2 (e)
C22	157.3 (e)	156.6 (e)	157.2 (e)
C11	169.6 (e)	171.8 (e)	171.5 (e)
C20	171.9 (e)	173.4 (e)	171.8 (e)

[a] All the assignments were based on the results of 2D ^1H-detected ^1H–^{13}C correlation and the attached proton test (APT).

gradient elution) to afford **32** (232 mg, 309 mg theoretical, 75%) as a colorless oil which solidified upon standing: mp 146–148 °C (50% EtOAc–hexane, white powder); $[\alpha]^{25}_D$ −120 (*c* 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz, 50 °C) mixture of two rotamers, δ 8.56 and 6.65 (two d, 1H, J = 6.6 Hz, Ala-NH), 7.09 and 7.04 (two d, 2H, J = 8.6 Hz, Tyr C2-H and C6-H), 6.81 and 6.78 (two d, 2H, J = 8.6 Hz, Tyr C3-H and C5-H), 6.96 and 6.69 (two d, 1H, J = 7.6 Hz, Ala-NH), 5.54 and 4.81 (two dd, 1H, J = 6.0, 10.6 Hz, Tyr$^{\alpha}$-H), 4.98 and 4.78 (two d, 1H, J = 7.8 Hz, Ala-NH), 4.88 and 4.70 (two p, 1H, J = 7.0 Hz, Ala$^{\alpha}$-H), 4.74 and 4.34 (two p, 1H, J = 6.6 Hz, Ala$^{\alpha}$-H), 4.55 and 4.11 (two p, 1H, J = 7.3 Hz, Ala$^{\alpha}$-H), 3.74 (s, 3H, Tyr $ArOCH_3$), 3.27 and 2.95 (two dd, 1H, J = 10.6, 14.8 Hz, Tyr$^{\beta}$-H_{α}), 3.18 and 3.00 (two dd, 1H, J = 3.6, 14.8 Hz, Tyr$^{\beta}$-H_{β}), 2.97, 2.94 and 2.90 (three s, 3H, Tyr-NCH_3), 1.60 and 1.53 (two d, 3H, J = 7.2 Hz, Ala$^{\beta}$-CH_3), 1.45 and 1.43 (two s, 9H, $CO_2C(CH_3)_3$), 1.31 and 1.27 (two d, 3H, J = 7.0 Hz, Ala$^{\beta}$-CH_3), 1.00 and 0.50 (two d, 3H, J = 6.8 Hz, Ala$^{\beta}$-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz, 50 °C) mixture of two rotamers, δ 173.3 and 172.7, 170.0 and 169.6, 169.3, 168.9 and 168.8, 158.7 and 158.4, 155.4, 142.3, 140.8, 139.8, 139.1, 138.3, 136.6, 130.4 and 129.9, 128.7 and 128.6, 114.4 and 114.0, 80.5, 62.5, 56.8, 55.3 and 55.2, 49.9 and 49.5, 48.4 and 47.9, 45.7 and 44.4, 33.0 and 32.5, 28.3 and 28.2, 18.5 and 17.8, 17.5 and 16.9, 16.7 and 16.2; IR (KBr) ν_{max} 3297, 2980, 2939, 1794, 1686, 1650, 1517, 1456, 1369, 1246, 1169, 1098, 1041, 995, 867, 826 cm^{-1}; FABHRMS (NBA-NaI) *m/e* 711.2440 (M^+ + Na, $C_{31}H_{37}F_5N_4O_8$ requires 711.2429). Anal. Calcd for $C_{31}H_{37}F_5N_4O_8$: C, 54.07; H, 5.38; N, 8.14. Found: C, 53.83; H, 5.72; N, 7.88.

***N*-[*N*-[(*tert*-Butyloxy)carbonyl]-*O*^{4}-methyl-L-tyrosyl]-L-alanine Methyl Ester (38).** A solution of **36**[54,55] (590 mg, 2.0 mmol) and L-alanine methyl ester hydrochloride salt (**37**, 280 mg, 2.0 mmol, 1.0 equiv) in DMF (15 mL) was treated with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI, 422 mg, 2.2 mmol, 1.1 equiv), 1-hydroxybenzotriazole (HOBt, 297 mg, 2.2 mmol, 1.1 equiv), and $NaHCO_3$ (376 mg, 4.0 mmol, 2.0 equiv) at 25 °C under Ar. The resulting reaction mixture was stirred at 25 °C under Ar for 10 h before H_2O (10 mL) and EtOAc (20 mL) were added. The solution was treated with 10% aqueous HCl (pH = 3.0), and the two layers were separated. The aqueous layer was extracted with EtOAc (3 × 15 mL), and the combined EtOAc extracts were washed with 10% aqueous HCl (10 mL), H_2O (10 mL), saturated aqueous $NaHCO_3$ (2 × 20 mL), H_2O (10 mL), and saturated aqueous NaCl (20 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 2 × 10 cm, 15–40% EtOAc–hexane gradient elution) afforded **38** (667 mg, 760 mg theoretical, 88%) as a colorless oil which solidified upon standing: mp 113–114 °C (30% EtOAc–hexane, white needles); $[\alpha]^{25}_D$ +6 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.09 (d, 2H, J = 8.6 Hz, C2-H and C6-H), 6.79 (d, 2H, J = 8.6 Hz, C3-H and C5-H), 6.50 (d, 1H, J = 6.9 Hz, Ala-NH), 5.03 (d, 1H, J = 7.6 Hz, Tyr-NHBOC), 4.49 (dq, 1H, J = 6.9, 7.2 Hz, Ala$^{\alpha}$-H), 4.30 (m, 1H, Tyr$^{\alpha}$-H), 3.75 (s, 3H, $ArOCH_3$), 3.69 (s, 3H, CO_2CH_3), 2.98 (m, 2H, Tyr$^{\beta}$-H), 1.39 (s, 9H, $CO_2C(CH_3)_3$), 1.32 (d, 3H, J = 7.2 Hz, Ala$^{\beta}$-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.8, 170.9, 158.6, 155.3, 130.4, 128.4, 114.0, 80.1, 55.6, 55.2, 52.4, 48.0, 37.5, 28.2, 18.3; IR (KBr) ν_{max} 3323, 2954, 2830, 1754, 1692, 1656, 1615, 1528, 1461, 1303, 1245, 1164, 1031, 990, 805, 677 cm^{-1}; FABHRMS (NBA) *m/e* 381.2020 (M^+ + H, $C_{19}H_{28}N_2O_6$ requires 381.2026). Anal. Calcd for $C_{19}H_{28}N_2O_6$: C, 60.00; H, 7.37; N, 7.37. Found: C, 59.88; H, 7.50; N, 7.29.

***N*-(*O*^{4}-Methyl-L-tyrosyl)-L-alanine Methyl Ester (39).** A solution of **38** (500 mg, 1.32 mmol) in CH_2Cl_2 (2.5 mL) was treated with trifluoroacetic acid (TFA, 2.5 mL) at 25 °C under Ar, and the resulting reaction mixture was stirred at 25 °C under Ar for 1 h. The volatiles were removed in vacuo, and the residue was treated with saturated aqueous $NaHCO_3$ (3 mL). The aqueous phase was extracted with EtOAc (3 × 10 mL). The combined EtOAc extracts were washed with saturated aqueous NaCl (4 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 3 × 8 cm, 0–5% CH_3OH-$CHCl_3$ gradient elution) afforded **39** (311 mg, 368 mg theoretical, 85%) as a colorless oil which solidified upon standing: mp 274–276 °C (dec, CH_3OH, fine white needles); $[\alpha]^{25}_D$ −56 (*c* 0.3, CH_3OH); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.72 (d, 1H, J = 7.6 Hz, Ala-NH), 7.12 (d, 2H, J = 8.6 Hz, C2-H and C6-H), 6.84 (d, 2H, J = 8.6 Hz, C3-H and C5-H), 4.57 (dq, 1H, J = 7.2, 7.6 Hz, Ala$^{\alpha}$-H), 3.78 (s, 3H, $ArOCH_3$), 3.73 (s, 3H, CO_2CH_3), 3.61 (dd, 1H, J = 4.2, 9.0 Hz, Tyr$^{\alpha}$-H), 3.15 (dd, 1H, J = 4.2, 13.8 Hz, Tyr$^{\beta}$-H_{β}), 2.69 (dd, 1H, J = 9.0, 13.8 Hz, Tyr$^{\beta}$-H_{α}), 1.38 (d, 3H, J = 7.2 Hz, Ala$^{\beta}$-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.8, 173.4, 158.6, 130.3, 129.4, 114.1, 56.2, 55.3, 52.4, 47.6, 39.8, 18.4; IR (KBr) ν_{max} 3430, 3195, 3061, 2908, 1667, 1615, 1512, 1461, 1338, 1256, 1107, 1036, 862, 837 cm^{-1}; FABHRMS (NBA) *m/e* 281.1492 (M^+ + H, $C_{14}H_{20}N_2O_4$ requires 281.1501). Anal. Calcd for $C_{14}H_{20}N_2O_4$: C, 60.00; H, 7.14; N, 10.00. Found: C, 60.38; H, 6.80; N, 10.10.

BOC-D-Ala-L-Ala-L-Tyr(OMe)-L-Ala-OMe (41). A solution of **39** (280 mg, 1.0 mmol) and BOCNH-D-Ala-L-Ala-OH[25] (**40**, 260 mg, 1.0 mmol, 1.0 equiv) in anhydrous DMF (5 mL) was treated with EDCI (211 mg, 1.1 mmol, 1.1 equiv) and HOBt (149 mg, 1.1 mmol, 1.1 equiv) at 25 °C under Ar. The resulting reaction mixture was stirred at 25 °C under Ar for 12 h before H_2O (5 mL) and EtOAc (10 mL) were added. The solution was treated with 10% aqueous HCl (pH = 3.0), and the two layers were separated. The aqueous was extracted with EtOAc (2 × 15 mL), and the combined EtOAc extracts were washed with H_2O (10 mL), saturated aqueous $NaHCO_3$ (2 × 10 mL), H_2O (10 mL), and saturated aqueous NaCl (10 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 3 × 8 cm, 20–50% EtOAc–hexane gradient elution) afforded **41** (461 mg, 522 mg theoretical, 88%) as a white solid: mp 154–156 °C (40% EtOAc–hexane, white powder); $[\alpha]^{25}_D$ −23 (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.11 (d, 2H, J = 8.6 Hz, C2-H and C6-H), 6.90 (br s, 1H, Ala-NH), 6.85 (br s, 1H, Ala-NH), 6.79 (d, 2H, J = 8.6 Hz, C3-H and

(54) Compound **36** was obtained by *N*-BOC protection of *O*^{4}-methyl-L-tyrosine (**35**) as white needles: mp 92–94 °C (40% EtOAc–hexane, white needles) [lit.[55a] mp 92–94 °C (EtOAc–hexane) and lit.[55b] mp 89–91 °C (toluene-hexane)]; $[\alpha]^{25}_D$ +42.2 (*c* 1, EtOH) [lit.[55a] $[\alpha]^{26}_D$ +42.9 and +42.0 (*c* 1, EtOH) and lit.[55b] $[\alpha]^{26}_D$ +30.6 and +33.2 (*c* 1, EtOH)]; ^1H NMR ($CDCl_3$, 400 MHz) mixture of two rotamers, δ 11.23 (br s, 1H, CO_2H), 7.09 (d, 2H, J = 8.4 Hz, C2-H and C6-H), 6.83 (d, 2H, J = 8.4 Hz, C3-H and C5-H), 6.39 and 4.95 (two d, 1H, J = 7.7 Hz, NHBOC), 4.57 and 4.34 (two m, 1H, Tyr$^{\alpha}$-H), 3.77 (s, 3H, OCH_3), 3.04 and 2.86 (two m, 2H, Tyr$^{\beta}$-H), 1.41 and 1.31 (two s, 9H, $CO_2C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ mixture of two rotamers, δ 176.7 and 176.2, 158.6, 155.4, 130.4, 127.7, 114.0, 80.2, 56.1 and 54.4, 55.2, 38.2 and 36.9, 28.3 and 28.0; IR (KBr) ν_{max} 3374, 2974, 2830, 1718, 1682, 1615, 1513, 1251, 1169, 1131, 933, 815 cm^{-1}.

