Total Synthesis of Cycloisodityrosine, RA-VII, Deoxybouvardin, and N^{29}-Desmethyl-RA-VII: Identification of the Pharmacophore and Reversal of the Subunit Functional Roles

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Abstract: Full details of a concise total synthesis of RA-VII (**1**) and deoxybouvardin (**2**) are described based on the implementation of an effective intramolecular Ullmann reaction as the key macrocyclization reaction in the preparation of the elusive 14-membered cycloisodityrosine subunit (**33**) of the bicyclic hexapeptides. Subsequent coupling of **34** to tetrapeptide **17** and macrocyclization with C^2–N^3 amide bond formation provided **1** and **2**. In efforts that address the key structural and conformational features of the agents that contribute to their antitumor activity, N^{29}-desmethyl-RA-VII was prepared and its chemical, conformational, and preliminary biological properties are detailed. The comparable conformational features of N^{29}-desmethyl-RA-VII and RA-VII including a characteristic cis C^{30}–N^{29} amide bond suggest that the tetrapeptide housed within the 18-membered ring induces the 14-membered cycloisodityrosine to adopt a conformation possessing an inherently disfavored cis secondary or tertiary amide. Moreover, in contrast to prior suppositions in which the rigid 14-membered ring of *N*-methylcycloisodityrosine has been suggested to serve the functional role of inducing a rigid, normally inaccessible conformation within the biologically relevant D-Ala-Ala-*N*-Me-Tyr-(OMe)-Ala tetrapeptide, experimental studies demonstrating that the intrinsic activity of the agents resides within the cycloisodityrosine subunit are presented. Thus, the results of the experimental studies require a reversal of the functional roles of the subunits of the agents in which it is the tetrapeptide housed within the 18-membered ring that potentiates the inherent biological properties and alters the conformation of cycloisodityrosine.

Bouvardin (**8**, NSC 259968) and deoxybouvardin (**2**), bicyclic hexapeptides isolated from *Bouvardia ternifolia* (Rubiacea) and unambiguously identified by single-crystal X-ray structure analysis (bouvardin)[1] and chemical correlation (deoxybouvardin),[1] constitute the initial members of a growing class of potent antitumor antibiotics including RA-I–RA-VII (**1–7**)[2–7] (Chart I). Studies of the antitumor properties of RA-VII revealed efficacious activity in a number of animal tumor models including the demonstration of complete cures in the solid-tumor colon adenocarcinoma 38.[8] Bouvardin and RA-VII have been shown to inhibit protein synthesis[8–10] through eukaryotic 80S ribosomal binding,[11] resulting in the inhibition of amino acyl-tRNA binding and peptidyl-tRNA translocation, and this is presently thought to be the site of action for the agent antitumor activity. Subsequent studies[12] have supported the early proposal[1] that the unusual isodityrosine-derived[15,16] 14-membered cyclophane subunit of the agents may serve to induce a rigid, normally inaccessible conformation within the 18-membered cyclic hexapeptide that in turn constrains the biologically relevant D-Ala-Ala-*N*-Me-Tyr-(OMe)-Ala tetrapeptide to a biologically active conformation. However, efforts to critically examine the origin of the importance of the cycloisodityrosine subunit have been hampered by the inaccessibility of such systems.[17–21] Synthetic efforts on **1–8** have been characterized by the failure of conventional macrolactamization techniques[18] or direct biaryl ether cyclization procedures including an intramolecular Ullmann reaction[20] and an intramolecular oxidative phenol coupling[12] to provide the elusive 14-

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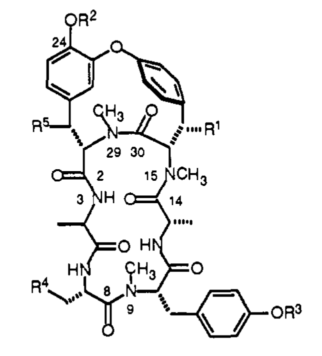
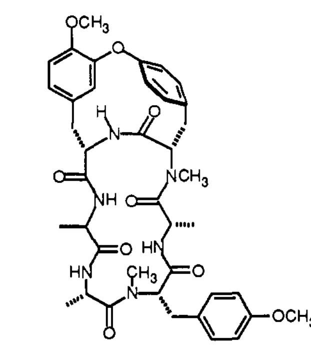
Chart I



	R^1	R^2	R^3	R^4	R^5	
1	H	Me	Me	H	H	RA-VII
2	H	H	Me	H	H	RA-V, deoxybouvardin
3	H	H	Me	OH	H	RA-I
4	H	Me	H	H	H	RA-II
5	H	Me	Me	OH	H	RA-III
6	H	Me	Me	H	OH	RA-IV
7	OH	Me	Me	H	H	RA-VI
8	OH	H	Me	H	H	bouvardin



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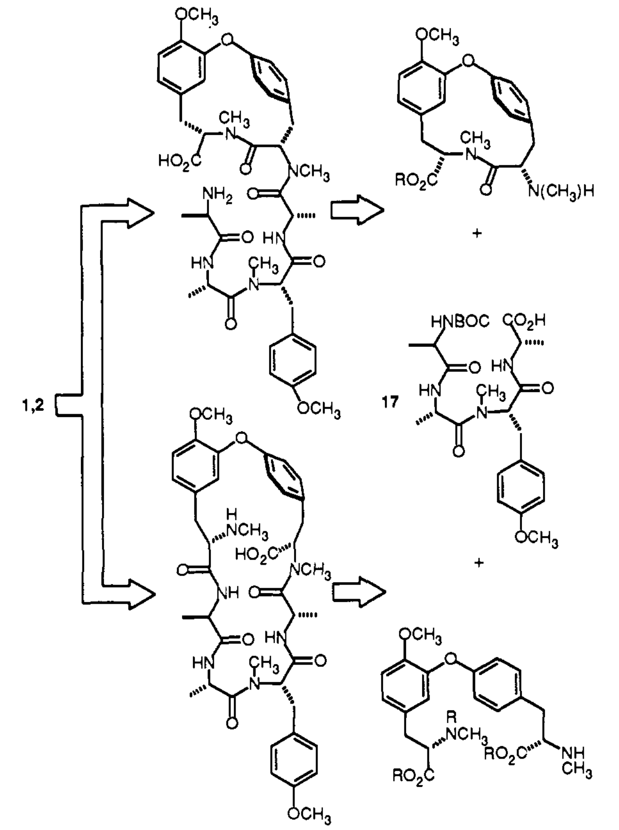
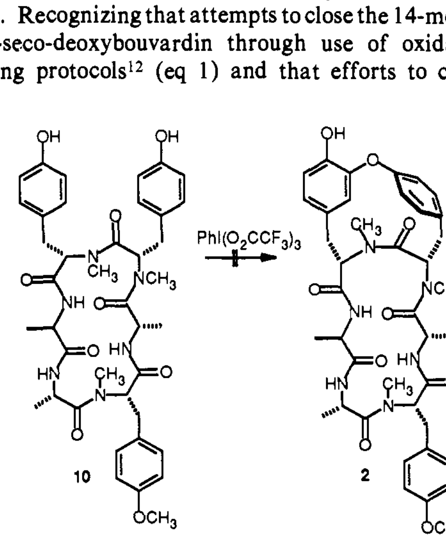
membered ring. Consequently, an indirect thallium trinitrate-promoted two-step method for achieving the intramolecular phenol coupling has been introduced by Yamamura and co-workers,[22-25] requires the use of dichloro- and dibromophenol coupling partners, and has been employed by Inoue and co-workers in the first total synthesis of RA-VII (**1**) and deoxybouvardin (**2**) albeit with the key steps proceeding in low yields (ca. 2–5%).[26,27]

Herein, we provide full details of the total synthesis of RA-VII (**1**) and deoxybouvardin (**2**) based on the successful implementation of an effective intramolecular Ullmann reaction[19] as the key macrocyclization reaction in the preparation of the 14-membered cycloisodityrosine **33**. Similarly, the synthesis of N^{29}-desmethyl-RA-VII (**9**) is detailed and its comparative chemical, conformational, and preliminary biological properties are described in efforts that further define unexpected structural and conformational features of the agents contributing to their biological properties.

Studies on the 14-Membered Ring Macrocyclization. Important in the strategic planning was the anticipation and early demonstration[28] that 18-membered or 26-membered macrocyclization in route to the natural products would be productively conducted with formation of a secondary amide. Of the three such sites available for macrocyclization, that conducted with closure of a hexapeptide at a D-amino acid amine terminus (C^2–N^3) could be anticipated to be most productive (Scheme I).[29-31] The remaining key to the synthesis was the stage and manner by which the elusive 14-membered ring, cycloisodityrosine, was to be introduced. Recognizing that attempts to close the 14-membered ring on *O*-seco-deoxybouvardin through use of oxidative phenol coupling protocols[12] (eq 1) and that efforts to close the 14-membered ring with C^3–O^2 bond formation had proven largely unsuccessful[20,26] (eq 2 and 3), we focused on efforts to form cycloisodityrosine through C^{11}–N^{10} amide bond formation.

Scheme I





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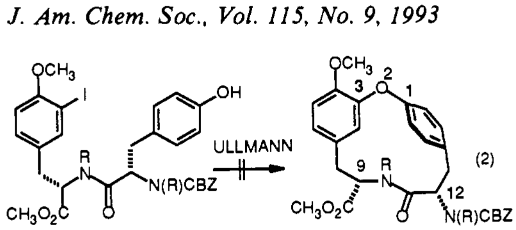
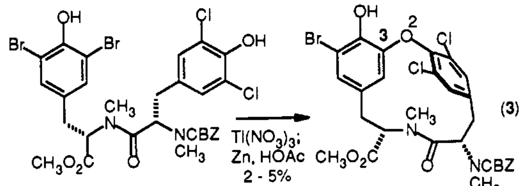


11a R=H
11b R=CH3



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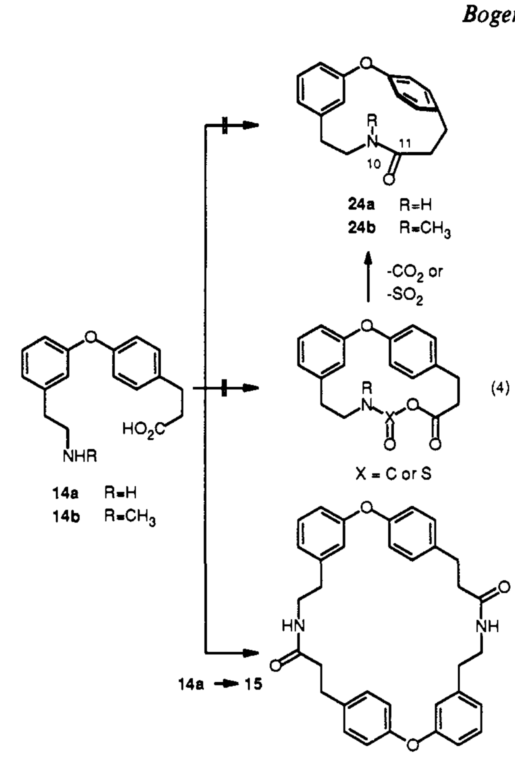

15 (14-56%)

Representative of such efforts, repeated attempts to close **14a,b** employing a range of macrolactamization procedures (DPPA,[30,31] EDCI, HOBt;[32] DCC;[26] pentafluorophenyl esters of ω-(*Z*)-amino carboxylic acids[33,34]) including polymer supported reagents (polystyrene-*i*-PrCl;[35] polystyrene–SO_2Cl[36,37]) failed to provide **24a,b** and provided **15** (14–56%) as the sole cyclization product[38] (eq 4). Additional unsuccessful efforts employing carbonyldiimidazole[39] or dipyridyl sulfite[40] in macrocyclizations that could proceed with initial 16-membered ring formation and subsequent collapse of an intermediate anhydride to the 14-membered ring ($-CO_2$, $-SO_2$) provided convincing evidence that the direct closure of the 14-membered ring with N^{10}–C^{11} bond formation may not be successful in our efforts.

Concurrent with these studies, we examined the potential of ring closure within the preformed 26-membered ring in hopes that the subsequent 14-membered ring cyclization may benefit from the entropic assistance of the transannular cyclization (Scheme II). Tetrapeptide **17**[28] was coupled with **16**[41] to provide the linear peptide **18**. Sequential deprotection of the carboxy and amine termini of **18** provided **19** which cleanly cyclized to the 26-membered ring upon exposure to diphenyl phosphorazidate (DPPA).[31] This clean pentultimate cyclization reaction to provide **20** proceeded in a manner comparable to that disclosed for a related 26-membered macrocyclization[28] and may benefit from ring closure at a D-amino acid terminus.[29,30] Deprotection of **20** and efforts to close the elusive 14-membered ring with transannular C^{30}–N^{29} amide bond formation (DPPA, 0 °C) failed to provide deoxybouvardin (**2**).

Convinced that attempts to close the 14-membered ring with C^{11}–N^{10} amide bond formation may not be implemented successfully in our hands, we elected to reexamine the C^3–O^2 and C^1–O^2 Ullmann macrocyclization reactions. On the basis of observations made on related intermolecular Ullmann reactions of functionalized tyrosine derivatives,[42] the C^1–O^2 closure could be anticipated to be more facile than C^3–O^2 bond formation as a consequence of the decelerating effect of the electron-donating substituent ortho to the aryl iodide necessarily present in a C^3–O^2 Ullmann closure. Consistent with prior observations,[17–20] attempts to close the C^3–O^2 bond through Ullmann condensation of **11a** derived from the commercially available 3-iodo-L-tyrosine and L-tyrosine have proven unsuccessful in our efforts to date[43] (eq 2). In sharp contrast, the intramolecular Ullmann reaction with C^1–O^2 bond formation proved synthetically viable for direct formation of the 14-membered diaryl ethers. Summarized in Table I are optimized results from the study of the macrocyclization of **23a–f**. Full details of this study have been described and routine macrocyclization conversions of 45–60% were realized under moderately dilute reaction conditions (0.004 M) with a full range of substrates including those bearing an alkoxy or hydroxy substituent ortho to the participating phenol.[19] The racemization of substrate **24f** observed in pyridine was suppressed with reactions conducted in collidine or dioxane. In addition to the improved conversions available through use of this procedure, the Ullmann reaction permits the use of readily available amino acids and directly provides the appropriately functionalized diaryl ethers without resorting to the use of the less accessible dichloro- or dibromophenols. With the viability of the key Ullmann macrocyclization established and modifications that effectively address potential substrate racemization in hand, its application in the total synthesis of **1** and **2** was pursued.

