of **7** δ 8.24 (d, J = 8 Hz, 1 H), 7.26 (t, J = 8 Hz, 1 H), 6.86 (t, J = 8 Hz, 1 H), 6.68 (d, J = 8 Hz, 1 H), 5.47 (d, J = 6 Hz, 1 H), 4.81 (d, J = 6 Hz, 1 H), 3.86 (dd, J = 9 Hz, 10 Hz, 1 H), 3.74 (m, 1 H), 3.03 (m, 1 H), 2.72 (t, J = 4 Hz, 1 H), 2.66–1.40 (m, 7 H); IR (KBr) 3392, 3263, 3045, 2972, 2929, 1612, 1594, 1575, 1553, 1489, 1439, 1405, 1337, 1129, 1117 cm^{-1}; HRMS, calcd for $C_{15}H_{16}N_2O_2$ 256.1212, found 256.1205.

Preparation of the Imino Epoxide 34. To a stirred solution of the tosylate **33** (100 mg, 0.257 mmol) in 10 mL of dry THF at 0 °C was added 0.26 mL of 1 M $LiN(TMS)_2$ in THF (0.26 mmol) dropwise, and the mixture was stirred for 0.5 h at 0 °C. At the end of the stirring 20 mL of EtOAc was added and the solution was washed with 5 mL of cold water and 5 mL of brine. After drying over Na_2SO_4 the solvents were evaporated off to give a white solid residue of the epoxide **34**. It was recrystallized from EtOAc–MeOH–hexane to afford 42 mg of the pure epoxide **34**: mp 151.5–153 °C (70.1% yield); NMR ($CDCl_3$) imino epoxide **34** δ 8.04 (d, J = 8 Hz, 1 H), 7.77 (s, 1 H), 7.53 (t, J = 8 Hz, 1 H), 7.34 (d, J = 8 Hz, 1 H), 7.33 (t, J = 8 Hz, 1 H), 4.13 (m, 1 H), 3.60 (m, 1 H), 2.97 (m, 1 H), 2.8–1.4 (m, 7 H), hydrate of **34** δ 8.10 (d, J = 8 Hz, 1 H), 7.23 (t, J = 8 Hz, 1 H), 6.80 (t, J = 8 Hz, 1 H), 6.75 (d, J = 8 Hz, 1 H), 5.38 (d, J = 6 Hz, 1 H), 4.40 (d, J = 6 Hz, 1 H), 3.88 (m, 2 H), 3.03 (m, 1 H), 2.8–1.4 (m, 8 H); IR (KBr) 3394, 3289, 2980, 2914, 2822, 1601, 1558, 1553, 1491, 1472, 1403, 1346, 1121 cm^{-1}; HRMS, CH_3OH adduct of **34**, calcd for $C_{16}H_{20}N_2O_3$ 288.1474, found 288.1464; hydrate of **34**, calcd for $C_{15}H_{18}N_2O_3$ 274.1318, found 274.1270; imino epoxide **34**, calcd for $C_{15}H_{16}N_2O_2$ 256,1212, found 256.1243.

Acknowledgment. We thank Dr. Joseph D. Calabrese for the determination of the X-ray structure, Mr. Dennis Sabol for technical assistance, and Ms. Theresa A. Bonnes for help with the manuscript and drawings.

Supplementary Material Available: Detailed X-ray crystal data for compound **32** (atomic coordinates, bond lengths, bond angles, etc.) (5 pages). Ordering information is given on any current masthead page.

Studies on the Total Synthesis of Bouvardin and Deoxybouvardin: Cyclic Hexapeptide Cyclization Studies and Preparation of Key Partial Structures

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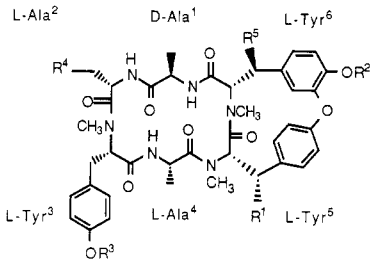
The total synthesis of *cyclo*-(D-Ala-Ala-*N*-Me-Tyr(OCH_3)-Ala-*N*-Me-Tyr-*N*-Me-Tyr) (**9**), *cyclo*-(D-Ala-Ala-*N*-Me-Tyr(OCH_3)-Ala-*N*-Me-Gly-*N*-Me-Gly) (**10**), and *cyclo*-(D-Ala-Ala-*N*-Me-Tyr(OCH_3)-Ala-*N*-$(CH_3)CH_2CH_2$-(*p*-C_6H_4)-O-(*m*-C_6H_4)$CH_2CH_2C(O)$) (**11**) are detailed and constitute the parent 18-membered (**9**, **10**) and 26-membered (**11**) monocyclic peptide skeletons of the exceptionally potent, naturally occurring, bicyclic hexapeptide antitumor antibiotics bouvardin (**1**), deoxybouvardin (**2**, RA-V), RA-I–RA-IV, RA-VI, and RA-VII. The preparation of *cyclo*-(D-Ala-Ala-*N*-Me-Tyr(OCH_3)-Ala) (**12**), a conformationally constrained 12-membered cyclic tetrapeptide constituting a monocyclic, skeletal substructure of the naturally occurring materials, is detailed. Macrocyclization studies revealed no apparent preference for 12-membered vs 18-membered vs 26-membered ring closure and each represent a macrocyclization reaction which is facilitated with closure conducted at a N-terminus D-amino acid site (D-Ala).

Bouvardin (**1**, NSC 259968) and deoxybouvardin (**2**), bicyclic hexapeptides isolated initially from *Bouvardia ternifolia* (Rubiacea) and unambiguously identified by single-crystal X-ray structure analysis (bouvardin) and chemical correlation (deoxybouvardin),[2] are the initial members of a class of selective, exceptionally potent antitumor antibiotics[2-4] now including the additional, provisionally named, bicyclic hexapeptides RA-I–RA-VII.[3-5]

Bouvardin (**1**) and related agents inhibit protein synthesis[6]



	R^1	R^2	R^3	R^4	R^5	
1	OH	H	CH_3	H	H	bouvardin[2]
2	H	H	CH_3	H	H	deoxybouvardin,[2] (RA-V)[3,4]
3	H	H	CH_3	OH	H	RA-I[3]
4	H	CH_3	H	H	H	RA-II[3]
5	H	CH_3	CH_3	OH	H	RA-III[3,4]
6	H	CH_3	CH_3	H	OH	RA-IV[3,4]
7	H	CH_3	CH_3	H	H	*O*-methyl deoxybouvardin, (RA-VII)[3,4]
8	OH	CH_3	CH_3	H	H	*O*-methyl bouvardin[5]

by binding to the eukaryotic 80S ribosome and subsequently inhibit EF1-dependent binding of aminoacyl-tRNA *and* EF2-dependent translocation of peptidyl-

(1) National Institutes of Health research career development award recipient, 1983–1988 (CA 01134). Alfred P. Sloan research fellow, 1985–1989.

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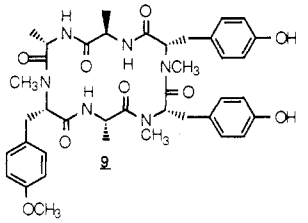
tRNA.[7] Consequently, the bouvardin-defined eukaryotic binding site has proven distinct from the well-defined cycloheximide and cryptopleurine 80S ribosomal binding sites currently established as effective binding sites for protein synthesis inhibition.[7] The unusual 14-membered para- and metacyclophane unit of the naturally occurring agents has been postulated to arise from the oxidative coupling of two adjacent L-tyrosine residues in cyclic hexapeptide precursors although the direct incorporation of naturally derived isodityrosine cannot be excluded.[2,3,8] The isodityrosine-derived 14-membered segment has been suggested to be responsible for attainment and/or maintenance of an active, normally inaccessible, conformation of the parent, cyclic hexapeptides.[5] In support of this, the parent 18-membered monocyclic hexapeptide **9** [*cyclo*-(D-Ala-Ala-*N*-Me-Tyr(OCH_3)-Ala-*N*-Me-Tyr-*N*-Me-Tyr), *O*-*seco*-deoxybouvardin][9a] has been shown to lack the antitumor and cytotoxic properties of deoxybouvardin while substantial functional group modification of the 14-membered para- and metacyclophane dipeptide segment of bouvardin and deoxybouvardin potentiate the biological properties of the naturally occurring agents.[3,4]

Herein, we provide full details of an effective, convergent preparation of Boc-D-Ala-Ala-*N*-Me-Tyr(OCH_3)-Ala-OCH_3 (**13a**) constituting the tetrapeptide segment of bouvardin (**1**), deoxybouvardin (**2**), *O*-methylbouvardin (**8**), *O*-methyldeoxybouvardin (**7**, RA-VII), and RA-IV. The preparation of the 18-membered (**9**, **10**) and 26-membered (**11**) cyclic peptides **9** [*cyclo*-(D-Ala-Ala-*N*-Me-Tyr-(OCH_3)-Ala-*N*-Me-Tyr-*N*-Me-Tyr)], **10** [*cyclo*-(D-Ala-Ala-*N*-Me-Tyr(OCH_3)-Ala-*N*-Me-Gly-*N*-Me-Gly)], and **11** [*cyclo*-(D-Ala-Ala-*N*-Me-Tyr(OCH_3)-Ala-N(CH_3)-CH_2CH_2(*p*-C_6H_4)-O-(*m*-C_6H_4)CH_2CH_2C(O))] constituting the two, parent monocyclic substructures of the bicyclic hexapeptide antitumor antibiotics are detailed in efforts that establish a preferred site and method for macrocyclization suited for implementation in the total synthesis of the naturally occurring materials.[9a,c] Comparative in vitro cytotoxic evaluation of the agents are described in efforts to establish the structural and conformational features of the bicyclic hexapeptides responsible for the potent, selected antitumor activity. The additional preparation of **12** [*cyclo*-(D-Ala-Ala-*N*-Me-Tyr(OCH_3)-Ala)], a conformationally constrained 12-membered cyclic tetrapeptide constituting a monocyclic, skeletal substructure of the naturally occurring materials is described.



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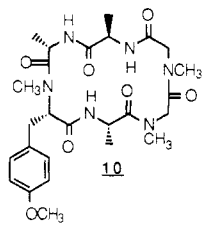

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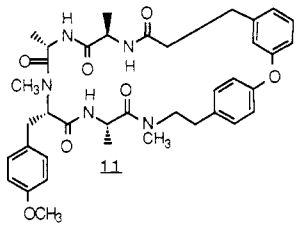

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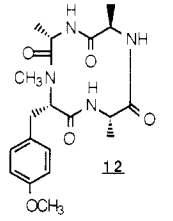
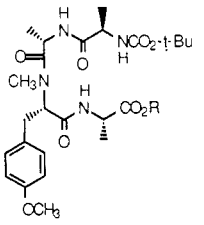


12



13a R=CH_3

13b R=H

Preparation of Boc-D-Ala-Ala-*N*-Me-Tyr(OCH_3)-Ala-OCH_3 (13a). Linear Tetrapeptide Segment of Bouvardin (1), Deoxybouvardin (2, RA-V), *O*-Methylbouvardin (8), *O*-Methyldeoxybouvardin (7, RA-VII), and RA-IV. In efforts complementary to those detailed by Bates and co-workers[9a] in which a linear, solution-phase synthetic approach to the preparation of Boc-D-Ala-Ala-*N*-Me-Tyr(OCH_3)-Ala-OCH_3 (**13a**) has been detailed, we have devised and implemented a convergent approach to the preparation of **13**, Scheme I.

Exhaustive methylation of *N*-Boc-L-tyrosine employing carefully controlled reaction conditions (3.3 equiv of NaH, 2.2 equiv of CH_3I, THF, 25 °C) comparable to *N*-methylation conditions detailed by Coggins and Benoiton[10] provided L-*N*-Boc-*N*-methyl-*O*-methyltyrosine (**14**, 90%) with minimal, competitive racemization.[11] Dicyclohexylcarbodiimide-promoted coupling of **14** with L-alanine methyl ester (**15**), deprotection (TFA, 25 °C, 0.5 h, 82%) of the dipeptide **16**, and subsequent dicyclohexylcarbodiimide-promoted coupling of *N*-Me-Tyr(OCH_3)-Ala-OCH_3 (**17**) with Boc-D-Ala-Ala (**20**), Scheme I, provided the linear tetrapeptide **13a** [Boc-D-Ala-Ala-*N*-Me-Tyr(OCH_3)-Ala-OCH_3] in a sequence that has proven amenable to a multigram-scale preparation of **13**. Competitive diketopiperazine formation, a common side reaction in the preparation of *N*-methyl amides,[12-14] was not observed in

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(8) More recent efforts have established the natural occurrence of piperazinomycin,[8a] OF4949-I–OF4949-IV,[8b] isodityrosine,[8c] and K-13,[8d] bearing the diaryl ether linked dityrosine (isodityrosine). These observations and the failure of synthetic efforts to effect diaryl ether formation of the monocyclic hexapeptide 9[9] suggest that diaryl ether coupling may precede cyclization (14-membered dipeptide, 18-/26-membered hexapeptide) and incorporation into the cyclic hexapeptide structure. (a) Piperazinomycin. Fermentation, isolation, characterization, biological properties: Tamai, S.; Kaneda, M.; Nakamura, S. *J. Antibiot.* **1982**, *35*, 1130. X-ray structure determination: Kaneda, M.; Tamai, S.; Nakamura, S.; Hirata, T.; Kushi, Y.; Suga, T. *J. Antibiot.* **1982**, *35*, 1137. Total synthesis: Nishiyama, S.; Nakamura, K.; Suzuki, Y.; Yamamura, S. *Tetrahedron Lett.* **1986**, *27*, 4481. Synthetic studies: Jung, M. E.; Rohloff, J. C. *J. Org. Chem.* **1985**, *50*, 4909. (b) OF4949-I–IV, aminopeptidase B inhibitors. Fermentation, isolation, and characterization: Sano, S.; Ikai, K.; Kuroda, H.; Nakamura, T.; Obayashi, A.; Ezure, Y.; Enomoto, H. *J. Antibiot.* **1986**, *39*, 1674. Structure determination: Sano, S.; Ikai, K.; Katayama, K.; Takesako, K.; Nakamura, T.; Obayashi, A.; Ezure, Y.; Enomoto, H. *J. Antibiot.* **1986**, *39*, 1685. Biosynthesis: Sano, S.; Ueno, M.; Katayama, K.; Nakamura, T.; Obayashi, A. *J. Antibiot.* **1986**, *39*, 1697. (c) Isodityrosine: Fry, S. C. *Biochem. J.* **1982**, *204*, 449. Cooper, J. B.; Varner, J. E. *Biochem. Biophys. Res. Commun.* **1983**, *112*, 161. (d) K-13 angiotensin I converting enzyme (ACE) inhibitor. Fermentation, isolation, and biological properties: Kase, H.; Kaneko, M.; Yamada, K. *J. Antibiot.* **1987**, *40*, 450. Structure determination: Yasuzawa, T.; Shirahata, K.; Sano, H. *J. Antibiot.* **1987**, *40*, 455.

