Design and Synthesis of a Conformational Analogue of Deoxybouvardin

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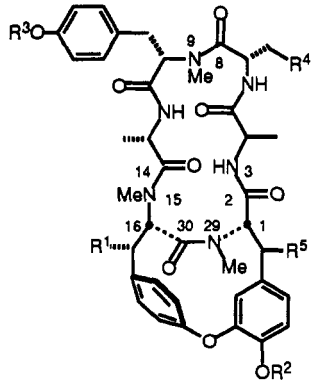
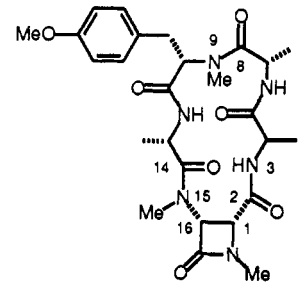
The design and synthesis of **9**, an analogue of deoxybouvardin (**2**), is detailed. The substitution of the β-lactam in **9** for the 14-membered *N*-methylcycloisodityrosine subunit of **2** serves to mimic the unusual β-turn possessing a central cis amide bond found in the natural product and restricts the accessible conformations of the remaining tetrapeptide (D-Ala-Ala-*N*(Me)-Tyr(OMe)-Ala) to those including that found in the solution and X-ray conformation of the natural product.

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) and chemical correlation (deoxybouvardin),[1] constitute the initial members of a growing class of potent antitumor antibiotics now including RA-I–RA-VII (**1–7**).[2–7] Bouvardin has been



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

shown to inhibit protein synthesis through eukaryotic 80S ribosomal binding, resulting in inhibition of aminoacyl-*t*RNA binding and peptidyl-*t*RNA translocation, and this is presently thought to be the site of action for the agent antitumor activity.[8–15] Early studies have supported the proposal that the 14-membered *N*-methylcycloisodityrosine 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.[1,15] Independent studies have shown the *O-seco*-deoxybouvardin (**10**)[16,17] and **11** lack the cytotoxic properties of deoxybouvardin, substantial functional group modification of the 14-membered cyclic dipeptide segment within bouvardin and deoxybouvardin potentiate the biological properties,[5–7] and an apparent modification within the tetrapeptide of bouvardin abolished activity.[18] Thus, the



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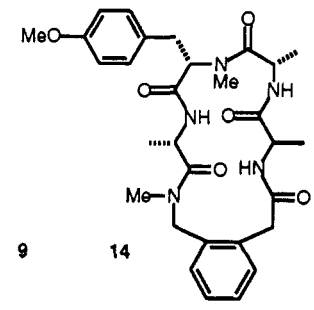

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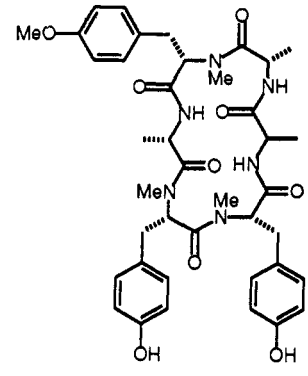

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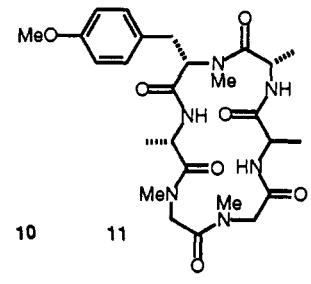

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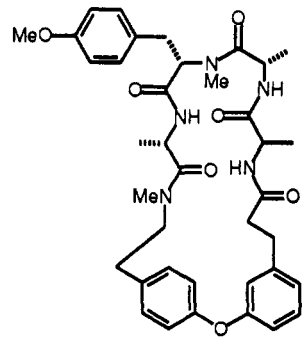

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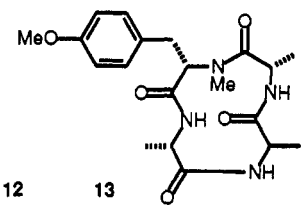

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initial observations have suggested that the 14-membered *N*-methylcycloisodityrosine subunit potentiates the cyto-

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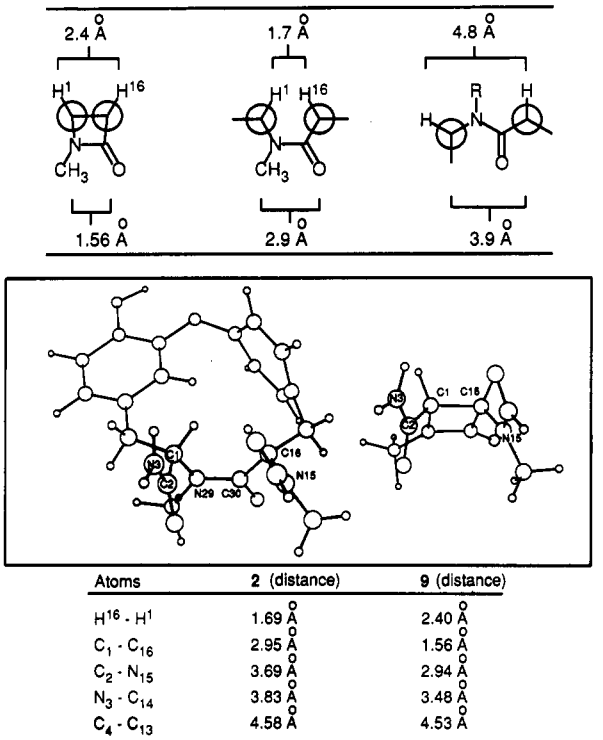
(1) Jolad, S. D.; Hoffmann, J. J.; Torrance, S. J.; Wiedhopf, R. M.; Cole, J. R.; Arora, S. K.; Bates, R. B.; Gargiulo, R. L.; Kriek, G. R. *J. Am. Chem. Soc.* **1977**, *99*, 8040.
(2) Itokawa, H.; Takeya, K.; Mori, N.; Sonobe, T.; Mihashi, S.; Hamanaka, T. *Chem. Pharm. Bull.* **1986**, *34*, 3762.
(3) Itokawa, H.; Takeya, K.; Mihara, K.; Mori, N.; Hamanaka, T.; Sonobe, T.; Iitaka, Y. *Chem. Pharm. Bull.* **1983**, *31*, 1424.
(4) Itokawa, H.; Takeya, K.; Mori, N.; Kidokoro, S.; Yamamoto, H. *Planta Med.* **1984**, *51*, 313.
(5) Itokawa, H.; Takeya, K.; Mori, N.; Sonobe, T.; Serisawa, N.; Hamanaka, T.; Mihashi, S. *Chem. Pharm. Bull.* **1984**, *32*, 3216.
(6) Itokawa, H.; Takeya, K.; Mori, N.; Takanashi, M.; Yamamoto, H.; Sonobe, T.; Kidokoro, S. *Gann.* **1984**, *75*, 929.
(7) Itokawa, H.; Takeya, K.; Mori, N.; Hamanaka, T.; Sonobe, T.; Mihara, K. *Chem. Pharm. Bull.* **1984**, *32*, 284.
(8) Tobey, R. A.; Orlicky, D. J.; Deaven, L. L.; Rall, L. B.; Kissane, R. J. *J. Cancer Res.* **1978**, *38*, 4415.