(55) (a) Kolodziejczyk, A. M.; Manning, M. *J. Org. Chem.* **1981**, *46*, 1944. (b) Mendelson, W. L.; Tickner, A. M.; Lantos, I. *J. Org. Chem.* **1983**, *48*, 4127.

Table 5. Comparison ^{1}H NMR **21**−**24**[a]

assignment	**21** $R^1 = R^2 = CH_3$	**22** $R^1 = H$, $R^2 = CH_3$	**23** $R^1 = CH_3$, $R^2 = H$	**24** $R^1 = R^2 = H$
C4-OCH_3	3.95 (s)	3.94 (s)	3.93 (s)	3.93 (s)
C5-H	6.81 (d; 8.4)	6.77 (d; 8.2)	6.80 (d; 8.3)	6.75 (d; 8.2)
C6-H	6.64 (dd; 2.2, 8.4)	6.69 (dd; 1.8, 8.2)	6.62 (dd; 2.2, 8.3)	6.57 (dd; 2.0, 8.2)
C8-Hα	2.93−3.05 (m)	2.80 (dd; 11.0, 16.3)	2.98 (dd; 12.0, 18.0)	2.67 (dd; 11.0, 16.6)
C8-Hβ	2.93−3.05 (m)	2.90 (dd; 1.3, 16.3)	3.06 (dd; 2.8, 18.0)	2.84 (d; 16.6)
C9-H	4.80 (dd; 2.0, 12.0)	4.20 (ddd; 1.3, 8.1, 10.8)	4.58 (dd, 2.8, 12.0)	4.07−4.15 (m)
C9-CO_2CH_3	3.66 (s)	3.66 (s)	3.66 (s)	3.66 (s)
N10-H		5.87 (d; 8.1)		5.87 (d; 7.4)
N10-CH_3	2.81 (s)		2.83 (s)	
C12-H	5.36 (dd; 5.0, 11.7)	4.58 (dd; 2.0, 12.0)	4.92 (ddd; 5.4, 9.8, 12.6)	4.07−4.15 (m)
C12 $N^α$-H			5.09 (d; 9.8)	5.17 (d; 9.2)
C12 $N^α$-CH_3	2.93 (s)	3.00 (s)		
$CO_2C(CH_3)_3$	1.49 (s)	1.51 (s)	1.46 (s)	1.44 (s)
C13-Hα	3.23 (t; 12.0)	3.27 (t; 12.0)	2.88 (dd; 12.0, 12.6)	2.86 (t; 12.2)
C13-Hβ	2.93−3.05 (m)	2.99 (m)	3.32 (dd; 5.4, 12.0)	3.25 (dd; 5.0, 12.2)
C15-H	7.29 (dd; 2.2, 8.3)	7.29 (dd; 2.2, 8.3)	7.22 (dd; 2.2, 8.3)	7.21 (dd; 2.1, 8.4)
C16−H	7.02 (dd; 2.2, 8.3)	6.98 (dd; 2.2, 8.3)	7.02 (dd; 2.2, 8.3)	6.98 (dd; 2.1, 8.4)
C17-H	7.04 (dd; 2.2, 8.3)	7.04 (dd; 2.2, 8.3)	7.05 (dd; 2.2, 8.3)	7.08 (dd; 2.1, 8.4)
C18-H	7.46 (dd; 2.2, 8.3)	7.44 (dd; 2.2, 8.3)	7.43 (dd; 2.2, 8.3)	7.40 (dd; 2.1, 8.4)
C19-H	4.75 (d; 2.2)	5.14 (d; 1.8)	4.73 (d; 2.2)	5.05 (d; 2.0)

[a] Listed are the chemical shifts in ppm (multiplicity, coupling constants in Hz). All the assignments were based on 2D ^{1}H−^{1}H NOESY and ^{1}H−^{1}H decoupling NMR experiments.

C5-H), 6.66 (d, 1H, J = 6.7 Hz, Tyr-NH), 5.09 (d, 1H, J = 5.2 Hz, NHBOC), 4.68 (dd, 1H, J = 7.8, 14.2 Hz, Tyr^α-H), 4.47 (p, 1H, J = 7.2 Hz, Ala^α-H), 4.39 (p, 1H, J = 7.0 Hz, Ala^α-H), 4.12 (p, 1H, J = 6.7 Hz, Ala^α-H), 3.75 (s, 3H, $ArOCH_3$), 3.70 (s, 3H, CO_2CH_3), 3.13 (dd, 1H, J = 4.8, 14.0 Hz, Tyr^β-H$_β$), 2.96 (dd, 1H, J = 7.9, 14.0 Hz, Tyr^β-H$_α$), 1.43 (s, 9H, $CO_2C(CH_3)_3$), 1.36 (d, 3H, J = 7.2 Hz, Ala^β-CH_3), 1.31 (d, 3H, J = 7.2 Hz, Ala^β-CH_3), 1.29 (d, 3H, J = 7.2 Hz, Ala^β-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.0, 172.8, 171.9, 170.5, 158.4, 155.5, 130.3, 128.6, 113.8, 80.0, 55.1, 54.1, 52.3, 50.1, 49.0, 48.0, 37.6, 28.3, 18.9, 18.7, 17.9; IR (KBr) $ν_{max}$ 3303, 2974, 2933, 1739, 1646, 1538, 1513, 1451, 1369, 1246, 1164, 1062, 1031, 856, 830, 790 cm^{-1}; FABHRMS (NBA-NaI) *m/e* 545.2580 (M^+ + Na, $C_{25}H_{38}N_4O_8$ requires 545.2587). Anal. Calcd for $C_{25}H_{38}N_4O_8$: C, 57.47; H, 7.28; N, 10.73. Found: C, 57.37; H, 7.27; N, 10.63.

BOC-D-Ala-L-Ala-L-Tyr(OMe)-L-Ala-OH (42). A solution of **41** (830 mg, 1.6 mmol) in THF−CH_3OH−H_2O (3:1:1, 15 mL) was treated with LiOH−H_2O (133.3 mg, 3.2 mmol, 2.0 equiv) at 25 °C under Ar, and the reaction mixture was stirred at 25 °C under Ar for 3 h. The organic solvents were removed under a stream of N_2 before H_2O (10 mL) and EtOAc (20 mL) were added to the residue. The solution was treated dropwise with 15% aqueous citric acid (0 °C, pH = 3). The two layers were separated, and the aqueous phase was extracted with EtOAc (2 × 20 mL). The combined EtOAc extracts were washed with H_2O (20 mL) and saturated aqueous NaCl (20 mL), dried ($MgSO_4$), and concentrated in vacuo. The crude product (790 mg) was recrystallized from 70% EtOAc−hexane to afford **42** (725 mg, 807 mg theoretical, 90%) as white needles: mp 173−176 °C (dec, 70% EtOAc−hexane, white needles); $[α]^{25}_D$ −18 (*c* 0.23, CH_3OH); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.80 (d, 1H, J = 4.8 Hz, Ala NH), 7.47 (d, 1H, J = 9.1 Hz, Ala NH), 7.45 (d, 1H, J = 6.4 Hz, Tyr NH), 7.17 (d, 2H, J = 8.6 Hz, C2-H and C6-H), 6.80 (d, 2H, J = 8.6 Hz, C3-H and C5-H), 6.45 (d, 1H, J = 3.8 Hz, NHBOC), 4.58 (dt, 1H, J = 4.0, 10.3 Hz, Tyr^α-H), 4.31 (p, 1H, J = 7.1 Hz, Ala^α-H), 4.16 (p, 1H, J = 6.6 Hz, Ala^α-H), 4.06 (p, 1H, J = 6.8 Hz, Ala^α-H), 3.73 (s, 3H, $ArOCH_3$), 3.26 (dd, 1H, J = 4.0, 14.2 Hz, Tyr^β-H$_β$), 2.86 (dd, 1H, J = 10.3, 14.2 Hz, Tyr^β-H$_α$), 1.41 (d, 3H, J = 7.2 Hz, Ala^β-CH_3), 1.40 (s, 9H, $CO_2C(CH_3)_3$), 1.30 (d, 3H, J = 7.0 Hz, Ala^β-CH_3), 1.19 (d, 3H, J = 7.2 Hz, Ala^β-CH_3); ^{13}C NMR (acetone-d_6, 100 MHz) δ 174.9, 173.8, 172.4, 171.8, 159.3, 156.8, 130.9, 128.7, 114.4, 79.8, 55.4, 55.0, 51.3, 50.6, 48.8, 36.9, 28.7, 17.7, 17.39, 17.37; IR (KBr) $ν_{max}$ 3303, 2974, 2933, 1723, 1651, 1513, 1451, 1369, 1246, 1164, 1027, 856, 826, 785 cm^{-1}; FABHRMS (NBA-NaI) *m/e* 509.2620 (M^+ + H, $C_{24}H_{36}N_4O_8$ requires 509.2611). Anal. Calcd for $C_{24}H_{36}N_4O_8$: C, 56.69; H, 7.09; N, 11.02. Found: C, 56.49; H, 7.24; N, 10.84.