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(38) For **15**: ^{1}H NMR (pyridine-d_5, 300 MHz) δ 8.48 (b s, 2 H, NH), 7.50–6.88 (m, 16 H, ArH), 3.55 (dd, 4 H, *J* = 6.8, 12.9 Hz, CH_2NH), 3.05 (t, 4 H, *J* = 6.4 Hz, CH_2Ar), 2.75 (t, 4 H, *J* = 6.8 Hz, CH_2Ar), 2.55 (t, 4 H, *J* = 6.4 Hz, CH_2CON); IR (neat) 3338, 3055, 2956, 2931, 2868, 1642, 1605, 1586, 1538, 1508, 1485, 1442, 1420, 1359, 1253, 1218, 1173, 1142, 1109, 1077, 1049, 1014, 969, 911, 830 cm^{-1}; EIMS *m/e* (relative intensity) 534 (M$^+$, 5), 267 (base); CIMS (isobutane) *m/e* 535 (M$^+$ + H, 33), 534 (M$^+$, base). For related observations with a closely related 15-membered biaryl ether lactone, see ref 51 and: Justus, K.; Steglich, W. *Tetrahedron Lett.* **1991**, *32*, 5781. Deshpande, V. H.; Gokhale, N. J. *Tetrahedron Lett.* **1992**, *33*, 4213.
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(41) Compound **16** was prepared following the method detailed in Scheme VII of ref 42. For **16**: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.33 (b s, 10 H, two PhH), 7.37–7.20 (m, 2 H, ArH), 7.09 (d, 2 H, *J* = 8 Hz, C$^{3'}$- and C$^{5'}$-H), 6.80 (d, 2 H, *J* = 8 Hz, C$^{2'}$- and C$^{6'}$-H), 5.11 (s, 2 H, PhCH$_2$O), 5.05 (s, 2 H, PhCH$_2$O), 4.64 (m, 1 H, CH$_2$C*H*N), 4.38 (m, 2 H, CO$_2$C*H*$_2$CH$_2$), 4.32 (m, 1 H, CH$_2$C*H*N), 3.64 (s, 3 H, OCH$_3$), 3.18 (s, 3 H, NCH$_3$), 3.09–2.72 (m, 4 H, two CHC*H*$_2$Ar), 2.80 (b s, 3 H, NCH$_3$), 1.01 (t, 2 H, *J* = 6 Hz, CH$_2$Si), 0.06 (s, 9 H, Si(CH$_3$)$_3$); IR (neat) ν_{max} 3680, 2950, 1734, 1718, 1700, 1654, 1610, 1560, 1501, 1390, 1274, 1172, 850, 737 cm^{-1}; CIMS (isobutane) *m/e* 641 (M$^+$ + H, base); CIHRMS *m/e* 641.6918 ($C_{37}H_{40}N_2O_8$ requires 641.6914).
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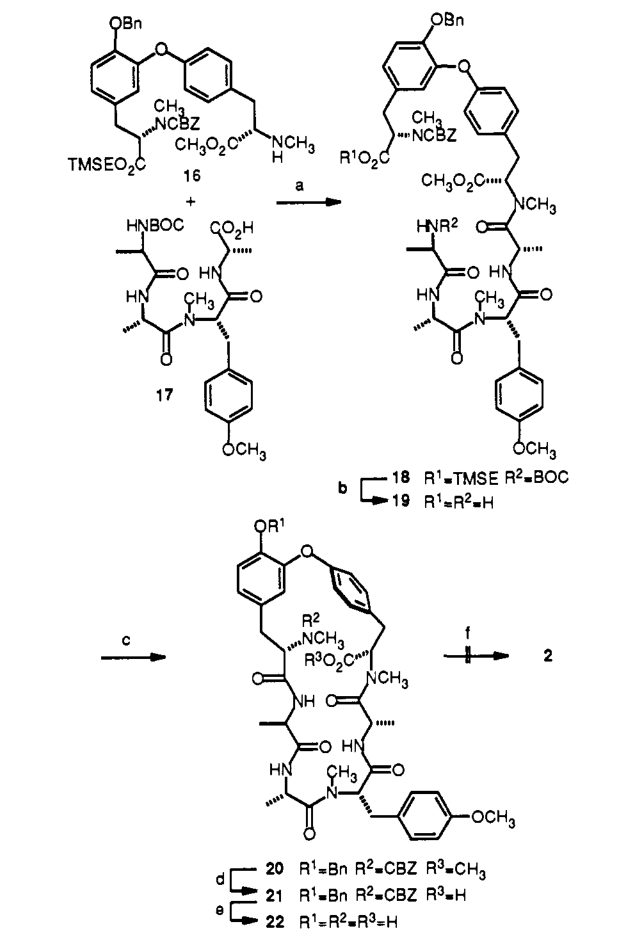
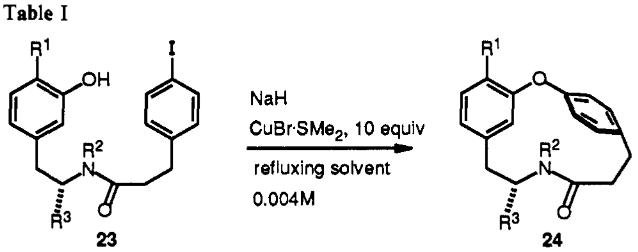
Scheme II[a]



[a] (a) 2.0 equiv of **17**, 2.0 equiv of EDCI, 2.0 equiv of HOBt·H_2O, DMF, 25 °C, 12 h, 52%. (b) 1.5 equiv of *n*-Bu_4NF, THF, 25 °C, 3 h; 3.0 M HCl/EtOAc, 25 °C, 1 h, 98%. (c) 1.5 equiv of DPPA, 5.0 equiv of $NaHCO_3$, DMF, 0 °C, 72 h, 56%. (d) 2.0 equiv of LiOH·H_2O, THF/MeOH/H_2O (3:1:1), 25 °C, 2 h, 88%. (e) 0.1 wt equiv of 10% Pd/C, 1 atm of H_2, CH_3OH, 25 °C, 6 h, 98%. (f) 1.5 equiv of DPPA, 5.0 equiv of $NaHCO_3$, DMF, 0 °C, 72 h.

Table I



	R^1	R^2	R^3	solvent	yield (%)	
23a	H	H	H	pyridine	**24a**	58
23b	H	CH_3	H	pyridine	**24b**	49
23c	OCH_3	H	H	pyridine	**24c**	46
23d	OCH_3	CH_3	H	pyridine	**24d**	45
23e	OH	H	CO_2CH_3	pyridine	**24e**	51
23f	OCH_3	H	CO_2CH_3	pyridine	**24f**	51
23f	OCH_3	H	CO_2CH_3	dioxane	**24f**	31
23f	OCH_3	H	CO_2CH_3	collidine	**24f**	50

Total Synthesis of RA-VII (1) and Deoxybouvardin (2). Single-step O- and N-methylation[44] of *N*-CBZ-3-acetyl-L-tyrosine methyl ester (**25**)[45] followed by Baeyer–Villiger oxidation and acid-catalyzed methanolysis of the resulting acetate provided the selectively protected *N*-methyl-L-DOPA derivative **28** (Scheme III). Catalytic hydrogenolysis of **28** served to remove the CBZ protecting group, and coupling of the resultant amine **29** with *N*-CBZ-*N*-methyl-4-iodo-L-phenylalanine (**31a**) provided **32a**. Subjection of **32a** to the conditions for effecting the strategic intramolecular Ullmann condensation reaction with macrocyclization provided **33a** (30%) without detectable evidence of racemization. Comparable to the efforts to prepare **32a**, coupling of *N*-BOC-*N*-methyl-4-iodo-L-phenylalanine (**31b**) with **29** and subjection of **32b** to the conditions of the Ullmann reaction provided **33b** in slightly lower conversions.

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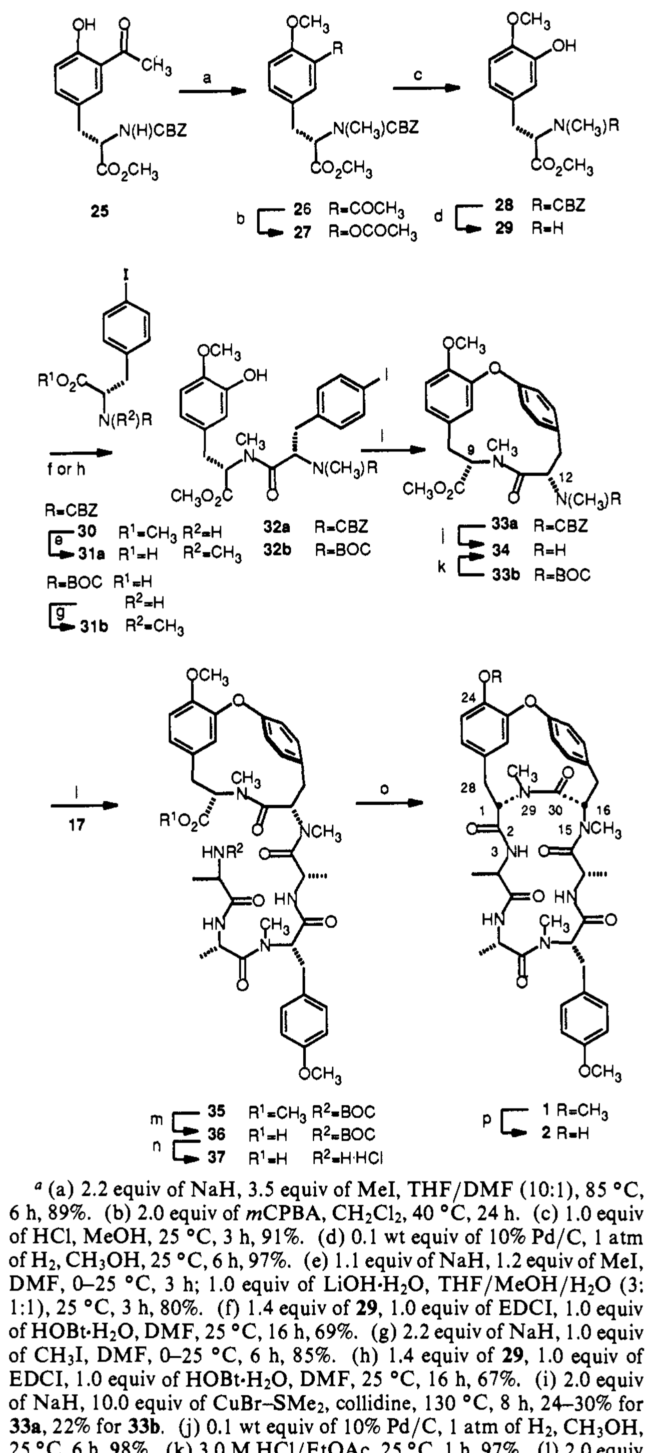
Scheme III[a]



[a] (a) 2.2 equiv of NaH, 3.5 equiv of MeI, THF/DMF (10:1), 85 °C, 6 h, 89%. (b) 2.0 equiv of *m*CPBA, CH_2Cl_2, 40 °C, 24 h. (c) 1.0 equiv of HCl, MeOH, 25 °C, 3 h, 91%. (d) 0.1 wt equiv of 10% Pd/C, 1 atm of H_2, CH_3OH, 25 °C, 6 h, 97%. (e) 1.1 equiv of NaH, 1.2 equiv of MeI, DMF, 0–25 °C, 3 h; 1.0 equiv of LiOH·H_2O, THF/MeOH/H_2O (3:1:1), 25 °C, 3 h, 80%. (f) 1.4 equiv of **29**, 1.0 equiv of EDCI, 1.0 equiv of HOBt·H_2O, DMF, 25 °C, 16 h, 69%. (g) 2.2 equiv of NaH, 1.0 equiv of CH_3I, DMF, 0–25 °C, 6 h, 85%. (h) 1.4 equiv of **29**, 1.0 equiv of EDCI, 1.0 equiv of HOBt·H_2O, DMF, 25 °C, 16 h, 67%. (i) 2.0 equiv of NaH, 10.0 equiv of CuBr–SMe_2, collidine, 130 °C, 8 h, 24–30% for **33a**, 22% for **33b**. (j) 0.1 wt equiv of 10% Pd/C, 1 atm of H_2, CH_3OH, 25 °C, 6 h, 98%. (k) 3.0 M HCl/EtOAc, 25 °C, 1 h, 97%. (l) 2.0 equiv of **17**, 2.0 equiv of EDCI, 2.0 equiv of HOBt·H_2O, DMF, 25 °C, 16 h, 53%. (m) 3.0 equiv of LiOH·H_2O, THF/MeOH/H_2O (3:1:1), 25 °C, 2 h. (n) 3.0 M HCl/EtOAc, 25 °C, 1 h, 92% from **33**. (o) 1.5 equiv of DPPA, 5 equiv of $NaHCO_3$, DMF, 0 °C, 72 h, 58%. (p) 2.0 equiv of BBr_3, CH_2Cl_2, −78 to 0 °C, 3 h, 57%.

Table II

agent	(conformation)[a]	relative energy[b] (kcal/mol)	coupling constants (Hz) (calculated or experimental)
			C^9-H; C^{12}-H
24b	(X-ray, trans)[c]		
	(1, trans)	0.0	
	(2, trans)	1.2	
	(3, cis)	3.0	
33a	(experimental, trans)[d]		2.2, 11.9; 4.7, 11.7
	(1, trans)	0.0	2.2, 11.7; 4.1, 11.7
	(2, trans)	1.1	
	(3, cis)	2.5	
24a	(1, trans)	0.0	
	(2, trans)	2.6	
	(3, cis)	4.7	
41	(experimental, trans)[d]		1.3, 10.8, 8.1; 2, 12
	(1, trans)	0.0	2.1, 10.9, 8.5; 2.2, 11.6
	(2, trans)	0.1	
	(10, cis)	5.5	
			C^1-H; C^{16}-H
8	(X-ray, cis)[e]		2.4, 11.8; 1.2
	(experimental, cis)[f]		3, 10.8; 1.8
	(1, cis)		2.0, 11.6; 2.1
1–2	(experimental, cis)[f]		3.9, 11.8; 3.2, 11.4
	(1, cis)	0.0	1.8, 11.5; 2.1, 11.5
	(2, trans)	3.0	1.8, 11.5; 4.7, 11.4
9	(experimental, cis)[f]		3.6, 10.4, 8; 3.2, 11.4
	(1, cis)	0.0	2.1, 11.7, –; 2.3, 11.6
	(2, trans)	2.3[g]	1.6, 11.3, 5.5; 4.1, 11.7

[a] Trans or cis C^{11}–N^{10} (**24, 33, 41**) or C^{30}–N^{29} (**1–2, 8–9**) amide bond. [b] MacroModel, OPLSA force field. [c] Reference 19. [d] 2D H^1–H^1 NOESY NMR confirmed the trans C^{11}–N^{10} amide, see text. [e] Reference 1. [f] 2D H^1–H^1 NOESY NMR confirmed the cis C^{30}–N^{29} amide, see text. [g] A slightly lower energy (relative E = 1.5 kcal) conformation possessing a trans amide and significantly altered tetrapeptide conformation was located in the exhaustive conformational search, see ref 56.