0022-3263/91/1956-5385$02.50/0

toxic and antitumor properties of the D-Ala-Ala-*N*(Me)-Tyr(OMe)-Ala segment of 1–8 by serving the scaffolding role of maintenance of an active, normally inaccessible conformation within the 18-membered ring. Herein, we detail the design and synthesis of the agent **9** lacking the 14-membered *N*-methylcycloisodityrosine structural subunit of 1–8 that was anticipated to share conformational characteristics with the deoxybouvardin 18-membered cyclic hexapeptide.[18]

Design of β-Lactam Analogue 9. In contrast to the classical β-turns[20-23] and their mimetics[24] in which the central residues ($i + 1$ and $i + 2$) of the β-turn (residues i to $i + 3$) are linked with a trans amide bond, one turn present in 1–8 contains a cis amide central to the turn that reverses the direction of the peptide chain. One important ramification of the introduction of the cis amide central to the turn is a significant reduction in the distance between the central residues α-carbons (2.9 versus 3.9 Å),[25] Figure 1. Thus, the replacement of the 14-membered *N*-methylcycloisodityrosine subunit of 1–8 with mimetics of classical β-turns would not be expected to accurately reproduce a key conformational feature of the agents (N^{29}–C^{30} cis amide) and may not convey the required conformational properties to the tetrapeptide subunit of the natural products. In initial efforts to reproduce the proposed functional role of the *N*-methylcycloisodityrosine subunit of 1–8 including the potential reactivity of C^{30}–N^{29} amide, we have elected to examine the agent **9**. In this agent, the *N*-methyl β-lactam serves to mimic the structure and potential reactivity of the deoxybouvardin N^{29}–C^{30} cis *N*-methyl amide[19] and the β-lactam linkage of the C^1–C^{16} carbons serves to restrict the number of accessible cyclic hexapeptide conformations to those including the unusual β-turn found in deoxybouvardin.

Exhaustive conformational searches of deoxybouvardin (**2**), the *N*-methyl β-lactam analogue **9**, **10**–**11**, and additional related agents[19] were conducted in which global and close low lying minima (<5.0 kcal) were located by use directed Monte Carlo sampling of starting conformations generated by random variations (0–180°) in two to six of the 18 available torsional angles in the 18-membered rings including the amide bonds themselves.[26-29] For each agent,



Atoms	2 (distance)	9 (distance)
H^{16} - H^1	1.69 Å	2.40 Å
C_1 - C_{16}	2.95 Å	1.56 Å
C_2 - N_{15}	3.69 Å	2.94 Å
N_3 - C_{14}	3.83 Å	3.48 Å
C_4 - C_{13}	4.58 Å	4.53 Å

Figure 1. Top: Newman projections of C1/C16 of **9**, deoxybouvardin (**2**), and a typical β-turn. Bottom: comparative models of the 14-membered *N*-methylcycloisodityrosine subunit of **2** (conformation no. 1) and the β-lactam subunit of **9** (conformation no. 8).

16 starting conformations were employed and constituted each accessible cis and trans permutation in the stereochemistry of the *N*-methyl amides present in **9**. Comparable but less complete results were obtained by conducting the conformational searches with random variations in the torsional angles (0–180 °C) of two–four of the available six amide bonds starting with conformations derived from the X-ray crystal structure of bouvardin. The results of the evaluation of the accessible conformations located within 5 kcal of the global minima are detailed in Table I. The manner in which the conformational searches were conducted (16 different starting conformations) and the number of times the low energy conformations were located suggest that the conformational space was exhaustively explored and that the relevant low energy conformations were located. Consistent with the solution conformations of 1–2 and 8,[15,30] the X-ray crystal structure of 8 bears a single cis *N*-methyl amide bond (C^{30}–N^{29}) located central to the 14-membered ring, Figure 1. The lowest energy conformation of deoxybouvardin has proven significantly more stable than additional low energy conformations located in the conformational searches and very close in structure to the X-ray crystal conformation (conformations 1 and 6 respectively, RMS = 0.40 Å),[31] sub-

(9) Johnson, R. K.; Chitnis, M. P. *Proc. Am. Assoc. Cancer Res.* **1978**, *19*, 218.
(10) Chitnis, M. P.; Alate, A. D.; Menon, R. S. *Chemotherapy (Basel)* **1981**, *27*, 126.
(11) Zalacain, M.; Zaera, E.; Vazquez, D.; Jiminez, A. *FEBS Lett.* **1982**, *148*, 95.
(12) Zaera, E.; Santamaria, F.; Vazquez, D.; Jiminez, A. *Curr. Microbiol.* **1983**, *9*, 259.
(13) Chitnis, M. P.; Joshi, S. S.; Gude, R. P.; Menon, R. S. *Chemotherapy (Basel)* **1982**, *28*, 209.
(14) Adwankar, M. K.; Chitnis, M. P. *Tumori* **1983**, *69*, 309.
(15) Bates, R. B.; Cole, J. R.; Hoffmann, J. J.; Kriek, G. R.; Linz, G. S.; Torrance, S. J. *J. Am. Chem. Soc.* **1983**, *105*, 1343.
(16) Bates, R. B.; Gin, G. L.; Hassen, M. A.; Hruby, V. J.; Janda, K. D.; Kriek, G. R.; Michaud, J.-P.; Vine, D. B. *Heterocycles* **1984**, *22*, 785.
(17) Boger, D. L.; Yohannes, D. *J. Org. Chem.* **1988**, *53*, 487.
(18) Petroski, R. J.; Bates, R. B.; Linz, G. S.; Rosazza, J. P. *J. Pharm. Sci.* **1983**, *72*, 1291.
(19) Boger, D. L.; Yohannes, D. *Synlett.* **1990**, 33.
(20) Venkatachalam, C. M. *Biopolymers* **1986**, *6*, 1425.
(21) Lewis, P. N.; Momany, F. A.; Scheraga, H. A. *Biochem. Biophys. Acta* **1973**, *303*, 211.
(22) Richardson, J. S. *Adv. Protein Chem.* **1981**, *34*, 167.
(23) Rose, G. D.; Gierasch, L. M.; Smith, J. A. *Adv. Protein Chem.* **1985**, *37*, 1.
(24) Olson, G. I.; Voss, M. E.; Hill, D. E.; Kahn, M.; Madison, V. S.; Cook, C. M. *J. Am. Chem. Soc.* **1990**, *112*, 323 and references cited therein.
(25) Kemp, D. S.; McNamara, P. *Tetrahedron Lett.* **1982**, *23*, 3761. Kemp, D. S.; Sun, E. T. *Tetrahedron Lett.* **1982**, *23*, 3759.
(26) Conformational searches were performed with the AMBER/OPLS[29] force field (OPLSA, MacroModel).[27,28]
(27) Still, W. C.; Mohmadi, F.; Richards, N. G. J.; Guida, W. C.; Lipton, M.; Liskamp, R.; Chang, G.; Hendrickson, T.; DeGunst, F.; Hasel, W. *MacroModel*, Version 2.5, Columbia University, New York, NY, 1990.
(28) Chang, G.; Guida, W. C.; Still, W. C. *J. Am. Chem. Soc.* **1989**, *111*, 4379.
(29) Jorgensen, W. L.; Tirado-Rives, J. *J. Am. Chem. Soc.* **1988**, *110*, 1657.
(30) Diagnostic of the cis C^{30}–N^{29} amide bond, an intense NOE crosspeak between C1-H and C16-H is observed in the 2D ^1H–^1H NOESY NMR spectrum of deoxybouvardin in solution ($CDCl_3$) and the NOE crosspeak between C1-H/N^{29}-CH_3 or C16-H/N^{29}-CH_3 diagnostic of a trans C^{30}–N^{29} amide bond is not observed, cf. Figure 1. Boger, D. L.; Yohannes, D.; Myers, J. B. Unpublished observations.