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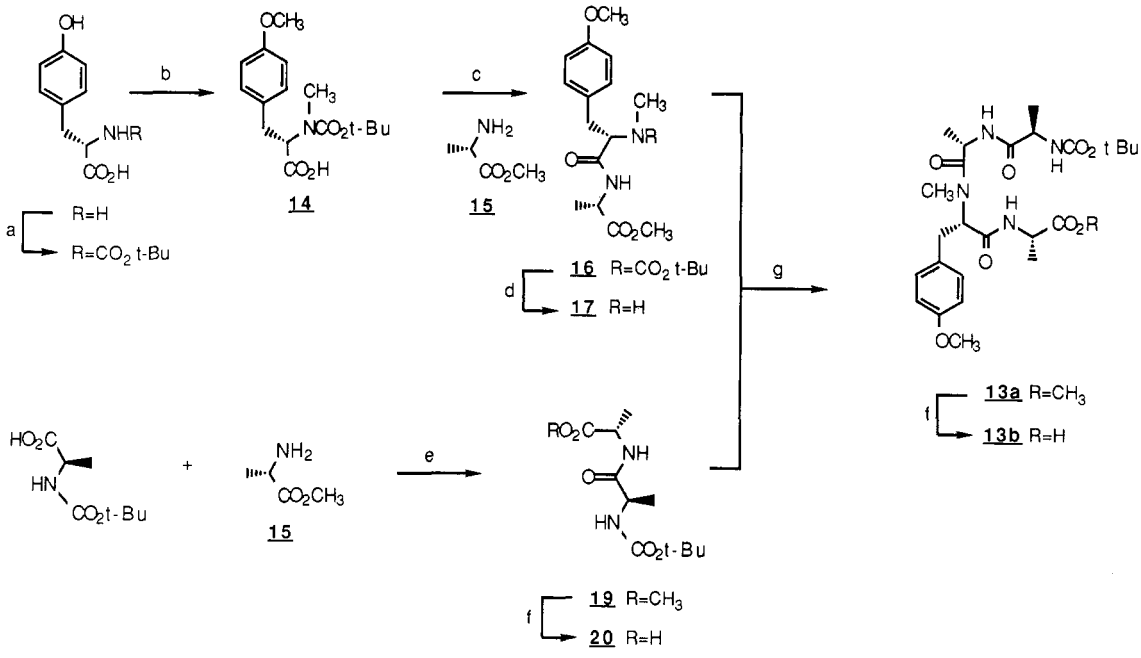
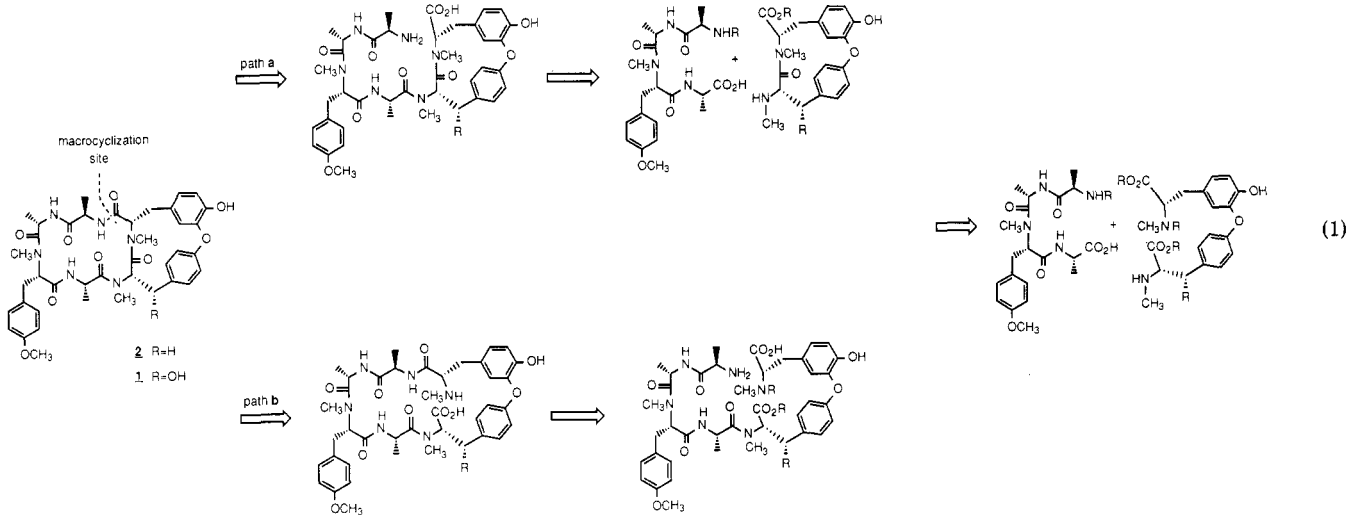
Scheme I[a]



[a] (a) 10 equiv of NaOH (1.0 M), 1.1 equiv of $(t\text{-BuOCO})_2O$, dioxane/H_2O (2:1), 25 °C, 1 h, 60%; (b) 3.3 equiv of NaH, 2.2 equiv of CH_3I, THF, 25 °C, 90%; (c) 1.0 equiv of **15**, 1.1 equiv of DCC, CH_2Cl_2, 25 °C, 24 h, 76%; (d) TFA, 25 °C, 0.5 h, 82%; (e) 1.0 equiv of **15**, 1.0 equiv of DCC, CH_2Cl_2, 25 °C, 36 h, 95%; (f) 3.0 equiv of LiOH, THF/MeOH/H_2O (3:1:1), 25 °C, 3 h, 78% for **20**; 82% for **13b**; (g) 1.0 equiv of DCC, CH_2Cl_2, 25 °C, 36 h, 71%.



(1)

the coupling of **20** [Boc-D-Ala-Ala] with **17** [*N*-Me-Tyr-(OCH_3)-Ala-OCH_3].

Preparation of *cyclo*-(D-Ala-Ala-*N*-Me-Tyr-(OCH_3)-Ala-*N*-Me-Gly-*N*-Me-Gly). 18-Membered Cyclic Hexapeptide Cyclization Studies. At the onset of the efforts on the total synthesis of bouvardin (**1**), deoxybouvardin (**2**), and structurally related naturally occurring and synthetic mono- and bicyclic hexapeptides several sites were available as apparent locations for macrocyclization and cyclic peptide formation. Recent, empirical observations have shown that subtle structural features may facilitate or decelerate cyclic peptide formation.[15] The well-documented rate deceleration of peptide bond formation accompanying amino substitution (e.g. *N*-methyl amide formation)[12-14] discourage attempts to promote macrocyclization and cyclic peptide formation at three of the six available bouvardin/deoxybouvardin peptide-bond sites. In addition, the empirically derived demonstration of the acceleration that accompanies macrocyclization and cyclic peptide formation of selected peptides bearing a D-amino acid at the amine terminus[16,17] suggested that macrocyclization and cyclic peptide formation may best be conducted at the D-Ala[1]/modified L-Tyr[6] site. However, it was not evident whether this macrocyclization may be best conducted with efforts to form the 18-membered cyclic hexapeptide by employing intermediates bearing the intact 14-membered para- and

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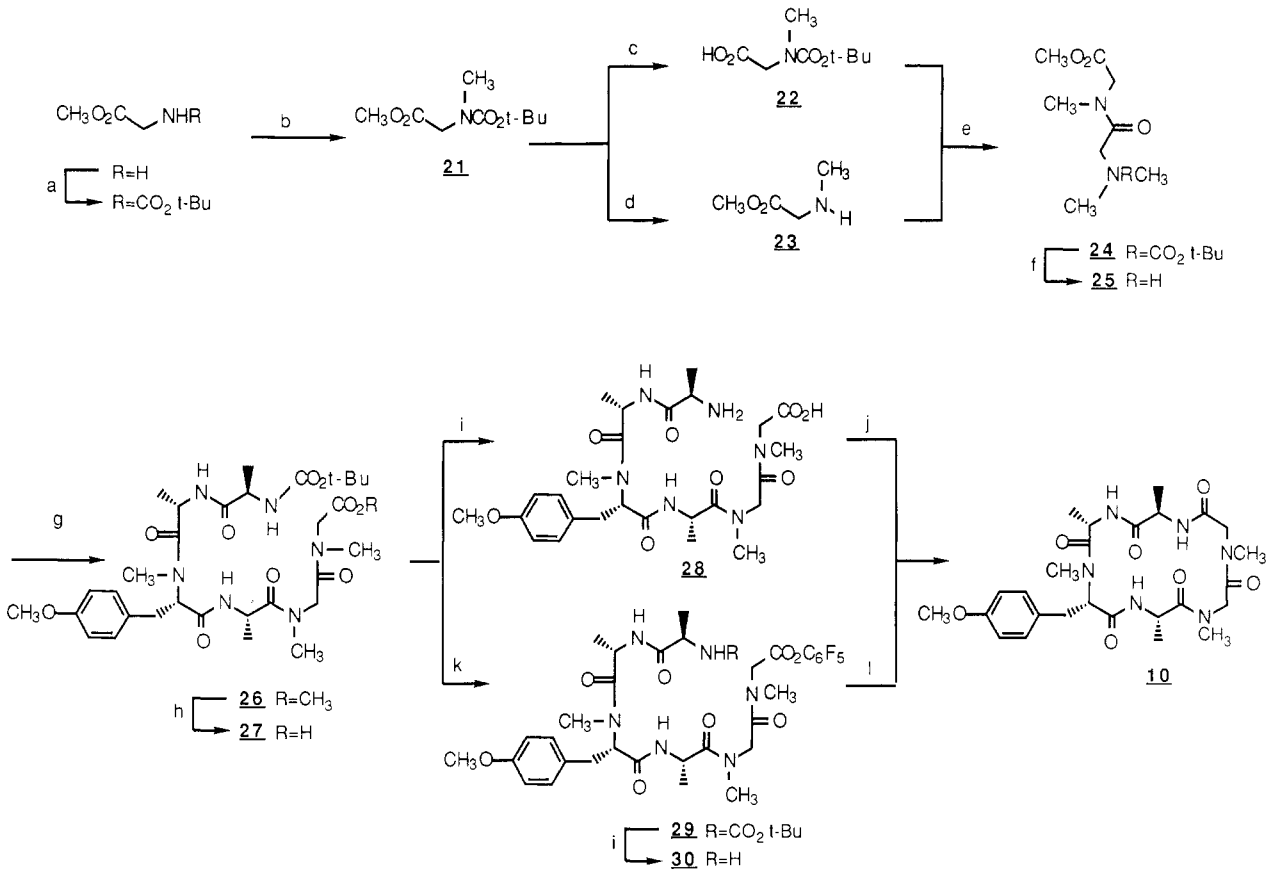
Scheme II[a]



[a] (a) 1.0 equiv of $(t\text{-BuOCO})_2O$, THF, 25 °C, 2 h, 91%; (b) 1.1 equiv of NaH, 3.0 equiv of CH_3I, THF/DMF (10:1), 80 °C, 18 h, 79%; (c) 3.0 equiv of LiOH, THF/MeOH/H_2O (3:1:1), 25 °C, 2.5 h, 66%; (d) TFA, 25 °C, 0.5 h, 59%; (e) 1.0 equiv of DCC, CH_2Cl_2, 25 °C, 24 h, 68%; (f) TFA/CH_2Cl_2 (1:1), 25 °C, 0.5 h, 69%; (g) 1.0 equiv of **13b**, 1.0 equiv of DCC, CH_2Cl_2, 25 °C, 24 h, 64%; (h) 3.0 equiv of LiOH, THF/MeOH/H_2O (3:1:1), 25 °C, 3 h, 86%; (i) TFA/CH_2Cl_2 (1:1), 25 °C, 2 h; (j) 1.3 equiv of DPPA, DMF, 0.008 M **28**, pH 7 (Et_3N), −20 °C, 48 h; 0 °C, 48 h, 64%; (k) 1.1 equiv of C_6F_5OH, 1.0 equiv of EDCI, CH_2Cl_2, 25 °C, 24 h, 65% from **26**; (l) **30** (DMF) addition (3–4 h) to pyridine, 0.0003 M, 90 °C, 8 h, 51% overall from **29**.

metacyclophane dipeptide (eq 1, path a) or conducted with penultimate macrocyclization with closure to a 26-membered cyclic peptide followed by final closure of the peptide bond constituting formation of the 14-membered para- and metacyclophane segment (eq 1, path b). In order to examine the feasibility and facility of the 18-membered cyclic hexapeptide cyclization reaction (eq 1, path a), the readily accessible, simplified linear hexapeptide **26** was prepared.

Dicyclohexylcarbodiimide-promoted coupling of the tetrapeptide **13b** with *N*-Me-Gly-*N*-Me-Gly-OCH_3 (**25**), prepared as detailed in Scheme II, provided the linear hexapeptide **26**. The potential, competitive intramolecular reactions, diketopiperazine formation, normally observed upon peptide *N*-methyl amide formation are not accessible to the linear tetrapeptide **13b** as a consequence of the ^{3}Tyr(OCH$_3$) N-methylation.

The linear hexapeptide **26** was subjected to two sets of cyclization procedures employing experimental conditions previously detailed as suitable, optimal approaches to cyclic peptide formation with closure conducted at a N-terminus D-amino acid site.[16,17] Consistent with expectations, closure of the linear hexapeptide as its free amino acid **28** in a reaction effected by diphenylphosphoryl azide (DPPA, diphenylphosphoroazidate) and conducted at near normal solution-phase concentrations (0.008 M substrate)[17] provided the cyclic 18-membered hexapeptide **10**; Scheme II, Table I. Alternatively, formation of the pentafluorophenyl ester **29** of the linear hexapeptide and subsequent cyclization of the liberated (TFA, 25 °C, 0.5 h) free amine of the linear hexapeptide active ester **30** employing solution-phase, high dilution techniques[16,18] provided the 18-membered cyclic hexapeptide **10**, Scheme II and Table I, identical in all respects with the material prepared in the diphenylphosphoryl azide promoted closure.

Table I. Macrocyclization Studies

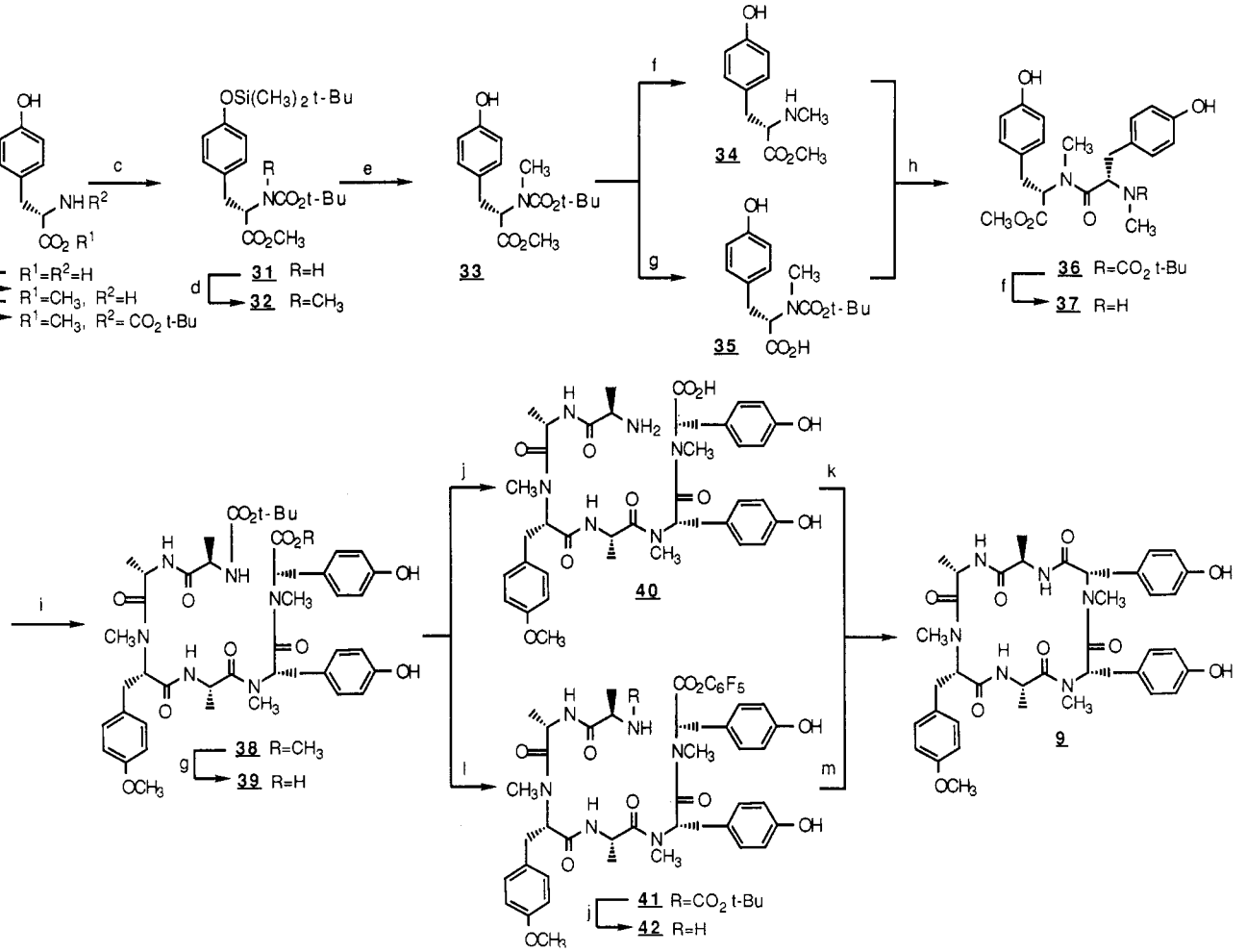
substrate[a]	method[b,c]	cyclic peptide	% yield[d]
28	A[b]	**10**	64 (9)
30	B[c]	**10**	51 (17)
40	A	**9**	56 (20)
42	B	**9**	48 (24)
53	A	**11**	61 (3)
55	B	**11**	49 (14)
56	A	**12**	68 (6)
58	B	**12**	51 (9)

[a] The trifluoroacetic acid salt of all substrates were employed. [b] A = 1.3 equiv of DPPA, DMF, 0.008 M in substrate, pH 7 ($NaHCO_3$), 0 °C, 72 h. [c] B = substrate in DMF added (3–8 h) to pyridine, 0.0003 M, 90 °C, 8 h. [d] All yields (overall for two steps from *tert*-butylcarbamate) are based on chromatographically homogeneous material isolated by chromatography (SiO_2). The yields in parentheses represents recovered, starting substrate.