In contrast to the natural products but consistent with expectations based on a conformational analysis, **33a,b** adopt a rigid solution conformation possessing a trans C^{11}–N^{10} amide bond. A conformational search of **33a** was conducted in which the global and close, low-lying minima (≤5 kcal) were located by use-directed Monte Carlo sampling of two starting conformations (cis and trans amides) with random variations (0–180°) in two to four of the available tortional angles excluding those originating in the aryl rings (MacroModel, OPLSA force field).[46,47] The search revealed a single, lowest energy conformation for **33a** which possessed a trans C^{11}–N^{10} amide bond that was greater than 1.1 kcal lower in energy than any other located conformation and 2.5 kcal lower in energy than a conformation possessing a cis amide bond (Table II). The calculated coupling constants for the C^9 and C^{12} hydrogens in this lowest energy conformation are 11.7, 2.2 Hz and 11.7, 4.1 Hz, respectively, and match the experimentally measured values of 11.9, 2.2 Hz and 11.7, 4.7 Hz. Unambiguous confirmation that **33a** adopts a solution conformation that possesses a trans amide was derived from 2D ^{1}H–^{1}H NOESY NMR. Strong NOE crosspeaks were observed for C^9-H/*N*-Me and C^{12}-H/*N*-Me and are uniquely diagnostic of the trans amide stereochemistry. Similarly, a C^9-H/C^{12}-H NOE crosspeak was not observed and would be uniquely diagnostic of the cis amide stereochemistry. Further supporting evidence that cycloisodityrosine and related agents exist in a conformation possessing a trans C^{11}–N^{10} *N*-methyl amide came from the single-crystal X-ray analysis of **24b**.[19] The X-ray structure of **24b** possesses a trans *N*-methyl amide and a backbone conformation identical to the lowest energy conformations located for **24b** (RMS = 0.17 Å) and **33a** (RMS = 0.38 Å) in our conformational searches.

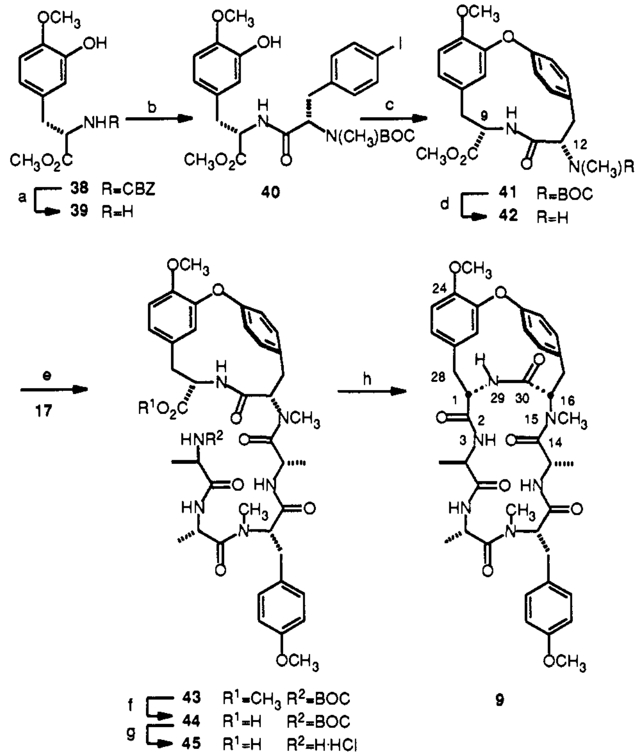
(46) Still, W. C.; Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Lipton, M.; Liskamp, R.; Chang, G.; Hendrickson, T.; DeGunst, F.; Hasel, W. *MACROMODEL*; Columbia University: New York, 1990; version 2.7.
(47) Chang, G.; Guida, W. C.; Still, W. C. *J. Am. Chem. Soc.* **1989**, *111*, 4379.

C^{12} Amine deprotection through CBZ hydrogenolysis and coupling of **34** with tetrapeptide **17**[28] provided **35**. Sequential methyl ester hydrolysis, *N*-BOC deprotection, and diphenyl phosphorazidate promoted macrocyclization with C^2–N^3 amide bond formation strategically conducted at a D-amino acid amine terminus under the improved reaction conditions[30] provided RA-VII [**1**, $[\alpha]^{22}_D$ −222° (*c* 0.1, $CHCl_3$)] identical in all compared respects with a sample of natural material, $[\alpha]^{21}_D$ −229° (*c* 0.1, $CHCl_3$).[48] Selective C^{24} methyl ether removal provided deoxybouvardin [**2**, $[\alpha]^{23}_D$ −219° (*c* 0.05, $CHCl_3$)] identical in all compared respects to a sample of natural material, $[\alpha]^{21}_D$ −225° (*c* 0.3, $CHCl_3$).[49]

Synthesis of N^{29}-Desmethyl-RA-VII (9). In efforts to assess the importance of the *N*-methyl cis amide bond central to the 14-membered ring, N^{29}-desmethyl-RA-VII (**9**) was prepared in a similar sequence relying on the Ullmann macrocyclization reaction for formation of the 14-membered ring. O-Methylation of *N*-CBZ-3-acetyl-L-tyrosine methyl ester followed by Baeyer–Villiger oxidation and subsequent methanolysis of the resulting acetate provided O^4-methyl-*N*-CBZ-L-DOPA methyl ester (**38**). Catalytic hydrogenolysis of **38** and coupling of the resultant amine **39** with *N*-BOC-*N*-methyl-4-iodophenylalanine (**31b**) provided **40** (Scheme IV). Subjection of **40** to the conditions for effecting the intramolecular Ullmann reaction provided **41** (30–39%) and optimal results in initial studies were obtained employing methylcopper[50,51] to stoichiometrically generate the cuprous phenoxide. Given the importance of the Ullmann macrocyclization and its unique success in providing the 14-membered biaryl ring system, we elected to examine the conversion of **40** to **41** in detail. In these studies, we have observed that the reaction reached an optimum conversion of 30–40% and that extending the reaction time to insure complete consumption of the starting material did not improve the yield. Further extended reaction times led to diminished yields indicating the consumption of product competitive with its generation. Key to the optimum yields recorded were the use of rigorously and freshly dried collidine, purified $CuBr$–SMe_2 complex, and careful degassing of the reaction solvent immediately prior to the conduct of the reaction. Because the dilute reaction conditions require the use of considerable solvent, the former and latter precautions may prove to be the most critical. Under such conditions, the reaction may be conducted conveniently with NaH/$CuBr$–SMe_2 (2 equiv/10 equiv) to provide **41** in 30–40%. Two additional reaction byproducts, **42** and **46**, could be isolated from the reaction mixtures in variable amounts, and both proved to be derived from the primary cyclization product. The first of the two reaction byproducts, amine **42**, could be eliminated by simply avoiding acidic conditions (10% aqueous HCl) generally employed to remove collidine from the reaction products in the course of the workup. The second byproduct **46** (5–20%) is derived from intramolecular N-acylation of the N^{10}–C^{11} amide by the C^{12} *tert*-butyl carbamate and proved to be a

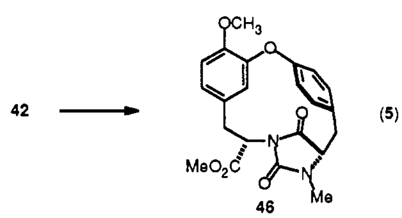
(48) Synthetic [$[\alpha]^{22}_D$ −222° (c = 0.1, $CHCl_3$); R_f 0.23 (48:50:2 pentane/CH_2Cl_2/MeOH)] and natural RA-VII [$[\alpha]^{21}_D$ −229° (c = 0.1, $CHCl_3$);[3] R_f 0.23 (48:50:2 pentane/CH_2Cl_2/MeOH)] proved indistinguishable by ^{1}H NMR ($CDCl_3$, 300 MHz), ^{13}C APT ($CDCl_3$, 75 MHz), IR (KBr), and EIMS. We thank Dr. Inaba of Tobishi Pharmaceutical Co., Ltd., Japan, for providing a generous sample of naturally occurring RA-VII and copies of spectra (^{1}H NMR, 200 MHz).
(49) Synthetic [$[\alpha]^{22}_D$ −219° (c = 0.05, $CHCl_3$); R_f 0.16 (48:50:2 pentane/CH_2Cl_2/MeOH)] and natural deoxybouvardin [$[\alpha]^{22}_D$ −225° (c = 0.03, $CHCl_3$); R_f 0.16 (48:50:2 pentane/CH_2Cl_2/MeOH)][3] proved indistinguishable by ^{1}H NMR ($CDCl_3$, 300 MHz), ^{13}C APT ($CDCl_3$, 75 MHz), IR (KBr), and EIMS. We thank Professor J. Hoffmann of the University of Arizona and Dr. Inaba of Tobishi Pharmaceutical Co., Ltd., Japan, for providing authentic comparison samples of naturally occurring deoxybouvardin.
(50) Whitesides, G. M.; Sadowski, J. S.; Liburn, J. *J. Am. Chem. Soc.* **1974**, *96*, 2829.
(51) Boger, D. L.; Sakya, S. M.; Yohannes, D. *J. Org. Chem.* **1991**, *56*, 4204. Boger, D. L.; Nomoto, Y.; Teegarden, B. R. *J. Org. Chem.*, in press.

Scheme IV[a]



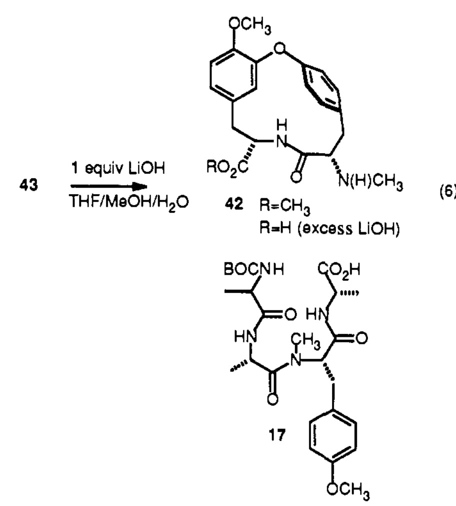
[a] (a) 0.1 wt equiv of 10% Pd/C, 1 atm of H_2, CH_3OH, 25 °C, 6 h, 99%. (b) 1.0 equiv of **31b**, 1.0 equiv of EDCI, 1.0 equiv of HOBt, DMF, 25 °C, 12 h, 92%. (c) 2.0 equiv of CH_3Cu, collidine, 130 °C, 9 h, 36%, or 2.0 equiv of NaH, 10 equiv of CuBr–SMe_2, collidine, 130 °C, 9 h, 30–39%. (d) 3.0 M HCl/EtOAc, 25 °C, 1 h, 100%. (e) 2.0 equiv of **17**, 3.0 equiv of EDCI, 3.0 equiv of HOBt, 8.0 equiv of $NaHCO_3$, DMF, 25 °C, 48 h, 71%. (f) 3 equiv of LiOH, 11 equiv of H_2O_2, THF/H_2O (3:1), 25 °C, 6 h. (g) 3.0 M HCl/EtOAc, 25 °C, 1.5 h, 97%. (h) 1.5 equiv of DPPA, 5.0 equiv of $NaHCO_3$, DMF, 0 °C, 72 h.

robust material (eq 5).[52,53] In general, attempts to avoid the generation of **46** through use of less basic reaction conditions (2 equiv of NaH, 10 equiv of CuBr–SMe_2, 0.004 M dioxane, 8 equiv of HMPA, reflux, 18 h, 10% **41**) provided predominantly recovered **40**.



Similar to **33**, **41** was found to possess a solution conformation with a trans C^{11}–N^{10} amide bond. A conformational search of **41** was conducted as described for **33** in which the global and close, low-lying minima were located and revealed two, nearly indistinguishable low-energy conformations greater than 0.7 kcal lower in energy than other located conformations and 5.5 kcal lower in energy than a conformation possessing a cis C^{11}–N^{10} amide (Table II). The two closely related low-energy conformations possess a trans C^{11}–N^{10} amide bond and the calculated C^9 and C^{12} coupling constants for the lowest energy conformation match the experimentally measured values. Unambiguous confirmation that **41** adopts a solution conformation possessing a trans amide was derived from 2D ^1H–^1H NMR in which strong NOE crosspeaks were observed for C^9-H/N-H and C^{12}-H/N-H diagnostic of a trans amide conformation and from the absence of a C^9-H/C^{12}-H NOE crosspeak diagnostic of a cis amide conformation.

Amine deprotection and coupling of **42** with tetrapeptide **17**[28] provided **43**. Efforts to conduct the methyl ester hydrolysis of **43** under standard conditions (1–3 equiv of LiOH, THF/MeOH/H_2O, 25 °C) resulted in facile C^{14}–N^{15} amide bond hydrolysis presumably the result of C^{30}–N^{29} amide deprotonation and intramolecular C^{14} O-acylation with C^{14}–N^{15} amide cleavage (eq 6). Consequently, hydrolysis of **43** was initially accomplished under the conditions of Fischer deesterification or more conveniently with the use of lithium peroxide and provided **44**. BOC deprotection and subsequent diphenyl phosphorazidate promoted macrocyclization with C^2–N^3 amide bond formation provided **9**, $[\alpha]^{22}_D$ −202° (*c* 0.5, $CHCl_3$).