BOC-D-Ala-L-Ala-L-Tyr(OMe)-L-Ala-OC$_6$F$_5$ (33). A suspension of **42** (117 mg, 0.2 mmol) in CH_2Cl_2 (2 mL) was treated with C_6F_5OH (36.9 mg, 0.2 mmol, 1.0 equiv) and EDCI (38.4 mg, 0.2 mmol, 1.0 equiv) at 25 °C under Ar. The resulting reaction mixture was then stirred at 25 °C under Ar for 4 h before the solvent was removed in vacuo. The residue was purified by flash chromatography (SiO_2, 1.5 × 5 cm, 40−60% EtOAc−hexane gradient elution) to afford **33** (116 mg, 135 mg theoretical, 86%) as a white solid: mp 168−170 °C (70% EtOAc−hexane, white powder); $[α]^{25}_D$ −42 (*c* 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.11 (d, 2H, J = 8.6 Hz, C2-H and C6-H), 7.00 (d, 1H, J = 6.2 Hz, Ala-NH), 6.86 (d, 1H, J = 8.1 Hz, Ala-NH), 6.77 (d, 2H, J = 8.6 Hz, C3-H and C5-H), 6.64 (d, 1H, J = 6.5 Hz, Tyr-NH), 5.01 (d, 1H, J = 6.7 Hz, NHBOC), 4.79 (p, 1H, J = 7.2 Hz, Ala^α-H), 4.71 (dd, 1H, J = 8.0, 14.2 Hz, Tyr^α-H), 4.37 (p, 1H, J = 7.0 Hz, Ala^α-H), 4.10 (p, 1H, J = 6.8 Hz, Ala^α-H), 3.73 (s, 3H, $ArOCH_3$), 3.16 (dd, 1H, J = 6.0, 14.2 Hz, Tyr^β-H$_β$), 2.98 (dd, 1H, J = 8.0, 14.2 Hz, Tyr^β-H$_α$), 1.56 (d, 3H, J = 7.2 Hz, Ala^β-CH_3), 1.44 (s, 9H, $CO_2C(CH_3)_3$), 1.31 (d, 3H, J = 7.3 Hz, Ala^β-CH_3), 1.29 (d, 3H, J = 7.2 Hz, Ala^β-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.0, 171.8, 171.0, 168.6, 158.6, 155.7, 142.4, 140.8, 139.8, 139.1, 138.3, 136.6, 130.2, 128.6, 114.0, 80.5, 55.2, 54.1, 50.5, 49.5, 48.0, 36.8, 28.3, 18.1, 17.9, 17.3; IR (KBr) $ν_{max}$ 3292, 2974, 2933, 1785, 1692, 1641, 1518, 1451, 1369, 1246, 1169, 1092, 1041, 995, 903, 744, 697 cm^{-1}; FABHRMS (NBA-NaI) *m/e* 675.2465 (M^+ + H, $C_{30}H_{35}F_5N_4O_8$ requires 675.2453). Anal. Calcd for $C_{30}H_{35}F_5N_4O_8$: C, 53.41; H, 5.19; N, 8.31. Found: C, 53.53; H, 5.23; N, 8.09.

Methyl 4-Methoxy-10-methyl-12(*S*)-methylamino-11-oxo-10-aza-2-oxatricyclo[12.2.2.1^{3,7}]nonadeca-3,5,7(19),14,16,17-hexaen-9(*S*)-carboxylate (43). A solution of **21**[23] (6.5 mg, 0.013 mmol) in 4 M HCl−EtOAc (0.5 mL) was stirred at 25 °C for 30 min. The volatiles were removed in vacuo, and the residue was treated with saturated aqueous $NaHCO_3$ (2 mL). The resulting aqueous solution was extracted with EtOAc (4 × 4 mL). The combined EtOAc extracts were washed with H_2O (2 mL) and saturated aqueous NaCl (2 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 1 × 2 cm, 0−8% CH_3OH−$CHCl_3$ gradient elution) afforded **43** (4.8 mg, 5.2 mg theoretical, 92%) as a clear yellow oil which solidified upon standing: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.40 (dd, 1H, J = 2.2, 8.3 Hz, C18-H), 7.20 (dd, 1H, J = 2.2, 8.3 Hz, C15-H), 7.05 (dd, 1H, J = 2.2, 8.3 Hz, C17-H), 7.03 (dd, 1H, J = 2.2, 8.3 Hz, C16-H), 6.81 (d, 1H, J = 8.3 Hz, C5-H), 6.63 (dd, 1H, J = 2.2, 8.3 Hz, C6-H), 4.73 (d, 1H, J = 2.2 Hz, C19-H), 4.64 (dd, 1H, J = 2.6, 12.6 Hz, C9-H), 3.94 (s, 3H, $ArOCH_3$), 3.91 (dd, 1H, J = 5.5, 10.8 Hz, C12-H), 3.70 (s, 3H, CO_2CH_3), 3.57 (dd, 1H, J = 5.5, 12.5 Hz, C13-H$_β$), 3.09 (dd, 1H, J = 2.6, 18.1 Hz, C8-H$_β$), 2.94 (dd, 1H, J = 12.6, 18.1 Hz, C8-H$_α$), 2.83 (dd, 1H, J = 10.8, 12.5 Hz, C13-H$_α$), 2.77 (s, 3H, N10−CH_3), 2.53 (s, 3H, C12-$NHCH_3$); IR (KBr) $ν_{max}$ 3447, 2919, 2848, 1738, 1652, 1555, 1533, 1516, 1459, 1266, 1212, 1161, 1125, 1069, 1023, 875, 839, 808 cm^{-1}; FABHRMS (NBA) *m/e* 399.1929 (M^+ + H, $C_{22}H_{26}N_2O_5$ requires 399.1920).

Methyl 4-Methoxy-12(*S*)-methylamino-11-oxo-10-aza-2-oxatricyclo-[12.2.2.1^{3,7}]nonadeca-3,5,7(19),14,16,17-hexaen-9(*S*)-carboxylate (44). As described for **21**, **22**[23] (4.9 mg, 0.01 mmol) afforded **44** (3.5 mg, 3.8 mg theoretical, 92%) as a colorless oil which solidified upon standing: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.34 (dd, 1H, J = 2.4, 8.2 Hz, C18-H), 7.20 (dd, 1H, J = 2.4, 8.2 Hz, C15-H), 7.06 (dd, 1H, J = 2.4, 8.2 Hz, C17-H), 6.99 (dd, 1H, J = 2.4, 8.2 Hz, C16-H), 6.77 (d, 1H, J = 8.2 Hz, C5-H), 6.58 (dd, 1H, J = 2.4, 8.2 Hz, C6-H), 5.53 (d, 1H, J = 6.2 Hz, N10-H), 5.05 (d, 1H, J = 2.4 Hz, C19-H), 4.16 (ddd, 1H, J = 1.4, 6.2, 11.3 Hz, C9-H), 3.93 (s, 3H, $ArOCH_3$), 3.70 (s, 3H, CO_2CH_3), 3.24 (dd, 1H, J = 4.8, 12.2 Hz, C12-H), 3.03 (dd, 1H, J = 4.8, 11.3, C13-H$_\beta$), 2.87 (dd, 1H, J = 1.4, 16.0 Hz, C8-H$_\beta$), 2.73 (dd, 1H, J = 11.3, 16.0 Hz, C8-H$_\alpha$), 2.70 (dd, 1H, J = 11.3, 12.2 Hz, C13-H$_\alpha$), 2.45 (s, 3H, C12-$NHCH_3$); IR (KBr) ν_{max} 3426, 3036, 2944, 1739, 1651, 1513, 1436, 1369, 1262, 1226, 1200, 1128, 1021, 980, 882, 836, 800, 728 cm^{-1}; FABHRMS (NBA-NaI) *m/e* 385.1770 (M^+ + H, $C_{21}H_{24}N_2O_5$ requires 385.1763).

Methyl 12(*S*)-Amino-4-methoxy-10-methyl-11-oxo-10-aza-2-oxatricyclo[12.2.2.1^{3,7}]nonadeca-3,5,7(19),14,16,17-hexaen-9(*S*)-carboxylate (45). As described for **21**, **23** (4.0 mg, 0.0083 mmol) afforded **45** (2.9 mg, 3.2 mg theoretical, 91%) as a clear yellow oil which solidified upon standing: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.34 (dd, 1H, J = 2.2, 8.3 Hz, C18−H), 7.22 (dd, 1H, J = 2.2, 8.3 Hz, C15-H), 7.02 (dd, 2H, J = 2.2, 8.3 Hz, C16- and C17-H), 6.80 (d, 1H, J = 8.3 Hz, C5-H), 6.63 (dd, 1H, J = 2.2, 8.3 Hz, C6-H), 4.77 (d, 1H, J = 2.2 Hz, C19-H), 4.66 (dd, 1H, J = 2.5, 12.4 Hz, C9-H), 4.05 (dd, 1H, J = 5.6, 11.0 Hz, C12-H), 3.94 (s, 3H, $ArOCH_3$), 3.69 (s, 3H, CO_2CH_3), 3.25 (dd, 1H, J = 5.6, 12.2 Hz, C13-H$_\beta$), 3.09 (dd, 1H, J = 2.5, 18.1 Hz, C8-H$_\beta$), 2.94 (dd, 1H, J = 12.4, 18.1 Hz, C8-H$_\alpha$), 2.81 (dd, 1H, J = 11.0, 12.2 Hz, C13-H$_\alpha$), 2.75 (s, 3H, N10-CH_3); IR (KBr) ν_{max} 3436, 2933, 2851, 1733, 1641, 1513, 1441, 1369, 1269, 1205, 1164, 1123, 1072, 1021, 872, 836, 800, 759 cm^{-1}; FABHRMS (NBA) *m/e* 385.1770 (M^+ + H, $C_{21}H_{24}N_2O_5$ requires 385.1763).

Methyl 12(*S*)-Amino-4-methoxy-11-oxo-10-aza-2-oxatricyclo-[12.2.2.1^{3,7}]nonadeca-3,5,7(19),14,16,17-hexaen-9(*S*)-carboxylate (46). As described for **21**, **24**[34] (5.0 mg, 0.01 mmol) afforded **46** (3.6 mg, 3.9 mg theoretical, 92%) as a clear yellow oil which solidified upon standing: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.34 (dd, 1H, J = 2.1, 8.3 Hz, C18-H), 7.19 (dd, 1H, J = 2.1, 8.3 Hz, C15-H), 7.10 (dd, 1H, J = 2.1, 8.3 Hz, C17-H), 6.99 (dd, 1H, J = 2.1, 8.3 Hz, C16-H), 6.77 (d, 1H, J = 8.3 Hz, C5-H), 6.58 (dd, 1H, J = 2.1, 8.3 Hz, C6-H), 6.26 (br s, 1H, N10-H), 5.07 (d, 1H, J = 2.1 Hz, C19-H), 4.09 (ddd, 1H, J = 1.4, 7.0, 11.4 Hz, C9-H), 3.93 (s, 3H, $ArOCH_3$), 3.73 (s, 3H, CO_2CH_3), 3.54 (m, 1H, C12-H), 3.22 (dd, 1H, J = 4.7, 12.4 Hz, C13-H$_\alpha$), 2.74−2.88 (m, 3H, C8-H_2, and C13-H$_\beta$); IR (KBr) ν_{max} 3436, 3046, 2954, 1718, 1667, 1590, 1513, 1436, 1415, 1267, 1225, 1205, 1128, 1021, 980, 882, 836, 805, 764 cm^{-1}; FABHRMS (NBA-CsI) *m/e* 503.0598 (M^+ + Cs, $C_{20}H_{22}N_2O_5$ requires 503.0583).