Table I[a]

conf	agent 2: ΔE (kcal), % pop., RMS (Å)	agent 2: amide stereochem.: C^{30}–N^{29}, C^{8}–N^{9}, C^{14}–N^{15}	agent 9: ΔE (kcal), % pop., RMS (Å)[c]	agent 9: amide stereochem.: C^{8}–N^{9}, C^{14}–N^{15}	agent 11: ΔE (kcal), % pop., RMS (Å)[c]	agent 10: ΔE (kcal), RMS (Å)
1	0.0, 34.8, 0.36	c, t, t	0.0, 34.3, 1.03 (42)	c, c	0.0, 22.6, 2.59 (26)	0.0, 2.71 (10)
2	0.3, 21.3, 1.35	c, c, c	0.09, 29.7, 1.03 (23)	c, c	0.0, 22.6, 1.65 (97)	
3	0.8, 9.4, 1.45	t, t, c	0.6, 13.6, 1.07 (4)	c, c	0.4, 11.3, 2.29 (20)	
4	1.0, 7.0, 1.31	c, t, c	0.9, 7.8, 0.89 (312)	t, c	0.5, 10.0, 2.56 (25)	
5	1.0, 7.0, 1.09	c, c, c	1.5, 3.2, 0.78 (124)	c, t	0.6, 8.9, 2.30 (36)	
6[b]	1.2, 4.9, 0.00	c, t, t	1.7, 2.2, 0.78 (125)	t, c	0.6, 8.9, 2.65 (8)	
7	1.2, 4.9, 1.39	t, t, t	1.8, 1.7, 0.88 (1)	c, t	0.8, 6.4, 1.48 (19)	
8	1.2, 4.9, 1.30	t, t, c	1.9, 1.6, 0.14 (32)	t, t	1.2, 3.0, 1.22 (3)	
9	1.5, 3.1, 1.46	t, t, t	1.9, 1.6, 0.89 (16)	c, t	1.2, 3.0, 0.82 (14)	
10	2.4, 0.7, 0.50	c, t, t	2.3, 0.8, 0.89 (45)	c, t	1.4, 2.3, 0.80 (39)	
11	2.7, 0.4, 0.86	t, c, t	2.7, 0.4, 0.92 (15)	c, c	2.0, 0.9, 1.28 (6)	
12	2.8, 0.4, 0.60	t, t, t	2.8, 0.4, 0.75 (7)	c, t	2.3, 0.5, 1.34 (48)	
13	2.8, 0.4, 1.34	t, t, t	2.9, 0.3, 0.93 (8)	t, t	2.8, 0.2, 1.35 (11)	
14	3.1, 0.2, 1.27	t, t, t	3.0, 0.2, 0.71 (31)	c, t	2.8, 0.2, 2.60 (139)	
15	3.4, 0.1, 1.36	c, t, c	3.3, 0.1, 0.72 (13)	t, t	2.9, 0.2, 2.60 (3)	
16	3.4, 0.1, 0.49	c, c, t	3.3, 0.1, 0.72 (2)	c, c	3.0, 0.2, 1.60 (3)	
17	3.5, 0.1, 1.32	c, c, c	3.4, 0.1, 0.70 (12)	t, c	3.4, 0.1, 2.29 (1)	
18	3.9, 0.06, 1.32	c, t, c			3.5, 0.1, 0.70 (22)	
19	4.1, 0.04, 0.43	t, t, t			3.6, 0.1, 1.58 (23)	
20	4.1, 0.04, 1.09	c, c, c			5.3, –, 0.08 (10)[d]	4.3, 0.07[d]
21	4.2, 0.04, 0.62	t, c, t				
22	4.4, 0.03, 1.20	t, c, c				
23	4.6, 0.02, 1.04	t, t, c				
24	4.8, 0.01, 1.25	t, c, c				
25	4.9, 0.01, 0.54	c, c, t				

[a] % pop. = calculated Boltzmann distribution at 37 °C. RMS = least square deviation for all atoms (2, 10–11) or C^{2}–N^{15} (9) of the 18-membered ring from that of the minimized structure of 2 derived from modification of the X-ray structure of bouvardin (2, conformation no. 6). [b] Conformation of 2 derived from direct minimization of the X-ray structure of bouvardin (RMS = 0.16 Å). [c] The number of times the conformation was located in the conformational search is shown in parentheses. [d] Conformation of 10–11 derived from modification and direct minimization of the X-ray structure of bouvardin.

Table II

conformation no. 2	conformation no. 9	RMS, Å	conformation no. 2	conformation no. 9	RMS, Å
1	8	0.40	11	12	0.32
2	2	0.43	11	14	0.30
2	3	0.41	12	8	0.39
3	4	0.42	13	13	0.24
4	6	0.23	17	3	0.42
5	1	0.36	19	8	0.17
5	2	0.38	20	1	0.09
5	3	0.40	20	2	0.31
6	8	0.14	20	3	0.35
7	13	0.24	22	1	0.31
8	6	0.25	23	4	0.30
9	15	0.40	23	6	0.39
11	5	0.35	25	14	0.40