Preparation of *O*-*seco*-Deoxybouvardin [*cyclo*-(D-Ala-Ala-*N*-Me-Tyr(OCH$_3$)-Ala-*N*-Me-Tyr-*N*-Me-Tyr)]. 18-Membered Cyclic Hexapeptide Cyclization Studies. The facility with which the macrocyclization reaction employed in the preparation of the 18-membered

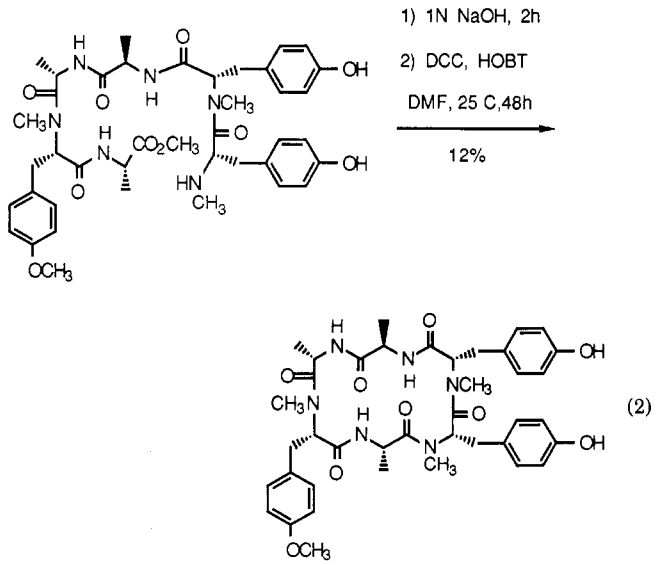
(18) The amine·CF_3CO_2H salt in DMF (0.09–0.01 M) at 25 °C was added dropwise (3–8 h, syringe pump) to a warm solution of pyridine (90 °C). The final concentration of substrate in pyridine was ≤0.0003 M.

Scheme III[a]



[a] (a) HCl (g), MeOH, 25 °C, 2 h, 95%; (b) 1.0 equiv of $(t\text{-BuOCO})_2O$, THF, 25 °C, 2 h, 72%; (c) 1.2 equiv of TBDMSCl, 2.5 equiv of imidazole, DMF, 25 °C, 6 h, 93%; (d) 1.1 equiv of NaH, 3.0 equiv of CH_3I, THF/DMF (10:1), 25 °C, 48 h, 87%; (e) AcOH/THF/H_2O (3:2:1), 25 °C, 8 h, 82%; (f) TFA/CH_2Cl_2 (1:1), 25 °C, 1.5 h, 78% for **34**; 68% for **37**; (g) 3.0 equiv of LiOH, THF/MeOH/H_2O (3:1:1), 25 °C, 12 h, 59% for **35**; 81% for **39**; (h) 1.0 equiv of EDCI, CH_2Cl_2, 25 °C, 12 h, 59%; (i) 1.0 equiv of **13b**, 1.0 equiv of EDCI, CH_2Cl_2, 25 °C, 20 h, 57%; (j) TFA/CH_2Cl_2 (1:1), 25 °C, 2 h; (k) 1.3 equiv of DPPA, DMF, 0.008 M in **40**, pH 7 ($NaHCO_3$), 0 °C, 72 h, 56% overall from **39**; (l) 1.1 equiv of C_6F_5OH, 1.0 equiv of EDCI, CH_2Cl_2, 25 °C, 24 h, 78%; (m) **42** (DMF) addition (3–4 h) to pyridine, 0.0003 M, 90 °C, 8 h, 48% overall from **41**.

cyclic hexapeptide **10** proceeded and the results of an alternative, comparative cyclic hexapeptide closure reported by Bates and co-workers[9a] in the preparation of *O-seco*-deoxybouvardin, eq 2, raised the concern that the



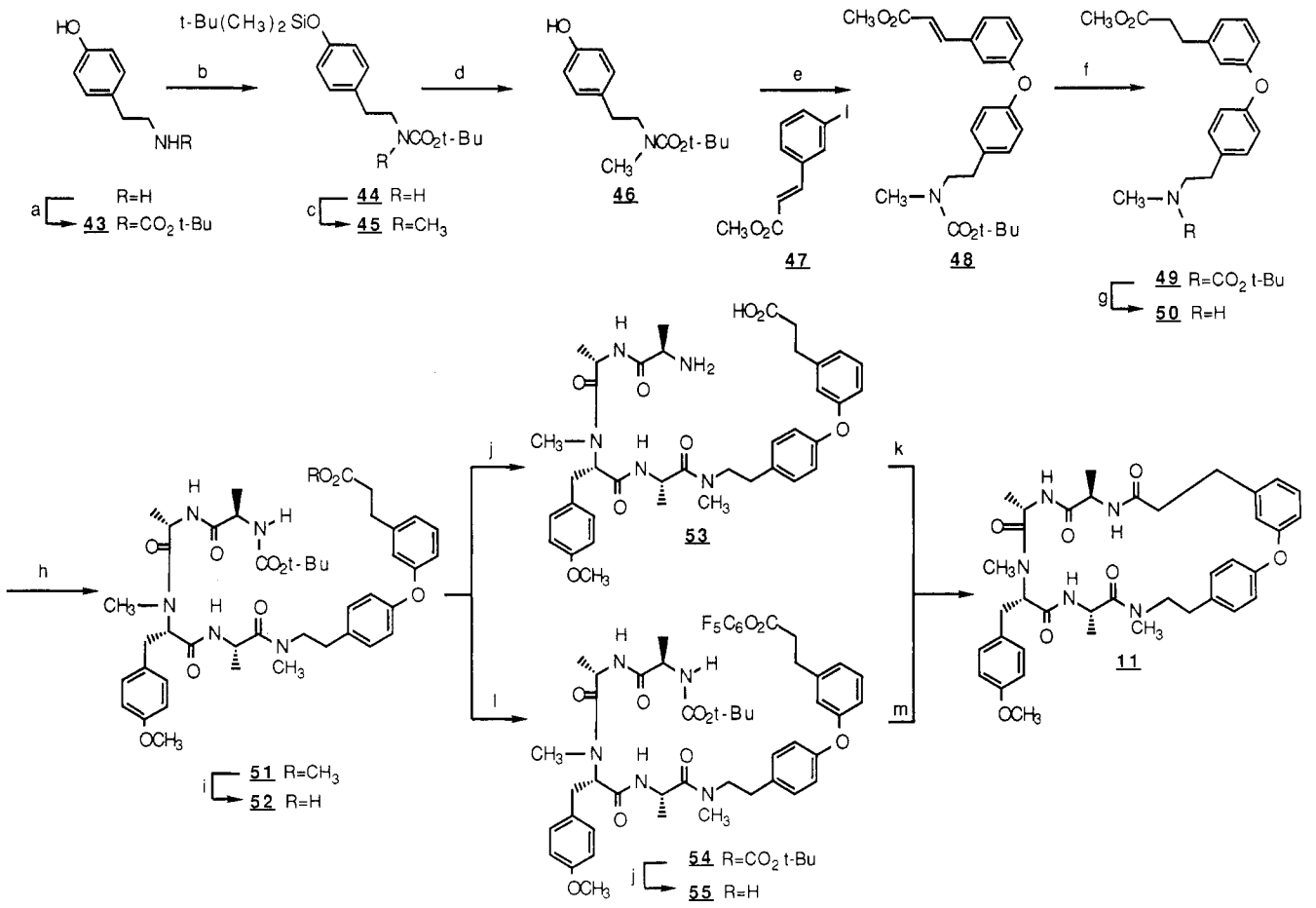
closure observed in the formation of **10** may not be applicable to the anticipated work with bouvardin and deoxybouvardin. Consequently, we elected to examine the 18-membered cyclic hexapeptide closure conducted at the *N*-terminus D-amino acid site (D-Ala[1]/L-Tyr[6]) in the formation of *O-seco*-deoxybouvardin (**9**) for direct comparison.

The preparation of L-*N*-Boc-*N*-Me-Tyr-*N*-Me-Tyr-OCH_3 (**36**) is detailed in Scheme III and complements the efforts of Bates and co-workers.[9a] *N*-Methylation of L-*N*-Boc-*O-tert*-butyldimethylsilyltyrosine methyl ester following a modified and improved Coggins–Benoiton procedure[10,19] and subsequent *O*-desilylation provided L-*N*-Boc-*N*-methyltyrosine methyl ester (**33**). 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide (EDCI) promoted coupling of L-*N*-methyltyrosine methyl ester (**34**) with L-*N*-Boc-*N*-methyltyrosine (**35**), both derived from **33**, provided **36**, Scheme III.

Coupling of the tetrapeptide **13b** with *N*-Me-Tyr-*N*-Me-Tyr-OCH_3 (**37**) provided the linear hexapeptide **38**. As previously observed, competitive diketopiperazine formation was not detected and may be attributed to the

(19) A ratio of 98.5:1.5 L:D-**33** was determined by chiral-phase HPLC analysis.

Scheme IV[a]



[a] (a) 1.0 equiv of $(t\text{-BuOCO})_2O$, THF, 25 °C, 2.5 h, 97%; (b) 1.2 equiv of TBDMSCl, 2.5 equiv of imidazole, DMF, 25 °C, 8 h, 98%; (c) 1.1 equiv of NaH, 3.0 equiv of CH_3I, THF/DMF (10:1), 80 °C, 18 h, 97%; (d) AcOH/THF/H_2O (3:2:1), 25 °C, 8 h, 87%; (e) 1.1 equiv of NaH, 1.0 equiv of CuBr, 2.0 equiv of **47**, pyridine, 115 °C, 12 h, 44%; (f) 0.1 wt equiv of 10% Pd–C, 3 atm H_2, MeOH, 25 °C, 12 h, 98%; (g) TFA/CH_2Cl_2 (1:1), 25 °C, 1.5 h, 94%; (h) 1.0 equiv of **13b**, 1.0 equiv of EDCI, CH_2Cl_2, 25 °C, 24 h, 65%; (i) 3.0 equiv of LiOH, THF/MeOH/H_2O (3:1:1), 35 °C, 6 h, 82%; (j) TFA/CH_2Cl_2 (1:1), 25 °C, 2 h; (k) 1.3 equiv of DPPA, DMF, 0.008 M in **53**, pH 7 ($NaHCO_3$), 0 °C, 72 h, 61% overall from **52**; (l) 1.1 equiv of C_6F_5OH, 1.0 equiv of EDCI, CH_2Cl_2, 25 °C, 24 h, 76%; (m) **55** (DMF) addition (2–3 h) to pyridine, 0.0003 M, 90 °C, 8 h, 49% overall from **54**.

^{3}Tyr(OCH_3) *N*-methylation. The linear hexapeptide **38** was subjected to the two sets of cyclization conditions employed in the preparation of cyclic hexapeptide **10**. Consistent with expectations, closure of the linear hexapeptide as its free amino acid **40** in a reaction effected by DPPA employing the improved ($NaHCO_3$)[17b] conditions for closure at near normal solution-phase concentrations (0.008 M substrate) provided the cyclic 18-membered hexapeptide **9**, Scheme III and Table I. In addition, formation of the pentafluorophenyl ester **41** and subsequent cyclization of the liberated (TFA, 25 °C, 0.5 h) free amine of the linear hexapeptide active ester **42** employing conventional solution-phase, high dilution[16,18] reaction conditions provided *O-seco*-deoxybouvardin (**9**) identical in all respects with the samples of **9** prepared by the DPPA-promoted cyclization and identical in comparable respects with authentic, synthetic material.[20]

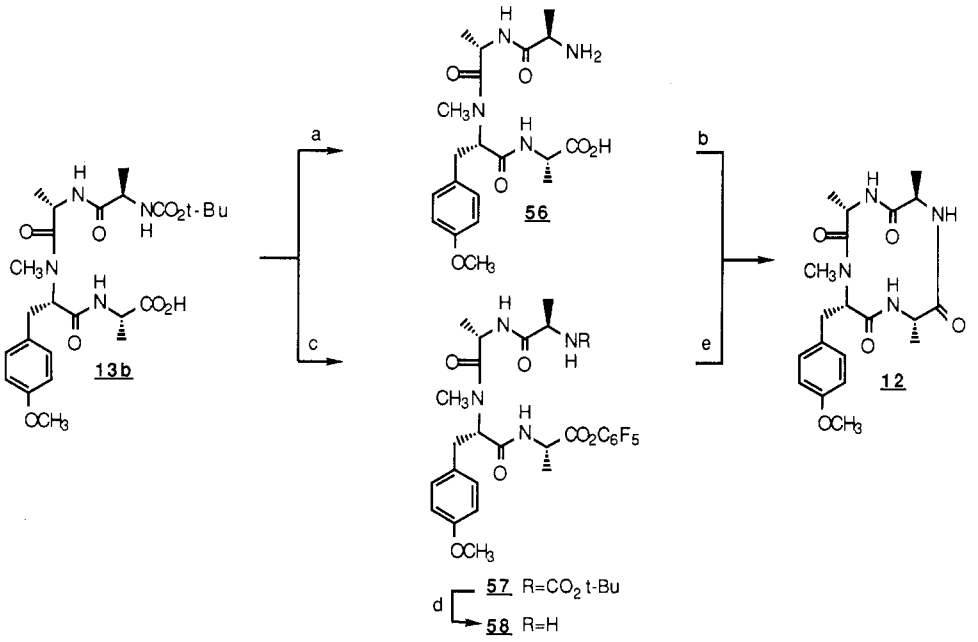
Preparation of *cyclo*-(D-Ala-Ala-*N*-Me-Tyr-(OCH_3)-Ala-*N*-Me-CH_2CH_2(*p*-C_6H_4)-*O*-(*m*-C_6H_4)-$CH_2CH_2C(O)$). 26-Membered Cyclic Peptide Cyclization Studies. In order to test the feasibility and facility for 26-membered cyclic peptide formation (eq 1, path b), the readily accessible, linear peptide **51** was examined. The preparation of **50**, [CH_3O-C(O)CH_2CH_2(*m*-C_6H_4)-*O*-(*p*-C_6H_4)$CH_2CH_2NHCH_3$], the simplified diaryl ether required as the coupling component necessary to examine cyclization of **51** with 26-membered cyclic peptide formation is detailed in Scheme IV. *N*-Methylation of *N*-Boc-*O*-(*tert*-butyldimethylsilyl)tyramine (**44**) employing a modified Coggins–Benoiton procedure[10] and subsequent *O*-desilylation provided *N*-Boc-*N*-methyltyramine (**46**). Copper(I)-promoted coupling of **46** with methyl *m*-iodocinnamate (**47**) under conditions optimized for diaryl ether formation[21] provided the diaryl ether **48**. Subjection of **48** to the conditions of catalytic hydrogenation provided the required diaryl ether **49**. Removal of the *tert*-butyloxycarbonyl protecting group (TFA/CH_2Cl_2, 1:1, 25 °C, 1.5 h, 94%) provided the diaryl ether free *N*-methyl amine **50**.

Coupling of the tetrapeptide **13b** with the *N*-methyl amine **50** provided the linear peptide **51**. The linear peptide **51** was subjected to the two sets of cyclization conditions employed in the preparation of the cyclic peptides **9** and **10**. Consistent with expectations, DPPA-promoted closure of the linear peptide as its free amino acid **53** employing the improved ($NaHCO_3$)[17b] conditions for closure effected at near normal solution-phase concentrations (0.008 M substrate) provided the cyclic 26-

(20) A sample of authentic,[9a] synthetic material was not available for direct comparison. The reported[9a] ^{1}H NMR and reported mp (280–290 °C)[9a] compare favorably with synthetic **9** (mp 290–292 °C).

(21) Whitesides, G. M.; Sadowski, J. S.; Lilburn, J. *J. Am. Chem. Soc.* **1974**, *96*, 2829.