BOC-D-Alanyl-L-alanyl-*O*^{4}-methyl-L-tyrosyl-L-alanyl-*N*-methyl-L-tyrosyl-*N*,*O*^{4}-dimethyl-L-tyrosine Cyclic 5^4→6^3 Ether, Methyl Ester (47). A solution of **43** (4 mg, 0.01 mmol) in anhydrous THF (0.5 mL) was treated with **33** (7.4 mg, 0.011 mmol, 1.1 equiv) at 25 °C under Ar. The resulting reaction mixture was warmed at 50 °C for 12 h. The solvent was removed under a stream of N_2, and the residue was purified by flash chromatography (SiO_2, 1 × 3 cm, 0−8% CH_3OH−$CHCl_3$ gradient elution) to afford **47** (7.2 mg, 8.9 mg theoretical, 81%) as a white solid: mp > 250 °C dec; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.47 (dd, 1H, J = 2.2, 8.3 Hz, Tyr$^{5\delta a}$-H), 7.29 (dd, 1H, J = 2.2, 8.3 H, Tyr$^{5\delta b}$-H), 7.12 (d, 2H, J = 8.6 Hz, Tyr$^{3\delta}$-H), 7.04 (dd, 1H, J = 2.2, 8.3 Hz, Tyr$^{5\epsilon a}$-H), 7.01 (dd, 1H, J = 2.2, 8.3 Hz, Tyr$^{5\epsilon b}$-H), 6.96 (br s, 1H, CONH), 6.83 (d, 2H, J = 8.6 Hz, Tyr$^{3\epsilon}$-H), 6.78 (d, 1H, J = 8.3 Hz, Tyr$^{6\epsilon}$-H), 6.70 (br s, 1H, CONH), 6.62 (dd, 1H, J = 2.2, 8.3 Hz, Tyr$^{6\delta a}$-H), 6.50 (br s, 1H, CONH), 5.69 (dd, 1H, J = 4.8, 11.8 Hz, Tyr$^\alpha$-H), 4.98 (br s, 1H, NHBOC), 4.84 (p, 1H, J = 7.0 Hz, Ala$^\alpha$-H), 4.73 (d, 1H, J = 2.2, Hz, Tyr$^{6\delta b}$-H), 4.61 (dd, 1H, J = 6.8, 13.6 Hz, Tyr$^\alpha$-H), 4.38 (p, 1H, J = 7.2 Hz, Ala$^\alpha$-H), 4.07 (m, 1H, Tyr$^\alpha$-H), 3.96 (m, 1H, Ala$^\alpha$-H), 3.93 (s, 3H, Tyr^6-OCH_3), 3.78 and 3.77 (two s, 3H, Tyr^3-OCH_3), 3.64 (s, 3H, CO_2CH_3), 3.23 (t, 1H, J = 11.6 Hz, Tyr$^\beta$-H), 3.12 and 3.11 (two s, 3H, NCH_3), 3.07−2.90 (m, 5H, Tyr$^\beta$-H), 2.76 and 2.71 (two s, 3H, NCH_3), 1.44 and 1.43 (two s, 9H, $CO_2C(CH_3)_3$), 1.33 (d, 3H, J = 7.0 Hz, Ala$^\beta$-CH_3), 1.29 (d, 3H, J = 6.8 Hz, Ala$^\beta$-CH_3), 1.24 (d, 3H, J = 7.0 Hz, Ala$^\beta$-CH_3); IR (KBr) ν_{max} 3448, 2963, 2872, 1733, 1698, 1650, 1518, 1459, 1369, 1246, 1159, 1099, 903, 795, 748 cm^{-1}; FABHRMS (NBA) *m/e* 889.4735 (M^+ + H, $C_{46}H_{60}N_6O_{12}$ requires 889.4347).

BOC-D-Alanyl-L-alanyl-*N*,*O*^{4}-dimethyl-L-tyrosyl-L-alanyl-L-tyrosyl-*N*,*O*^{4}-dimethyl-L-tyrosine Cyclic 5^4→6^3 Ether, Methyl Ester (48). A solution of **45** (2.9 mg, 0.0076 mmol) in anhydrous THF (0.5 mL) was treated with **32** (5.7 mg, 0.0083 mmol, 1.1 equiv) at 25 °C under Ar. The resulting reaction mixture was stirred at 25 °C for 2 h under Ar. The solvent was removed under a stream of N_2, and the residue was purified by flash chromatography (SiO_2, 1 × 3 cm, 0−8% CH_3OH−$CHCl_3$ gradient elution) to afford **48** (6.4 mg, 7.4 mg theoretical, 87%) as a white solid: mp > 250 °C dec; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.01 (d, 1H, J = 8.0 Hz, CONH), 7.51 (br s, 1H, CONH), 7.45 (dd, 1H, J = 2.1, 8.2 Hz, Tyr$^{5\delta a}$-H), 7.29 (dd, 1H, J = 2.1, 8.2 Hz, Tyr$^{5\delta b}$-H), 7.24 (dd, 1H, J = 2.1, 8.2 Hz, Tyr$^{5\epsilon a}$-H), 7.18 (d, 1H, J = 7.6 Hz, CONH), 7.09 and 7.08 (two d, 2H, J = 8.6 Hz, Tyr$^{3\delta}$-H), 7.05 (dd, 1H, J = 2.1, 8.2 Hz, Tyr$^{5\epsilon b}$-H), 6.83 and 6.81 (d, 2H, J = 8.6 Hz, Tyr$^{3\epsilon}$-H), 6.80 (d, 1H, J = 8.2 Hz, Tyr$^{6\epsilon a}$-H), 6.62 (dd, 1H, J = 2.1, 8.2 Hz, Tyr$^{6\delta a}$-H), 5.40 (br s, 1H, NHBOC), 5.14 (m, 1H, Tyr$^\alpha$-H), 4.92 and 4.75 (two dd, 1H, J = 3.2, 11.5 Hz, Tyr$^\alpha$-H), 4.73 (d, 1H, J = 2.1 Hz, Tyr$^{6\delta b}$-H), 4.61 (dd, 1H, J = 2.6, 12.0 Hz, Tyr$^\alpha$-H), 4.45 (p, 1H, J = 7.4 Hz, Ala$^\alpha$-H), 4.31 (p, 1H, J = 6.6 Hz, Ala$^\alpha$-H), 4.04 (p, 1H, J = 7.2 Hz, Ala$^\alpha$-H), 3.94 (s, 3H, Tyr^6-OCH_3), 3.78 and 3.74 (two s, 3H, Tyr^3-OCH_3), 3.63 and 3.55 (two s, 3H, CO_2CH_3), 3.39−3.25 (m, 3H, Tyr$^{3\beta}$-, Tyr$^{5\beta}$- and Tyr$^{6\beta}$-H), 3.10−2.85 (m, 3H, Tyr$^{3\beta}$-, Tyr$^{5\beta}$-, and Tyr$^{6\beta}$-H), 2.92 and 2.89 (two s, 3H, Tyr-NCH_3), 2.81 and 2.80 (two s, 3H, Tyr-NCH_3), 1.43 and 1.41 (two s, 9H, $CO_2C(CH_3)_3$), 1.39 and 1.38 (two d, 3H, J = 6.8 Hz, Ala$^\beta$-CH_3), 1.33 (d, 3H, J = 7.2 Hz, Ala$^\beta$-CH_3), 1.19 (d, 3H, J = 7.0 Hz, Ala$^\beta$-CH_3); IR (KBr) ν_{max} 3436, 2984, 2851, 1656, 1636, 1512, 1462, 1359, 1246, 1205, 1162, 1129, 1071, 1031, 980, 806 cm^{-1}; FABHRMS (NBA) *m/e* 889.4365 (M^+ + H, $C_{46}H_{60}N_6O_{12}$ requires 889.4347).

BOC-D-Alanyl-L-alanyl-*O*^{4}-methyl-L-tyrosyl-L-alanyl-L-tyrosyl-*N*,*O*^{4}-dimethyl-L-tyrosine Cyclic 5^4→6^3 Ether, Methyl Ester (49). Following the procedure detailed for **48**, **45** (3.0 mg, 0.0078 mmol) and **33** (5.8 mg, 0.0086 mmol, 1.1 equiv) afforded **49** (6.0 mg, 6.8 mg theoretical, 88%) as a white solid: mp > 250 °C dec; ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.57−6.20 (m, 14H), 5.42 (br s, 1H, NHBOC), 4.93 (dd, 1H, J = 7.8, 14.2 Hz, Tyr$^\alpha$-H), 4.81 (d, 1H, J = 2.2 Hz, Tyr$^{6\delta b}$-H), 4.74 (m, 1H, Tyr$^\alpha$-H), 4.45 (m, 1H), 4.28 (p, 1H, J = 7.4 Hz, Ala$^\alpha$-H), 4.18−4.02 (m, 2H), 3.87 and 3.77 (two s, 3H, Tyr^6-OCH_3), 3.75 and 3.74 (two s, 3H, Tyr^3-OCH_3), 3.64 and 3.59 (two s, 3H, CO_2CH_3), 3.28−2.63 (m, 6H, Tyr$^{3\beta}$-, Tyr$^{5\beta}$- and Tyr$^{6\beta}$-H), 2.82 and 2.79 (two s, 3H, Tyr^6-NCH_3), 1.41 and 1.40 (two s, 9H, $CO_2C(CH_3)_3$), 1.36 (d, 3H, J = 7.1 Hz, Ala$^\beta$-CH_3), 1.27 (d, 3H, J = 7.2 Hz, Ala$^\beta$-CH_3), 1.20 (d, 3H, J = 7.2 Hz, Ala$^\beta$-CH_3); IR (KBr) ν_{max} 3297, 2980, 2928, 1734, 1696, 1635, 1512, 1451, 1364, 1246, 1164, 1128, 1026, 867, 837, 800, 704 cm^{-1}; FABHRMS (NBA) *m/e* 875.4180 (M^+ + H, $C_{45}H_{58}N_6O_{12}$ requires 875.4191).