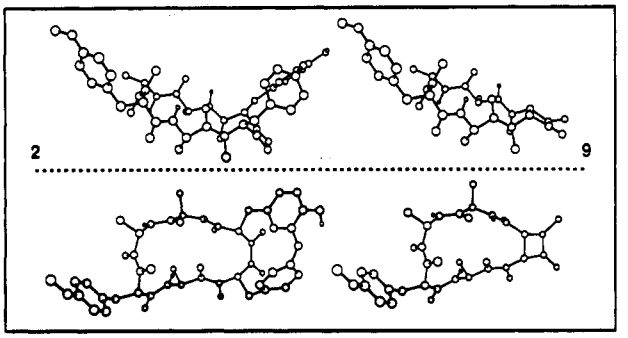


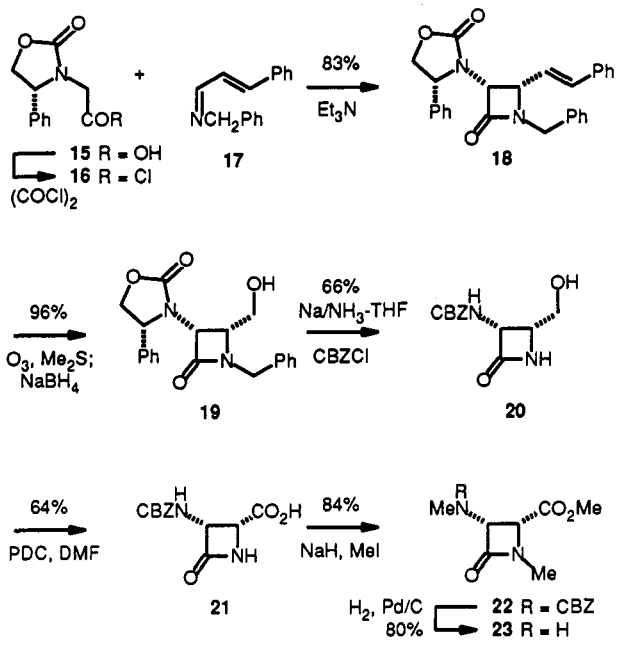
Figure 2. Side and top views of the conformations of 2 and 9 derived from modification of the X-ray structure of bouvardin and minimization (MacroModel, OPLS-A force field; Table I, 2 (conformation no. 6), 9 (conformation no. 8); RMS = 0.14 Å).

stantially more stable than the lowest energy conformation located possessing a trans C^{30}–N^{29} amide bond, and the set of low energy conformations proved conformationally distinct from a large number of low energy conformations available to 10–11. For each of the agents, calculation of a Boltzmann distribution (37 °C) among the accessible conformations revealed that agent 9 possessed a substantially restricted set of available conformations and that the ensemble of conformations available to 2 were well represented by the ensemble of accessible conformations of 9, Table II. In contrast to 10–11, one conformation among the restricted set of accessible conformations for 9 possessed conformational characteristics similar to that found in the low energy and X-ray crystal structure conformations of bouvardin and deoxybouvardin, Figure 2. Further examination of this accessible conformation of 9 (conformation no. 8) revealed that although significant perturbations in the cyclic hexapeptide of 9 from that of 1–2 may be found in the region adjacent to the β-lactam (C1–C16 distance = 1.56 Å versus 2.95 Å, C2–N15 distance = 2.94 Å versus 3.69 Å), the tetrapeptide region from N3 through C14 remains relatively unperturbed (N3–C14 distance = 3.48 Å versus 3.83 Å, C4–C13 distance = 4.53 versus 4.58 Å), Figure 1. In particular, the spacial location of the tetrapeptide α-substituents on C4, C7, C10, and C13 in this conformation are not displaced by the constriction of the C1–C16 distance found in 9. Thus, the examination of 9, which lacks the 14-membered *N*-methylcycloisodityrosine subunit of 1–8 but possesses an ensemble of accessible conformations comparable to those accessible to 1–8 including a conformation comparable to that of the low energy conformation of the natural products, was expected to address directly the supposition that the inherent biological properties of the agents may be found within the tetrapeptide.

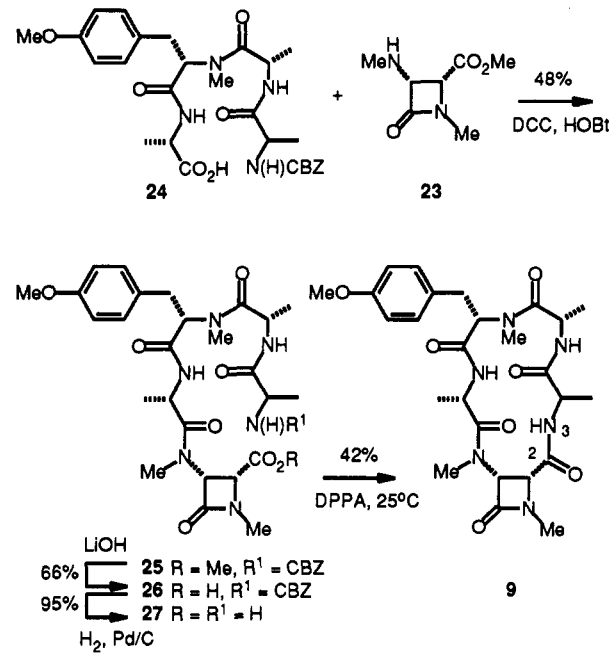
Synthesis of (1*R*,16*R*)-Cyclo-(D-Ala-Ala-*N*(Me)-Tyr(OMe)-Ala-*N*(Me)-(cCHCON(Me)CH)-CO) (9).

(31) The lowest energy conformation of deoxybouvardin located in the conformational search differs only slightly from the X-ray crystal conformation (conformation no. 6, Table I) with small perturbations in the N^{9}–C^{10}–C^{11}–N^{12} and C^{11}–N^{12}–C^{13}–C^{14} torsional angles.

Scheme I



Scheme II



As established in prior studies,[17] macrocyclization of the 16-membered ring to provide **9** directly was expected to be most productively conducted at a secondary amide site. Of the three such sites available, that which could be conducted with a D-amino acid amine terminus (C^2–N^3 amide) was anticipated to potentially be most productive.[32,33] Thus, pentultimate coupling of β-lactam **23** with the suitably protected tetrapeptide CBZNH-D-Ala-Ala-*N*(Me)-Tyr(OMe)-Ala-OH (**24**)[17] followed by final C^2–N^3 amide bond formation through macrocyclization was anticipated to provide the desired agent **9**.