Scheme V[a]



[a] (a) TFA/CH_2Cl_2 (1:1), 25 °C, 1.5 h, 84%; (b) 1.3 equiv of DPPA, DMF, 0.008 M in **56**, pH 7 ($NaHCO_3$), 0 °C, 72 h, 68%; (c) 1.0 equiv of C_6F_5OH, 1.0 equiv of EDCI, CH_2Cl_2, 25 °C, 24 h, 67%; (d) TFA/CH_2Cl_2 (1:1), 25 °C, 1.2 h; (e) **58** (DMF) addition (8 h) to pyridine, 0.0003 M, 90 °C, 8 h, 51% overall from **57**.

membered peptide (**11**), Scheme IV and Table I. In addition, formation of the pentafluorophenyl ester **54** and subsequent cyclization of the liberated (TFA, CH_2Cl_2, 25 °C, 2 h) free amine of the linear hexapeptide active ester **55** employing conventional, solution-phase, high dilution reaction conditions[16,18] provided the cyclic peptide **11** identical in all respects with the sample of **11** prepared in the diphenylphosphoroazidate-promoted cyclization.

Preparation of *cyclo*-(D-Ala-Ala-Tyr(OCH_3)-Ala). 12-Membered Cyclic Tetrapeptide Cyclization. The linear tetrapeptide **13** was subjected to the two sets of cyclization conditions employed in the preparation of the cyclic peptides **9–11**. Diphenylphosphoroazidate-promoted closure of the linear tetrapeptide as its free amino acid **56** employing the improved ($NaHCO_3$)[17b] conditions for closure effected at near normal solution-phase concentrations (0.008 M substrate) provided the cyclic 12-membered tetrapeptide **12**, Scheme V and Table I. Alternatively, formation of the pentafluorophenyl ester **57** and subsequent cyclization of the liberated (TFA, CH_2Cl_2, 25 °C, 1.2 h) free amine of the linear tetrapeptide active ester **58** under high dilution, solution-phase reaction conditions[16,18] provided the cyclic tetrapeptide **12** identical in all respects with the sample of **12** prepared by the diphenylphosphoroazidate-promoted cyclization.

In Vitro Cytotoxic Activity. The cyclic peptides **9–12** were subjected to comparative evaluation for in vitro cytotoxic activity[22] by employing four cell culture assays: B16 (mouse melanoma),[23,24] L-1210 (mouse lymphocytic leukemia),[23] 9PS (P388 mouse leukemia),[25] and 9KB (human epidermoid carcinoma of the nasopharynx).[25] The results, inhibitory concentration for 50% cell growth relative to untreated controls (IC_{50}, μg/mL), are detailed in Table II.[22] Consistent with the observations reported by Bates and co-workers[9a] *O-seco*-deoxybouvardin (**9**) lacked detectable, observable cytotoxic activity, confirming the apparent requirement for the bouvardin/deoxybouvardin cyclic 14-membered dipeptide diaryl ether linkage. Consistent with this observation, the cyclic hexapeptide **10** [*cyclo*-(D-Ala-Ala-*N*-Me-Tyr(OCH_3)-Ala-*N*-Me-Gly-*N*-Me-Gly)] lacking the 14-membered para- and metacyclophane segment of deoxybouvardin lacked observable cytotoxic activity. In contrast, the 26-membered cyclic peptide **11** [*cyclo*-(D-Ala-Ala-*N*-Me-Tyr(OCH_3)-Ala-N(CH_3)-CH_2CH_2(*p*-C_6H_4)-O-(*m*-C_6H_4)CH_2CH_2C(O)] possessing the intact monocyclic skeleton of bouvardin/deoxybouvardin as well as the 12-membered cyclic peptide **12** possessing only the D-Ala-Ala-*N*-Me-Tyr(OCH_3)-Ala segment of bouvardin/deoxybouvardin exhibited observable, albeit marginal, cytotoxic activity. The comparative cytotoxic properties of **11** and **12**, the inactivity of **9** and **10**, coupled with reports of the successful substantial functional group modifications of the 14-membered cyclophane dipeptide segment of the naturally occurring materials with full maintenance of the cytotoxic/antitumor properties suggest that the bouvardin/deoxybouvardin 14-membered cyclic dipeptide unit potentiates the cytotoxic and antitumor properties of the D-Ala-Ala-*N*-Me-Tyr(OCH_3)-Ala segment of the naturally occurring bicyclic hexapeptides. Such observations are consistent with the potential that the

Table II. In Vitro Cytotoxic Activity (IC_{50}, μg/mL)[22]

	9PS(P388)[25]	9KB[25]	L-1210[23]	B16[23,24]
9	>100	>100	>20	>20
10	>100	>100	>20	>20
11	5	47	>20	>20
12	13	41	>20	>20

(22) IC_{50} (μg/mL, Inhibitory Concentration for 50% cell growth relative to untreated control) values for B16 mouse melanoma and L-1210 mouse leukemia cell culture (ATCC CCL-219) were determined by Professor Paul Kitos, Department of Biochemistry, University of Kansas, Lawrence, Ks 66045-2500, by employing a previously detailed procedure.[23,24] IC_{50} (μg/mL) values for P388 (9PS) mouse leukemia and 9KB cell culture were determined under the supervision of Linda Jacobsen, Purdue Cancer Center Cell Culture Lab, Purdue University, following the protocols established by the National Institutes of Health, National Cancer Institute.[25]

(23) Boger, D. L.; Mitscher, L. A.; Mullican, M. D.; Drake, S. D.; Kitos, P. A. *J. Med. Chem.* **1985**, *28*, 1543.

(24) Donoso, J. A.; Himes, R. H. *Cancer Biochem. Biophys.* **1984**, *7*, 133.

(25) Geran, R. I.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep.* **1972**, *3* (2), 17–20, 59–61.

isodityrosine-derived 14-membered para- and meta-cyclophane provides maintenance of an active, otherwise inaccessible conformation of this segment of the naturally occurring antitumor antibiotics.

Experimental Section

Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded on Varian FT80, Varian XL-200, General Electric QE-300, and Nicolet NT-470 spectrophotometers and chemical shifts are reported in parts per million relative to internal tetramethylsilane (0.00 ppm). Infrared spectra (IR) were recorded on a Perkin-Elmer 1420 spectrometer and a Perkin-Elmer 1710 Fourier transform spectrometer. Melting points (mp) were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. Electron impact mass spectra (EIMS) and chemical ionization mass spectra (CIMS) were recorded on a Finnigan 4000 spectrometer. High-resolution mass spectra (HRMS) and fast atom bombardment mass spectra (FABMS) were recorded on a Kratos MS-50 spectrometer. Flash chromatography[26a] was performed on 230–400-mesh silica gel. Preparative centrifugal thin-layer chromatography (PCTLC)[26b] was performed on a Harrison Model 7924 Chromatotron, using Merck silica gel 60 PF_{154} containing $CaSO_4 \cdot {}^1/_2H_2O$ binder. Chiral-phase HPLC analysis was performed on a Gilson Model 320 dual pump chromatograph equipped with an ISCO V^4 variable wavelength absorbance detector (254 nm) employing a J. T. Baker Baker Bond DNBPG (covalent) chiral column. Reverse-phase HPLC analysis was performed on the same system employing a Whatman Partisil PXS 10/25 ODS-2 reverse-phase column. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl. Methanol (MeOH) was distilled from magnesium methoxide. Methylene chloride (CH_2Cl_2) was distilled from phosphorus pentoxide. Pyridine was distilled from barium oxide. Dimethylformamide (DMF) and triethylamine (Et_3N) were distilled from calcium hydride and stored over KOH pellets. All extraction and chromatographic solvents [ethyl acetate (EtOAc), hexane, and methylene chloride (CH_2Cl_2)] were distilled prior to use. Di-*tert*-butyl dicarbonate [$(BOC)_2O$], diphenylphosphoroazidate (DPPA), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI), dicyclohexylcarbodiimide (DCC), pentafluorophenol, glycine methyl ester hydrochloride, tyramine, L-tyrosine, L-alanine, and D-alanine hydrochloride were obtained from the Aldrich Chemical Company. 1-Hydroxybenzotriazole (HOBT) was obtained from the Pierce Chemical Company. All reactions requiring anhydrous conditions were performed under an atmosphere of nitrogen (N_2) or argon.

Boc-*N*-Me-Tyr(OMe)-OH (14). A solution of Boc-Tyr-OH (5.30 g, 18.8 mmol) and methyl iodide (2.57 mL, 41.4 mmol, 2.2 equiv) in 80 mL of THF was cooled to 0 °C and sodium hydride (50% oil dispersion, 2.97 g, 62.0 mmol, 3.3 equiv) was added. The resulting reaction mixture was stirred at 0 °C (1 h) and then at 25 °C (16 h). The excess sodium hydride was quenched by the dropwise addition of 10 mL of THF/H_2O (1:1) and the solvents were removed in vacuo. The residue was diluted with 30 mL of water and washed with pentane (2 × 30 mL). The aqueous phase made acidic with solid citric acid (pH 2) and was extracted with EtOAc (3 × 40 mL). The combined extracts were washed with saturated aqueous NaCl, dried ($MgSO_4$), and concentrated in vacuo. Short column chromatography (SiO_2, 5 × 25 cm, Et_2O) afforded Boc-*N*-Me-Tyr(OMe)[9a] (**14**, 5.34 g, 5.84 g theoretical yield, 90%) as a yellow oil. **14**: $[\alpha]^{22}_D$ −16.9° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 300 MHz, ppm) 7.18 and 7.12 (two d, 2 H, *J* = 9 Hz, Tyr C2-H and C6-H), 6.85 (d, 2 H, *J* = 9 Hz, Tyr C3-H and C5-H), 4.58 (two t, 1 H, *J* = 5 Hz, CH_2CHN), 3.80 (s, 3 H, OCH_3), 3.24 and 3.13 (two dd, 1 H each, *J* = 15, 5 Hz, $CHHCHN$ and $CHHCHN$), 2.76 and 2.68 (two s, 3 H, NCH_3), 1.43 and 1.38 (two s, 9 H, *t*-Boc CH_3); IR (neat) ν_{max} 2976, 2934, 1741, 1698, 1613, 1585, 1514, 1456, 1393, 1368, 1330, 1301, 1249, 1177, 1110, 1074, 1036, 963, 863, 818, 765 cm^{-1}; CIMS (isobutane), *m/e* (relative intensity) 310 (M^+ + H, 2), 254 (54), 210 (base); HRMS, *m/e* 309.1572 ($C_{16}H_{23}NO_5$ requires 309.1576). Chiral-phase HPLC analysis revealed a 99:1 ratio of L:D-**14**: t_R 28 min/32 min, 2.0 mL/min, 10% 2-propanol–hexane.

Boc-*N*-Me-Tyr(OMe)-Ala-OMe (16). A steady stream of ammonia gas was passed through a suspension of the hydrochloride salt of alanine methyl ester (**15**, 4.86 g, 34.9 mmol) in 60 mL of CH_2Cl_2 at 25 °C for 2–5 min. The precipitated ammonium chloride was collected by filtration and the filtrate was added to a solution of **14** (10.8 g, 34.9 mmol, 1.0 equiv), DCC (7.18 g, 34.9 mmol, 1.0 equiv), and HOBT (533 mg, 3.49 mmol, 0.1 equiv) in 30 mL of CH_2Cl_2 at 0 °C. The reaction mixture was allowed to warm to 25 °C and was stirred for 24 h. The reaction mixture was filtered through Celite (CH_2Cl_2) and concentrated in vacuo. Short column chromatography (SiO_2, 5 × 20 cm, Et_2O) afforded **16** (10.9 g, 14.3 g theoretical yield, 76%) as a yellow oil: $[\alpha]^{22}_D$ −17.4° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 300 MHz, ppm) 7.15 and 7.09 (two d, 2 H, *J* = 9 Hz, Tyr C2-H and C6-H), 6.84 (d, 2 H, *J* = 9 Hz, Tyr C3-H and C5-H), 4.24 (m, 2 H, Tyr $^\alpha$CH and Ala $^\alpha$CH), 3.82 (s, 3 H, Tyr(OCH_3)), 3.80 (br s, 3 H, CO_2CH_3), 2.80 (m, 2 H, Tyr $^\beta CH_2$), 1.40 (two s, 9 H, *t*-Boc CH_3); IR (neat) ν_{max} 3855, 3753, 3714, 3678, 3631, 3355, 2932, 2855, 1737, 1701, 1612, 1584, 1514, 1452, 1390, 1367, 1301, 1248, 1152, 1108, 1036, 803, 772 cm^{-1}; CIMS (NH_3), *m/e* (relative intensity), 395 (M^+ + H, 10), 263 (98), 164 (base); CIHRMS, *m/e* 395.4575 ($C_{20}H_{30}N_2O_6$ requires 395.4360). Reverse-phase HPLC analysis: >98%, t_R 21 min, 2.0 mL/min, 0–16% methanol–water gradient elution (0.6%/min).

H-*N*-Me-Tyr(OMe)-Ala-OMe (17). A solution of **16** (7.70 g, 18.7 mmol) in 50 mL of trifluoroacetic acid was stirred for 30 min (25 °C). The volatiles were removed in vacuo. The residue was dissolved in 10 mL of CH_2Cl_2 and the solution was poured onto 200 mL of 0.10 N HCl. The CH_2Cl_2 layer was separated and the aqueous phase was extracted with 10 mL of CH_2Cl_2. The aqueous layer was made basic (pH 10) with the addition of solid K_2CO_3 and was extracted with CH_2Cl_2 (3 × 75 mL). The combined extracts were dried ($MgSO_4$) and concentrated in vacuo. Short column chromatography (SiO_2, 7 × 25 cm, 0–10% MeOH–CH_2Cl_2 gradient elution) afforded **17** (4.78 g, 5.82 g theoretical yield, 82%) as a yellow oil which solidified upon standing: mp 99–100 °C (methanol, fine white needles); $[\alpha]^{22}_D$ −24.6° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 300 MHz, ppm) 7.07 (d, 2 H, *J* = 9 Hz, Tyr C2-H and C6-H), 6.87 (d, 2 H, *J* = 9 Hz, Tyr C3-H and C5-H), 5.74 (br s, 1 H, Ala NH), 4.20 (t, 1 H, *J* = 8 Hz, Ala $^\alpha$CH), 3.92 (q, 1 H, *J* = 8.4 Hz, Tyr $^\alpha$CH), 3.82 and 3.75 (two s, 3 H, CO_2CH_3), 3.79 (s, 3 H, Tyr(OCH_3)), 3.32 and 3.14 (two dd, 1 H each, *J* = 16, 4 Hz, Tyr $^\beta CH_2$), 3.09 (s, 3 H, NCH_3), 0.58 (d, 3 H, *J* = 7 Hz, Ala $^\beta CH_3$); IR (KBr) ν_{max} 3490, 3278, 2936, 2270, 1742, 1679, 1618, 1511, 1475, 1449, 1402, 1333, 1300, 1244, 1182, 1163, 1112, 1053, 1025, 887, 837, 818, 790, 758, 735 cm^{-1}; CIMS (isobutane), *m/e* (relative intensity) 295 (M^+ + H, 9), 263 (base); CIHRMS, *m/e* 295.1650 ($C_{15}H_{22}N_2O_4$ requires 295.1658).

Boc-D-Ala-Ala-OMe (19):[27] $[\alpha]^{22}_D$ −12.4° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 300 MHz, ppm) 6.69 (br d, 1 H, *J* = 5 Hz, Ala NH), 4.94 (br d, 1 H, *J* = 5 Hz, D-Ala NH), 4.57 (apparent p, 1 H, *J* = 7 Hz, D-Ala $^\alpha$CH), 4.19 (apparent p, 1 H, *J* = 7 Hz, Ala $^\alpha$CH), 3.74 (s, 3 H, OCH_3), 1.45 (s, 9 H, *t*-Boc CH_3), 1.40 (d, 3 H, *J* = 7 Hz, Ala $^\beta CH_3$), 1.35 (d, 3 H, *J* = 7 Hz, D-Ala $^\beta CH_3$); IR (neat) ν_{max} 3855, 3321, 2980, 1742, 1690, 1670, 1518, 1454, 1367, 1292, 1248, 1214, 1165, 1100, 1056, 1022, 984, 952, 861, 759 cm^{-1}; CIMS (NH_3), *m/e* (relative intensity) 275 (M^+ + H, 8), 168 (base); CIHRMS, *m/e* 275.1599 ($C_{12}H_{22}N_2O_5$ requires 275.1607). Reverse-phase HPLC: 98.6%, t_R 20 min, 2.0 mL/min, 0–16% methanol–water gradient elution (0.5%/min).