Comparative Conformational and Biological Properties. The X-ray crystal structure of bouvardin (**8**) revealed that the three secondary amides as well as the C^8–N^9 and C^{14}–N^{15} *N*-methyl amides possess the trans stereochemistry while the C^{30}–N^{29} *N*-methyl amide central to the 14-membered ring possesses a cis amide conformation in an unusual type VI β-turn.[54] The X-ray structure conformation of **8** has been unambiguously assigned to the single, predominant (ca. 85–95%) solution conformation of **8**, and the diagnostic ^1H NMR coupling constants within the rigid 14-membered ring match those calculated from the X-ray crystal structure (Table II). A second, spectroscopically detectable conformation of **8** (ca. 5–15%) is observed and has been attributed to an additional conformation within the flexible portion of the 18-membered ring (cis C^8–N^9 or C^{14}–N^5 *N*-methyl amide) rather than the rigid 14-membered ring.[1] Similar observations have been made with **1**–**7**.[55] More diagnostic of the solution

(52) For **46**: pale-yellow oil which solidified upon standing; mp 143–146 °C; ^1H NMR ($CDCl_3$, 250 MHz) δ 7.21 (dd, 1 H, *J* = 2.2, 8.4 Hz, C^{15}-H), 7.12 (dd, 1 H, *J* = 2.4, 8.2 Hz, C^{18}-H), 7.02 (dd, 1 H, *J* = 2.1, 8.3 Hz, C^{16}-H), 6.91 (dd, 1 H, *J* = 2.4, 8.4 Hz, C^{17}-H), 6.74 (d, 1 H, *J* = 8.3 Hz, C^5-H), 6.62 (dd, 1 H, *J* = 1.9, 8.2 Hz, C^6-H), 4.79 (d, 1 H, *J* = 1.8 Hz, C^{19}-H), 4.44 (dd, 1 H, *J* = 1.7, 12.0 Hz, C^{12}-H), 4.18 (t, 1 H, *J* = 3.4 Hz, C^9-H), 4.03 (dd, 1 H, *J* = 5.2, 16.7 Hz, C^{13}-H), 3.90 (s, 3 H, $ArOCH_3$), 3.69 (s, 3 H, CO_2CH_3), 3.22 (t, 2 H, *J* = 3.8 Hz, C^8-H), 3.10 (s, 3 H, NCH_3), 2.98 (dd, 1 H, *J* = 1.7, 16.3 Hz, C^{13}-H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 172.1, 169.8, 158.7, 147.3, 133.4, 130.7, 130.6, 129.7, 124.7, 124.2, 121.9, 115.8, 111.6, 63.0, 56.1, 55.4, 53.2, 33.2, 30.2, 28.0; IR (neat) ν_{max} 2890, 2880, 1744, 1715, 1518, 1438, 1405, 1395, 1263, 1219, 1208, 1130 cm^{-1}; FABHRMS (NBA) *m/e* 410.1474 ($C_{22}H_{22}N_2O_6$ requires 410.1478).

(53) Compound **46** proved to be chemically unreactive and treatment with 3.25 M HCl/EtOAc (25 °C, 24 h), 8 M HCl/CH_3OH (25 °C, 72 h), and aqueous HCl/THF solutions provided no reaction. Treatment with NaOMe (2 equiv) in MeOH (25 °C, 24 h) provided the two regioisomeric five-membered-ring cleavage products.

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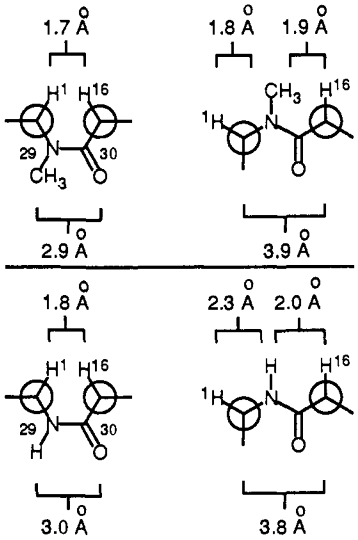


Figure 1. Newman projections of the C^1–N^{29}–C^{30}–C^{16} cis and trans amides of **1** and **9** illustrating the origin of the diagnostic ^{1}H–^{1}H NOEs. Distances were taken from low-energy conformations of **1** and **9** possessing the cis and trans amide stereochemistry (MacroModel, OPLSA force field).

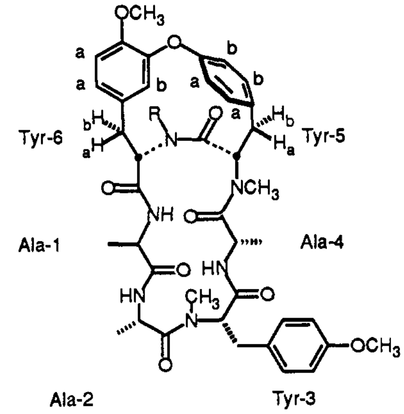
structure of the agents is a characteristic, strong NOE observed between C^1-H and C^{16}-H. Within the X-ray crystal structure of bouvardin and in the low-energy conformations of **1** and **2**, the C^1-H/C^{16}-H proton–proton distance is only 1.7–1.8 Å. Accordingly, the C^1-H/C^{16}-H crosspeak in the 2D ^{1}H–^{1}H NOESY NMR spectrum of **1** constitutes the strongest observed NOE crosspeak. Expectedly absent are NOE crosspeaks between C^1-H or C^{16}-H and N^{29}-CH_3 that would be present if **1** adopted a trans C^{30}–N^{29} amide bond. In the conformation of **1** possessing a trans C^{30}–N^{29} amide, the C^1-H/C^{16}-H proton–proton distance is approximately 4.9 Å and the methyl group of N^{29}-CH_3 lies directly between the two hydrogens with C^1-H and C^{16}-H/N^{29}-CH_3 proton–proton distances of 1.8–1.95 Å (Figure 1). Thus, the presence of a strong C^1-H/C^{16}-H NOE crosspeak in the 2D ^{1}H–^{1}H NOESY NMR spectrum is uniquely diagnostic of an agent in a solution conformation possessing a cis N^{29}–C^{30} amide bond while the presence of strong C^1-H/N^{29}-R and C^{16}-H/N^{29}-R (R = CH_3 or H) NOE crosspeaks may be considered uniquely diagnostic of an agent in a solution conformation possessing a trans N^{29}–C^{30} amide bond. In contrast to the simple 14-membered ring of the cycloisodityrosine derivatives **24a–f**, **33**, and **41**, which exist in a conformation possessing a trans *N*-methyl or NH amide, the 14-membered rings of **1–8** have adopted a conformation possessing the inherently disfavored C^{30}–N^{29} cis *N*-methyl amide bond.

Extensive and repetitive conformational searches[56] of **1** and **2** have provided results consistent with the experimental observations. The lowest energy conformation located for the agents possesses the X-ray structure conformation. In addition, the results of the conformational searches have suggested that the C^{14}–N^{15} *N*-methyl amide as well as the three secondary amides will exist predominantly or exclusively in the trans conformation, that conformations possessing the cis and trans C^8–N^9 *N*-methyl amides may possess comparable energies, and that significant differences exist in the relative stabilities of the experimentally preferred cis versus trans C^{30}–N^{29} *N*-methyl amide bond. For the natural products **1** and **2**, conformations possessing the cis C^{30}–N^{29} *N*-methyl amide bond proved to be substantially more stable than the comparable conformation located possessing a trans C^{30}–N^{29} amide, $\Delta E = \geq 3.0$ kcal.

The examination of the conformational properties of **9** (*N*^{29}-desmethyl-RA-VII) was anticipated to more clearly define the inherent N^{29}–C^{30} amide stereochemical preference and its

(56) Boger, D. L.; Myers, J. B. *J. Org. Chem.* **1991**, *56*, 5385.

Table III. ^{1}H NMR Chemical Shifts (500 MHz, $CDCl_3$, ppm) and Coupling Constants (Hz, in Parentheses) for **1** and **9**[a]



signal	1	9
Ala$^{4\beta}$	1.12 d (6.6)	1.11 d (6.6)
*Ala$^{1\beta}$	1.30 d (6.9)	1.30 d (6.9)
*Ala$^{2\beta}$	1.35 d (6.9)	1.34 d (6.9)
Tyr$^{5\beta a}$	2.63 dd (2.9, 11.4)	2.63 dd (3, 11)
Tyr^6-NMe	2.69 s	
Tyr^3-NMe	2.84 s	2.83 s
Tyr$^{6\beta a}$	2.98 dd (19, 3.9)	3.01 dd (19, 4.1)
Tyr$^{6\beta b}$	3.14 dd (19, 11.8)	3.17 (dd, 19, 11)
Tyr^5-NMe	3.13 s	3.13 s
Tyr$^{3\beta}$	3.36 m (2 H)	3.35 m (2 H)
Tyr$^{3\alpha}$	3.59 dd (5.4, 10.2)	3.60 dd (5, 11)
Tyr$^{5\beta b}$	3.67 dd (8.5, 11.3)	3.67 dd (8, 11)
Tyr^3-*O*-Me	3.79 s	3.78 s
Tyr^6-*O*-Me	3.93 s	3.93 s
Tyr$^{6\delta b}$	4.32 d (2.2)	4.76 d (2.2)
*Ala$^{1\alpha}$	4.34 p (7)	4.32 p (7)
Tyr$^{6\alpha}$	4.55 dd (3.9, 11.8)	4.55 ddd (3.6, 8, 10.4)
*Ala$^{4\alpha}$	4.74 p (7.2)	4.74 p (7)
*Ala$^{2\alpha}$	4.86 p (7)	4.85 p (7)
Tyr$^{5\alpha}$	5.41 dd (3.2, 11.4)	5.41 dd (3.2, 11.4)
Tyr^6-NH		5.83 d (8)
*Ala^2-NH	6.08 d (8.5)	6.08 d (8.5)
*Ala^1-NH	6.41 d (6.6)	6.40 d (6.6)
Tyr$^{6\delta a}$	6.58 dd (1.9, 8.3)	6.60 dd (2.2, 8.4)
*Ala^4-NH	6.70 d (7.7)	6.70 d (8)
Tyr$^{6\epsilon a}$	6.80 d (8.4)	6.78 d (8.4)
Tyr$^{3\epsilon}$	6.83 d (8.6)	6.80 d (8.5)
Tyr$^{5\epsilon b}$	6.87 dd (2.4, 8.5)	6.83 dd (2, 8)
Tyr$^{3\delta}$	7.05 d (8.6)	7.02 d (8.5)
Tyr$^{5\epsilon a}$	7.20 dd (2.4, 8.4)	7.19 dd (2, 8)
Tyr$^{5\delta b}$	7.27 dd (2.3, 8.5)	7.25 dd (2, 8)
Tyr$^{5\delta a}$	7.42 dd (2.4, 8.4)	7.40 dd (2, 8)

[a] The * represents reassignments from that presented in ref 1 based on 2D ^{1}H–^{1}H NOESY NMR.

potential origin. *N*^{29}-Desmethyl-RA-VII (**9**) and RA-VII (**1**) displayed remarkably similar spectroscopic properties (Tables III and IV). Like **1**, **9** was found to exist predominantly in one solution conformation with the presence of a second, spectroscopically detectable conformation being observed albeit in minor amounts (ca. 5–15%). Moreover, the comparable spectroscopic behavior and relative amounts of the minor conformations detected with **1**, **2**, **8** (ca. 5–15%), and **9** (ca. 5–15%) including the appearance of the same perturbed signals suggest that the minor conformations are derived from an alternative conformation within the flexible portion of the 18-membered ring, *i.e.* cis versus trans C^8–N^9 *N*-methyl amide, rather than within the rigid 14-membered ring.[1,55] Pertinent to the lack of potential perturbation to the 14-membered ring within *both* **1** and **9**, **9** exhibited coupling constants consistent with a conformation possessing a cis C^{30}–N^{29} amide bond. Consistent with the experimental observations, a conformational search of **9** revealed that the lowest energy conformation of **9** located possesses the cis C^{30}–N^{29} secondary amide central to the 14-membered ring and an overall conformation comparable to that of the X-ray conformation of

Table IV. APT ^{13}C NMR Chemical Shifts (75 MHz, $CDCl_3$, ppm) for **1** and **9**

signal	1	9
$Ala^{2\beta}$	16.7 (o)	16.6 (o)
$Ala^{4\beta}$	18.6 (o)	18.4 (o)
$Ala^{1\beta}$	20.8 (o)	21.0 (o)
Tyr^{6}-NMe	29.4 (o)	
Tyr^{5}-NMe	30.6 (o)	30.3 (o)
$Tyr^{3\beta}$	32.7 (e)	32.7 (e)
$Tyr^{6\beta}$	35.5 (e)	35.5 (e)
$Tyr^{5\beta}$	37.0 (e)	36.7 (e)
Tyr^{3}-NMe	39.8 (o)	39.9 (o)
$Ala^{2\alpha}$	44.6 (o)	44.4 (o)
$Ala^{4\alpha}$	46.5 (o)	46.6 (o)
$Ala^{1\alpha}$	47.9 (o)	48.3 (o)
$Tyr^{5\alpha}$	54.3 (o)	54.1 (o)
Tyr^{3}-*O*-Me	55.3 (o)	55.3 (o)
Tyr^{6}-*O*-Me	56.2 (o)	56.1 (o)
$Tyr^{6\alpha}$	57.4 (o)	57.5 (o)
$Tyr^{3\alpha}$	68.4 (o)	68.3 (o)
$Tyr^{6\epsilon\,a}$	112.3 (o)	112.9 (o)
$Tyr^{6\delta\,b}$	113.4 (o)	113.5 (o)
$Tyr^{3\epsilon}$	114.0 (o)	114.2 (o)
$Tyr^{6\delta\,a}$	120.9 (o)	121.0 (o)
$Tyr^{5\epsilon\,b}$	124.3 (o)	124.3 (o)
$Tyr^{5\epsilon\,a}$	125.9 (o)	126.0 (o)
$Tyr^{6\gamma}$	128.1 (e)	128.2 (e)
$Tyr^{3\delta}$	130.3 (o)	130.2 (o)
$Tyr^{3\gamma}$	130.7 (e)	130.5 (e)
$Tyr^{5\delta\,a}$	131.0 (o)	130.9 (o)
$Tyr^{5\delta\,b}$	132.8 (o)	132.8 (o)
$Tyr^{5\gamma}$	135.2 (e)	135.1 (e)
$Tyr^{6\zeta}$	146.5 (e)	146.6 (e)
$Tyr^{6\epsilon\,b}$	153.1 (e)	153.2 (e)
$Tyr^{5\zeta}$	158.3 (e)	158.3 (e)
$Tyr^{3\zeta}$	158.4 (e)	158.5 (e)
Tyr^{3}-CO	168.1 (e)	169.7 (e)
Tyr^{5}-CO	169.4 (e)	169.4 (e)
Tyr^{6}-CO	170.9 (e)	170.9 (e)
Ala^{4}-CO	171.8 (e)	171.7 (e)
Ala^{1}-CO	172.3 (e)	172.4 (e)
Ala^{2}-CO	172.6 (e)	172.6 (e)

bouvardin. Like **1** and **2**, the conformations located possessing a cis C^{30}–N^{29} amide bond proved to be more stable than the comparable conformation located with a trans C^{30}–N^{29} bond although the relative stability of the cis versus trans amide was somewhat diminished, $\Delta E \geq 1.5$ kcal. Thus, in marked contrast to the simple 14-membered ring of **41** possessing a trans C^{11}–N^{10} secondary amide central to the cycloisodityrosine structure, *the 14-membered cycloisodityrosine ring of* **9** *has adopted a preferred conformation possessing the inherently disfavored cis C^{30}–N^{29} secondary amide.*