The β-lactam **23** possessing the unnatural β-lactam 3*R*,4*R* absolute configuration and exhaustive N-methylation was prepared through adoption of the Evans asymmetric β-lactam synthesis[34-36] employing optically active oxazolidinones as chiral auxilaries in a diastereoselective[37-39] Staudinger [2 + 2] ketene–imine cycloaddition reaction. Thus, [2 + 2] cycloaddition of imine **17** with (*R*)-(4-phenyloxazolidinyl)ketene generated in situ by low temperature triethylamine treatment of the crude acid chloride **16** provided the β-lactam **18** in 83% yield (95:5, 3*R*,4*R*:3*S*,4*S*), Scheme I. Following the Evans procedure, ozonolysis of the carbon–carbon double bond of the pure major diastereomer **18** followed by sodium borohydride reductive workup of the liberated aldehyde (Me_2S) provided the alcohol **19** in near quantitative yield. Dissolving metal reductive cleavage of the oxazolidinone and the *N*-benzyl group proved technically challenging and in our hands proved to be most reliably conducted with Na/NH_3–THF/*t*-BuOH following closely the protocol described by Evans (Li/NH_3–THF/*t*-BuOH). Immediate protection of the liberated primary amine as its carbobenzyloxy (CBZ) derivative provided **20**. Direct PDC oxidation of the primary alcohol in DMF (64%) followed by exhaustive O- and N-methylation of the crystalline carboxylic acid **21** under the nonracemization alkylation conditions of Coggins and Benoiton[40] provided **22** in excellent yield (84%). Minimal epimerization of the C3 or C4 centers was observed (<5%)[41] through this sequence and the exhaustive methylation was most effectively accomplished at 0 °C with 3.1 equiv of sodium hydride. Alternative methods of oxidation of **20** to provide **21** were investigated and no oxidation was observed with AgO or PtO_2/O_2 and low conversions (25 °C) with subsequent decomposition (60 °C) precluded the use of buffered (NaH_2PO_4) or basic $KMnO_4$.[42]

Catalytic hydrogenolysis of **22** served to remove the CBZ protecting group and the resultant free amine **23** was coupled with the tetrapeptide **24**[17] to provide **25**, Scheme

Table III. In Vitro Cytotoxic Activity, L1210 (IC_{50}, μg/mL)[a]

2, deoxybouvardin	0.002
9	50
10	>100
11	>100
12	20
13	20
14	25

[a] Inhibitory concentration for 50% cell growth relative to untreated controls, L1210 mouse lymphocytic leukemia cell culture, see: Boger, D. L.; Yasuda, M.; Mitscher, L. A.; Drake, S. D.; Kitos, P. A.; Thompson, S. C. *J. Med. Chem.* **1987**, *30*, 1918.

(32) Rich, D. H.; Bhatnagar, P.; Mathiaparanam, P.; Grant, J. A.; Tam, J. P. *J. Org. Chem.* **1978**, *43*, 296.
(33) Brady, S. F.; Freidinger, R. M.; Paleveda, W. J.; Colton, C. D.; Homnick, C. F.; Whitter, W. L.; Curley, P.; Nutt, R. R.; Veber, D. F. *J. Org. Chem.* **1987**, *52*, 764. Brady, S. F.; Varga, S. L.; Freidinger, R. M.; Schwenk, D. A.; Mendlowski, M.; Holly, F. W.; Veber, D. F. *J. Org. Chem.* **1979**, *44*, 3101.
(34) Evans, D. A.; Sjogren, E. B. *Tetrahedron Lett.* **1985**, *26*, 3783.
(35) Evans, D. A.; Sjogren, E. B. *Tetrahedron Lett.* **1985**, *26*, 3787.
(36) Bodurow, C. C.; Boyer, B. D.; Brennan, J.; Bunnell, C. A.; Burks, J. E.; Carr, M. A.; Doecke, C. W.; Eckrich, T. M.; Fisher, J. W.; Gardner, J. P.; Graves, B. J.; Hines, P.; Hoying, R. C.; Jackson, B. G.; Kinnick, M. D.; Kochert, C. D.; Lewis, J. S.; Luke, W. D.; Moore, L. L.; Morin, J. M., Jr.; Nist, R. L.; Prather, D. E.; Sparks, D. L.; Vladuchick, W. C. *Tetrahedron Lett.* **1989**, *30*, 2321.
(37) Thomas, R. C. *Tetrahedron Lett.* **1989**, *30*, 5239.
(38) Cooper, R. D. G.; Daugherty, B. W.; Boyd, D. B. *Pure Appl. Chem.* **1987**, *59*, 485.
(39) Hubschwerlen, C.; Schmid, G. *Helv. Chim. Acta* **1983**, *66*, 2206.
(40) Coggins, J. R.; Benoiton, N. L. *Can. J. Chem.* **1971**, *49*, 1968.
(41) The extent of epimerization was easily determined by comparison of the integration of the diagnostic ^1H NMR signals for H3 at 5.5 and 5.4 ppm for **22** to the corresponding signals at 5.1 and 4.9 ppm for the epimerized trans β-lactam.
(42) AgO: Syper, L. *Tetrahedron Lett.* **1967**, 4193. Clarke, T. G.; Hampson, N. A.; Lee, J. B.; Morley, J. R.; Scanlon, B. *Tetrahedron Lett.* **1968**, 5685. PtO_2/O_2: Maurer, P. J.; Takahata, H.; Rapoport, H. *J. Am. Chem. Soc.* **1984**, *106*, 1095. Buffered $KMnO_4$: Abiko, A.; Roberts, J. C.; Takemasa, T.; Masamune, S. *Tetrahedron Lett.* **1986**, *27*, 4537. $KMnO_4$: Ciufolini, M. A.; Swaminethan, S. *Tetrahedron Lett.* **1989**, *30*, 3027.

II. Sequential methyl ester hydrolysis and CBZ hydrogenolysis followed by subjection of the resultant amino acid **27** to diphenyl phosphorazidate promoted macrocyclization[33] (0 °C, 72 h, 42%) provided **9** in good yield.[43]

Evaluation of 9. Provided the tetrapeptide of **1–8** proved to be the pharmacophore of the naturally occuring agents, **9** was anticipated to display potent cytotoxic activity albeit at a potentially reduced level from that of **1–8** (10–100×). Consequently, we were initially disappointed when the agent failed to display cytotoxic activity at a level commonly accepted to represent the observation of biological activity (≤4 μg/mL), Table III. The agent **9** displayed cytotoxic activity at a level comparable to **12–14**, greater than that of the inactive agents **10–11**, and proved to be 25000× less potent than deoxybouvardin. Although there are several explanations that may accommodate the observations including potential inaccuracies in the modeling studies (relative energies, unlocated minima), the relative importance of the perturbations in the C1/C16 region of the 18-membered ring, assurance that the low energy versus an alternative accessible conformation within the tetrapeptide of **1–8** may prove relevant, or that the ensemble of conformations available to **2** are well represented by the ensemble of conformations available to **9**, recent observations made in related studies suggest an unanticipated but more germane interpretation. That is that **9** does in fact represent an adequate analogue of the accessible conformations within the tetrapeptide of **1–2** but *that the tetrapeptide lacks inherent biological activity.* This successful demonstration of the lack of activity of the tetrapeptide even when its accessible conformations are restricted to include those found in the natural products coupled with the unanticipated observation of potent cytotoxic activity with simple derivatives of the 14-membered *N*-methylcycloisodityrosine[44] lacking the tetrapeptide requires that the functional roles of the agents subunits be reassigned.[45] We suggest that it is the inactive tetrapeptide housed within the 18-membered ring that potentiates the inherent biological activity of *N*-methylcycloisodityrosine.