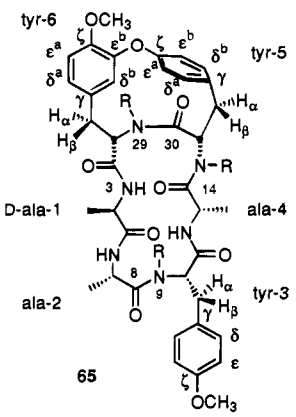
BOC-D-Alanyl-L-alanyl-*O*^{4}-methyl-L-tyrosyl-L-alanyl-*N*-methyl-L-tyrosyl-*O*^{4}-methyl-L-tyrosine Cyclic 5^4→6^3 Ether, Methyl Ester (50). Following the procedure detailed for **47**, **44** (3.5 mg, 0.0091 mmol) and **33** (6.8 mg, 0.01 mmol, 1.1 equiv) afforded **50** (6.6 mg, 8.0 mg theoretical, 83%) as a white solid: mp > 250 °C dec; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.41 (dd, 1H, J = 2.1, 8.2 Hz, tyr$^{5\delta a}$-H), 7.26 (dd, 1H, J = 2.1, 8.2 Hz, Tyr$^{5\delta b}$-H), 7.11 (d, 2H, J = 8.6 Hz, Tyr$^{3\delta}$-H), 7.08 (dd, 1H, J = 2.1, 8.2 Hz, Tyr$^{5\epsilon a}$-H), 7.01 (br s, 1H, CONH), 6.97 (dd, 1H, J = 2.1, 8.2 Hz, Tyr$^{5\epsilon b}$-H), 6.82 (d, 2H, J = 8.6 Hz, Tyr$^{3\epsilon}$-H), 6.75 (d, 1H, J = 8.2 Hz, Tyr$^{6\epsilon a}$-H), 6.66 (d, 1H, J = 7.8 Hz, CONH), 6.58 (dd, 1H, J = 2.1, 8.2 Hz, Tyr$^{6\delta a}$-H), 6.50 (br s, 1H, CONH), 6.20 (br s, 1H, CONH), 5.22 (br s, 1H, CONH), 5.19 (d, 1H, J = 2.1 Hz, Tyr$^{6\delta b}$-H), 4.96 (dd, 1H, J = 4.4, 11.7 Hz, Tyr$^\alpha$-H), 4.85 (p, 1H, J = 7.2 Hz, Ala$^\alpha$-H), 4.58 (m, 1H, Tyr$^\alpha$-H), 4.40 (p, 1H, J = 7.1 Hz, Ala$^\alpha$-H), 4.25−4.05 (m, 2H, Tyr$^\alpha$-H and Ala$^\alpha$-H), 3.93 (s, 3H, Tyr^6-OCH_3), 3.78 (s, 3H, Tyr^3-OCH_3), 3.62 (s, 3H, CO_2CH_3), 3.29 (t, 1H, J = 7.9 Hz, Tyr$^{3\beta}$-, Tyr$^{5\beta}$-, or Tyr$^{6\beta}$-H), 3.18 (s, 3H, Tyr^5-NCH_3), 3.15−2.70 (m, 5H, Tyr$^{3\beta}$-, Tyr$^{5\beta}$- and Tyr$^{6\beta}$-H), 1.47−1.26 (m, 18H, $CO_2C(CH_3)_3$ and three Ala$^\beta$-CH_3); IR (KBr) ν_{max} 3415, 2923, 2851, 1739, 1651, 1513, 1456, 1369, 1246, 1161, 1128, 1026, 882, 835 cm^{-1}; FABHRMS (NBA-CsI) *m/e* 875.4170 (M^+ + H, $C_{45}H_{58}N_6O_{12}$ requires 875.4191).

BOC-D-Alanyl-L-alanyl-*N,O*^{4}-dimethyl-L-tyrosyl-L-alanyl-L-tyrosyl-*O*^{4}-methyl-L-tyrosine Cyclic 5^4→6^3 Ether, Methyl Ester (51). Following the procedure detailed for **48**, **46** (3.6 mg, 0.0097 mmol) and **32** (7.4 mg, 0.011 mmol, 1.1 equiv) afforded **51** (7.4 mg, 8.5 mg theoretical, 87%) as a white solid: mp > 250 °C dec; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.14 (br s, 1H, CONH), 7.62 (br s, 1H, CONH), 7.42 (dd, 1H, J = 2.1, 8.2 Hz, Tyr$^{5\delta a}$-H), 7.30 (dd, 1H, J = 2.1, 8.2 Hz, Tyr$^{5\delta b}$-H), 7.21 (dd, 1H, J = 2.1, 8.2 Hz, Tyr$^{5\epsilon a}$-H), 7.08 (d, 2H, J = 8.6 Hz, Tyr$^{3\delta}$-H), 7.04 (br s, 1H, CONH), 6.97 (dd, 1H, J = 2.1, 8.2 Hz, Tyr$^{5\epsilon b}$-H), 6.81 (d, 2H, J = 8.6 Hz, Tyr$^{3\epsilon}$-H), 6.74 (d, 1H, J = 8.2 Hz, Tyr$^{6\epsilon a}$-H), 6.57 (dd, 1H, J = 2.1, 8.2 Hz, Tyr$^{6\delta a}$-H), 6.20 (br s, 1H, CONH), 5.52 (br s, 1H, CONH), 5.10 (d, 1H, J = 2.1 Hz, Tyr$^{6\delta b}$-H), 4.90 (m, 1H, Tyr$^{\alpha}$-H), 4.71 (p, 1H, J = 6.8 Hz, Ala$^{\alpha}$-H), 4.47 (m, 1H), 4.32 (m, 1H), 4.15−4.05 (m, 2H), 3.92 (s, 3H, Tyr^6-OCH_3), 3.77 and 3.74 (two s, 3H, Tyr^3-OCH_3), 3.62 and 3.58 (two s, 3H, CO_2CH_3), 3.40−3.19 (m, 3H, Tyr$^{3\beta}$-, Tyr$^{5\beta}$- and Tyr$^{6\beta}$-H), 2.91−2.70 (m, 3H, Tyr$^{3\beta}$-, Tyr$^{5\beta}$- and Tyr$^{6\beta}$-H), 2.82 (s, 3H, Tyr^5-NCH_3), 1.54−1.16 (m, 18H, $CO_2C(CH_3)_3$ and three Ala$^{\beta}$-CH_3); IR (KBr) ν_{max} 3307, 2978, 2949, 1733, 1712, 1692, 1650, 1512, 1456, 1369, 1246, 1164, 1128, 1026, 836, 805 cm^{-1}; FABHRMS (NBA) *m/e* 875.4223 (M^+ + H, $C_{45}H_{58}N_6O_{12}$ requires 875.4191).

BOC-D-Alanyl-L-alanyl-*O*^{4}-methyl-L-tyrosyl-L-alanyl-L-tyrosyl-*O*^{4}-methyl-L-tyrosine Cyclic 5^4→6^3 Ether, Methyl Ester (52). Following the procedure detailed for **48**, **46** (3.3 mg, 0.0089 mmol) and **33** (6.6 mg, 0.0088 mmol, 1.1 equiv) afforded **52** (6.8 mg, 7.7 mg theoretical, 88%) as a white solid: mp > 250 °C dec; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.50−6.60 (m, 15H), 5.81−4.10 (m, 8H), 3.95 (s, 3H, tyr^6-OCH_3), 3.70 (br s, 6H, Tyr^3-OCH_3 and CO_2CH_3), 3.40−2.70 (m, 6H, three Tyr$^{\beta}$-H), 1.50−1.20 (m, 18H, $CO_2C(CH_3)_3$ and three Ala$^{\beta}$-CH_3); IR (KBr) ν_{max} 3291, 2963, 2922, 1717, 1692, 1635, 1512, 1451, 1364, 1246, 1164, 1128, 1066, 1030, 830, 799, 702 cm^{-1}; FABHRMS (NBA) *m/e* 861.4030 (M^+ + H, $C_{44}H_{56}N_6O_{12}$ requires 861.4034).

Cyclo(D-alanyl-L-alanyl-*N,O*^{4}-dimethyl-L-tyrosyl-L-alanyl-*N*-methyl-L-tyrosyl-*O*-methyl-L-tyrosyl) cyclic 5^4→6^3 ether (*N*^{29}-desmethyl RA-VII, 14): mp > 300 °C dec; $[\alpha]^{22}_D$ −202 (*c* 0.05, $CHCl_3$); ^{1}H NMR[56] ($CDCl_3$, 300 MHz) δ 7.40 (dd, 1H, J = 2.0, 8.0 Hz, Tyr$^{5\delta a}$-H), 7.25 (dd, 1H, J = 2.0, 8.0 Hz, Tyr$^{5\delta b}$-H), 7.19 (dd, 1H, J = 2.0, 8.0 Hz, Tyr$^{5\epsilon a}$-H), 7.02 (d, 2H, J = 8.5 Hz, Tyr$^{3\delta}$-H), 6.83 (dd, 1H, J = 2.0, 8.0 Hz, Tyr$^{5\epsilon b}$-H), 6.80 (d, 2H, J = 8.5 Hz, Tyr$^{3\epsilon}$-H), 6.78 (d, 1H, J = 8.5 Hz, Tyr$^{6\epsilon a}$-H), 6.70 (d, 1H, J = 8.0 Hz, Ala^4-NH), 6.60 (dd, 1H, J = 2.2, 8.4 Hz, Tyr$^{6\delta a}$-H), 6.40 (d, 1H, J = 6.6 Hz, Ala^1-NH), 6.08 (d, 1H, J = 8.5 Hz, Ala^2-NH), 5.83 (d, 1H, J = 8.0 Hz, Tyr^6-NH), 5.41 (dd, 1H, J = 3.2, 11.4 Hz, Tyr$^{5\alpha}$-H), 4.85 (p, 1H, J = 7.0 Hz, ala$^{2\alpha}$-H), 4.76 (d, 1H, J = 2.2 Hz, Tyr$^{6\delta b}$-H), 4.74 (p, 1H, J = 7.2 Hz, Ala$^{4\alpha}$-H), 4.55 (ddd, 1H, J = 4.0, 8.0, 10.0 Hz, Tyr$^{6\alpha}$-H), 4.32 (p, 1H, J = 7.0 Hz, Ala$^{1\alpha}$-H), 3.93 (s, 3H, Tyr^6-OCH_3), 3.78 (s, 3H, Tyr^3-OCH_3), 3.67 (dd, 1H, J = 8.0, 11.0 Hz, Tyr$^{5\beta}$-H$_\alpha$), 3.60 (dd, 1H, J = 5.0, 11.0 Hz, Tyr$^{3\alpha}$-H), 3.35 (m, 2H, Tyr$^{3\beta}$-H), 3.17 (dd, 1H, J = 11.0, 19.0 Hz, Tyr$^{6\beta}$-H$_\alpha$), 3.13 (s, 3H, Tyr^5-NCH_3), 3.01 (dd, 1H, J = 4.1, 19.0 Hz, Tyr$^{6\beta}$-H$_\beta$), 2.83 (s, 3H, Tyr^3-NCH_3), 2.63 (dd, 1H, J = 3.0, 11.0 Hz, Tyr$^{5\beta}$-H$_\beta$), 1.34 (d, 3H, J = 6.9 Hz, Ala$^{2\beta}$-CH_3), 1.30 (d, 3H, J = 6.9 Hz, Ala$^{1\beta}$-CH_3), 1.11 (d, 3H, J = 6.6 Hz, Ala$^{4\beta}$-CH_3); ^{13}C NMR[56] ($CDCl_3$, 75 MHz) δ 172.6, 172.4, 171.7, 170.9, 169.7, 169.4, 158.5, 158.3, 153.2, 146.6, 135.1, 132.8, 130.9, 130.5, 130.2, 128.2, 126.0, 124.3, 121.0, 114.2, 113.5, 112.9, 68.3, 57.5, 56.1, 55.3, 54.1, 48.3, 46.6, 44.4, 39.9, 36.7, 35.5, 32.7, 30.3, 21.0, 18.4, 16.6; IR (KBr) ν_{max} 3390, 2930, 1638, 1586, 1445, 1412, 1380, 1262, 1250, 1180, 1159, 1094, 966, 838, 732 cm^{-1}; FABHRMS (NBA) *m/e* 757.3753 ($C_{40}H_{48}N_6O_9$ requires 753.3561).

(56) The ^{1}H NMR and ^{13}C NMR numbering system is illustrated with structure **65**.