These surprising results require a reinterpretation of the origin of the conformational properties of **1–9**. In contrast to the initial suggestion that the rigid 14-membered ring of cycloisodityrosine serves the scaffolding role of inducing a rigid, normally inaccessible conformation within the biologically relevant tetrapeptide housed in the 18-membered ring,[1,55] the experimental results illustrate *that it is the tetrapeptide that induces a rigid, normally inaccessible conformation within the 14-membered cycloisodityrosine ring.*

Moreover, but consistent with this reinterpretation of the origin of the conformational properties of the agents, **9** was found to possess potent cytotoxic activity ca. two times greater than that of the natural products, Table V. In addition, the simple cycloisodityrosine derivatives **33** and **41** exhibited potent in vitro cytotoxic activity and were found to be only 10–30 times less potent than the natural products.[57] Thus, *N*-methyl cycloisodityrosine possesses inherent cytotoxic activity at a level comparable to that of the natural products and potentially constitutes the pharmacophore.[58] These observations in conjunction with those of related studies[56,57] suggest that it is the tetrapeptide housed within the 18-membered ring that potentiates the inherent biological properties and alters the conformation of cycloisodityrosine.

Table V. In Vitro Cytotoxic Activity

agent	IC_{50} (μg/mL; L1210, B16)[a]	
1 (RA-VII)	0.002	0.003
2 (deoxybouvardin)	0.002	0.003
9 (N^{29}-desmethyl-RA-VII)	0.001	0.002
33a	0.04	0.09
33b	0.03	0.04
41	0.06	0.1
24a–f	>100	

[a] Inhibitory concentration (IC_{50}) for 50% inhibition of cell growth relative to untreated controls, L1210 leukemia and B16 melanoma cell culture assays, ref 57.

Experimental Section[59]

3-Acetyl-*N,O*-dimethyl-*N*-[(phenylmethoxy)carbonyl]-L-tyrosine Methyl Ester (26). A solution of **25**[45] (7.34 g, 19.8 mmol) in 60 mL of THF/DMF (10:1) was treated with CH_3I (9.83 g, 4.31 mL, 69.3 mmol, 3.5 equiv) and cooled to 0 °C before the addition of NaH (60% dispersion in mineral oil, 1.74 g, 43.5 mmol, 2.2 equiv). The reaction mixture was stirred for 10 min (25 °C) and warmed at reflux (85 °C bath) for 6 h. The cooled reaction mixture was poured over 10% aqueous HCl (60 mL) and extracted with EtOAc (100 mL). The organic phase was washed with 10% aqueous HCl (2 × 60 mL) and saturated aqueous NaCl (100 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 4 × 20 cm, 30% EtOAc/hexane) afforded **26** (7.02 g, 7.89 g theoretical yield, 89%) as a pale-yellow oil: $[\alpha]^{22}_D$ –49.5° (*c* 0.37, MeOH); ^1H NMR ($CDCl_3$, 300 MHz) of major rotomer δ 7.58 (d, 1 H, *J* = 2.4 Hz, C^2-H), 7.35 (m, 5 H, PhH), 7.20 (dd, 1 H, *J* = 2.4, 8.4 Hz, C^6-H), 6.87 (d, 1 H, *J* = 8.4 Hz, C^5-H), 5.10 (b s, 2 H, CH_2Ph), 4.97 (dd, 1 H, *J* = 5.3, 10.6 Hz), 3.90 (s, 3 H, $ArOCH_3$), 3.80 (s, 3 H, CO_2CH_3), 3.30 (dt, 2 H, *J* = 5.3, 14.6 Hz, $CHCH_2$), 2.84 (s, 3 H, NCH_3), 2.60 (s, 3 H, $COCH_3$); IR (neat) ν_{max} 3035, 2954, 1730, 1681, 1570, 1495, 1420, 1381, 1350, 1245, 1151, 1060, 1021, 818, 739 cm^{-1}; CIMS (isobutane) *m/e* 400 (M^+ + H, base); EIHRMS *m/e* 399.1682 ($C_{22}H_{24}NO_6$ requires 399.1682).

3-Hydroxy-*N,O*-dimethyl-*N*-[(phenylmethoxy)carbonyl]-L-tyrosine Methyl Ester (28). A solution of **26** (1.22 g, 3.06 mmol) in 10 mL of dry CH_2Cl_2 was treated with *m*-chloroperbenzoic acid (*m*CPBA, 80–85% grade, 0.8 g, 3.7 mmol, 1.2 equiv) and warmed at 40 °C. After 12 h, an additional 0.80 equiv of *m*CPBA (0.52 g) was added and the reaction mixture was stirred an additional 12 h (40 °C). The cooled reaction mixture was concentrated in vacuo, dissolved in EtOAc (30 mL), washed with saturated aqueous $NaHCO_3$ (5 × 50 mL) and saturated aqueous NaCl (50 mL), dried ($MgSO_4$), and concentrated in vacuo. Crude **27** was added to a solution of methanolic HCl prepared by dropwise addition of CH_3COCl (0.216 mL, 3.06 mmol, 1.0 equiv) to 10 mL of CH_3OH at 0 °C. The reaction mixture was stirred at 25 °C (3 h) and concentrated in vacuo. Flash chromatography (SiO_2, 3 × 15 cm, 30% EtOAc/hexane) afforded **28** (1.03 g, 1.14 g theoretical yield, 91%) as a clear, pale-yellow oil: $[\alpha]^{22}_D$ –13.8° (*c* 0.24, MeOH); ^1H NMR ($CDCl_3$, 200 MHz) mixture of two rotamers δ 7.35 (m, 5 H, PhH), 6.85–6.68 (m, 3 H, Ar-H), 5.10 and 5.05 (two s, 2 H, CH_2Ph), 4.95–4.78 (m, 1 H, CH_2CH), 3.86 (s, 3 H, $ArOCH_3$), 3.75 and 3.65 (two s, 3 H, CO_2CH_3), 3.44–2.95 (m, 2 H, CH_2CH), 2.82 (b s, 3 H, NCH_3); IR (neat) ν_{max} 3370, 2950, 2841, 1740, 1700, 1601, 1510, 1450, 1404, 1319, 1270, 1129, 914, 855, 764, 700 cm^{-1}; EIMS *m/e* 373 (M^+, 5), 208 (47), 137 (32), 91 (base); CIMS (isobutane) *m/e* 374 (M^+ + H, base); EIHRMS *m/e* 373.1529 ($C_{20}H_{23}NO_6$ requires 373.1525).

(57) Boger, D. L.; Yohannes, D.; Myers, J. B. *J. Org. Chem.* **1992**, *57*, 1319. Boger, D. L.; Myers, J. B.; Yohannes, D.; Kitos, P. A.; Suntornwat, O.; Kitos, J. C. *BioMed. Chem. Lett.* **1991**, *1*, 313.

(58) The structural similarity of the 14-membered biaryl subunit of RA-VII and combretastatin D1 and D2, mitotic inhibitors through tubulin binding,[51] suggested the agents may act at a tubulin-binding site. However, RA-VII exhibited no evidence of mitotic inhibition in human T222 cell cycle specificity testing. Similar observations with bouvardin have been described.[8] We thank Dr. H. Pearce and Dr. P. Mahn of Eli Lilly and Co. for conducting this assay.

(59) Chiral HPLC analysis was conducted on a Gilson Model 320 dual-pump chromatograph equipped with an ISCO V[4] variable-wavelength absorbance detector (254 nm unless otherwise indicated) employing a J. T. Baker Bond DNBPG (covalent) chiral column.

4-Iodo-*N*-methyl-*N*-[(phenylmethoxy)carbonyl]-L-phenylalanine (31a). A solution of **30** (3.66 g, 8.34 mmol) in 40 mL of dry DMF was cooled to 0 °C and treated with NaH (60% oil dispersion, 0.367 g, 9.17 mmol, 1.1 equiv). After 5 min, CH_3I (1.41 g, 0.62 mL, 10.0 mmol, 1.2 equiv) was added and the reaction mixture was stirred at 25 °C (3 h). The reaction mixture was poured over 10% aqueous HCl (50 mL) and extracted with EtOAc (60 mL). The EtOAc layer was washed with water (3 × 100 mL) and saturated aqueous NaCl (60 mL), dried ($MgSO_4$), and concentrated in vacuo. The resulting oil was dissolved in THF/MeOH/H_2O (3:1:1, 20 mL) and was treated with LiOH·H_2O (0.350 g, 8.34 mmol, 1.0 equiv). The reaction mixture was stirred at 25 °C (3 h), poured over 10% aqueous HCl (20 mL), and extracted with EtOAc (3 × 10 mL). The combined EtOAc extracts were washed with saturated aqueous NaCl (30 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 3 × 15 cm, 50% EtOAc/hexane) afforded **31a** (2.92 g, 3.66 g theoretical yield, 80%) as a white solid: mp 100–102 °C (EtOAc, white flakes); $[\alpha]^{22}_D$ −3.4° (*c* 0.125, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) of major rotomer δ 7.58 (d, 2 H, *J* = 8 Hz, C^3- and C^5-H), 7.37 (m, 5 H, PhH), 6.95 (d, 2 H, *J* = 8 Hz, C^2- and C^6-H), 5.13 (s, 2 H, C*H*$_2$Ph), 4.86 (dd, 1 H, *J* = 10, 6 Hz, C*H*CH$_2$), 3.27 (dt, 2 H, *J* = 12, 6 Hz, CHC*H*$_2$), 2.80 (s, 3 H, NCH_3); IR (KBr) ν_{max} 3330, 2929, 1718, 1670, 1616, 1559, 1540, 1517, 1470, 1395, 1369, 1163, 838, 775 cm^{-1}; EIMS *m/e* 439 (M^+, 31), 424 (8), 395 (22), 312 (16), 304 (18), 91 (base); CIMS (isobutane) *m/e* 440 (M^+ + H, base); EIHRMS *m/e* 439.2506 ($C_{18}H_{18}INO_4$ requires 439.2508).

3-Hydroxy-*N*,*O*-dimethyl-*N*-[4-iodo-*N*-methyl-*N*-[(phenylmethoxy)-carbonyl]-L-phenylalanyl]-L-tyrosine Methyl Ester (32a). A solution of **28** (1.11 g, 2.97 mmol) in 10 mL of dry MeOH was treated with 10% Pd/C (0.11 g, 10% wt equiv) and stirred under an atmosphere of H_2 at 25 °C (6 h). The reaction mixture was filtered through Celite (MeOH wash), concentrated in vacuo, and dried thoroughly under vacuum to afford crude **29**, which was used without purification in the following reaction. A solution of **29** (0.703 g, 2.97 mmol, 1.0 equiv) in 5 mL of dry DMF was added to a solution of **31a** (0.93 g, 2.1 mmol), EDCI (0.41 g, 2.1 mmol, 1.0 equiv), and HOBt·H_2O (0.29 g, 2.1 mmol, 1.0 equiv) in 5 mL of DMF, and the resulting reaction solution was stirred at 25 °C (16 h). The reaction mixture was poured over 10% aqueous HCl (30 mL) and extracted with EtOAc (2 × 15 mL). The combined EtOAc layer was washed with 10% aqueous HCl (3 × 30 mL), saturated aqueous $NaHCO_3$ (3 × 30 mL), and saturated aqueous NaCl (30 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 3 × 25 cm, 15–30% EtOAc/hexane) afforded **32a** (0.966 g, 1.39 g theoretical yield, 69%) as a viscous pale-yellow oil: $[\alpha]^{22}_D$ −47.8° (*c* 0.27, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.60–7.45 (m, 2 H, $C^{2'}$- and $C^{6'}$-H), 7.36 (b s, 5 H, PhH), 7.24 (m, 2 H, $C^{3'}$- and $C^{5'}$-H), 7.01–6.60 (m, 3 H, C^2-, C^5-, and C^6-H), 5.20 (b s, 1 H, Ar-OH), 5.08 (m, 2 H, C*H*$_2$Ph), 4.95 (m, 1 H, CH$_2$C*H*), 4.79 (m, 1 H, CH$_2$C*H*), 3.80 (four s, 3 H, $ArOCH_3$), 3.75, 3.70, 3.69, and 3.66 (four s, 3 H total, CO_2CH_3), 3.18 (m, 2 H, CHC*H*$_2$Ar), 2.95, 2.90, 2.86, and 2.80 (four s, 3 H total, NCH_3), 2.79, 2.77, 2.75, and 2.70 (four s, 3 H total, NCH_3), 2.65–2.55 (m, 2 H, CHC*H*$_2$-Ar); IR (neat) ν_{max} 3300, 2936, 2346, 1736, 1700, 1654, 1560, 1542, 1508, 1488, 1458, 1400, 1266, 1008 cm^{-1}; CIMS (isobutane) *m/e* 661 (M^+ + H); CIHRMS *m/e* 661.1398 ($C_{30}H_{33}N_2O_7I$ requires 661.1411).

Chiral-phase HPLC analysis of **32a** revealed a 95:5 ratio of diastereomers; t_R 10.2 min/11.3 min, respectively; 1.0 mL/min, 25% EtOAc/hexane elution, 258-nm detection.