Experimental Section

(*R*)-(4-Phenyl-2-oxo-3-oxazolidinyl)acetic Acid (15). A flame-dried, N_2-flushed flask was charged with NaH (950 mg, 23.7 mmol, 60% in oil). The NaH was washed with dry hexane (3 × 5 mL), suspended in 10 mL of dry THF, and cooled to 0 °C. A solution of (*R*)-4-phenyloxazolidone[34] (3.0 g, 18.4 mmol) in 100 mL of THF was added, and the mixture was stirred for 4 h. Ethyl bromoacetate (4.2 g, 25 mmol) was added and the resulting mixture was allowed to stir at 0 °C for 1 h and at 25 °C for 4 h. The reaction mixture was diluted with water, LiOH–H_2O (2.3 g, 54.8 mmol, 3.0 equiv) was added, and the mixture was allowed to stir at 25 °C for 2.5 h. The mixture was washed with ethyl acetate (2 × 20 mL), acidified to pH 2 with the addition of 10% aqueous HCl, and extracted with ethyl acetate (4 × 20 mL). The organic extracts were combined, washed with 10% aqueous HCl (1 × 10 mL) and saturated aqueous NaCl (1 × 5 mL), dried ($MgSO_4$), and concentrated in vacuo. Chromatography (SiO_2, 5 × 13 cm, 60% ethyl acetate–hexane eluant) afforded **15** (3.62 g, 4.07 g theoretical, 89%): mp 107–108 °C (lit.[34] mp 106–108 °C); $[\alpha]^{21}_D$ −170° (*c* 1.3, $CHCl_3$); ^1H NMR ($CDCl_3$, 200 MHz, ppm) 7.41 (m, 3 H), 7.34 (m, 2 H), 5.07 (t, 1 H, *J* = 8.4 Hz), 4.74 (t, 1 H, *J* = 8.3 Hz), 4.33 (d, 1 H, *J* = 18.4 Hz), 4.18 (t, 1 H, *J* = 8.3 Hz), 3.42 (d, 1 H, *J* = 18.4 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) 173.1, 159.1, 136.5, 129.6, 129.5, 127.4, 70.4, 60.0, 42.7; IR (KBr) ν_{max} 2924 (br), 1752 (C=O oxazolidinone), 1654 (C=O carboxylic acid) cm^{-1}.

(3*R*,3′*R*,4′*R*)-3-[2′-Oxo-4′-(2-phenylethenyl)-1′-(phenylmethyl)-3′-azetidinyl]-4-phenyl-2-oxazolidinone (18). Oxalyl chloride (3.05 g, 24 mmol, 1.5 equiv) was added to a solution of **15** (3.5 g, 15.8 mmol) in 50 mL of dry toluene, and the reaction mixture was warmed at 60 °C for 3 h. The solvent and excess oxalyl chloride were removed under vacuum and the resulting crude acid chloride **16** was dissolved in 50 mL of dry CH_2Cl_2 and cooled to −78 °C. Dry triethylamine (3.4 mL, 24 mmol, 1.5 equiv) was added to the −78 °C solution of **16**. After 15 min, **17** (3.85 g, 17.4 mmol, 1.1 equiv) dissolved in toluene (32 mL) was added to the solution, the −78 °C dry ice/acetone bath was replaced with a 0 °C ice–water bath, and the reaction mixture was allowed to stir at 0 °C for 3 h. The reaction mixture was poured into water (35 mL) and acidified to pH = 2 with the addition of 10% aqueous HCl. The aqueous solution was extracted with ethyl acetate (4 × 25 mL) and the combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo. Chromatography (SiO_2, 5 × 13 cm, 10% ethyl acetate–methylene chloride eluant) afforded **18** (5.6 g, 6.7 g theoretical, 83%): $[\alpha]^{21}_D$ −58.8° (*c* 1.3, $CHCl_3$); mp 186–187 °C (lit.[34] mp 186–187 °C); ^1H NMR ($CDCl_3$, 500 MHz, ppm) 7.43–7.12 (m, 8 H, Ar), 7.10 (dd, 2 H, *J* = 7.9, 1.7 Hz, Ar), 6.49 (d, 1 H, *J* = 15.8 Hz, =C*H*Ph), 5.87 (dd, 1 H, *J* = 15.8, 8.9 Hz, C*H*=CHPh), 4.89 (dd, 1 H, *J* = 8.8, 7.3 Hz, C*H*HCHPh), 4.55 (t, 1 H, *J* = 9.0 Hz, CH*H*CHPh), 4.49 (d, 1 H, *J* = 5.1 Hz, H3′), 4.49 (d, 1 H, *J* = 15.1 Hz, NCH_2Ph), 4.14 (dd, 1 H, *J* = 8.4, 5.5 Hz, H4′), 4.11 (dd, 1 H, *J* = 8.8, 7.3 Hz, CHPh), 4.07 (d, 1 H, *J* = 15.1 Hz, NCH_2Ph); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm) 163.6, 158.1, 137.5, 137.1, 135.9, 135.4, 129.6, 129.6, 128.9, 128.8, 128.6, 128.6, 128.0, 127.9, 127.0, 123.6, 70.8, 63.0, 60.9, 60.0, 44.9; IR (KBr) ν_{max} 1761 (C=O, β-lactam), 1732 (C=O, oxazolidinone) cm^{-1}.

(4*R*,3′*R*,4′*R*)-3-[4′-(Hydroxymethyl)-2′-oxo-1′-(phenylmethyl)-3′-azetidinyl]-4-phenyl-2-oxazolidinone (19). A solution of **18** (3.2 g, 7.54 mmol) in 50 mL of CH_2Cl_2 was cooled to −78 °C and O_3/O_2 was passed through the solution (45 min) until it sustained the characteristic blue color of ozone. Excess dimethyl sulfide (2 mL) was added, the dry ice/acetone bath was removed, and the solution was stirred for 1 h before the solvent was removed in vacuo. A solution of the resulting white solid in 25 mL of ethanol cooled to 0 °C was treated with sodium borohydride (300 mg, 7.9 mmol). The reaction mixture was allowed to stir at 0 °C for 1 h and room temperature for 4 h. Aqueous HCl (10%, 15 mL) was added and the mixture was extracted with ethyl acetate (4 × 15 mL). The organic extracts were combined, dried ($MgSO_4$), and concentrated in vacuo to afford a white solid. Chromatography (SiO_2, 5 × 13 cm, 70% ethyl acetate–hexane eluant) afforded **19** (2.55 g, 2.66 g theoretical, 96%) as a white solid: mp 159–160 °C (lit.[34] mp 159–160 °C); $[\alpha]^{20}_D$ −108° (*c* 0.68, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz, ppm) 7.47–7.41 (m, 4 H, Ar), 7.26–7.24 (m, 4 H, Ar), 7.02–7.01 (m, 2 H, Ar), 5.11 (dd, 1 H, *J* = 9.2, 6.4 Hz, CH*H*CHPh), 4.75 (t, 1 H, *J* = 9.2 Hz, C*H*HCHPh), 4.55 (d, 1 H, *J* = 4.6 Hz, H3′), 4.38 (d, 1 H, *J* = 15.2 Hz, NC*H*HPh), 4.32 (dd, 1 H, *J* = 6.4, 9.0 Hz, CHHC*H*Ph), 4.25 (d, 1 H, *J* = 15.2 Hz, NCH*H*Ph), 3.74–3.70 (m, 1 H, H4′), 3.50–3.45 (m, 1 H, C*H*HOH), 3.34–3.31 (m, 1 H, CH*H*OH); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm) 163.6, 158.7, 137.7, 135.5, 129.6, 129.5, 128.9, 128.2, 127.9, 127.8, 71.2, 60.4, 59.9, 58.9, 45.3; IR (KBr) ν_{max} 3432 (NH, OH), 1741 (C=O) cm^{-1}; CIMS (isobutane), *m/e* 353 (M^+ + H, base).