Boc-D-Ala-Ala-OH (20). Lithium hydroxide monohydrate (2.53 g, 60.6 mmol, 3.0 equiv) was added to a solution of **19** (5.80 g, 20.1 mmol) in 50 mL of THF/MeOH/H_2O (3:1:1) at 25 °C. The reaction mixture was stirred for 3 h (25 °C). The reaction mixture was diluted with water (20 mL) and extracted with EtOAc (20 mL). The aqueous phase was poured onto 10% aqueous HCl (50 mL) and was extracted with EtOAc (3 × 30 mL). The combined organic extracts were washed with saturated aqueous NaCl, dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 5 × 15 cm, 60% EtOAc–hexane eluant) afforded **20** (4.37 g, 5.57 g theoretical yield, 78%) as a colorless viscous oil which solidified on standing: mp 156–157 °C (EtOAc–hexane, colorless cubes); $[\alpha]^{22}_D$ −19.7° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 300 MHz, ppm)

(26) (a) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923. (b) Stahl, E.; Mueller, J. *Chromatographia* **1982**, *15*, 493.

(27) Ariyoshi, Y. *Bull. Chem. Soc. Jpn.* **1984**, *57*, 3197.

7.01 (br s, 1 H, Ala NH), 5.18 (br s, 1 H, D-Ala NH), 4.60 (m, 1 H, D-Ala $^{\alpha}$CH), 4.44 (m, 1 H, Ala $^{\alpha}$CH), 1.48 (d, 3 H, J = 7 Hz, Ala $^{\beta}CH_3$), 1.47 (s, 9 H, t-Boc CH_3), 1.40 (d, 3 H, J = 7 Hz, D-Ala $^{\beta}CH_3$); IR (KBr) ν_{max} 3855, 3840, 3678, 3344, 3049, 2977, 2934, 2517, 2025, 1680, 1535, 1456, 1389, 1368, 1336, 1310, 1253, 1226, 1167, 1120, 1073, 1040, 1022, 957, 931, 864, 837, 786, 753 cm^{-1}; CIMS (isobutane), m/e (relative intensity) 261 (M^+ + H, 9), 205 (base), 161 (11); CIHRMS, m/e 261.1439 ($C_{11}H_{20}N_2O_5$ requires 261.1450).

Boc-D-Ala-Ala-*N*-Me-Tyr(OMe)-Ala-OMe (13a). A solution of **20** (4.64 g, 15.8 mmol) and DCC (3.17 g, 15.8 mmol, 1.0 equiv) in 90 mL of CH_2Cl_2 at 0 °C was treated with **17** (4.78 g, 15.8 mmol, 1.0 equiv) and HOBT (236 mg, 1.58 mmol, 0.1 equiv) in 20 mL of CH_2Cl_2, and the resulting reaction mixture was stirred for 36 h (0 °C). The reaction mixture was filtered through Celite and the filtrate was concentrated in vacuo. Flash chromatography (SiO_2, 5 × 25 cm, 80–100% EtOAc–hexane gradient elution) afforded **13a** (6.25 g, 8.73 g theoretical yield, 71%) as a clear, crystalline solid:[9a,c] mp 142–143 °C (MeOH, colorless cubes); $[\alpha]^{22}_D$ −44.2° (c 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm) 8.28 (d, 1 H, J = 8 Hz, NH), 7.16 and 7.10 (two d, 2 H, J = 9 Hz, Tyr C2-H and C6-H), 6.87 and 6.85 (two d, 2 H, J = 9 Hz, Tyr C3-H and C6-H), 6.60 (d, 1 H, J = 8 Hz, NH), 5.09 (two d, 1 H, J = 8 Hz, t-Boc NH), 4.80 (t, 1 H, J = 7 Hz, $^{\alpha}$CH), 4.52 (t, 1 H, J = 7 Hz, $^{\alpha}$CH), 4.34 (t, 1 H, J = 7 Hz, $^{\alpha}$CH), 4.18 (t, 1 H, J = 7 Hz, $^{\alpha}$CH), 3.80 and 3.76 (two s, 3 H, CO_2CH_3), 3.78 (s, 3 H, Tyr-(OCH_3)), 3.10 (m, 2 H, Tyr $^{\beta}CH_2$), 2.95 and 2.88 (two s, 3 H, NCH_3), 1.45 and 1.43 (two s, 9 H, t-Boc CH_3), 1.37 and 1.35 (two d, 3 H, J = 7 Hz, Ala $^{\beta}CH_3$), 1.29 and 1.27 (two d, 3 H, J = 7 Hz, Ala $^{\beta}CH_3$), 0.46 (d, 3 H, J = 7 Hz, Ala $^{\beta}CH_3$); IR (KBr) ν_{max} 3855, 3290, 3064, 2980, 2935, 1746, 1714, 1654, 1514, 1454, 1411, 1367, 1302, 1249, 1174, 1089, 1068, 1033, 858, 825, 806, 783, 738 cm^{-1}; CIMS (isobutane), m/e (relative intensity) 537 (M^+ + H, 4), 434 (base), 378 (13). Reverse-phase HPLC: >98%, t_R 17 min, 2.0 mL/min, 0–10% methanol–water gradient elution (0.5%/min).

Boc-D-Ala-Ala-*N*-Me-Tyr(OMe)-Ala-OH (13b). Lithium hydroxide monohydrate (88 mg, 2.07 mmol, 3.0 equiv) was added to a solution of **13a** (373 mg, 0.69 mmol) in 3 mL of THF/MeOH/H_2O (3:1:1) at 25 °C, and the resulting reaction mixture was stirred for 3 h (25 °C). The reaction solution was poured onto water (3 mL) and extracted with EtOAc (1 mL). The aqueous phase was poured onto 10% aqueous HCl (3 mL) and extracted with EtOAc (3 × 3 mL). The combined aqueous acid extracts were washed with saturated aqueous NaCl, dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 2 × 10 cm, 2–5% MeOH–CH_2Cl_2 gradient elution) afforded **13b** (298 mg, 363 mg theoretical yield, 82%) as a white solid: mp 159–160 °C (EtOH, white plates); $[\alpha]^{22}_D$ −42.2° (c 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm) 7.11 (d, 2 H, J = 9 Hz, Tyr C2-H and C6-H), 6.86 (d, 2 H, J = 9 Hz, Tyr C3-H and C5-H), 4.55 (t, 1 H, J = 7 Hz, $^{\alpha}$CH), 3.80 (s, 3 H, OCH_3), 3.28 (br d, 2 H, Tyr $^{\beta}CH_2$), 2.96 and 2.92 (two s, 3 H, NCH_3), 2.09 (d, 3 H, Ala $^{\beta}CH_3$), 1.42 (br s, 9 H, t-Boc CH_3), 1.30 (d, 3 H, Ala $^{\beta}CH_3$), 0.52 (br s, 3 H, Ala $^{\beta}CH_3$); IR (KBr) ν_{max} 3855, 3296, 2980, 2936, 1718, 1654, 1514, 1457, 1393, 1368, 1301, 1249, 1176, 1104, 1034, 825, 738 cm^{-1}; CIMS (isobutane), m/e (relative intensity) 523 (M^+ + H, 3), 479 (4), 434 (23), 323 (29), 316 (base).

Boc-*N*-Me-Gly-*N*-Me-Gly-OMe (24). A solution of **22**[28] (772 mg, 4.09 mmol) in 1–2 mL of CH_2Cl_2 at 0 °C was treated with DCC (841 mg, 4.09 mmol, 1.0 equiv) and **23**·CF_3CO_2H (892 mg, 4.09 mmol, 1.0 equiv). The resulting reaction mixture was stirred at 25 °C (48 h), filtered through Celite (CH_2Cl_2), and concentrated in vacuo. Flash chromatography (SiO_2, 2 × 15 cm, 25% EtOAc–hexane eluant) afforded **24**[28] (762 mg, 1.12 g theoretical yield, 68%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz, ppm) 3.99 (s, 2 H, CH_2), 3.90 (s, 2 H, CH_2), 3.74 (s, 3 H, OCH_3), 2.93 (s, 3 H, NCH_3), 2.92 (s, 3 H, NCH_3), 1.47 and 1.42 (two s, 9 H, t-Boc CH_3); IR (neat) ν_{max} 2979, 2935, 1756, 1702, 1666, 1559, 1484, 1455, 1395, 1369, 1302, 1250, 1154, 1062, 973, 873, 777, 633 cm^{-1}. Reverse-phase HPLC: 97.2%, t_R 11 min, 2.0 mL/min, 0–8% methanol–water gradient elution (0.5%/min).

Boc-D-Ala-Ala-*N*-Me-Tyr(OMe)-*N*-Me-Gly-*N*-Me-Gly-OMe (26). A solution of **25** (61 mg, 0.37 mmol) prepared from **24** (TFA/CH_2Cl_2, 1:1, 25 °C, 0.5 h) in 1 mL of CH_2Cl_2 was added to a solution of **13b** (193 mg, 0.37 mmol, 1.0 equiv), DCC (76 mg, 0.37 mmol, 1.0 equiv), and HOBT (6 mg, 0.04 mmol, 0.1 equiv) in 2 mL of CH_2Cl_2 at 0 °C. The resulting reaction mixture was allowed to warm to 25 °C and was stirred for 24 h. The reaction mixture was filtered through Celite (CH_2Cl_2) and concentrated in vacuo. Flash chromatography (SiO_2, 2 × 20 cm, 1–5% MeOH/CH_2Cl_2 gradient elution) afforded **26** (150 mg, 246 mg theoretical yield, 64%) as a yellow oil: $[\alpha]^{22}_D$ −28.7° (c 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm) 7.16 and 7.10 (two d, 2 H, J = 9 Hz, Tyr C2-H and C6-H), 6.88 and 6.84 (two d, 2 H, J = 9 Hz, Tyr C3-H and C5-H), 3.77 and 3.73 (two s, 3 H, CO_2CH_3), 3.80 (s, 3 H, Tyr(OCH_3), 3.19 and 3.16 (two s, 3 H, NCH_3), 3.16 and 3.12 (two s, 3 H, NCH_3), 2.98 and 2.92 (two s, 3 H, NCH_3), 1.45 and 1.44 (two s, 9 H, t-Boc CH_3), 1.47 (d, 3 H, J = 7 Hz, Ala $^{\beta}CH_3$), 1.36 (d, 3 H, J = 7 Hz, Ala $^{\beta}CH_3$); IR (neat) ν_{max} 3753, 3678, 3652, 3631, 3302, 2979, 2934, 2281, 1752, 1712, 1648, 1514, 1451, 1411, 1367, 1301, 1249, 1214, 1177, 1107, 1033, 858, 824 cm^{-1}; EIMS, m/e (relative intensity) 608 (1), 508 (3), 434 (6), 334 (19), 204 (31), 161 (34), 121 (25), 104 (27), 44 (base); CIMS (isobutane), m/e (relative intensity) 608 (39), 508 (93), 434 (85), 334 (23), 290 (18), 225 (base). Reverse-phase HPLC: >99%, t_R 24 min, 2.0 mL/min, 0–16% methanol–water gradient elution (0.5%/min).

Boc-D-Ala-Ala-*N*-Me-Tyr(OMe)-Ala-*N*-Me-Gly-*N*-Me-Gly-OH (27). A solution of **26** (108 mg, 0.16 mmol) in 2 mL of THF/MeOH/H_2O (3:1:1) at 25 °C was treated with lithium hydroxide monohydrate (21 mg, 0.48 mmol, 3.0 equiv), and the reaction mixture was stirred for 3 h (25 °C). The reaction mixture was poured onto 2 mL of 10% aqueous HCl and extracted with CH_2Cl_2 (3 × 6 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo. Flash chromatography (SiO_2, 2 × 15 cm, 2–5% MeOH–CH_2Cl_2) afforded **27** (90 mg, 104 mg theoretical yield, 86%) as a white solid: mp 187–189 °C; $[\alpha]^{22}_D$ −21.7° (c 0.9, MeOH); IR (KBr) ν_{max} 3854, 3839, 3802, 3745, 3690, 3676, 3650, 3630, 3301, 2979, 2934, 1717, 1637, 1559, 1541, 1514, 1457, 1418, 1367, 1248, 1176, 1104, 1034 cm^{-1}; EIMS, m/e (relative intensity) 434 (1), 387 (1), 320 (1), 315 (1), 204 (2); CIMS (isobutane), m/e (relative intensity) 650 (1), 629 (1), 612 (1), 594 (1), 566 (1), 550 (2), 449 (8), 388 (45), 342 (39), 225 (base).

***cyclo*-(D-Ala-Ala-*N*-Me-Tyr(OCH_3)-Ala-*N*-Me-Gly-*N*-Me-Gly) (10): Method A.** A solution of **27** (111 mg, 0.17 mmol) in 1 mL of TFA/CH_2Cl_2 (1:1) at 25 °C was stirred for 2 h (25 °C). The volatiles were removed in vacuo to afford the trifluoroacetic acid salt of **28** as a hygroscopic, crystalline solid. For **28**·CF_3CO_2H: $[\alpha]^{22}_D$ −29.0° (c 1.1, MeOH); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm), 7.06 (br d, 2 H, J = 9 Hz, Tyr C2-H and Tyr C6-H), 6.86 and 6.78 (two d, 2 H, J = 9 Hz, Tyr C3-H and Tyr C5-H), 3.70 and 3.78 (two s, 3 H, Tyr (OCH_3)), 3.20 and 3.18 (two s, 3 H, NCH_3), 3.02 and 2.98 (two s, 3 H, NCH_3), 2.90 and 2.88 (two s, 3 H, NCH_3), 1.40–1.20 (m, 9 H, three Ala $^{\beta}CH_3$); IR (neat) ν_{max} 3350, 2934, 1734, 1684, 1653, 1636, 1559, 1541, 1516, 1458, 1419, 1252, 1180, 1037, 799, 723 cm^{-1}.

A solution of the trifluoroacetic acid salt of **28** (112 mg, 0.17 mmol) in 12 mL of DMF was cooled to −20 °C. The pH was adjusted to 7.2 with the addition of triethylamine (estimated by spotting moistened narrow range pHydrion indicator paper). Diphenylphosphoroazidate (diphenylphosphoryl azide, DPPA, 49 μL, 0.22 mmol, 1.3 equiv) was added and the reaction mixture was stirred at −20 °C (48 h) and 0 °C (48 h). The solvent was removed in vacuo and the residue was diluted with water (2 mL) and extracted with EtOAc (3 × 2 mL). The combined organic extracts were washed with water (6 mL) and saturated aqueous NaCl, dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 2 × 20 cm, 2–10% MeOH/CH_2Cl_2 gradient solution) afforded **10** (59 mg, 93 mg theoretical yield, 64%) as a tan solid: mp 175–177 °C (MeOH, yellow needles); $[\alpha]^{22}_D$ −41.2° (c 0.8, MeOH); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm), 7.16 and 7.08 (two d, 2 H, J = 9 Hz, Tyr C2-H and Tyr C6-H), 6.84 (d, 2 H, J = 9 Hz, Tyr C3-H and Tyr C5-H), 3.80 and 3.79 (two s, 3 H, Tyr(OCH_3)), 3.06 and 3.00 (two s, 3 H, NCH_3), 2.98 and 2.92 (two s, 3 H, NCH_3), 2.90 and 2.84 (two s, 3 H, NCH_3), 1.40–1.20 (m, 9 H, three Ala $^{\beta}CH_3$); IR (KBr) ν_{max} 3302, 3059, 2982, 2935, 2362, 2341, 1653, 1584, 1514, 1449, 1410, 1374, 1302, 1248, 1180, 1104, 1035, 956, 823, 733 cm^{-1}; CIMS (isobutane), m/e (relative intensity) 476 (56), 163 (base), 134 (15), 121 (75); FABMS (DMSO:H_2O:glycerol:thioglycerol, 5:5:1:1), m/e 567 (M^+ + Na − H), 545 (M^+ − H). Reverse-phase HPLC: 97%, t_R 20 min, 2.0 mL/min, 0–10%

(28) Mauger, A. B.; Rzeszotarski, W. J.; Ford, R. A. *Org. Magn. Reson.* **1973**, *5*, 231.

methanol–water gradient elution (0.5%/min).