3-Hydroxy-*N*,*O*-dimethyl-*N*-[4-iodo-*N*-methyl-*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-L-tyrosine Methyl Ester (32b). The coupling of **29** and **31b** was conducted as detailed for **32a** to afford **32b** (67%) as a yellow oil: $[\alpha]^{22}_D$ −52.0° (*c* 0.18, CH_3OH); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.65–7.50 (m, 2 H, $C^{2'}$- and $C^{6'}$-H), 7.38 (b s, 5 H, PhH), 7.29 (m, 2 H, $C^{3'}$- and $C^{5'}$-H), 7.08–6.65 (m, 3 H, C^2-, C^5-, and C^6-H), 5.20 (b s, 1 H, ArOH), 5.11 (m, 2 H, C*H*$_2$Ph), 5.02 (m, 1 H, CH$_2$C*H*), 4.80 (m, 1 H, CH$_2$C*H*), 3.86 (m, 3 H, $ArOCH_3$), 3.70 (m, 3 H, CO_2CH_3), 3.22 (m, 2 H, CHC*H*$_2$Ar), 2.97, 2.89, 2.88, and 2.82 (four s, 3 H total, NCH_3), 2.81, 2.77, 2.73, and 2.69 (four s, 3 H total, NCH_3), 2.67–2.55 (m, 2 H, CHC*H*$_2$Ar), 1.40 (m, 9 H, $CO_2C(CH_3)_3$); IR (neat) ν_{max} 3568, 2926, 1734, 1700, 1684, 1654, 1576, 1560, 1542, 1508, 1498, 1490, 1458, 1396, 1256, 806 cm^{-1}; CIMS (isobutane) *m/e* 627 (M^+ + H, base); CIHRMS *m/e* 627.1560 ($C_{27}H_{25}N_2O_7I$ requires 627.1569).

Chiral-phase HPLC analysis of **32b** revealed a 94:6 ratio of diastereomers; t_R 9.6 min/10.5 min, respectively; 1.0 mL/min, 25% EtOAc/hexane solution, 258-nm detection.

Methyl 4-Methoxy-12-[*N*-methyl-*N*-[(phenylmethoxy)carbonyl]amino]-N^{10}-methyl-11-oxo-2-azatricyclo[12.2.2.1^{3,7}]nonadeca-3,5,7(19),14,16,-17-hexaene-9-carboxylate (33a). A solution of **32a** (0.178 g, 0.268 mmol) in 1 mL of dry collidine was added to a 0 °C suspension of NaH (60% dispersion in mineral oil, 22 mg, 0.54 mmol, 2.0 equiv) in 1 of mL dry collidine followed by the addition of CuBr–SMe_2 (0.522 g, 2.68 mmol, 10.0 equiv). After 0.5 h (25 °C), the reaction mixture was diluted with collidine (70 mL) and warmed to 130 °C (bath) for 9 h. The cooled reaction mixture was poured over 10% aqueous HCl (30 mL) and extracted with EtOAc (30 mL). The EtOAc extract was washed with 10% aqueous HCl (3 × 50 mL), saturated aqueous NH_4Cl (2 × 30 mL), and saturated aqueous NaCl (30 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 3 × 15 cm, 30–60% EtOAc/hexane) afforded **33a** (0.04 g, 0.14 g theoretical yield, 30%) as a clear yellow oil: $[\alpha]^{22}_D$ −22.1° (*c* 0.12, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 7.48 (dd, 1 H, *J* = 3, 8 Hz, C^{15}- or C^{18}-H), 7.38 (m, 5 H, PhH), 7.31 (dd, 1 H, *J* = 3, 8 Hz, C^{18}- or C^{15}-H), 7.05 (dd, 1 H, *J* = 2.4, 8.4 Hz, C^{16}- or C^{17}-H), 7.03 (dd, 1 H, *J* = 2.4, 8.3 Hz, C^{17}- or C^{16}-H), 6.81 (d, 1 H, *J* = 8.3 Hz C^5-H), 6.64 (dd, 1 H, *J* = 2, 8.4 Hz, C^6-H), 5.42 (dd, 1 H, *J* = 4.7, 11.7 Hz, C^{12}-H), 5.23 and 5.17 (two d, 1 H each, *J* = 12.5 Hz, C*H*HPh and CH*H*Ph), 4.80 (dd, 1 H, *J* = 2.2, 11.9 Hz, C^9-H), 4.74 (d, 1 H, *J* = 2.5 Hz, C^{19}-H), 3.95 (s, 3 H, $ArOCH_3$), 3.65 (s, 3 H, CO_2CH_3), 3.30–2.81 (m, 4 H, C^8- and C^{13}-H_2), 3.03 (s, 3 H, NCH_3), 2.82 (b s, 3 H, NCH_3); IR (neat) ν_{max} 2926, 1772, 1734, 1718, 1700, 1684, 1654, 1648, 1636, 1560, 1542, 1518, 1508, 1490, 1474, 1458, 1266 cm^{-1}; EIMS *m/e* (relative intensity) 532 (M^+, 1), 473 (1), 397 (13), 367 (7), 350 (2), 91 (base); CIMS (isobutane) *m/e* 533 (M^+ + H, base); CIHRMS *m/e* 533.2278 ($C_{30}H_{32}N_2O_7$ requires 533.2288).

The 2D ^1H–^1H NOESY NMR spectrum of **33a** ($CDCl_3$, 500 MHz) displayed diagnostic NOE crosspeaks for C^{15}-H/C^{16}-H, C^{15}-H/C^{13}-H_β, C^{15}-H/C^{12}-H, C^{18}-H/C^{19}-H, C^{18}-H/C^{17}-H, C^{18}-H/C^{13}-H_α, C^{17}-H/C^{19}-H, C^5-H/C^4-OCH_3, C^5-H/C^6-H, C^6-H/C^8-H_α, C^6-H/C^8-H_β, C^{19}-H/C^{12}-H, C^{19}-H/N^{10}-CH_3, C^9-H/C^{13}-H_β, C^9-H/N^{10}-CH_3, C^9-H/C^8-H_β, C^{13}-H_α/C^{13}-H_β, C^{13}-H_β/C^{12}-H, C^{13}-H_α/N^{10}-CH_3, and C^{12}-H/N^{10}-CH_3.

Chiral-phase HPLC analysis of **33a** revealed a single peak; t_R 8.3 min; 1.0 mL/min, 25% EtOAc/hexane elution, 258-nm detection.

Methyl 4-Methoxy-12-[*N*-methyl-*N*-[(1,1-dimethylethoxy)carbonyl]-amino]-N^{10}-methyl-11-oxo-2-azatricyclo[12.2.2.1^{3,7}]nonadeca-3,5,7(19),-14,16,17-hexaene-9-carboxylate (33b). The cyclization of **32b** was conducted as detailed for **32a** to afford **33b** (22%)[60] as a pale-yellow solid: mp 36–44 °C; $[\alpha]^{22}_D$ −23° (*c* 0.24, MeOH); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.29 (two d, 2 H total, *J* = 8 Hz, C^{15}- and C^{18}-H), 7.03 (two d, 2 H total, *J* = 8 Hz, C^{16}- and C^{17}-H), 6.81 (d, 1 H, *J* = 8 Hz, C^5-H), 6.64 (dd, 1 H, *J* = 2.2, 8 Hz, C^6-H), 5.39 (dd, 1 H, *J* = 5, 11.7 Hz, C^{12}-H), 4.80 (dd, 1 H, *J* = 2, 12 Hz, C^9-H), 4.75 (d, 1 H, *J* = 2.2 Hz, C^{19}-H), 3.95 (s, 3 H, $ArOCH_3$), 3.67 (s, 3 H, CO_2CH_3), 3.30–2.78 (m, 4 H, C^8- and C^{13}-H_2), 2.93 (s, 3 H, NCH_3), 2.81 (b s, 3 H, NCH_3), 1.49 (b s, 9 H, $CO_2C(CH_3)_3$); IR (KBr) ν_{max} 2925, 1772, 1734, 1718, 1700, 1654, 1618, 1576, 1560, 1540, 1508, 1491, 1436, 1340 cm^{-1}; CIMS (isobutane) *m/e* 499 (M^+ + H, base); CIHRMS *m/e* 499.2424 ($C_{27}H_{34}N_2O_7$ requires 499.2444).

Chiral-phase HPLC analysis of **33b** revealed a single peak; t_R 7.9 min; 1.0 mL/min, 25% EtOAc/hexane elution, 258-nm detection.

BOC-D-alanyl-L-alanyl-*N*,*O*-dimethyl-L-tyrosyl-L-alanyl-*N*-methyl-L-tyrosyl-*N*,*O*-dimethyl-L-tyrosine Cyclic $5^4 \rightarrow 6^3$ Ether, Methyl Ester (35). A solution of **33a** (16.0 mg, 0.030 mmol) in 1 mL of dry THF was stirred with 10% Pd/C (2 mg, 13% wt equiv) under an atmosphere of H_2 at 25 °C (6 h). The reaction mixture was filtered through Celite (THF), concentrated in vacuo, and dried under vacuum to afford **34** (11.5 mg, 0.029 mmol), which was used directly in the following reaction.

A solution of **34** (11.5 mg, 0.029 mmol) in 0.5 mL of dry DMF was added to a solution of **17** (28.0 mg, 0.058 mmol, 2.0 equiv), EDCI (11.0 mg, 0.058 mmol, 2.0 equiv), and HOBt·H_2O (8.0 mg, 0.058 mmol, 2.0 equiv) in 0.5 mL of dry DMF at 25 °C. The reaction mixture was stirred at 25 °C (12 h), poured over water (5 mL), and extracted with EtOAc (2 × 3 mL). The combined EtOAc extract was washed with 10% aqueous HCl (3 mL), saturated aqueous $NaHCO_3$ (3 × 5 mL), and saturated aqueous NaCl (5 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 1 × 10 cm, Et_2O) afforded **35** (13.9 mg, 26.2 mg theoretical, 53%) as a clear yellow oil: ^1H NMR ($CDCl_3$, 300 MHz) δ 7.40 (dd, 1 H, *J* = 2, 8 Hz, Tyr^5-ArH), 7.21 (dd, 1 H, *J* = 2, 8 Hz, Tyr^5-ArH), 7.11 (dd, 2 H, *J* = 2, 8 Hz, Tyr^5-ArH), 7.05 and 7.02 (two d, 2 H, *J* = 6.5 Hz, Tyr^3-ArH), 6.82 and 6.80 (two d, 2 H, *J* = 6.5 Hz, Tyr^3-ArH), 6.80 (d, 1 H, *J* = 8 Hz, Tyr^{6a}), 6.64 (dd, 1 H, *J* = 2, 8 Hz, Tyr^{6b}), 5.45 (b s, 1 H, NH), 4.83 (dd, *J* = 4, 10 Hz, α-H), 4.74 (d, 1 H, *J* = 2.1 Hz, Tyr^{6a}), 4.65 (dd, 1 H, *J* = 3, 12 Hz, α-H), 4.35 (p, 1 H, *J*

(60) Workup as detailed for **41** could be expected to further improve on this conversion.

= 6 Hz, α-H), 4.18 (m, 1 H, α-H), 4.00 (dd, 1 H, *J* = 5, 10 Hz, α-H), 3.95 (s, 3 H, Tyr^{6}-*O*-Me), 3.78 and 3.76 (two s, 3 H, Tyr^{3}-O-Me), 3.69 (s, 3 H, CO_2CH_3), 3.40 (dd, 1 H, *J* = 5, 10 Hz, α-H), 3.30–2.70 (m, 6 H, Tyr^{3,5,6}-β-H), 2.93 (s, 3 H, NCH_3), 2.79 (s, 3 H, NCH_3), 2.44 (s, 3 H, NCH_3), 1.42 (s, 9 H, $OC(CH_3)_3$), 1.30 (d, 3 H, *J* = 6.8 Hz, Ala-CH_3), 1.28 (d, 3 H, *J* = 6.8 Hz, Ala-CH_3), 0.45 (d, 3 H, *J* = 6.8 Hz, Ala-CH_3); IR (neat) ν_{max} 3650, 3270, 1772, 1734, 1718, 1700, 1684, 1654, 1636, 1560, 1542, 1508, 1474, 1458, 1248 cm^{-1}.

Cyclo(D-alanyl-L-alanyl-*N,O*-dimethyl-L-tyrosyl-L-alanyl-*N*-methyl-L-tyrosyl-*N,O*-dimethyl-L-tyrosyl) Cyclic 5^4 → 6^3 Ether (RA-VII; 1). A solution of **35** (0.015 g, 0.017 mmol) in 0.5 mL of a 0.1 M solution of LiOH in THF/MeOH/H_2O (3:1:1) was stirred at 25 °C (2 h). The reaction mixture was quenched with the addition of 0.5 mL of 10% aqueous HCl, and the mixture was extracted with EtOAc (3 × 0.5 mL). The combined organic layers were concentrated in vacuo and dried thoroughly to afford **36** (IR 3350, 1717, 1700, 1661 cm^{-1}). The crude acid **36** was stirred in 3 N HCl/EtOAc (1 mL) at 25 °C (1 h). The volatiles were removed in vacuo to afford **37** (0.013 g, 0.014 g theoretical yield, 92%) as a solid (IR 3350, 1698, 1660 cm^{-1}), which was used directly in the following reaction.