(3*R*,4*R*)-4-(Hydroxymethyl)-2-oxo-3-[[(phenylmethoxy)carbonyl]amino]azetidine (20). A THF/*t*-BuOH (10:1, 9 mL) solution of **19** (637 mg, 1.8 mmol) was added to a −78 °C solution of Na (315 mg, 13.7 mmol, 7.6 equiv) in NH_3 (30 mL). The excess Na was quenched after 3 min with the addition of solid NH_4Cl (481 mg, 9.0 mmol, 5 equiv) and the ammonia was allowed to distill off at −33 °C under a stream of N_2. The resulting white solid was dried in vacuo, dissolved in 5 mL of H_2O, briefly acidified to pH = 2 with the addition of 10% aqueous HCl, and subsequently

(43) Initial attempts to prepare **9** through coupling of **23** with BOCNH-D-Ala-Ala-*N*(Me)-Tyr(OMe)-Ala-OH followed by uneventful methyl ester hydrolysis, a problematic acid-catalyzed *N*-BOC deprotection (TFA, CH_2Cl_2 or HCl, EtOAc), and subsequent DPPA cyclization suffered from competitive acid-catalyzed reactions of the β-lactam.

(44) Boger, D. L.; Yohannes, D.; Myers, J. B.; Kito, P. A.; Suntornwat, O.; Kito, J. C. *Biomed. Chem. Lett.* **1991**, *1*, 313. The CBZ derivative of *N*-methylcycloisodityrosine methyl ester exhibits an IC_{50} value of 0.04 μg/mL (L1210).

(45) The evidence suggesting the inherent importance of the tetrapeptide has been derived indirectly through evaluation of the agent obtained from microbial demethylation of bouvardin to provide *O*-desmethylbouvardin (demethylation of Tyr^3-OMe), which proved inactive, refs 15 and 18. Since this report, the isolation and evaluation of RA-II (*O*-desmethyl RA-VII) suggests that the results of the earlier report should be reexamined.

made basic (pH = 8) with the addition of solid $NaHCO_3$. Benzyl chloroformate (1.5 g, 8.8 mmol, 5 equiv) was added to the aqueous mixture. After 4 h, the reaction mixture was extracted with 20% isopropyl alcohol–chloroform (10 × 20 mL). The organic extracts were combined, washed with saturated aqueous NaCl (1 × 10 mL), dried ($MgSO_4$), and concentrated in vacuo. Chromatography (SiO_2, 3 × 13 cm, EtOAc eluant) afforded **20** (298 mg, 450 mg theoretical, 66%): mp 128–130 °C (lit.[37] mp 127–128 °C); $[\alpha]^{20}_D$ −8.4° (*c* 1.14, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz, ppm) 7.30 (br s, 5 H, Ar), 6.15 (br s, 1 H, NH), 5.90 (d, 1 H, *J* = 9.7 Hz, H3), 5.20 (m, 1 H, NH), 5.10 (s, 2 H, CH_2Ph), 3.90 (m, 2 H, CH_2OH), 3.70 (m, 1 H, H4), 2.60 (m, 1 H, OH); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm) 170.5, 156.5, 130.1, 128.7, 128.4, 128.3, 67.3, 59.7, 59.1, 54.7; IR (KBr) ν_{max} 3298, 1759, 1704, 1558, 1326, 1272 cm^{-1}; CIMS (isobutane), *m/e* 251 (M^+ + H, base).

(3*R*,4*R*)-2-Oxo-3-[[(phenylmethoxy)carbonyl]amino]azetidine-4-carboxylic Acid (21). A solution of **20** (49 mg, 0.20 mmol) in 2 mL of dry DMF under argon was treated with PDC (450 mg, 1.2 mmol, 5 equiv) and stirred for 48 h. Water (2 mL) was added, and the solution was extracted extensively with EtOAc (10 × 10 mL). The organic extracts were combined, dried ($MgSO_4$), and concentrated in vacuo. The residue was dissolved in saturated aqueous $NaHCO_3$ (1 mL) and washed with EtOAc. The aqueous layer was acidified to pH = 1 with the addition of 10% aqueous HCl and extracted with EtOAc (5 × 5 mL). The organic extracts were combined, dried ($MgSO_4$), and concentrated in vacuo. Chromatography (SiO_2, 1 × 12 cm, acetic acid–methanol–ethyl acetate, 1:3:96) provided **3** (34 mg, 53 mg theoretical, 64%) as a white solid: mp 167 °C dec, $[\alpha]^{21}_D$ −55.7° (*c* 2.3, MeOH); ^1H NMR (DMF-d_7, 200 MHz, ppm) 7.4–7.25 (m, 5 H, Ar), 5.36 (dd, 1 H, *J* = 5.6, 11.6 Hz, H3), 5.19 (br s, 2 H, CH_2Ph), 4.46 (d, 1 H, *J* = 5.6 Hz, H4); ^{13}C NMR (acetone-d_6, 50 MHz, ppm) 170.7, 166.7, 156.1, 137.4, 128.7, 128.2, 128.0, 66.4, 62.2, 55.2; IR (KBr) ν_{max} 3358, 3318 (NH), 2966 (br, COOH), 1756 (C=O, β-lactam), 1718, 1684 (CO_2H) cm^{-1}; FABMS (glycerol), *m/e* (relative intensity) 265 (M^+ + H, 16), 154 (base); FABHRMS (glycerol), *m/e* 265.0833 ($C_{12}H_{13}N_2O_5$ requires 265.0824).