***cyclo*-(D-Ala-Ala-*N*-Me-Tyr(OCH_3)-Ala-*N*-Me-Gly-*N*-Me-Gly) (10): Method B.** A solution of **27** (98 mg, 0.147 mmol) in 1 mL of CH_2Cl_2 was cooled to 0 °C and treated sequentially with EDCI (44 mg, 0.147 mmol, 1.0 equiv) and pentafluorophenol (27 mg, 0.147 mmol, 1.0 equiv). The reaction mixture was allowed to warm to 25 °C and was stirred for 24 h (25 °C). The reaction mixture was diluted with CH_2Cl_2 (4 mL) and washed with water (2 × 5 mL). The CH_2Cl_2 solution was dried ($MgSO_4$) and concentrated in vacuo. Flash chromatography (SiO_2, 2 × 15 cm, 5% MeOH/CH_2Cl_2 eluant) afforded **29** (79 mg, 122 mg theoretical yield, 65%) as a yellow oil: $[\alpha]^{22}_D$ −36.4° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm) 7.12 and 7.08 (two d, 2 H, *J* = 9 Hz, Tyr C2-H and Tyr C6-H), 6.82 (d, 2 H, *J* = 9 Hz, Tyr C3-H and C5-H), 3.80 (s, 3 H, Tyr(OCH_3)), 3.28 (m, 3 H, NCH_3), 2.92 (m, 6 H, two NCH_3), 1.44 and 1.42 (two s, 9 H, *t*-Boc CH_3), 1.38 (d, 3 H, *J* = 7 Hz, Ala $^\beta CH_3$), 1.30 (d, 3 H, *J* = 7 Hz, Ala $^\beta CH_3$); IR (neat) ν_{max} 3313, 2932, 2854, 1793, 1717, 1701, 1684, 1653, 1648, 1559, 1541, 1522, 1458, 1419, 1367, 1249, 1172, 1101, 1027, 1004 cm^{-1}.

A solution of **29** (60 mg, 0.073 mmol) in 1 mL of TFA/CH_2Cl_2 (1:1) at 25 °C was stirred for 2 h (25 °C). Removal of the volatiles in vacuo afforded the trifluoroacetic acid salt of **30** which was used directly in the following reaction. For **30**·CF_3CO_2H: $[\alpha]^{22}_D$ −32.6° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm), 7.08 (d, 2 H, *J* = 9 Hz, Tyr C2-H and C6-H), 6.86 (two d, 2 H, *J* = 9 Hz, Tyr C3-H and C5-H), 3.80 and 3.78 (two s, 3 H, Tyr(OCH_3)).

A solution of **30**·CF_3CO_2H (61 mg, 0.073 mmol) in 1 mL of dry DMF at 25 °C was added dropwise over 4 h (using a motor driven syringe pump) to a warm (90 °C) solution of pyridine (243 mL). The reaction mixture was stirred for an additional 4 h (90 °C). The solvent was removed in vacuo and the residue was dissolved in 3 mL of EtOAc. The EtOAc solution was washed with water (3 × 1 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 2 × 20 cm, 2–10% MeOH/CH_2Cl_2 gradient elution) afforded **10** (26 mg, 40 mg theoretical yield, 51%) as a tan solid identical in all respects with that described above.

Boc-*N*-Me-Tyr-OCH_3 (33). A solution of *N*-*tert*-butoxycarbonyl-L-tyrosine methyl ester[29] (6.70 g, 23.7 mmol) in 5 mL of DMF was added to a solution of *tert*-butyldimethylsilyl chloride (4.08 g, 27.0 mmol, 1.2 equiv) and imidazole (3.60 g, 49.9 mmol, 2.5 equiv) in 30 mL of DMF at 25 °C.[30] The resulting solution was stirred for 6 h (25 °C). The reaction mixture was diluted with 150 mL of EtOAc and the solution was washed with water (2 × 150 mL), dried ($MgSO_4$), and concentrated in vacuo to afford **31** (8.64 g, 9.28 g theoretical yield, 93%) as a yellow oil which was used directly in the following reaction. For **31**: $[\alpha]^{22}_D$ −6.2° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 300 MHz, ppm) 6.98 (d, 2 H, *J* = 9 Hz, C2-H and C6-H), 6.76 (d, 2 H, *J* = 9 Hz, C3-H and C5-H), 4.96 (d, 1 H, *J* = 8 Hz, NH), 4.55 (q, 1 H, *J* = 8 Hz, CH_2CHNH), 3.69 (s, 3 H, OCH_3), 3.04 and 2.96 (two dd, 2 H, *J* = 16, 8 Hz, $CHHCHNH$ and $CHHCHNH$), 1.42 (s, 9 H, *t*-Boc CH_3), 0.97 (s, 9 H, Si-*t*-BuCH_3), 0.18 (s, 6 H, Si$(CH_3)_2$); IR (neat) ν_{max} 3214, 2430, 1748, 1701, 1561, 1472, 1443, 1392, 1378, 1313, 1252, 1160, 970, 873, 776 cm^{-1}; CIMS (isobutane), *m/e* (relative intensity) 410 (M^+ + H, 2), 354 (base), 310 (99); CIHRMS, *m/e* 410.2316 ($C_{21}H_{35}NO_5Si$ requires 410.2363).

Sodium hydride (60% oil dispersion, 960 mg, 24.0 mmol, 1.0 equiv) was carefully added to a solution of methyl iodide (4.48 mL, 72.0 mmol, 3.0 equiv) and **31** (9.80 g, 24.0 mmol) in 100 mL of dry DMF at 0 °C. The reaction mixture was allowed to warm to 25 °C and was stirred 48 h. The reaction mixture was poured onto water (100 mL) and extracted with EtOAc (3 × 100 mL). The combined organic extracts were washed with water (3 × 100 mL) and saturated aqueous NaCl, dried ($MgSO_4$), and concentrated in vacuo. Short column chromatography (SiO_2, 5 × 5 cm, EtOAc) afforded **32** (8.83 g, 10.2 g theoretical yield, 87%) as a clear, viscous oil: $[\alpha]^{22}_D$ −9.2° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 300 MHz, ppm) 7.06 and 7.03 (two d, 2 H, *J* = 9 Hz, C2-H and C6-H), 6.78 and 6.75 (two d, 2 H, *J* = 9 Hz, C3-H and C5-H), 3.74 and 3.73 (two s, 3 H, Tyr(OCH_3)), 2.70 (two br d, 3 H, NCH_3), 1.45, 1.44, 1.40 and 1.36 (four s, 9 H, *t*-Boc CH_3), 1.00 (s, 9 H, Si-*t*-BuCH_3), 0.18 (s, 6 H, Si$(CH_3)_2$). Chiral-phase HPLC: 98.5:1.5 L:D-**32**; t_R 12 min/15 min, 2.0 mL/min, 10% 2-propanol–hexane.

A solution of **32** (8.80 g, 20.8 mmol) in 150 mL of AcOH/THF/H_2O (3:1:1) was stirred for 8 h at 25 °C. The solvents were removed in vacuo and the residue was mixed with 75 mL of saturated aqueous NaCl. Solid K_2CO_3 was carefully added until the solution was basic (pH 10) and the mixture was extracted with EtOAc (3 × 100 mL). The combined organic extracts were washed with saturated aqueous NaCl, dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 5 × 20 cm, 30% EtOAc/hexane eluant) afforded **33** (5.25 g, 6.41 g theoretical yield, 82%) as a white crystalline solid: mp 109–110 °C (EtOAc–hexane, white plates); $[\alpha]^{22}_D$ −7.4° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 300 MHz, ppm) 7.03 and 6.98 (two d, 2 H, *J* = 9 Hz, C2-H and C6-H), 6.74 (d, 2 H, *J* = 9 Hz, C3-H and C5-H), 3.75 and 3.70 (two s, 3 H, Tyr(OCH_3)), 2.73 (s, 3 H, NCH_3), 1.59, 1.42 and 1.38 (three s, 9 H, *t*-Boc CH_3); IR (KBr) ν_{max} 3355, 2976, 2931, 1744, 1671, 1616, 1596, 1560, 1517, 1481, 1439, 1394, 1368, 1340, 1225, 1167, 1104, 1028, 820, 801, 774 cm^{-1}; EIMS, *m/e* (relative intensity), 309 (M^+, 10), 253 (51), 246 (16), 239 (16), 236 (base), 222 (61), 208 (84); CIMS (isobutane), *m/e* (relative intensity) 310 (M^+ + H, 6), 254 (60), 240 (27), 210 (100), 196 (10); CIHRMS, *m/e* 310.1660 ($C_{16}H_{23}NO_5$ requires 310.1654). Chiral-phase HPLC: 98.5:1.5 L:D-**33**; t_R 18 min/23 min, 2.0 mL/min, 10% 2-propanol–hexane.

H-*N*-Me-Tyr-OMe (34). A mixture of TFA/CH_2Cl_2 (1:1; 5 mL) was added to **33** (500 mg, 1.63 mmol) at 25 °C and the reaction mixture was stirred for 1.5 h (25 °C). The volatiles were removed in vacuo and the residue was diluted with 5% aqueous $NaHCO_3$ (5 mL). The aqueous mixture was extracted with EtOAc (3 × 5 mL) and the combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo to afford **34** (264 mg, 339 mg theoretical yield, 78%): mp 109–111 °C (MeOH, yellow needles, lit.[9a] mp 109–111 °C); $[\alpha]^{22}_D$ −9.2° (*c* 1.1, MeOH); ^{1}H NMR ($CDCl_3$, 300 MHz, ppm) 7.05 (d, 2 H, *J* = 9 Hz, C2-H and C6-H), 6.74 (d, 2 H, *J* = 9 Hz, C3-H and C5-H), 3.68 (s, 3 H, Tyr(OCH_3)), 3.42 (t, 1 H, *J* = 8 Hz, CH_2CHN), 2.89 (d, 2 H, *J* = 8 Hz, CH_2CHN), 2.37 (s, 3 H, NCH_3); IR (KBr) ν_{max} 3630, 3300, 2924, 2855, 1727, 1654, 1613, 1595, 1560, 1542, 1516, 1458, 1369, 1256, 1220, 1205, 1172, 1106, 1032, 984, 827 cm^{-1}.

Boc-*N*-Me-Tyr-OH (35). Lithium hydroxide monohydrate (615 mg, 14.6 mmol, 3.0 equiv) was added to a solution of **33** (1.51 g, 4.88 mmol) in 13 mL of THF/MeOH/H_2O (3:1:1) at 25 °C. The reaction mixture was stirred for 1.2 h (25 °C). The reaction solution was extracted with EtOAc (1 × 5 mL) and the aqueous phase was poured onto 10% aqueous HCl (15 mL) and extracted with EtOAc (3 × 10 mL). The combined organic extracts were washed with saturated aqueous NaCl, dried ($MgSO_4$), and concentrated in vacuo. Short column chromatography (SiO_2, 5 × 20 cm, Et_2O) afforded **35** (1.35 g, 1.43 g theoretical yield, 95%) as a white, amorphous solid: mp 140–142 °C (MeOH, white needles, lit.[9a] mp 141–144 °C); $[\alpha]^{22}_D$ −7.0° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 300 MHz, ppm) 7.06 and 7.02 (two d, 2 H, *J* = 9 Hz, C2-H and C6-H), 6.74 (d, 2 H, *J* = 9 Hz, C3-H and C5-H), 2.74 and 2.70 (two s, 3 H, NCH_3), 1.43 and 1.36 (two s, 9 H, *t*-Boc CH_3); IR (KBr) ν_{max} 3854, 3330, 2929, 1718, 1670, 1616, 1559, 1541, 1517, 1475, 1457, 1395, 1369, 1229, 1163, 1104, 1063, 964, 838, 775 cm^{-1}; EIMS *m/e* (relative intensity) 295 (M^+, 1), 239 (4), 164 (32), 107 (66), 57 (base); CIMS (isobutane), *m/e* (relative intensity) 296 (M^+ + H, 7), 240 (base), 296 (89); HRMS, *m/e* 295.1418 ($C_{15}H_{21}NO_5$ requires 295.1420).

Boc-*N*-Me-Tyr-*N*-Me-Tyr-OMe (36). A solution of **35** (675 mg, 2.29 mmol) in 10 mL of CH_2Cl_2 was treated with EDCI (679 mg, 2.29 mmol, 1.0 equiv) and the resulting reaction mixture was stirred at 25 °C (5–10 min). A solution of **34** (478 mg, 2.29 mmol, 1.0 equiv) in 2 mL of CH_2Cl_2 was added. The reaction mixture was stirred for an additional 12 h (25 °C), washed with water (3 × 15 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 5 × 15 cm, 5% MeOH–CH_2Cl_2) afforded **36** (656 mg, 1.11 g theoretical yield, 59%) as a white foam: mp 52–55 °C; $[\alpha]^{22}_D$ −6.8° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm) broad absorptions at 7.01 (4 H, C2-H and C6-H), 6.75 (4 H, C3-H and C5-H), 4.78 (1 H, $^\alpha$CH), 4.20 (1 H, $^\alpha$CH), 3.74 (3 H, CO_2CH_3), 2.96 (3 H, NCH_3), 2.80 (3 H, NCH_3), 1.42 (9 H, *t*-Boc CH_3); IR (KBr) ν_{max} 3855, 3823, 3746, 3677, 3651, 3346, 2927, 1741, 1709, 1670, 1616, 1596, 1517, 1448, 1394, 1368, 1226, 1169, 830 cm^{-1}; EIMS, *m/e* (relative intensity), 487 (M^+, 1), 387 (2), 210 (20), 178 (11), 164 (16), 150 (32), 116 (26), 107 (31), 102 (47), 57

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(64), 41 (base); CIMS (isobutane), m/e (relative intensity) 487 ($M^+ + H$, 1), 401 (26), 387 (base), 373 (33), 355 (27), 341 (13); HRMS, m/e 486.5732 ($C_{28}H_{34}N_2O_7$ requires 486.5741). Reverse-phase HPLC: 97.2%, t_R 18 min, 2.0 mL/min, 0–6% methanol–water gradient elution (0.5%/min).

H-*N*-Me-Tyr-*N*-Me-Tyr-OMe (37). A mixture of TFA/CH_2Cl_2 (1:1, 5 mL) was added to **36** (182 mg, 0.37 mmol) at 25 °C and the reaction mixture was stirred for 1.5 h (25 °C). The solvents were removed in vacuo and the residue was diluted with 5% aqueous $NaHCO_3$ (5 mL). The aqueous solution was extracted with EtOAc (3 × 5 mL), the combined extracts were dried ($MgSO_4$), and the solvents were removed in vacuo. Short column chromatography (SiO_2, 1 × 5 cm, 10% MeOH–CH_2Cl_2) afforded **37** (98 mg, 144 mg theoretical yield, 68%) as a colorless oil which was used directly in the following reaction.

Boc-D-Ala-Ala-*N*-Me-Tyr(OMe)-Ala-*N*-Me-Tyr-*N*-Me-Tyr-OMe (38). A solution of **13b** (135 mg, 0.259 mmol) in 1 mL of CH_2Cl_2 at 25 °C was treated sequentially with EDCI (76 mg, 0.259 mmol, 1.0 equiv) and **37** (100 mg, 0.259 mmol). The reaction mixture was stirred for 20 h (25 °C) and was poured into water (2 mL) and extracted with EtOAc (3 × 2 mL). The combined extracts were washed with saturated aqueous NaCl, dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 2 × 15 cm, 5% MeOH–CH_2Cl_2) afforded **38** (134 mg, 235 mg theoretical yield, 57%) as a yellow oil: $[\alpha]^{22}_D$ −41.2° (c 0.9, MeOH); ^1H NMR ($CDCl_3$, 200 MHz, ppm) 7.3–6.7 (m, 12 H, Ar H), 3.78 (s, 3 H, Tyr(OCH_3)), 3.50 (s, 3 H, CO_2CH_3), 2.94 (m, 9 H, three NCH_3), 1.42 and 1.41 (two s, 9 H, t-Boc CH_3); IR (neat) ν_{max} 3300, 2979, 2936, 2837, 1762, 1720, 1653, 1514, 1456, 1411, 1367, 1301, 1249, 1203, 1169, 1134, 1067, 1034, 952, 895, 856, 823, 734 cm^{-1}. Reverse-phase HPLC: 96.9%, t_R 22 min, 2.0 mL/min, 0–10% methanol–water gradient elution (0.2%/min).