Cyclo(D-alanyl-L-alanyl-*O*^{4}-methyl-L-tyrosyl-L-alanyl-*N*-methyl-L-tyrosyl-*N,O*^{4}-dimethyl-L-tyrosyl) Cyclic 5^4→6^3 Ether (*N*^{9}-Desmethyl RA-VII, 15). A solution of **47** (8.9 mg, 0.01 mmol) in THF−CH_3OH−H_2O (3:1:1, 0.5 mL) was treated with LiOH−H_2O (1.3 mg, 0.03 mmol, 3.0 equiv) at 25 °C under Ar, and the resulting reaction mixture was stirred at 25 °C for 8 h. The organic solvents were removed under a stream of N_2, and the residue was treated with H_2O (1 mL), EtOAc (2 mL) and with 15% aqueous citric acid (pH 3.0). The two layers were separated, and the aqueous phase was extracted with EtOAc (4 × 2 mL). The combined EtOAc extracts were washed with H_2O (2 mL) and saturated aqueous NaCl (2 mL), dried ($MgSO_4$), and concentrated in vacuo to afford **53** (8.0 mg, 8.7 mg theoretical, 92%) as a white solid (FABHRMS (NBA) *m/e* 875.4188; M^+ + H, $C_{45}H_{58}N_6O_{12}$ requires 875.4191) which was used directly in the following reaction without purification.

A solution of **53** (8.0 mg, 0.0091 mmol) in 4 M HCl−EtOAc (0.5 mL) was stirred at 0 °C for 10 min and 25 °C for 50 min. The volatiles were removed in vacuo, and the residue was dried thoroughly under vacuum to afford **59**-HCl (7.4 mg, 7.4 mg theoretical, 100%) as a white solid (FABHRMS (NBA) *m/e* 775.3659; M^+ + H, $C_{40}H_{50}N_6O_{10}$ requires 775.3667) which was used directly in the next reaction.

A solution of **59**-HCl (7.0 mg, 0.0086 mmol) in anhydrous DMF (3.0 mL) was cooled to 0 °C and treated with $NaHCO_3$ (7.3 mg, 0.086 mmol, 10.0 equiv) and diphenylphosphoryl azide (DPPA, 4.7 mg, 3.7 μL, 0.017 mmol, 2.0 equiv) under Ar. The resulting reaction mixture was stirred at 4 °C for 48 h before the solvent was removed in vacuo. The residue was then treated with H_2O (2 mL) and EtOAc (3 mL), and the aqueous layer was extracted with EtOAc (4 × 3 mL). The combined EtOAc extracts were washed with H_2O (2 mL) and saturated aqueous NaCl (2 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 0.5 × 10 cm, 0−7% CH_3OH−$CHCl_3$ gradient elution) afforded **15** (4.2 mg, 6.5 mg theoretical, 65%) as a white powder: mp > 250 °C; $[\alpha]^{25}_D$ −123 (*c* 0.2, 50% CH_3OH−$CHCl_3$) [lit.[13] $[\alpha]^{25}_D$ −127 (*c* 0.3, 50% CH_3OH−$CHCl_3$); ^{1}H NMR[56] (DMSO-d_6, 400 MHz) δ 8.18 (d, 1H, J = 7.8 Hz, Ala^4-NH), 8.16 (d, 1H, J = 7.3 Hz, Ala^2-NH), 8.02 (d, 1H, J = 5.6 Hz, D-Ala^1-NH), 7.43 (dd, 1H, J = 2.2, 8.3 Hz, Tyr$^{5\delta a}$-H), 7.42 (d, 1H, J = 9.4 Hz, Tyr^3-NH), 7.25 (dd, 1H, J = 2.2, 8.3 Hz, Tyr$^{5\delta b}$-H), 7.09 (d, 2H, J = 8.7 Hz, Tyr$^{3\delta}$-H), 7.05 (dd, 1H, J = 2.2, 8.3 Hz, Tyr$^{5\epsilon a}$-H), 6.93 (dd, 1H, J = 2.2, 8.3 Hz, Tyr$^{5\epsilon b}$-H), 6.90 (d, 1H, J = 8.4 Hz, Tyr$^{6\epsilon a}$-H), 6.80 (d, 2H, J = 8.7 Hz, Tyr$^{3\epsilon}$-H), 6.67 (dd, 1H, J = 1.9, 8.4 Hz, Tyr$^{6\delta a}$-H), 5.47 (dd, 1H, J = 4.6, 11.5 Hz, Tyr$^{5\alpha}$-H), 4.63 (dd, 1H, J = 1.8, 12.2 Hz, Tyr$^{6\alpha}$-H), 4.58 (d, 1H, J = 1.9 Hz, Tyr$^{6\delta b}$-H), 4.37 (m, 1H, Tyr$^{3\alpha}$-H), 4.28 (p, 1H, J = 6.8 Hz, Ala$^{4\alpha}$-H), 4.11 (p, 1H, J = 6.1, Hz, Ala$^{1\alpha}$-H), 3.96 (p, 1H, J = 7.3 Hz, Ala$^{2\alpha}$-H), 3.81 (s, 3H, Tyr^6-OCH_3), 3.69 (s, 3H, Tyr^3-OCH_3), 3.11 (t, 1H, J = 11.5 Hz, Tyr$^{5\beta}$-H$_\alpha$), 3.05 (dd, 1H, J = 12.2, 17.8 Hz, Tyr$^{6\beta}$-H$_\alpha$), 2.93 (dd, 1H, J = 4.4, 11.4 Hz, Tyr$^{3\beta}$-H$_\beta$), 2.86 (dd, 1H, J = 4.6, 11.5 Hz, Tyr$^{5\beta}$-H$_\beta$), 2.82 (s, 3H, Tyr^5-NCH_3), 2.73 (dd, 1H, J = 1.8, 17.8 Hz, Tyr$^{6\beta}$-H$_\beta$), 2.72 (s, 3H, Tyr^6-NCH_3), 2.66 (dd, 1H, J = 9.8, 11.4 Hz, Tyr$^{3\beta}$-H$_\alpha$), 1.17 (d, 3H, J = 6.6 Hz, Ala$^{4\beta}$-CH_3), 1.13 (d, 3H, J = 6.9 Hz, D-Ala$^{1\beta}$-CH_3), 1.04 (d, 3H, J = 7.4 Hz, Ala$^{2\beta}$-CH_3); ^{13}C NMR[56] (DMSO-d_6, 100 MHz) δ 172.0, 170.9, 170.2, 169.8, 169.0, 168.8, 158.0, 157.5, 152.2, 145.8, 135.1, 132.7, 130.7, 130.4, 130.2, 129.4, 125.7, 123.8, 121.0, 114.3, 113.4, 112.6, 56.6, 55.7, 54.9, 54.5, 53.1, 48.5, 47.1, 45.5, 35.7, 34.8, 33.7, 30.1, 29.0, 20.5, 18.5, 16.7; IR (KBr) ν_{max} 3422, 2958, 2854, 1649, 1513, 1460, 1415, 1382, 1264, 1210, 1128, 1097, 1075, 1031, 964, 912, 867, 804, 794 cm^{-1}; FABHRMS (NBA) *m/e* 757.3569 (M^+ + H, $C_{40}H_{48}N_6O_9$ requires 757.3561).

Cyclo(D-alanyl-L-alanyl-*N,O*^{4}-dimethyl-L-tyrosyl-L-alanyl-L-tyrosyl-*N,O*^{4}-dimethyl-L-tyrosyl)Cyclic 5^4→6^3 Ether (*N*^{15}-Desmethyl RA-VII, 16). As described for **15**, **48** (5.9 mg, 0.0066 mmol) provided **54** (5.3 mg, 5.8 mg theoretical, 91%) as a white solid (FABHRMS (NBA) *m/e* 875.4165; M^+ + H, $C_{45}H_{58}N_6O_{12}$ requires 875.4191), **60**-HCl (4.9 mg, 4.9 mg theoretical, 100%) as a white solid (FABHRMS (NBA)

m/e 775.3640; $M^+ + H$, $C_{40}H_{50}N_6O_{10}$ requires 775.3667), and **16** (2.6 mg, 4.4 mg theoretical, 59%) as a white powder: mp > 250 °C dec; $[\alpha]^{25}_D$ −102 (*c* 0.1, $CHCl_3$); ^1H NMR[56] ($CDCl_3$, 400 MHz) mixture of two conformers (conformer A:conformer B = 66:34) δ 8.41 and 8.03 (two d, 1H, *J* = 7.2 Hz, CONH), 7.41 and 7.39 (two dd, 1H, *J* = 2.1, 8.3 Hz, Tyr$^{5\delta a}$-H), 7.30 and 7.21 (two dd, 1H, *J* = 2.1, 8.3 Hz, Tyr$^{5\delta b}$-H), 7.11 and 7.10 (d, 2H, *J* = 8.6 Hz, Tyr$^{3\delta}$-H), 7.09 (dd, 1H, *J* = 2.1, 8.3 Hz, Tyr$^{5\epsilon a}$-H), 7.10−7.02 (m, 3H, CONH), 6.86 and 6.85 (two dd, 1H, *J* = 2.1, 8.3 Hz, Tyr$^{5\epsilon b}$-H), 6.82 and 6.80 (two d, 2H, *J* = 8.6 Hz, Tyr$^{3\epsilon}$-H), 6.78 (d, 2H, *J* = 8.3 Hz, Tyr$^{6\epsilon a}$-H), 6.65 and 6.64 (two dd, 1H, *J* = 2.1, 8.3 Hz, Tyr$^{6\delta a}$-H), 4.90 and 4.80 (two p, 1H, *J* = 6.0 Hz, Ala$^{2\alpha}$-H), 4.74 and 4.48 (two dd, 1H, *J* = 2.2, 12.6 Hz, Tyr$^{6\alpha}$-H), 4.63 and 3.48 (two dd, 1H, *J* = 6.7, 8.3 Hz, Tyr$^{3\alpha}$-H), 4.68 and 4.55 (two d, 1H, *J* = 2.1 Hz, Tyr$^{6\delta b}$-H), 4.42 and 4.40 (two p, 1H, *J* = 7.4 Hz, Ala$^{1\alpha}$-H), 4.25 and 4.21 (two p, 1H, *J* = 7.1 Hz, Ala$^{4\alpha}$-H), 3.94 and 3.93 (two s, 3H, Tyr^6-OCH_3), 3.83 and 3.79 (two p, 1H, *J* = 7.2 Hz, Tyr$^{5\alpha}$-H), 3.78 and 3.76 (two s, 3H, Tyr^3-OCH_3), 3.34 (m, 2H, Tyr$^{3\beta}$-H), 3.31 and 3.24 (two dd, 1H, *J* = 2.2, 16.5 Hz, Tyr$^{6\beta}$-H$_\beta$), 3.15 and 3.14 (two t, 1H, *J* = 11.9 Hz, Tyr $^{5\beta}$-H$_\alpha$), 3.04 and 2.73 (two s, 3H, Tyr^3-NCH_3), 2.98 and 2.96 (two dd, 1H, *J* = 12.6, 16.5 Hz, Tyr$^{6\beta}$-H$_\alpha$), 2.89 and 2.87 (two s, 3H, Tyr^6-NCH_3), 2.86 (m, 1H, Tyr$^{5\beta}$-H$_\beta$), 1.66 and 1.53 (two d, 3H, *J* = 7.2 Hz, Ala$^{4\beta}$-CH_3), 1.34 and 1.30 (two d, 3H, *J* = 7.2 Hz, Ala$^{1\beta}$-CH_3), 1.29 and 0.78 (two d, 3H, *J* = 7.0 Hz, Ala$^{2\beta}$-CH_3); IR (KBr) ν_{max} 3423, 2958, 2853, 1654, 1638, 1560, 1513, 1458, 1420, 1383, 1263, 1214, 1129, 1097, 1075, 1029, 968, 913, 868, 803, 745 cm^{-1}; FABHRMS (NBA) *m/e* 757.3540 ($M^+ + H$, $C_{40}H_{48}N_6O_9$ requires 757.3561).