A solution of **37** (0.013 g, 0.014 mmol) in 2 mL of freshly distilled DMF was cooled to 0 °C and treated with $NaHCO_3$ (7.0 mg, 0.070 mmol, 5.0 equiv) and diphenyl phosphoroazidate (DPPA, 4 mg, 3 μL, 0.021 mmol, 1.5 equiv). The reaction mixture was stirred for 72 h (0 °C), poured over water (3 mL), and extracted with EtOAc (3 × 3 mL). The combined EtOAc extracts were washed with water (2 × 1 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 0.5 × 10 cm, 48:50:2 pentane/CH_2Cl_2/MeOH) afforded RA-VII (**1**, 6.2 mg, 10.8 mg theoretical, 58%) as a white powder: mp >300 °C (dec); $[\alpha]^{22}_D$ −222° (*c* 0.1, $CHCl_3$) [lit.[3] $[\alpha]^{21}_D$ −229° (*c* 0.1, $CHCl_3$)]; R_f 0.23 (48:50:2 pentane/CH_2Cl_2/MeOH); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.42 (dd, 1 H, *J* = 2.4, 8.4 Hz, Tyr$^{5\delta_a}$), 7.27 (dd, 1 H, *J* = 2.3, 8.5 Hz, Tyr$^{5\delta_b}$), 7.20 (dd, 1 H, *J* = 2.4, 8.4 Hz, Tyr$^{5\epsilon_a}$), 7.05 (d, 2 H, *J* = 8.6 Hz, Tyr$^{3\delta}$), 6.87 (dd, 1 H, *J* = 2.4, 8.5 Hz, Tyr$^{5\epsilon_b}$), 6.83 (d, 2 H, *J* = 8.6 Hz, Tyr$^{3\epsilon}$), 6.80 (d, 1 H, *J* = 8.4 Hz, Tyr$^{6\epsilon_a}$), 6.70 (d, 1 H, *J* = 7.7 Hz, Ala^4-NH), 6.58 (dd, 1 H, *J* = 1.9, 8.3 Hz, Tyr$^{6\delta_a}$), 6.41 (d, 1 H, *J* = 6.6 Hz, Ala^1-NH), 6.08 (d, 1 H, *J* = 8.5 Hz, Ala^2-NH), 5.41 (dd, 1 H, *J* = 3.2, 11.4 Hz, Tyr$^{5\alpha}$), 4.86 (p, 1 H, *J* = 7 Hz, Ala$^{2\alpha}$), 4.74 (p, 1 H, *J* = 7.2 Hz, Ala$^{4\alpha}$), 4.55 (dd, 1 H, *J* = 3.9, 11.8 Hz, Tyr$^{6\alpha}$), 4.34 (p, 1 H, *J* = 7 Hz, Ala$^{1\alpha}$), 4.32 (d, 1 H, *J* = 2.2 Hz, Tyr$^{6\delta_b}$), 3.93 (s, 3 H, Tyr^6-*O*-Me), 3.79 (s, 3 H, Tyr^3-*O*-Me), 3.67 (dd, 1 H, *J* = 8.5, 11.3 Hz, Tyr$^{5\beta_b}$), 3.59 (dd, 1 H, *J* = 5.4, 10.2 Hz, Tyr$^{3\alpha}$), 3.36 (m, 2 H, Tyr$^{3\beta}$), 3.14 (dd, 1 H, *J* = 19, 11.8 Hz, Tyr$^{6\beta_b}$), 3.13 (s, 3 H, Tyr^5-*N*-Me), 2.98 (dd, 1 H, *J* = 19, 3.9 Hz, Tyr$^{6\beta_a}$), 2.84 (s, 3 H, Tyr^3-*N*-Me), 2.69 (s, 3 H, Tyr^6-*N*-Me), 2.63 (dd, 1 H, *J* = 2.9, 11.4 Hz, Tyr$^{5\beta_a}$), 1.35 (d, 3 H, *J* = 6.9 Hz, Ala$^{2\beta}$), 1.30 (d, 3 H, *J* = 6.9 Hz, Ala$^{1\beta}$), 1.12 (d, 3 H, *J* = 6.6 Hz, Ala$^{4\beta}$); ^{13}C APT ($CDCl_3$, 75 MHz) Table IV; IR (KBr) ν_{max} 3500, 3390, 2932, 1636, 1586, 1514, 1446, 1412, 1376, 1340, 1264, 1248, 1210, 1180, 1160, 1128, 1094, 1032, 966, 944, 902, 866, 838, 802, 732 cm^{-1}; CIMS (isobutane) *m/e* 771 (M^+ + H, 8), 263 (73), 235 (base); FABMS (glycerol/thioglycerol, 1:1) *m/e* 771 (M^+ + H, 37), 149 (base); FABHRMS *m/e* 771.3727 ($C_{41}H_{50}N_6O_9$ requires 771.3718).

The 2D ^1H–^1H NOESY NMR spectrum ($CDCl_3$, 500 MHz) of **1** displayed the following diagnostic NOE crosspeaks: Tyr$^{5\delta_a}$/Tyr$^{5\epsilon_a}$, Tyr$^{5\delta_a}$/Tyr$^{5\alpha}$, Tyr$^{5\delta_a}$/Tyr$^{5\beta_a}$, Tyr$^{5\delta_b}$/Tyr$^{5\epsilon_b}$, Tyr$^{5\epsilon_a}$/Tyr$^{6\delta_b}$, Tyr$^{3\epsilon}$/Tyr$^{3\delta}$, Tyr$^{3\delta}$/Tyr$^{3\beta}$, Tyr$^{6\delta_a}$/Tyr$^{6\epsilon_a}$, Tyr^6-*O*-Me/Tyr$^{6\epsilon_a}$, Tyr^3-*O*-Me/Tyr$^{3\epsilon}$, Ala-4α/Ala^4-NH, Tyr^3-*N*-Me/Ala^4-NH, Tyr$^{6\alpha}$/Ala^1-NH, Tyr$^{5\alpha}$/Tyr$^{6\alpha}$ (most intense NOE in spectrum), Tyr$^{5\alpha}$/Tyr$^{5\beta_a}$, Tyr$^{6\alpha}$/Tyr$^{6\delta_b}$, Tyr^6-*N*-Me/Tyr$^{6\delta_b}$, Tyr^5-*N*-Me/Ala$^{4\alpha}$, Ala$^{4\alpha}$/Ala$^{4\beta}$, Tyr^3-*N*-Me/Ala-2α, Ala-2α/Ala-2β, Ala-1α/Ala-1β, Ala-4β/Ala-1α, Tyr^3-*N*-Me/Tyr$^{3\alpha}$, Tyr$^{5\beta_b}$/Tyr$^{5\beta_a}$, Tyr$^{5\beta_b}$/Tyr^5-*N*-Me, and Tyr$^{3\alpha}$/Tyr$^{3\beta}$.

Cyclo(D-alanyl-L-alanyl-*N,O*-dimethyl-L-tyrosyl-L-alanyl-*N*-methyl-L-tyrosyl-*N*-methyl-L-tyrosyl) Cyclic 5^4 → 6^3 Ether (Deoxybouvardin; 2). A solution of RA-VII (**1**, 3.1 mg, 0.004 mmol) in dry CH_2Cl_2 was cooled to −78 °C and treated with BBr_3 (1.0 M solution in CH_2Cl_2, 8 μL, 2.0 equiv). The reaction mixture was warmed gradually to room temperature over 3 h, poured over H_2O (1 mL), and extracted with Et_2O/THF/MeOH (1:1:1, 2 × 2 mL). The combined extracts were concentrated in vacuo. Flash chromatography (SiO_2, 0.5 × 4 cm, 5% MeOH/EtOAc) afforded **2** (1.7 mg, 3.0 mg theoretical, 57%) as a white solid: mp >300 °C dec; $[\alpha]^{22}_D$ −219° (*c* 0.05, $CHCl_3$) [lit.[3] $[\alpha]^{21}_D$ −225° (*c* 0.3, $CHCl_3$)]; R_f 0.16 (48:50:2 pentane/CH_2Cl_2/MeOH); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.43 (dd, 1 H, *J* = 2.0, 8.3 Hz, Tyr$^{5\delta_a}$), 7.27 (dd, 1 H, *J* = 2.1, 8.0 Hz, Tyr$^{5\delta_b}$), 7.21 (dd, 1 H, *J* = 2.3, 8.3 Hz, Tyr$^{5\epsilon_a}$), 7.04 (d, 2 H, *J* = 8.5 Hz, Tyr$^{3\delta}$), 6.86 (dd, 1 H, *J* = 2, 8 Hz, Tyr$^{5\epsilon_b}$), 6.82 (d, 2 H, *J* = 8.5 Hz, Tyr$^{3\epsilon}$), 6.80 (d, 1 H, *J* = 8 Hz, Tyr$^{6\epsilon_a}$), 6.69 (d, 1 H, *J* = 7.6 Hz, Ala^4-NH), 6.52 (dd, 1 H, *J* = 1.8, 8.4 Hz, Tyr$^{6\delta_a}$), 6.41 (d, 1 H, *J* = 6.6 Hz, Ala-1 NH), 6.01 (d, 1 H, *J* = 8.5 Hz, Ala^2-NH), 5.56 (s, 1 H, Tyr^6-OH), 5.43 (dd, 1 H, *J* = 2.5, 11 Hz, Tyr$^{5\alpha}$), 4.84 (p, 1 H, *J* = 7.1 Hz, Ala$^{2\alpha}$), 4.75 (p, 1 H, *J* = 7.1 Hz, Ala$^{4\alpha}$), 4.54 (dd, 1 H, *J* = 3.9, 11.9 Hz, Tyr$^{6\alpha}$), 4.35 (p, 1 H, *J* = 7 Hz, Ala$^{1\alpha}$), 4.33 (d, 1 H, *J* = 1.8 Hz, Tyr$^{6\delta_b}$), 3.80 (s, 3 H, Tyr^3-*O*-Me), 3.69 (dd, 1 H, *J* = 8, 11.0 Hz, Tyr$^{5\beta_b}$), 3.59 (dd, 1 H, *J* = 5.5, 10.2 Hz, Tyr$^{3\alpha}$), 3.35 (m, 2 H, Tyr$^{3\beta}$), 3.14 (dd, 1 H, *J* = 19, 11 Hz, Tyr$^{6\beta_a}$), 3.12 (s, 3 H, Tyr^5-*N*-Me), 3.00 (dd, 1 H, *J* = 3.9, 19 Hz, Tyr$^{6\beta_b}$), 2.85 (s, 3 H, Tyr^3-*N*-Me), 2.69 (s, 3 H, Tyr^6-*N*-Me), 2.63 (dd, 1 H, *J* = 2.3, 11.0 Hz, Tyr$^{5\beta_a}$), 1.36 (d, 3 H, *J* = 6.9 Hz, Ala$^{2\beta}$), 1.31 (d, 3 H, *J* = 6.9 Hz, Ala$^{1\beta}$), 1.12 (d, 3 H, *J* = 6.6 Hz, Ala$^{4\beta}$); IR (KBr) ν_{max} 3677, 3651, 3420, 3301, 2950, 1660, 1514, 1457, 1376, 1249, 1178, 1130, 1100, 971, 940, 866, 802, 735 cm^{-1}; CIMS (isobutane) *m/e* 757 (M^+ + H, base); FABMS (glycerol/thioglycerol, 1:1) *m/e* 757 (M^+ + H, 27), 154 (base); FABHRMS *m/e* 757.3545 ($C_{40}H_{48}N_6O_9$ requires 757.3569).

3-Hydroxy-*O*-methyl-*N*-[4-iodo-*N*-methyl-*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanyl]-L-tyrosine Methyl Ester (40). A solution of **39**[19] (0.325 g, 1.44 mmol), EDCI (0.275 g, 1.44 mmol), HOBt·H_2O (0.194 g, 1.44 mmol), and **31b** (0.583 g, 1.44 mmol) in 10 mL of dry DMF was stirred at 25 °C (12 h). The reaction mixture was poured into H_2O (30 mL) and extracted with EtOAc (30 mL). The organic phase was washed with 5% aqueous HCl (3 × 30 mL), saturated aqueous $NaHCO_3$ (3 × 30 mL), and saturated aqueous NaCl (30 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 3 × 20 cm, 30% EtOAc/hexane) afforded **40** (0.815 g, 0.881 g theoretical, 92%) as a white foam: $[\alpha]^{22}_D$ −38.9° (*c* 1.05, MeOH); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.60 (d, 2 H, *J* = 8 Hz, C$^{3'}$- and C$^{5'}$-H), 6.95 (b d, 2 H, *J* = 8 Hz, C$^{2'}$- and C$^{6'}$-H), 6.76 (d, 1 H, *J* = 8 Hz, C^5-H), 6.73 (d, 1 H, *J* = 1 Hz, C^2-H), 6.66 (dd, 1 H, *J* = 8, 1 Hz, C^6-H), 5.70 (d, 1 H, *J* = 6 Hz, NH), 4.88–4.70 (m, 2 H, CH_2CHNH and $CH_2CHN(CH_3)$), 3.87 (s, 3 H, $ArOCH_3$), 3.74 (two s, 3 H, CO_2CH_3), 3.32–2.72 (m, 4 H, 2 × ArCH_2CH), 2.64 and 2.59 (two b s, 3 H total, NCH_3), 1.38 (b s, 9 H, $CO_2C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 171.8 (e), 146.5 (e), 145.9 and 144.7 (e), 137.6 (o), 131.2 (o), 128.6 (o), 126.3 (e), 124.5 (e), 120.6 (e), 117.0 (o), 115.4 (o), 112.1 (e), 110.9 (e), 91.7 (o), 80.7 (o), 58.9 (e), 56.0 (o) and 55.8 (o), 52.4 (o), 37.0 (e), 34.4 and 33.1 (o), 30.4 and 30.3 (e), 27.9 (o); IR (neat) ν_{max} 3400, 2972, 1742, 1702, 1656, 1502, 1484, 1440, 1390, 1366, 1320, 1274, 1144, 1008, 806 cm^{-1}; CIMS (isobutane) *m/e* 613 (M^+ + H, base); CIHRMS *m/e* 613.1399 ($C_{26}H_{33}IN_2O_7$ requires 613.1411).

Anal. Calcd: C, 50.99; H, 5.43; N, 4.57. Found: C, 50.62; H, 5.38; N, 4.31.