Boc-D-Ala-Ala-*N*-Me-Tyr(OMe)-Ala-*N*-Me-Tyr-*N*-Me-Tyr-OH (39). A solution of **38** (69 mg, 0.076 mmol) in 1 mL of THF/MeOH/H_2O (3:1:1) at 25 °C was treated with lithium hydroxide monohydrate (10 mg, 0.228 mmol, 3.0 equiv) and the reaction mixture was stirred for 1.2 h (25 °C). The reaction mixture was poured onto 10% aqueous HCl (1 mL) and extracted with EtOAc (3 × 3 mL). The combined extracts were washed with saturated aqueous NaCl, dried ($MgSO_4$), and concentrated in vacuo. Short column chromatography (SiO_2, 2 × 10 cm, 1% MeOH–CH_2Cl_2) afforded **39** (62 mg, 68 mg theoretical yield, 91%) as a white solid: mp 182–187 °C (MeOH–H_2O, white needles); $[\alpha]^{22}_D$ −22.0° (c 1.0, MeOH); ^1H NMR ($CDCl_3$, 200 MHz, ppm) 7.14 (m, 12 H, Ar H), 3.80 (s, 3 H, Tyr(OCH_3)), 3.00 and 2.92 (two s, NCH_3), 1.46 (br s, 9 H, t-Boc CH_3), 1.36 (d, 3 H, J = 7 Hz, Ala $^\beta CH_3$), 1.28 (d, 3 H, J = 7 Hz, Ala $^\beta CH_3$).

***cyclo*-(D-Ala-Ala-*N*-Me-Tyr(OMe)-Ala-*N*-Me-Tyr-*N*-Me-Tyr) (9): Method A.** A solution of **39** (62 mg, 0.071 mmol) in 2 mL of TFA/CH_2Cl_2 (1:1) at 25 °C was stirred for 2 h. The volatiles were removed in vacuo to afford the trifluoroacetic acid salt of **40** as an extremely hygroscopic crystalline solid which was used directly in the following reaction. For **40**·CF_3CO_2H: $[\alpha]^{22}_D$ −22.6° (c 1.0, MeOH); IR (neat) ν_{max} 3279, 2937, 2347, 1654, 1542, 1515, 1458, 1341, 1302, 1250, 1204, 1036, 955, 823, 800, 724 cm^{-1}.

A solution of **40**·CF_3CO_2H (64 mg, 0.071 mmol) in 9 mL of dry DMF was cooled to 0 °C and treated sequentially with $NaHCO_3$ (30 mg, 0.350 mmol, 5.0 equiv) and DPPA (31 μL, 0.093 mmol, 1.3 equiv). The reaction mixture was stirred at 0 °C (72 h) and then was concentrated in vacuo. The residue was diluted with water (2 mL) and extracted with EtOAc (3 × 3 mL). The combined organic extracts were washed with water (5 mL) and saturated aqueous NaCl, dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 2 × 20 cm, 2–10% MeOH–CH_2Cl_2 gradient elution) afforded **9** (31 mg, 55 mg theoretical yield, 56%) as a clear yellow oil which solidified on standing: mp 290–292 °C (MeOH, light yellow needles, lit.[9a] mp 280–290 °C); $[\alpha]^{22}_D$ −32.7° (c 1.1, MeOH); ^1H NMR ($CDCl_3$, 470 MHz, ppm) broad absorptions at 7.1 and 6.8 (Ar H), 3.8 (Tyr(OCH_3)), 2.8 (NCH_3), 1.5–1.2 (Ala $^\beta CH_3$); IR (KBr) ν_{max} 3677, 3651, 3301, 2929, 2855, 1638, 1514, 1457, 1413, 1376, 1301, 1249, 1178, 1102, 1034, 959, 823, 755 cm^{-1}; EIMS, m/e (relative intensity) 421 (1), 408 (1), 338 (1), 307 (1), 249 (5), 167 (14), 149 (80), 129 (11), 121 (98), 71 (base); CIMS (isobutane), m/e (relative intensity), 684 (7), 672 (19), 670 (base); FABMS (DMSO:H_2O:glycerol:thioglycerol, 5:5:1:1), m/e 781 (M^+ + Na, weak). Reverse-phase HPLC: 88% (initial product isolated by chromatography); 96% (product after recrystallization), t_R 25 min, 2.0 mL/min, 0–10% methanol–water gradient elution (0.5%/min), 10–14% methanol–water gradient elution (0.6%/min).

***cyclo*-(D-Ala-Ala-*N*-Me-Tyr(OMe)-Ala-*N*-Me-Tyr-*N*-Me-Tyr) (9): Method B.** A solution of **39** (60 mg, 0.067 mmol) in 1 mL of CH_2Cl_2 at 25 °C was treated sequentially with pentafluorophenol (14.3 mg, 0.074 mmol, 1.0 equiv) and EDCI (20 mg, 0.067 mmol, 1.0 equiv). The reaction mixture was stirred for 24 h (25 °C) and then was poured onto water (2 mL) and extracted with EtOAc (3 × 3 mL). The combined extracts were washed with saturated aqueous NaCl, dried ($MgSO_4$), and concentrated in vacuo. Short column chromatography (SiO_2, 2 × 10 cm, 5% MeOH–CH_2Cl_2) afforded **41** (55 mg, 71 mg theoretical yield, 78%) as a clear yellow oil: $[\alpha]^{22}_D$ −25.4° (c 1.0, MeOH); IR (neat) ν_{max} 3312, 2980, 2935, 1717, 1654, 1515, 1457, 1412, 1392, 1368, 1301, 1249, 1167, 1102, 1035, 955, 824, 735 cm^{-1}.

A solution of **41** (55 mg, 0.052 mmol) in 2 mL of TFA/CH_2Cl_2 (1:1) at 25 °C was stirred for 2 h (25 °C). The volatiles were removed in vacuo to afford the trifluoroacetic acid salt of **42** as an extremely hygroscopic, crystalline solid which was used directly in the following reaction.

A solution of **42**·CF_3CO_2H (56 mg, 0.052 mmol) in 1 mL of dry DMF was added dropwise over 3–4 h (using a motor driven syringe pump) to a warm (90 °C) solution of pyridine (173 mL). The reaction mixture was stirred an additional 4 h (90 °C). The solvent was removed in vacuo and the residue was dissolved in 2 mL of EtOAc. The EtOAc solution was washed with water (3 × 1 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 2 × 20 cm, 2–10% MeOH–CH_2Cl_2 gradient solution) afforded **9** (23 mg, 49 mg theoretical yield, 48%) as a clear yellow oil which solidified on standing.

***N-tert*-Butoxycarbonyl-*N*-methyl-2-(4-hydroxyphenyl)-ethylamine (46).** A solution of *N*-Boc-tyramine (**43**, 5.00 g, 21.1 mmol) in 16 mL of dry DMF was added dropwise (2–3 min) to a solution of imidazole (3.59 g, 52.7 mmol, 2.5 equiv) and *tert*-butyldimethylsilyl chloride (3.82 g, 25.3 mmol, 1.2 equiv) in 10 mL of DMF[30] at 0 °C under nitrogen. The reaction mixture was allowed to warm to 25 °C and was stirred for 24 h. The reaction mixture was poured onto water and was extracted with EtOAc (3 × 20 mL). The combined EtOAc extracts were washed with H_2O (3 × 20 mL) and saturated aqueous NaCl, dried (Na_2SO_4), and concentrated in vacuo to afford **44** (7.25 g, 7.41 g theoretical yield, 98%) as a yellow oil which was used without further purification. For **44**: ^1H NMR ($CDCl_3$, 80 MHz, ppm) 7.05 (d, 2 H, J = 9 Hz, C2-H and C6-H), 4.45 (br s, 1 H, NH), 3.32 (dd, 2 H, J = 6, 12 Hz, CH_2NH), 2.70 (t, 2 H, J = 6 H, $ArCH_2$), 1.47 (s, 9 H, t-Boc CH_3), 1.00 (s, 9 H, Si-t-BuCH_3), 0.22 (s, 6 H, $Si(CH_3)_2$); IR (neat) ν_{max} 3346, 2932, 1688, 1613, 1513, 1452, 1392, 1367, 1252, 1168, 1051, 915, 829, 781 cm^{-1}; EIMS, m/e (relative intensity) 351 (M^+, 2), 295 (6), 234 (13), 177 (14), 120 (29), 57 (base); CIMS (isobutane), m/e (relative intensity) 351 (M^+, 1), 296 (48), 182 (base); HRMS, m/e 351.5690 ($C_{19}H_{33}NO_3Si$ requires 351.5710).

A solution of **44** (7.40 g, 21.1 mmol) in 50 mL of THF/DMF (10:1) at 0 °C under nitrogen was treated sequentially with methyl iodide (3.54 mL, 63.3 mmol, 3.0 equiv) and sodium hydride (50% oil dispersion, 1.09 g, 21.1 mmol, 1.08 equiv) and the reaction mixture was stirred for 10 min (0 °C). The reaction mixture was warmed at reflux (85 °C bath temperature) under nitrogen for 23 h. The reaction mixture was poured onto 10% aqueous HCl (50 mL) and the mixture was extracted with EtOAc (3 × 50 mL). The combined organic extracts were washed with saturated aqueous NaCl, dried ($MgSO_4$), and concentrated in vacuo to afford **45** as a yellow oil which was used directly in the following reaction.

Silyl ether **45** (7.5 g, 20.5 mmol) was dissolved in 60 mL of AcOH/THF/H_2O (3:1:1) and the reaction mixture was stirred at 25 °C for 72 h. The reaction mixture was made basic with the addition of solid K_2CO_3 (pH 10) and was extracted with EtOAc (5 × 50 mL). The combined extracts were washed with saturated aqueous $NaHCO_3$ (3 × 100 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 5 × 25 cm, 30% EtOAc–hexane eluant) afforded **46** (4.71 g, 5.29 g theoretical yield, 89%) as a colorless oil: ^1H NMR ($CDCl_3$, 200 MHz, ppm) 7.02 (d, 2 H, J = 9 Hz, C2-H and C6-H), 6.78 (d, 2 H, J = 9 Hz, C3-H and C5-H), 4.60 (br s, 1 H, OH), 3.35 (t, 2 H, J = 6 Hz, $ArCH_2$),

2.81 (s, 3 H, NCH_3), 2.70 (t, 2 H, J = 6 Hz, CH_2NCH_3), 1.41 (s, 9 H, t-Boc CH_3); IR (neat) ν_{max} 3330, 3010, 2965, 2931, 2863, 1957, 1712, 1613, 1594, 1518, 1480, 1451, 1395, 1362, 1261, 1245, 1222, 1163, 1134, 1098, 1050, 1031, 1012, 958, 912, 877, 828, 773 cm^{-1}; CIMS (isobutane), m/e (relative intensity) 252 (M^+ + H, 10), 196 (base); HRMS, m/e 251.1517 ($C_{14}H_{21}NO_3$ requires 251.1521).

Methyl 3-[3-(4-(2-(*tert*-Butoxycarbonylmethylamino)ethyl)phenoxy)phenyl]propenoate (48). A solution of **46** (260 mg, 1.04 mmol, 2.02 equiv) in 0.5 mL of pyridine was added dropwise to a cooled (0 °C) slurry of sodium hydride (60% dispersion in mineral oil, 50.0 mg, 1.04 mmol, 2.02 equiv) in 0.5 mL of pyridine under nitrogen. Cuprous bromide (150 mg, 1.04 mmol, 2.02 equiv) was added and the reaction mixture was warmed to 25 °C and was stirred for 0.5 h. Methyl 3-iodocinnamate[31] (**47**, 150 mg, 0.518 mmol) was added and the reaction mixture warmed at reflux (130 °C bath temperature, 12 h). The reaction mixture was cooled, poured over 10% aqueous HCl (10 mL), and extracted with EtOAc (3 × 15 mL). The combined organic extracts were washed with saturated aqueous NH_4Cl and saturated aqueous NaCl, dried ($MgSO_4$), and concentrated in vacuo. Chromatography (PCTLC, 1 mm SiO_2, 20% Et_2O–hexane eluant) afforded **48** (140 mg, 230 mg theoretical yield, 61%) as a yellow oil: ^1H NMR ($CDCl_3$, 200 MHz, ppm) 7.69 (d, 1 H, J = 16 Hz, ArC*H*=CH), 7.42–6.96 (m, 8 H, Ar H), 6.42 (d, 1 H, J = 16 Hz, C*H*CO_2CH_3), 3.80 (s, 3 H, OCH_3), 3.47 (t, 2 H, J = 6 Hz, ArCH_2), 2.86 (br s, 3 H, NCH_3), 2.83 (t, 2 H, J = 6 Hz, CH_2NCH_3), 1.43 (s, 9 H, t-Boc CH_3); IR (neat) ν_{max} 3030, 2982, 2930, 2864, 1709, 1692, 1635, 1576, 1501, 1460, 1440, 1385, 1326, 1327, 1270, 1241, 1166, 1042, 1002, 975, 857, 788 cm^{-1}; EIMS, m/e (relative intensity) 411 (M^+, 2), 355 (22), 338 (4), 306 (base); CIMS (isobutane), m/e (relative intensity) 412 (M^+ + H, 1), 370 (40), 356 (base); HRMS, m/e 411.2035 ($C_{24}H_{29}NO_5$ requires 411.2046).

Methyl 3-[3-(4-(2-(*tert*-Butoxycarbonylmethylamino)ethyl)phenoxy)phenyl]propanoate (49). A solution of **48** (97 mg, 0.240 mmol) in MeOH (1 mL) at 25 °C was treated with 10% palladium on carbon (10 mg, 0.1 wt equiv) and placed under an atmosphere of hydrogen (30 psi, Parr hydrogenation apparatus). After 12 h (25 °C), the reaction mixture was filtered through Celite (MeOH) and concentrated in vacuo. Short column chromatography (SiO_2, 2 × 10 cm, Et_2O) afforded **49** (95 mg, 96 mg theoretical yield, 98%) as a yellow oil: ^1H NMR ($CDCl_3$, 200 MHz, ppm) 7.3–6.8 (m, 8 H, Ar H), 3.70 (s, 3 H, OCH_3), 3.46 (t, 2 H, J = 6 Hz, CH_2CH_2N), 2.96 (t, 2 H, J = 6 Hz, $CH_2CH_2CO_2CH_3$), 2.87 (br s, 3 H, NCH_3), 2.82 (t, 2 H, J = 6 Hz, CH_2NCH_3), 2.64 (t, 2 H, J = 6 Hz, $CH_2CO_2CH_3$), 1.44 (s, 9 H, t-Boc CH_3); IR (neat) ν_{max} 3855, 2975, 2927, 1737, 1695, 1605, 1585, 1506, 1485, 1448, 1392, 1365, 1249, 1216, 1168, 1035, 884, 831, 772 cm^{-1}; EIMS, m/e (relative intensity) 413 (M^+, 1), 359 (19), 325 (13), 309 (base); CIMS (isobutane), m/e (relative intensity) 414 (M^+ + H, 1), 371 (39), 357 (base); HRMS, m/e 413.2126 ($C_{24}H_{31}NO_5$ requires 413.2202).

Methyl 3-[3-[4-(2-(Methylamino)ethyl)phenoxy]phenyl]propanoate (50). A solution of **49** (215 mg, 0.537 mmol) in 4 mL of TFA/CH_2Cl_2 (1:1) was stirred at 25 °C for 1.5 h. The solvents were removed in vacuo and the residue was diluted with 4 mL of 5% aqueous $NaHCO_3$. The aqueous solution was extracted with EtOAc (3 × 5 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent was removed in vacuo. Short column chromatography (SiO_2, 1 × 5 cm, Et_2O) afforded **50** (142 mg, 152 mg theoretical yield, 94%) as a clear yellow solid (mp 149–153 °C, EtOH) which was used directly in the following reaction.