Cyclo(D-alanyl-L-alanyl-*O*^{4}-methyl-L-tyrosyl-L-alanyl-L-tyrosyl-*N*,*O*^{4}-dimethyl-L-tyrosyl) Cyclic 5^4→6^3 Ether (*N*^{9},*N*^{15}-Desmethyl RA-VII, 17). As described for **15**, **49** (5.8 mg, 0.0066 mmol) provided **55** (5.3 mg, 5.7 mg theoretical, 93%) as a white solid (FABHRMS (NBA) *m/e* 861.4045; $M^+ + H$, $C_{44}H_{56}N_6O_{12}$ requires 861.4034), **61**-HCl (4.9 mg, 4.9 mg theoretical, 100%) as a white solid (FABHRMS (NBA) *m/e* 761.3530; $M^+ + H$, $C_{39}H_{48}N_6O_{10}$ requires 761.3511), and **17** (2.8 mg, 4.5 mg theoretical, 62%) as a white powder: mp > 250 °C dec; $[\alpha]^{25}_D$ +96 (*c* 0.1, $CHCl_3$); ^1H NMR[56] ($CDCl_3$, 400 MHz) δ 8.38 (d, 1H, *J* = 5.7 Hz, Tyr^5-NH), 7.68 (d, 1H, *J* = 2.8 Hz, Ala^2-NH), 7.50 (dd, 1H, *J* = 2.2, 8.4 Hz, Tyr$^{5\delta a}$-H), 7.41 (d, 1H, *J* = 6.4 Hz, Ala^4-NH), 7.24 (dd, 1H, *J* = 2.2, 8.4 Hz, Tyr$^{5\delta b}$-H), 7.21 (d, 1H, *J* = 8.2 Hz, D-Ala^1-NH), 7.13 (d, 2H, *J* = 8.6 Hz, Tyr$^{3\delta}$-H), 7.11 (dd, 1H, *J* = 2.2, 8.4 Hz, Tyr$^{5\epsilon a}$-H), 7.07 (dd, 1H, *J* = 2.2, 8.4 Hz, Tyr$^{5\epsilon b}$-H), 6.83 (d, 1H, *J* = 8.4 Hz, Tyr$^{6\epsilon a}$-H), 6.80 (d, 2H, *J* = 8.6 Hz, Tyr$^{3\epsilon}$-H), 6.67 (dd, 1H, *J* = 2.2, 8.4 Hz, Tyr$^{6\delta a}$-H), 6.02 (d, 1H, *J* = 10.2 Hz, Tyr^3-NH), 5.00 (ddd, 1H, *J* = 4.8, 10.2, 11.4 Hz, Tyr$^{3\alpha}$-H), 4.67 (ddd, 1H, *J* = 5.7, 5.8, 10.8 Hz, Tyr$^{5\alpha}$-H), 4.57 (d, 1H, *J* = 2.2 Hz, Tyr$^{6\delta b}$-H), 4.45 (dd, 1H, *J* = 2.1, 12.4 Hz, Tyr$^{6\alpha}$-H), 4.37 (dq, 1H, *J* = 7.0, 8.2 Hz, D-Ala$^{1\alpha}$-H), 3.95 (s, 3H, Tyr^6-OCH_3), 3.86 (dq, 1H, *J* = 2.8, 7.3 Hz, Ala$^{2\alpha}$-H), 3.74 (s, 3H, Tyr^3-OCH_3), 3.72 (dq, 1H, *J* = 6.4, 7.3 Hz, Ala$^{4\alpha}$-H), 3.62 (dd, 1H, *J* = 4.8, 14.4 Hz, Tyr$^{3\beta}$-H$_\beta$), 3.46 (dd, 1H, *J* = 5.8, 12.2 Hz, Tyr$^{5\beta}$-H$_\beta$), 3.39 (dd, 1H, *J* = 2.1, 18.2 Hz, Tyr$^{6\beta}$-H$_\beta$), 3.04 (dd, 1H, *J* = 11.4, 14.4 Hz, Tyr$^{3\beta}$-H$_\alpha$), 2.96 (dd, 1H, *J* = 12.4, 18.2 Hz, Tyr$^{6\beta}$-H$_\alpha$), 2.94 (s, 3H, Tyr^6-NCH_3), 2.89 (dd, 1H, *J* = 10.8, 12.2 Hz, Tyr$^{5\beta}$-H$_\alpha$), 1.78 (d, 3H, *J* = 7.3 Hz, Ala$^{4\beta}$-CH_3), 1.36 (d, 3H, *J* = 7.0 Hz, D-Ala$^{1\beta}$-CH_3), 1.02 (d, 3H, *J* = 7.3 Hz, Ala$^{2\beta}$-CH_3); ^{13}C NMR[56] ($CDCl_3$, 100 MHz) δ 172.6, 172.2, 171.8, 170.8, 169.4, 168.3, 158.5, 158.3, 153.2, 146.6, 135.2, 131.9, 130.9, 130.2, 130.0, 129.3, 125.9, 124.3, 121.2, 113.7, 113.2, 112.1, 60.2, 56.2, 56.0, 55.2, 53.4, 52.5, 51.9, 48.0, 37.9, 35.4, 32.5, 29.5, 16.7, 15.5, 13.8; IR (KBr) ν_{max} 3394, 3282, 2933, 2851, 1657, 1586, 1544, 1514, 1444, 1410, 1262, 1247, 1215, 1129, 1096, 1051, 1031, 969, 884, 805, 728 cm^{-1}; FABHRMS (NBA) *m/e* 743.3390 ($M^+ + H$, $C_{39}H_{46}N_6O_9$ requires 743.3405).

Cyclo(D-alanyl-L-alanyl-*O*^{4}-methyl-L-tyrosyl-L-alanyl-*N*-methyl-L-tyrosyl-*O*^{4}-methyl-L-tyrosyl) Cyclic 5^4→6^3 Ether (*N*^{9},*N*^{29}-Desmethyl RA-VII, 18). As described for **15**, **50** (6.2 mg, 0.007 mmol) provided **56** (5.8 mg, 6.1 mg theoretical, 95%) as a white solid (FABHRMS (NBA) *m/e* 861.4006; $M^+ + H$, $C_{44}H_{56}N_6O_{12}$ requires 861.4034), **62**-HCl (5.3 mg, 5.3 mg theoretical, 100%) as a white solid (FABHRMS (NBA) *m/e* 761.3510; $M^+ + H$, $C_{39}H_{48}N_6O_{19}$ requires 761.3510), and **18** (3.2 mg, 4.6 mg theoretical, 70%) as a white powder: mp > 250 °C dec; $[\alpha]^{25}_D$ +92 (*c* 0.15, $CHCl_3$); ^1H NMR[56] (15% CD_3OD−$CDCl_3$, 400 MHz) (conformer A:conformer B ≥ 98:2) δ 7.36 (dd, 1H, *J* = 2.2, 8.3 Hz, Tyr$^{5\delta a}$-H), 7.10 (dd, 1H, *J* = 2.2, 8.3 Hz, Tyr$^{5\delta b}$-H), 7.00 (d, 2H, *J* = 8.6 Hz, Tyr$^{3\delta}$-H), 6.99 (dd, 1H, *J* = 2.2, 8.3 Hz, Tyr$^{5\epsilon a}$-H), 6.82 (dd, 1H, *J* = 2.2, 8.3 Hz, Tyr$^{5\epsilon b}$-H), 6.67 (d, 2H, *J* = 8.6 Hz, Tyr$^{3\epsilon}$-H), 6.64 (d, 1H, *J* = 8.3 Hz, Tyr$^{6\epsilon a}$-H), 6.48 (dd, 1H, *J* = 2.2, 8.3 Hz, Tyr$^{6\delta a}$-H), 4.89 (d, 1H, *J* = 2.2 Hz, Tyr$^{6\delta b}$-H), 4.69 (dd, 1H, *J* = 4.2, 12.2 Hz, Tyr$^{5\alpha}$-H), 4.33 (q, 1H, *J* = 6.6 Hz, Ala$^{4\alpha}$-H), 4.27 (dd, 1H, *J* = 6.0, 9.3 Hz, Tyr$^{3\alpha}$-H), 4.03 (q, 1H, *J* = 7.1 Hz, Ala$^{2\alpha}$-H), 3.79 (s, 3H, Tyr^6-OCH_3), 3.78 (q, 1H, *J* = 7.5 Hz, D-Ala$^{1\alpha}$-H, partially overlapped with Tyr^6-OCH_3 and Tyr$^{6\alpha}$-H), 3.76 (dd, 1H, *J* = 2.0, 10.6 Hz, Tyr$^{6\alpha}$-H, partially overlapped with Tyr^6-OCH_3 and D-Ala$^{1\alpha}$-H), 3.60 (s, 3H, Tyr^3-OCH_3), 3.08 (dd, 1H, *J* = 11.9, 12.2 Hz, Tyr$^{5\beta}$-H$_\alpha$), 2.93 (dd, 1H, *J* = 6.0, 14.1 Hz, Tyr$^{3\beta}$-H$_\beta$), 2.87 (s, 3H, Tyr^5-NCH_3), 2.85 (dd, 1H, *J* = 4.2, 11.9 Hz, Tyr$^{5\beta}$-H$_\beta$), 2.77 (dd, 1H, *J* = 9.3, 14.1 Hz, Tyr$^{3\beta}$-H$_\alpha$), 2.70 (dd, 1H, *J* = 10.6, 16.8 Hz, Tyr$^{6\beta}$-H$_\alpha$), 2.60 (dd, 1H, *J* = 2.0, 16.8 Hz, Tyr$^{6\beta}$-H$_\beta$), 1.20 (d, 3H, *J* = 6.6 Hz, Ala$^{4\beta}$-CH_3), 1.15 (d, 3H, *J* = 7.5 Hz, D-Ala$^{1\beta}$-CH_3), 1.12 (d, 3H, *J* = 7.1 Hz, Ala$^{2\beta}$-CH_3); ^{13}C NMR[56] ($CDCl_3$, 100 MHz) δ (for major conformer) 173.6, 172.1, 171.0, 170.0, 169.3, 168.2, 154.8, 153.8, 152.4, 145.6, 134.5, 133.0, 131.4, 130.3, 130.1, 130.0, 124.7, 124.5, 121.5, 113.9, 113.8, 111.6, 57.3, 56.1, 55.3, 55.2, 54.9, 50.7, 49.6, 47.3, 35.5, 34.9, 33.9, 30.0, 17.5, 17.1, 16.4; IR (KBr) ν_{max} 3448, 3282, 2934, 2851, 1655, 1586, 1542, 1514, 1445, 1415, 1262, 1247, 1194, 1129, 1096, 1031, 969, 883, 806, 728 cm^{-1}; FABHRMS (NBA) *m/e* 743.3428 ($M^+ + H$, $C_{39}H_{46}N_6O_9$ requires 743.3405).