Methyl 4-Methoxy-12-[*N*-methyl-*N*-[(1,1-dimethylethoxy)carbonyl]-amino]-11-oxo-2-azatricyclo[12.2.2.1^{3,7}]nonadeca-3,5,7(19),14,16,17-hexaene-9-carboxylate (41). Method A: Methyllithium (1.4 M solution in hexane, 1.6 mL, 2.2 mmol) was added dropwise to a solution of CuI-$(SBu_2)_2$ (0.816 g, 2.2 mmol) in 20 mL of Et_2O at −78 °C. The bright-yellow slurry was stirred well, and the solid was collected by centrifugation. The Et_2O was decanted, and the yellow precipitate was triturated at −78 °C with 20 mL of Et_2O. The Et_2O was removed, and the yellow precipitate was dissolved in 7 mL of collidine at −78 °C. A solution of **40** (0.540 g, 0.88 mmol) in 1 mL of dry collidine was added to the solution of methylcopper in collidine at −78 °C, and the reaction mixture was stirred for 1 h at 25 °C. Collidine (200 mL) was added, and the reaction mixture was warmed at 130 °C (bath) for 9 h. The cooled reaction mixture was concentrated in vacuo. Flash chromatography (SiO_2, 2 × 15 cm, 30% EtOAc/hexane) afforded **41** (0.154 g, 0.427 g theoretical, 36%) as a clear yellow oil which solidified on standing: mp 149–152 °C; $[\alpha]^{22}_D$ −6.7° (*c* 0.018, MeOH); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.44 (b d, 1 H, *J* = 8 Hz, C^{15}-H), 7.29 (dd, 1 H, *J* = 2.2, 8.3 Hz, C^{18}-H), 7.10 (dd, 1 H, *J* = 2.3, 8.3 Hz, C^{16}-H), 6.98 (b d, 1 H, *J* = 8 Hz, C^{17}-H), 6.77 (d, 1 H, *J* = 8.2 Hz, C^5-H), 6.69 (dd, 1 H, *J* = 1.8, 8.2 Hz, C^6-H), 5.87 (b d, 1 H, *J* = 8.1 Hz, NH), 5.14 (b d, 1 H, *J* = 1.4 Hz, C^{19}-H), 4.58 (dd, 1 H, *J* = 2, 12 Hz, C^{12}-H), 4.20 (ddd, 1 H, *J* = 1.3, 8.1, 10.8 Hz, C^9-H), 3.94 (s, 3 H, $ArOCH_3$), 3.66 (s, 3 H, CO_2CH_3), 3.27 (t, 1 H, *J* = 12 Hz, C^{13}-H_αH), 3.00 (s, 3 H, NCH_3), 2.99 (m, 1 H, C^{13}-HH_β, obscured by NCH_3), 2.90 (dd, 1 H, *J* = 1.3, 16.3 Hz, C^8-H_α), 2.80 (dd, 1 H, *J* = 11.0, 16.3 Hz, C^8-H_β), 1.51 (s, 9 H, $CO_2C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 171.9 (e), 169.6 (e), 157.3 (e), 152.6 (e), 147.0 (e), 135.0 (e), 133.7 (o), 124.7 (o), 121.9 (o), 114.9 (o), 111.7 (o), 80.9 (e), 61.4 (o), 56.3 (o), 53.5 (o), 52.6 (o), 35.6 (e), 34.7 (e), 29.7 (o), 28.6 (o); IR (neat) ν_{max} 3300, 2926, 1772, 1734, 1718, 1700, 1684, 1654, 1636, 1560, 1540, 1522, 1508, 1490, 1474, 1458, 1396, 1364, 1262, 1130 cm^{-1}; EIMS *m/e* (relative intensity) 484 (M^+, 5), 428 (8), 383 (14), 353 (17), 298 (16), 282 (11), 227 (12), 57 (base); CIMS (isobutane) *m/e* 485 (M^+ + H, base); EIHRMS *m/e* 484.2210 ($C_{26}H_{32}N_2O_7$ requires 484.2210).

^{1}H NMR ($CDCl_3$) with irradiation at 5.87 ppm (NH) led to the collapse of the signal at 5.14 ppm (C^9-H) to a dd; irradiation at 5.14 ppm (C^9-H) led to the collapse of the signal at 5.87 ppm (NH) to a broadened singlet and to the collapse of the signals at 2.90 (C^8-H_α) and 2.80 (C^8-H_β) to doublets. The ^{1}H–^{1}H NOESY NMR ($CDCl_3$) displayed diagnostic NOE crosspeaks for C^{15}-H/C^{16}-H, C^{15}-H/C^{12}-H, C^{15}-H/C^{13}-H_β, C^{18}-H/C^{17}-H, C^{18}-H/C^{13}-H_α, C^{19}-H/C^{16}-H, C^{19}-H/C^{17}-H, C^{19}-H/NH, C^4-*O*-CH_3/C^5-H, C^5-H/C^6-H, C^6-H/C^8-H_α, C^{12}-H/NH, C^9-H/NH, C^8-H_β/NH, C^9-H/C^{19}-H, C^{19}-H/C^8-H_β, C^{13}-H_β/C^{12}-H, C^9-H/C^8-H_α, C^{13}-H_β/C^{13}-H_α, and C^8-H_α/C^8-H_β.

Method B: A solution of **40** (250 mg, 0.41 mmol) in 1 mL of dry collidine was added dropwise to a suspension of NaH (60% oil dispersion in mineral oil, 33 mg, 0.82 mmol, 2.0 equiv) in 1 mL of dry collidine under Ar at 0 °C, and the solution was allowed to stir for 10 min. The solution was treated with CuBr–SMe_2 (858 mg, 4.1 mmol, 10.0 equiv) and allowed to stir at 25 °C for 50 min before the mixture was diluted with dry degassed collidine to 0.004 M (100 mL) and warmed at 130 °C (bath) for 9 h. The cooled reaction mixture was concentrated in vacuo. The resulting residue was dissolved in EtOAc (30 mL) and saturated aqueous NH_4Cl (30 mL), and the aqueous phase was extracted with EtOAc (3 × 30 mL). The combined organic extracts were washed with saturated aqueous NH_4Cl (3 × 30 mL) and saturated aqueous NaCl (2 × 30 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 2.5 × 15 cm, 20–40% EtOAc/hexane) afforded **41** (58.4 mg, 30%), recovered **40** (43.6 mg, 17%), and **46** (13.4 mg, 8%).[52]

BOC-D-alanyl-L-alanyl-*N,O*-dimethyl-L-tyrosyl-L-alanyl-*N*-methyl-L-tyrosyl-*O*-methyl-L-tyrosine Cyclic $5^4 \rightarrow 6^3$ Ether, Methyl Ester (43). A solution of **41** (23 mg, 0.047 mmol) in 1.5 mL of 3.0 M HCl/EtOAc was stirred at 25 °C (50 min). The reaction mixture was concentrated in vacuo, and the residue was triturated with Et_2O (3 × 1 mL) and dried under vacuum. The amine hydrochloride **42**·HCl in 0.5 mL of DMF was added to a solution of **17** (25 mg, 0.047 mmol, 1.0 equiv), HOBt·H_2O (20 mg, 0.14 mmol, 3.0 equiv), EDCI (28 mg, 0.14 mmol, 3.0 equiv), and $NaHCO_3$ (32 mg, 0.38 mmol, 8 equiv) in 0.16 mL of DMF at 25 °C, and the mixture was stirred for 48 h (25 °C). The reaction mixture was poured over H_2O (5 mL), extracted with EtOAc (4 × 5 mL), washed with 5% aqueous HCl (3 × 3 mL), aqueous saturated $NaHCO_3$ (3 × 3 mL), H_2O, and saturated aqueous NaCl, dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 1 × 10 cm, 0–4% EtOH/EtOAc) afforded **43** (30 mg, 42 mg theoretical, 71%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.43 (b d, 1 H, *J* = 8 Hz, Tyr^5-ArH), 7.22 (d, 2 H, *J* = 8 Hz, Tyr^5-ArH), 7.10 (d, 1 H, *J* = 8 Hz, Tyr^5-ArH), 7.05 and 7.03 (two d, 2 H, *J* = 7 Hz, Tyr^3-ArH), 6.80 (d, 2 H, *J* = 7 Hz, Tyr^3-ArH), 6.75–6.50 (m, 2 H, Tyr^6-ArH), 5.30–4.80 (m, 4 H, 4 × α-H), 5.18 (b s, 1 H, Tyr^{6a}), 4.20 (m, 2 H, 2 × α-H), 3.96 and 3.94 (two s, 3 H, Tyr^6-*O*-Me), 3.75 (s, 3 H, Tyr^3-*O*-Me), 3.30 (m, 1 H, α-H), 3.29 and 3.25 (two s, 3 H, NCH_3), 3.20–2.70 (m, 6 H, Tyr^{3,5,6}-β-H), 2.91 and 2.85 (two s, 3 H, NCH_3), 1.41 (b s, 9 H, $OC(CH_3)_3$), 1.27 (d, 3 H, *J* = 7 Hz, Ala-CH_3), 1.09 (d, 3 H, *J* = 7 Hz, Ala-CH_3), 0.45 (d, 3 H, *J* = 7 Hz, Ala-CH_3); IR (neat) ν_{max} 3300, 2928, 1733, 1714, 1672, 1638, 1514, 1458, 1366, 1250, 1174, 1130, 1030 cm^{-1}; FABMS (glycerol/thioglycerol) *m/e* 889 (M^+ + H, base); FABHRMS (NBA–CsI) *m/e* 1021.3375 (M + Cs^+, $C_{46}H_{60}N_6O_{12}$ requires 1021.3324).

Cyclo(D-alanyl-L-alanyl-*N,O*-dimethyl-L-tyrosyl-L-alanyl-*N*-methyl-L-tyrosyl-*O*-methyl-L-tyrosyl) Cyclic $5^4 \rightarrow 6^3$ Ether (N^{29}-desmethyl RA-VII; 9). A solution of **43** (8.0 mg, 0.009 mmol) in 10 μL of THF was treated with a solution of LiOOH (10 μL, 2.7 M solution of LiOH in 30% H_2O_2) at 0 °C. The reaction mixture was stirred for 6 h (25 °C). The reaction mixture was quenched with the addition of solid sodium bisulfite (5.6 mg, 0.54 mmol, 6 equiv) followed by addition of H_2O (0.5 mL). The reaction mixture was diluted with THF (1 mL), and the organic phase was separated, washed with saturated aqueous NaCl (1 mL), dried (Na_2SO_4), and concentrated in vacuo to afford **44** (6.8 mg, 7.9 mg theoretical, 86%) as a yellow oil (IR 3349, 1718, 1700, 1658 cm^{-1}; FABHRMS (NBA) *m/e* 873.4098, M – H^+, $C_{45}H_{58}N_6O_{12}$ requires 873.4034.) which was used directly in the following reaction without purification.

A solution of **44** (6.4 mg, 0.008 mmol) in 3.0 M HCl/EtOAc (0.5 mL) was stirred at 25 °C (1 h). The volatiles were removed in vacuo, and the residue was dried thoroughly under vacuum to afford the amino acid hydrochloride salt **45**·HCl (IR 3350, 1670, 1638 cm^{-1}). A solution of **45**·HCl in 1.3 mL of dry DMF was cooled to 0 °C and treated with $NaHCO_3$ (4.0 mg, 0.04 mmol, 5.0 equiv) and DPPA (3.5 mg, 2.6 μL, 0.012 mmol, 1.5 equiv). The reaction mixture was stirred at 0 °C (72 h), poured over H_2O (2 mL), and extracted with EtOAc (3 × 2 mL). The combined EtOAc extracts were washed with H_2O (2 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 0.5 × 10 cm, 48:50:2 pentane/CH_2Cl_2/MeOH) afforded **9** (2.1 mg) as a tan powder: mp >300 °C dec; $[\alpha]^{22}_D$ –202° (*c* 0.05, $CHCl_3$); R_f 0.69 (48:50:2 pentane/CH_2Cl_2/MeOH); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.40 (dd, 1 H, *J* = 2, 8 Hz, Tyr$^{5\delta_a}$), 7.25 (dd, 1 H, *J* = 2, 8 Hz, Tyr$^{5\delta_b}$), 7.19 (dd, 1 H, *J* = 2, 8 Hz, Tyr$^{5\epsilon_a}$), 7.02 (d, 2 H, *J* = 8.5 Hz, Tyr$^{3\delta}$), 6.83 (dd, 1 H, *J* = 2, 8 Hz, Tyr$^{5\epsilon_b}$), 6.80 (d, 2 H, *J* = 8.5 Hz, Tyr$^{3\epsilon}$), 6.78 (d, 1 H, *J* = 8.4 Hz, Tyr$^{6\epsilon_a}$), 6.70 (d, 1 H, *J* = 8 Hz, Ala^4-NH), 6.60 (dd, 1 H, *J* = 2.2, 8.4 Hz, Tyr$^{6\delta_a}$), 6.40 (d, 1 H, *J* = 6.6 Hz, Ala^1-NH), 6.08 (d, 1 H, *J* = 8.5 Hz, Ala^2-NH), 5.83 (d, 1 H, *J* = 8 Hz, Tyr^6-NH), 5.41 (dd, 1 H, *J* = 3.2, 11.4 Hz, Tyr$^{5\alpha}$), 4.85 (p, 1 H, *J* = 7 Hz, Ala$^{2\alpha}$), 4.76 (d, 1 H, *J* = 2.2 Hz, Tyr$^{6\delta_b}$), 4.74 (p, 1 H, *J* = 7.2 Hz, Ala$^{4\alpha}$), 4.55 (ddd, 1 H, *J* = 4, 8, 10 Hz, Tyr$^{6\alpha}$), 4.32 (p, 1 H, *J* = 7 Hz, Ala$^{1\alpha}$), 3.93 (s, 3 H, Tyr^6-*O*-Me), 3.78 (s, 3 H, Tyr^3-*O*-Me), 3.67 (dd, 1 H, *J* = 8, 11 Hz, Tyr$^{5\beta_b}$), 3.60 (dd, 1 H, *J* = 5, 11 Hz, Tyr$^{3\alpha}$), 3.35 (m, 2 H, Tyr$^{3\beta}$), 3.17 (dd, 1 H, *J* = 19, 11 Hz, Tyr$^{6\beta_b}$), 3.13 (s, 3 H, Tyr^5-*N*-Me), 3.01 (dd, 1 H, *J* = 19, 4.1 Hz, Tyr$^{6\beta_a}$), 2.83 (s, 3 H, Tyr^3-*N*-Me), 2.63 (dd, 1 H, *J* = 3, 11 Hz, Tyr$^{5\beta_a}$), 1.34 (d, 3 H, *J* = 6.9 Hz, Ala$^{2\beta}$), 1.30 (d, 3 H, *J* = 6.9 Hz, Ala$^{1\beta}$), 1.11 (d, 3 H, *J* = 6.6 Hz, Ala$^{4\beta}$); ^{13}C NMR (Table IV); IR (KBr) ν_{max} 3390, 2930, 1638, 1586, 1445, 1412, 1380, 1262, 1250, 1180, 1159, 1094, 966, 838, 732 cm^{-1}; FABMS (glycerol/thioglycerol, 1:1) *m/e* 757 (M^+ + H, 89), 164 (base); FABHRMS *m/e* 757.3753 ($C_{40}H_{48}N_6O_9$ requires 757.3561).

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