Boc-D-Ala-Ala-*N*-Me-Tyr(OMe)-Ala-*N*(CH_3)CH_2CH_2(*p*-C_6H_4)-O-(*m*-C_6H_4)$CH_2CH_2CO_2CH_3$ (51). A solution of **50** (0.150 g, 0.487 mmol) in 1 mL of CH_2Cl_2 was added to a solution of **13b** (0.254 g, 0.487 mmol, 1.0 equiv) and EDCI (0.145 g, 0.487 mmol, 1.0 equiv) in 2 mL of CH_2Cl_2 at 23 °C. The reaction mixture was stirred for 24 h (25 °C), poured onto water (3 mL), and extracted with EtOAc (3 × 3 mL). The combined organic extracts were washed with water (3 × 1 mL) and saturated aqueous NaCl, dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 2 × 20 cm, 5% MeOH–CH_2Cl_2) afforded **51** (282 mg, 433 mg theoretical yield, 65%) as a pale yellow solid: mp 159–162 °C (EtOH, white plates); $[\alpha]^{22}_D$ −42.2° (c 0.9, MeOH); ^1H NMR ($CDCl_3$, 200 MHz, ppm) 7.92 (d, 1 H, J = 8 Hz, NH), 7.3–6.80 (m, 12 H, Ar H), 5.30 (d, 1 H, J = 8 Hz, NH), 4.88 (d, 1 H, J = 8 Hz, NH), 3.75 (s, 3 H, Tyr(OCH_3)), 3.66 (s, 3 H, CO_2CH_3), 2.94 and 2.91 (two s, 3 H, NCH_3), 2.86 and 2.87 (two s, 3 H, NCH_3), 2.64 (t, 2 H, $CH_2CO_2CH_3$), 1.43 and 1.41 (two s, 9 H, t-Boc CH_3), 1.33 (d, 3 H, J = 7 Hz, Ala $^\beta CH_3$), 1.25 (d, 3 H, J = 7 Hz, Ala $^\beta CH_3$), 0.49 (d, 3 H, J = 7 Hz, Ala $^\beta CH_3$); IR (neat) ν_{max} 3855, 3287, 2984, 1735, 1670, 1586, 1509, 1487, 1449, 1368, 1302, 1250, 1202, 1176, 1034, 832, 798, 721 cm^{-1}; EIMS, m/e (relative intensity) 615 (1), 485 (1), 449 (1), 391 (16), 279 (8), 225 (75), 149 (54), 57 (base); CIMS (isobutane), m/e (relative intensity) 615 (1), 578 (1), 520 (1), 505 (1), 449 (base), 416 (52), 225 (67), 186 (30), 136 (96), 120 (77). Reverse-phase HPLC: >99%, t_R 18 min, 2.0 mL/min, 0–12% methanol–water gradient elution (0.5%/min).

Boc-D-Ala-Ala-*N*-Me-Tyr(OMe)-Ala-*N*(CH_3)CH_2CH_2(*p*-C_6H_4)-O-(*m*-C_6H_4)$CH_2CH_2CO_2H$ (52). A solution of **51** (282 mg, 0.341 mmol) in 2 mL of THF/MeOH/H_2O (3:1:1) at 25 °C was treated with lithium hydroxide monohydrate (45 mg, 1.02 mmol, 3.0 equiv). The reaction mixture was warmed to 35 °C and was stirred for 6 h. The reaction mixture was cooled and poured onto 10% aqueous HCl (1 mL) and extracted with EtOAc (3 × 2 mL). The combined organic extracts were washed with saturated aqueous NaCl, dried ($MgSO_4$), and concentrated in vacuo. Short column chromatography (SiO_2, 2 × 10 cm, 10% MeOH–CH_2Cl_2) afforded **52** (230 mg, 281 mg theoretical yield, 82%) as a white powder: mp 172–174 °C; $[\alpha]^{22}_D$ −36.7° (c 1.0, MeOH); ^1H NMR ($CDCl_3$, 200 MHz, ppm) 7.4–6.8 (m, 12 H, Ar H), 4.58 (m, $^\alpha CH$), 3.80 and 3.79 (two s, 3 H, Tyr(OCH_3)), 3.00 and 2.96 (two s, 3 H, NCH_3), 2.96 and 2.92 (two s, 3 H, NCH_3), 1.44 and 1.42 (two s, 9 H, t-Boc CH_3), 1.34 and 1.32 (two d, 3 H, J = 7 Hz, Ala $^\beta CH_3$), 1.26 (d, 3 H, J = 7 Hz, Ala $^\beta CH_3$); IR (KBr) ν_{max} 3855, 3753, 3677, 3296, 2980, 2935, 1718, 1654, 1514, 1488, 1457, 1394, 1368, 1301, 1250, 1171, 1070, 1035, 953, 912, 824, 797, 720 cm^{-1}; EIMS, m/e (relative intensity) 434 (1), 423 (1), 378 (1), 360 (1), 249 (2), 194 (7), 164 (5), 121 (12), 44 (base); CIMS (isobutane), m/e (relative intensity) 731 (3), 718 (9), 704 (4), 618 (18), 600 (11), 586 (16), 576 (67), 562 (33), 399 (12), 385 (base), 371 (43), 316 (66), 263 (20), 234 (12).

***cyclo*-(D-Ala-Ala-*N*-Me-Tyr-Ala-N(CH_3)CH_2CH_2(*p*-C_6H_4)-O-(*m*-C_6H_4)$CH_2CH_2C(O)$) (11): Method A.** A solution of **52** (86 mg, 0.107 mmol) in 1 mL of TFA/CH_2Cl_2 (1:1) at 25 °C was stirred for 2 h. The solvents were removed in vacuo to afford the crude trifluoroacetic acid salt of **53** as an extremely hygroscopic, crystalline solid which was used directly in the following reaction. For **53**·CF_3CO_2H: mp 182–185 °C (EtOH–hexane); $[\alpha]^{22}_D$ −26.4° (c 1.0, MeOH); ^1H NMR ($CDCl_3$, 200 MHz, ppm) 7.4–6.8 (m, 12 H, Ar H), 3.78 (s, 3 H, Tyr(OCH_3)), 2.62 (t, 2 H, CH_2CO_2H), 1.34 (d, 3 H, J = 7 Hz, Ala $^\beta CH_3$), 0.48 (d, J = 7 Hz, Ala $^\beta CH_3$); IR (neat) ν_{max} 3903, 3854, 3839, 3822, 3802, 3752, 3735, 3712, 3690, 3676, 3650, 3630, 3288, 2926, 2855, 2363, 2344, 1781, 1735, 1684, 1653, 1637, 1577, 1559, 1541, 1507, 1489, 1457, 1420, 1170, 1030, 983, 798, 723 cm^{-1}.

A solution of the trifluoroacetic acid salt of **53** (87 mg, 0.107 mmol) in 13.3 mL of DMF was cooled to 0 °C and sequentially treated with $NaHCO_3$ (45 mg, 0.535 mmol, 5.0 equiv) and DPPA (31 μL, 0.139 mmol, 1.3 equiv). The reaction mixture was stirred at 0 °C for 72 h. The reaction mixture was concentrated in vacuo and the residue was diluted with water (2 mL) and extracted with EtOAc (3 × 3 mL). The combined organic extracts were washed with water (5 mL) and saturated aqueous NaCl, dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 2 × 20 cm, 10% MeOH–CH_2Cl_2 eluant) afforded **11** (45 mg, 73 mg theoretical yield, 61%) as a clear yellow oil which solidified on standing: mp 142–145 °C (EtOH–CH_2Cl_2, light yellow needles); $[\alpha]^{22}_D$ −41.2° (c 1.0, MeOH); ^1H NMR ($CDCl_3$, 200 MHz, ppm) 8.06 (d, 1 H, J = 8 Hz, NH), 7.4–6.6 (m, 12 H, Ar H), 3.78 and 3.70 (two s, 3 H, Tyr(OCH_3)), 3.01 and 2.6 (two s, 3 H, NCH_3), 2.96 and 2.90 (two s, 3 H, NCH_3), 1.38 and 1.32 (two d, 3 H, J = 7 Hz, Ala $^\beta CH_3$), 1.24 and 1.20 (two d, 3 H, J = 7 Hz, Ala $^\beta CH_3$); IR (KBr) ν_{max} 3855, 3879, 3803, 3746, 3736, 3691, 3677, 3650, 3630, 3314, 2922, 2851, 2473, 1718, 1636, 1559, 1541, 1507, 1458, 1249, 1176, 1035, 799 cm^{-1}; CIMS (isobutane), m/e (relative intensity) 686 (M^+ + H, 12), 672 (82), 654 (13), 390 (15), 316 (43). Reverse-phase HPLC: >97%, t_R 18 min, 2.0 mL/min, 0–10% methanol–water gradient elution (0.5%/min).

***cyclo*-(D-Ala-Ala-*N*-Me-Tyr(OMe)-Ala-*N*-(CH_3)CH_2CH_2-(*p*-C_6H_4)-O-(*m*-C_6H_4)$CH_2CH_2C(O)$) (11): Method B.** A so-

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lution of **52** (100 mg, 0.124 mmol) in 1 mL of CH_2Cl_2 at 0 °C was treated sequentially with EDCI (37 mg, 0.124 mmol) and pentafluorophenol (25 mg, 0.137 mmol, 1.1 equiv). The reaction mixture was warmed to 25 °C and was stirred for 24 h. The reaction mixture was diluted with CH_2Cl_2 (5 mL) and was washed with water (3 × 2 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuo. Short column chromatography (SiO_2, 2 × 15 cm, 7% MeOH–CH_2Cl_2 eluant) afforded **54** (91 mg, 120 mg theoretical yield, 76%) as a yellow oil: $[\alpha]^{22}_D$ −37.1° (*c* 1.1, MeOH); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm) 7.4–6.7 (m, 12 H, Ar H), 3.80 (br s, 3 H, Tyr(OCH_3)), 1.45 (br s, 9 H, *t*-Boc CH_3), 1.34 (d, 3 H, *J* = 7 Hz, Ala $^\beta CH_3$), 1.26 (d, 3 H, *J* = 7 Hz, Ala $^\beta CH_3$); IR (neat) ν_{max} 3854, 3839, 3752, 3676, 3650, 3312, 2978, 2935, 2668, 1752, 1638, 1521, 1448, 1419, 1368, 1248, 1172, 1112, 1005, 855, 825, 788, 735 cm^{-1}.

A solution of **54** (91 mg, 0.094 mmol) in 1 mL of TFA/CH_2Cl_2 (1:1) at 25 °C was stirred for 2 h. The solvents were removed in vacuo to afford the crude trifluoroacetic acid salt of **55** as a hygroscopic, crystalline solid which was used directly in the following reaction.

A solution of the trifluoroacetic acid salt of **55** (92 mg, 0.094 mmol) in 1 mL of DMF was added dropwise over 2–3 h (using a motor driven syringe pump) to a warm (90 °C) solution of pyridine (313 mL). The resulting reaction mixture was stirred for an additional 5 h (90 °C). The solvent was removed in vacuo and the residue dissolved in 2 mL of EtOAc. The EtOAc solution was washed with water (3 × 1 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 2 × 20 cm, 5–10% MeOH–CH_2Cl_2 eluant) afforded **11** (31 mg, 64 mg theoretical yield, 49%) as a clear yellow oil which solidified on standing.

***cyclo*-(D-Ala-Ala-*N*-Me-Tyr(OMe)-Ala) (12): Method A.** A solution of **13b** (35 mg, 0.067 mmol) in 1 mL of TFA/CH_2Cl_2 (1:1) at 25 °C was stirred 1.5 h. The solvents were removed in vacuo to afford the trifluoroacetic acid salt of **56** as an extremely hygroscopic, crystalline solid which was used directly in the following reaction. For **56**·CF_3CO_2H: $[\alpha]^{22}_D$ −21.6° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm) 7.05 (d, 2 H, *J* = 9 Hz, C2-H, and C6-H), 6.86 (m, 2 H, C3-H and C5-H), 3.80 (br s, 3 H, Tyr(OCH_3)), 1.6–1.2 (m, Ala $^\beta CH_3$); IR (neat) ν_{max} 3802, 3650, 3630, 2929, 1718, 1670, 1654, 1637, 1559, 1541, 1515, 1458, 1420, 1250, 1201, 1141, 1034, 799, 722 cm^{-1}.

A solution of the trifluoroacetic acid salt of **56** (56 mg, 0.067 mmol) in 0.4 mL of DMF was cooled to 0 °C and treated sequentially with $NaHCO_3$ (28 mg, 0.335 mmol, 5 equiv) and DPPA (19 μL, 0.087 mmol, 1.3 equiv). The reaction mixture was stirred for 72 h at 0 °C. The solvent was removed in vacuo and the residue was diluted with water (1 mL) and extracted with EtOAc (3 × 2 mL). The combined organic extracts were washed with water (2 × 2 mL) and saturated aqueous NaCl, dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 2 × 15 cm, 7% MeOH–CH_2Cl_2 eluant) afforded **12** (18 mg, 27 mg theoretical yield, 68%) as a yellow oil which solidified on standing: mp 149–152 °C (MeOH–H_2O, light yellow needles); $[\alpha]^{22}_D$ −19.9° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm) 7.44 (d, 1 H, *J* = 8 Hz, NH), 7.18 and 7.12 (two s, 2 H, *J* = 9 Hz, C2-H and C6-H), 6.87 and 6.85 (two s, 2 H, *J* = 9 Hz, C3-H and C5-H), 6.40 (d, 1 H, *J* = 8 Hz, NH), 6.18 and 6.12 (two d, 1 H, *J* = 8 Hz, NH), 4.60 (m, 4 H, $^\alpha$CH), 3.80 (s, 3 H, Tyr(OCH_3)), 3.04 and 2.95 (two s, 3 H, NCH_3), 1.38 (d, 3 H, *J* = 7 Hz, Ala $^\beta CH_3$), 1.29 (d, 3 H, *J* = 7 Hz, Ala $^\beta CH_3$), 1.19 (d, *J* = 7 Hz, Ala $^\beta CH_3$); IR (KBr) ν_{max} 3754, 3290, 3062, 2984, 2936, 1655, 1514, 1492, 1452, 1406, 1378, 1301, 1249, 1208, 1179, 1108, 1033, 919, 824, 778, 735 cm^{-1}; CIMS (isobutane), *m/e* (relative intensity) 405 (M^+ + H, 1), 334 (base), 283 (42). Reverse-phase HPLC: 97.8%, t_R 12 min, 2.0 mL/min, 0–12% methanol–water gradient elution (0.5%/min).

***cyclo*-(D-Ala-Ala-*N*-Me-Tyr(OMe)-Ala) (12): Method B.** A solution of **13b** (54 mg, 0.104 mmol) in 1 mL of CH_2Cl_2 at 0 °C was treated sequentially with EDCI (31 mg, 0.104 mmol, 1.0 equiv) and pentafluorophenol (19 mg, 0.104 mmol, 1.0 equiv). The reaction mixture was allowed to warm to 25 °C and was stirred for 24 h. The reaction mixture was diluted with CH_2Cl_2 (3 mL), washed with water (3 × 2 mL), dried ($MgSO_4$), and concentrated in vacuo. Short column chromatography (SiO_2, 2 × 15 cm, 3% MeOH–CH_2Cl_2 eluant) afforded **57** (48 mg, 72 mg theoretical yield, 67%) as a yellow oil: $[\alpha]^{22}_D$ −22.9° (*c* 1.2, MeOH); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm) 7.10 and 7.06 (two d, 2 H, *J* = 9 Hz, C2-H and C6-H), 6.86 and 6.80 (two d, 2 H, *J* = 9 Hz, C3-H and C5-H), 3.78 (s, 3 H, Tyr(OCH_3)), 3.00 and 2.86 (two s, 3 H, NCH_3), 1.68 and 1.63 (two d, 3 H, *J* = 7 Hz, Ala $^\beta CH_3$), 1.30 and 1.26 (two d, 3 H, *J* = 7 Hz, Ala $^\beta CH_3$), 0.42 (d, *J* = 7 Hz, Ala $^\beta CH_3$); IR (neat) ν_{max} 3286, 2937, 1793, 1685, 1654, 1636, 1519, 1457, 1368, 1256, 1167, 1100, 996 cm^{-1}.

A solution of **57** (48 mg, 0.069 mmol) in 2 mL of TFA/CH_2Cl_2 (1:1) at 25 °C was stirred for 1.2 h. The solvents were removed in vacuo to afford the trifluoroacetic acid salt of **58** as a hygroscopic, crystalline solid which was used directly in the following reaction.

A solution of the trifluoroacetic acid salt of **58** (49 mg, 0.069 mmol) in 5 mL of DMF was added dropwise over 8 h (using a motor driven syringe pump) to a warm (90 °C) solution of pyridine (230 mL). After the addition was complete the solvent was removed in vacuo and the residue was dissolved in 1 mL of EtOAc. The EtOAc solution was washed with water (3 × 1 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 1 × 20 cm, 5% MeOH–CH_2Cl_2 eluant) afforded **12** (14 mg, 28 mg theoretical yield, 50%) as a yellow oil which solidified on standing.

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Synthesis of Various Branched Triribonucleoside Diphosphates by Site-Specific Modification of a Diphenylcarbamoyl-Protected Guanine Residue

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Three branched triribonucleotides, consisting of an adenosine linked at 3′ to a cytidine and at 2′ to a guanosine or to a 2-aminopurine ribonucleoside bearing on its 6-position a phenylthio or a dimethylamino group, have been synthesized from a common precursor. These compounds, which may prove to be useful for understanding RNA splicing, were unambiguously characterized by NMR and mass spectra analysis as well as by enzymatic hydrolysis.

It is now established that, during the splicing of eukaryotic messenger RNA precursors, the intervening sequences are excised in the form of lariat or tailed circular RNA molecules.[1] The branch point of these lariat

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