Cyclo(D-alanyl-L-alanyl-*N*,*O*^{4}-dimethyl-L-tyrosyl-L-alanyl-L-tyrosyl-*O*^{4}-methyl-L-tyrosyl) Cyclic 5^4→6^3 Ether (*N*^{15},*N*^{29}-Desmethyl RA-VII, 19). As described for **15**, **51** (7.4 mg, 0.0085 mmol) provided **57** (6.8 mg, 7.3 mg theoretical, 93%) as a white solid (FABHRMS (NBA) *m/e* 861.4001; $M^+ + H$, $C_{44}H_{56}N_6O_{12}$ requires 861.4034), **63**-HCl (6.0 mg, 6.0 mg theoretical, 100%) as a white solid (FABHRMS (NBA) *m/e* 761.3543; $M^+ + H$, $C_{39}H_{48}N_6O_{19}$ requires 761.3510), and **19** (4.3 mg, 5.4 mg theoretical, 80%) as a white powder: mp > 250 °C dec; $[\alpha]^{25}_D$ −117 (*c* 0.2, $CHCl_3$); ^1H NMR[56] ($CDCl_3$, 400 MHz) mixture of two conformers (conformer A: conformer B = 62:38) δ 8.22 and 7.89 (two d, 1H, *J* = 6.6 Hz, Tyr^5-CONH), 7.41 and 7.36 (two dd, 1H, *J* = 2.4, 8.3 Hz, Tyr$^{5\delta a}$-H), 7.13 and 7.11 (two dd, 1H, *J* = 2.4, 8.3 Hz, Tyr$^{5\delta b}$-H), 7.08 and 7.05 (two d, 2H, *J* = 8.6 Hz, Tyr$^{3\delta}$-H), 7.07 and 7.04 (two br s, 1H, Tyr^6-CONH), 7.01 and 7.00 (two dd, 1H, *J* = 2.4, 8.3 Hz, Tyr$^{5\epsilon a}$-H), 6.98 and 6.97 (two dd, 1H, *J* = 2.4, 8.3 Hz, Tyr$^{5\epsilon b}$-H), 6.96 and 6.94 (two br s, 1H, Ala^1-CONH), 6.87 and 6.85 (two br s, 1H, Ala^4-CONH), 6.82 and 6.81 (two d, 2H, *J* = 8.6 Hz, Tyr$^{3\epsilon}$-H), 6.76 and 6.72 (two d, 1H, *J* = 8.3 Hz, Tyr$^{6\epsilon a}$-H), 6.59 and 6.49 (two dd, 1H, *J* = 2.0, 8.3 Hz, Tyr$^{6\delta a}$-H), 6.20 and 6.19 (two br s, 1H, Ala^2-CONH), 5.00 and 3.84 (two dd, 1H, *J* = 6.4, 10.4 Hz, Tyr$^{3\alpha}$-H), 4.94 and 4.75 (two d, 1H, *J* = 2.0 Hz, Tyr$^{6\delta b}$-H), 4.53 and 4.11 (two p, 1H, *J* = 6.5 Hz, Ala$^{1\alpha}$-H), 4.50 and 4.13 (two dd, 1H, *J* = 2.2, 12.8 Hz, Tyr$^{6\alpha}$-H), 4.46 and 4.45 (two p, 1H, *J* = 6.4 Hz, Ala$^{2\alpha}$-H), 4.28 and 4.26 (two p, 1H, *J* = 6.0 Hz, Ala$^{4\alpha}$-H), 3.98 and 3.77 (two p, 1H, *J* = 7.3 Hz, Tyr$^{5\alpha}$-H), 3.934 and 3.928 (two s, 3H, Tyr^6-OCH_3), 3.78 and 3.75 (two s, 3H, Tyr^3-OCH_3), 3.44 and 3.12 (two t, 1H, *J* = 12.0 Hz, Tyr$^{5\beta}$-H$_\alpha$), 3.26−3.17 (m, 2H, Tyr$^{3\beta}$-H), 3.19 and 2.98 (two dd, 1H, *J* = 7.8, 12.0 Hz, Tyr$^{5\beta}$-H$_\beta$), 3.16 and 2.58 (two dd, 1H, *J* = 11.0, 16.8 Hz, Tyr$^{6\beta}$-H$_\alpha$), 2.96 and 2.72 (two s, 3H, Tyr^3-NCH_3), 2.94 and 2.84 (two d, 1H, *J* = 16.8 Hz, Tyr$^{6\beta}$-H$_\beta$), 1.60 and 1.56 (two d, 3H, *J* = 7.2 Hz, Ala$^{4\beta}$-CH_3), 1.35 and 0.67 (two d, 3H, *J* = 6.9 Hz, Ala$^{2\beta}$-CH_3), 1.29 and 1.28 (two d, 3H, *J* = 7.2 Hz, Ala$^{1\beta}$-CH_3); IR (KBr) ν_{max} 3448, 3323, 2932, 2851, 1654, 1648, 1586, 1541, 1514, 1448, 1420, 1383, 1301, 1263, 1247, 1163, 1129, 1098, 1031, 969, 886, 836, 806, 728 cm^{-1}; FABHRMS (NBA) *m/e* 743.3416 ($M^+ + H$, $C_{39}H_{46}N_6O_9$ requires 743.3405).

Cyclo(D-alanyl-L-alanyl-*O*^{4}-methyl-L-tyrosyl-L-alanyl-L-tyrosyl-*O*^{4}-methyl-L-tyrosyl) Cyclic 5^4→6^3 Ether (*N*^{9},*N*^{15},*N*^{29}-Desmethyl RA-VII, 20). As described for **15**, **52** (8.0 mg, 0.0093 mmol) provided **58** (7.1 mg, 7.9 mg theoretical, 90%) as a white solid (FABHRMS (NBA) *m/e* 847.3880; $M^+ + H$, $C_{43}H_{54}N_6O_{12}$ requires 847.3878), **64**-HCl (6.6 mg, 6.6 mg theoretical, 100%) as a white solid (FABHRMS (NBA) *m/e* 747.3380; $M^+ + H$, $C_{38}H_{46}N_6O_{10}$ requires 747.3354), and **20** (4.7 mg, 6.0 mg theoretical, 78%) as a white powder: mp > 250 °C dec; $[\alpha]^{25}_D$ +109 (*c* 0.1, CH_3OH); ^1H NMR[56] (15% CD_3OD−$CDCl_3$, 400 MHz) δ 7.31 (dd, 1H, *J* = 2.2, 8.3 Hz, Tyr$^{5\delta a}$-H), 7.04 (dd, 1H, *J* = 2.2, 8.3 Hz, Tyr$^{5\delta b}$-H), 7.00 (dd, 1H, *J* = 2.2, 8.3 Hz, Tyr$^{5\epsilon a}$-H), 6.94

(d, 2H, J = 8.6 Hz, Tyr$^{3\delta}$-H), 6.84 (dd, 1H, J = 2.2, 8.3 Hz, Tyr$^{5\epsilon b}$-H), 6.66 (d, 2H, J = 8.6 Hz, Tyr$^{3\epsilon}$-H), 6.63 (d, 1H, J = 8.3 Hz, Tyr$^{6\epsilon a}$-H), 6.47 (dd, 1H, J = 2.0, 8.3 Hz, Tyr$^{6\delta a}$-H), 4.73 (d, 1H, J = 2.0 Hz, Tyr$^{6\delta b}$-H), 4.26 (dd, 1H, J = 4.7, 11.2 Hz, Tyr$^{3\alpha}$-H), 4.18 (q, 1H, J = 7.0 Hz, Ala$^{2\alpha}$-H), 4.08 (dd, 1H, J = 5.1, 11.8 Hz, Tyr$^{5\alpha}$-H), 3.92 (q, 1H, J = 7.2 Hz, Ala$^{1\alpha}$-H), 3.84 (q, 1H, J = 7.2 Hz, Ala$^{4\alpha}$-H), 3.78 (s, 3H, Tyr^{6}-OCH_3), 3.67 (dd, 1H, J = 2.2, 9.4 Hz, Tyr$^{6\alpha}$-H), 3.61 (s, 3H, Tyr^{3}-OCH_3), 3.21 (dd, 1H, J = 4.7, 14.2 Hz, Tyr$^{3\beta}$-H$_\beta$), 3.09 (dd, 1H, J = 5.1, 12.2 Hz, Tyr$^{5\beta}$-H$_\beta$), 2.90 (dd, 1H, J = 11.2, 14.2 Hz, Tyr$^{3\beta}$-H$_\alpha$), 2.79 (dd, 1H, J = 11.2, 12.2 Hz, Tyr$^{5\beta}$-H$_\alpha$), 2.64 (dd, 1H, J = 2.2, 16.6 Hz, Tyr$^{6\beta}$-H$_\beta$), 2.60 (dd, 1H, J = 9.4, 16.6 Hz, Tyr$^{6\beta}$-H$_\alpha$), 1.39 (d, 3H, J = 7.2 Hz, Ala$^{1\beta}$-CH_3), 1.17 (d, 3H, J = 7.0 Hz, Ala$^{2\beta}$-CH_3), 1.02 (d, 3H, J = 7.2 Hz, Ala$^{4\beta}$-CH_3); ^{13}C NMR[56] (DMSO-d_6, 100 MHz) δ 172.7, 171.7, 171.4, 171.1, 170.6, 169.9, 157.8, 156.9, 152.1, 146.0, 134.5, 132.5, 131.9, 130.9, 130.5, 130.2, 124.6, 124.1, 120.9, 115.0, 113.6, 112.0, 57.5, 57.1, 55.8, 55.4, 55.0, 49.5, 48.7, 47.9, 36.8, 35.5, 34.6, 19.0, 18.9, 17.0; IR (KBr) ν_{max} 3293, 2975, 2932, 1637, 1515, 1449, 1367, 1249, 1208, 1165, 1130, 1096, 1069, 1031, 976, 885, 837, 799, 707 cm^{-1}; FABHRMS (NBA) *m/e* 729.3250 (M^+ + H, $C_{38}H_{44}N_6O_9$ requires 729.3248).

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Supporting Information Available: Further ^{1}H NMR data on **15**−**20** in additional solvents and a listing of 1D decoupling and 2D ^{1}H−^{1}H NOEs for **15**−**20**, copies of comparison ^{1}H NMR spectra of **23**, **43**−**46** ($CDCl_3$) and **8**, **14**−**20** ($CDCl_3$, 15% CD_3OD−$CDCl_3$, and DMSO-d_6), and two tables (Tables 6 and 7) of ^{13}C NMR chemical shifts and assignments for **8**, **14**−**20** (37 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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