Methyl (3*R*,4*R*)-1-Methyl-2-oxo-3-[[(phenylmethoxy)carbonyl]methylamino]azetidine-4-carboxylate (22). A solution of **21** (200 mg, 0.76 mmol) in 10 mL of dry DMF cooled to 0 °C was treated sequentially with methyl iodide (0.7 mL, 11.2 mmol, 15 equiv) and NaH (60% in oil, 92 mg, 2.3 mmol, 3.1 equiv). After 0.5 h, the cold bath was removed and the solution was allowed to stir at 20 °C for an additional 10.5 h. The reaction mixture was poured into water and extracted with EtOAc (3 × 15 mL). The organic extracts were combined, washed with saturated aqueous $NaHCO_3$ (2 × 5 mL), water (2 × 5 mL), and saturated aqueous NaCl (1 × 5 mL), dried ($MgSO_4$), and concentrated in vacuo. Chromatography (SiO_2, 1 × 13 cm, 40% ethyl acetate–hexane eluant) afforded **22** (195 mg, 232 mg theoretical, 84%) as an oil: $[\alpha]^{20}_D$ +45.2° (*c* 2.3, $CHCl_3$); ^1H NMR ($CDCl_3$, 200 MHz, ppm) 7.36 (br s, 5 H, Ar) 5.50 and 5.40 (two d, 1 H, *J* = 5.5 and 5.3 Hz, H3), 5.14 (m, 2 H, CH_2Ph), 4.32 and 4.26 (two d, 1 H, *J* = 5.5 and 5.3 Hz, H4), 3.67 and 3.62 (two s, OCH_3, 3 H), 2.98 and 2.95 (two s, 3 H, NCH_3), 2.94 (s, 3 H, N^1CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm) 169.4 and 169.2, 165.0 and 164.5, 156.1 and 155.4, 136.3, 136.1, 128.6, 128.4, 128.3 and 128.1, 67.7, 67.2 and 67.1, 59.5 and 59.2, 52.4 and 52.2, 32.8, 27.8 and 27.7; IR (neat) ν_{max} 2954, 1772, 1706 cm^{-1}, CIMS (isobutane), *m/e* 307 (M^+ + H, base); CIHRMS, *m/e* 307.1294 ($C_{15}H_{19}N_2O_5$ requires 307.1294).

Methyl (3*R*,4*R*)-1-Methyl-3-(methylamino)-2-oxoazetidine-4-carboxylate (23). A solution of **23** (259 mg, 0.82 mmol) in 10 mL of THF was treated with 10% Pd/C (50 mg), placed under 1 atm of H_2, and stirred for 8 h (25 °C). The catalyst was filtered off through Celite and the filtrate was concentrated in vacuo. Chromatography (SiO_2, 2 × 12 cm, 20% ethyl acetate–methylene chloride eluant) afforded **23** as a clear oil (113 mg, 145 mg theoretical, 80%): $[\alpha]^{22}_D$ +25.5° (*c* 0.96, $CHCl_3$); ^1H NMR ($CDCl_3$, 200 MHz, ppm) 4.28 (m, 1 H), 4.20 (d, 1 H, *J* = 4.8 Hz), 3.74 (s, 3 H, OCH_3), 2.80 (s, 3 H, NCH_3), 2.41 (s, 3 H, NCH_3); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm) 170.2, 168.3, 72.0, 60.9, 52.2, 30.1, 27.3; IR (neat) ν_{max} 2931, 1752, 1735 cm^{-1}; EIHRMS, *m/e* 172.0854 ($C_7H_{12}N_2O_3$ requires 172.0845).

Methyl (3*R*,4*R*)-3-[(CBZNH-D-alanyl-L-alanyl-*N*(Me)-L-tyrosyl(OMe)-L-alanyl)methylamino]-1-methyl-2-oxoazetidine-4-carboxylate (25). A dry, cold (0 °C) DMF solution of **23** (49 mg, 0.29 mmol) and CBZNH-D-Ala-Ala-*N*(Me)-Tyr-(OMe)-Ala-OH[17] (**24**, 244 mg, 0.44 mmol) was treated with DCC (146 mg, 0.71 mmol, 2.4 equiv) and HOBt–H_2O (85 mg, 0.63 mmol, 2.1 equiv). After 2 h, the cold bath was removed and the reaction mixture was allowed to warm to 25 °C and stirred for an additional 12 h. The mixture was poured into 5 mL of EtOAc and filtered to remove dicyclohexylurea, and the organic layer was washed with 10% aqueous HCl (2 × 1 mL), saturated aqueous $NaHCO_3$ (2 × 1 mL), and saturated aqueous NaCl (1 × 2 mL), dried ($MgSO_4$), and concentrated in vacuo. Chromatography (SiO_2, 1 × 14 cm, 3% methanol–methylene chloride) afforded **25** (93 mg, 194 mg theoretical, 48%): ^1H NMR ($CDCl_3$, 200 MHz, ppm) 7.38 (br s, 5 H, Ar), 7.08 (two d, 2 H, *J* = 6.1 Hz, Tyr-δ), 6.82 (two d, 2 H, *J* = 9.2 Hz, Tyr-ε), 6.69 (m, 1 H, NH), 5.78 (m, 1 H, NH), 5.43 (m, 2 H, H3 and NH), 5.12 (br s, 2 H, CH_2Ph), 4.45–4.38 (m, 3 H, two CH_3CH and H4), 4.28 (m, 1 H, CH_3CH), 3.82 (s, 3 H, OCH_3), 3.78 (3 H, OCH_3), 3.30 (m, 1 H, Tyr-α), 3.10 (m, 2 H, Tyr-β), 2.96 and 2.93 (two s, 3 H, NCH_3), 2.88 and 2.79 (two s, 3 H, NCH_3), 2.69 and 2.67 (two s, 3 H, NCH_3), 1.34 (m, 9 H, CH_3); IR (neat) ν_{max} 1768 (β-lactam), 1718, 1648 cm^{-1}; CIMS (isobutane), *m/e* (relative intensity) 711 (13), 102 (base); FABHRMS (glycerol), *m/e* 711.3353 ($C_{35}H_{47}N_6O_{10}$ requires 711.3354).

(1*R*,16*R*)-Cyclo-(D-Ala-Ala-*N*(Me)-Tyr(OMe)-Ala-*N*-(Me)-(cCHCON(Me)CH)CO) (9). A solution of **25** (31 mg, 0.045 mmol) in THF/MeOH/H_2O (3:1:1, 0.5 mL) was treated with LiOH–H_2O (3.8 mg, 0.09 mmol, 2.0 equiv) and the resulting solution was stirred at 25 °C for 3 h. The mixture was washed with EtOAc (1 × 2 mL), acidifed to pH 2, and extracted with EtOAc (3 × 5 mL). The latter organic layers were combined, washed with saturated aqueous NaCl, and dried ($MgSO_4$). Chromatography (SiO_2, 1 × 10 cm, 5% ethanol–ethyl acetate) afforded the carboxylic acid (20 mg, 30 mg theoretical, 66%). A solution of the carboxylic acid (20 mg, 0.03 mmol) in 1.0 mL of MeOH was treated with 10% Pd/C (5 mg), placed under 1 atm of H_2, and stirred for 8 h at 25 °C. The catalyst was removed by filtration through Celite and the filtrate was concentrated in vacuo to afford the crude amino acid (19 mg). The crude amino acid (19 mg, 0.03 mmol) was dissolved in 5 mL of dry DMF, cooled to 0 °C, and treated sequentially with $NaHCO_3$ (12.6 mg, 0.15 mmol, 5 equiv) and diphenyl phosphorazidate (20.4 mg, 0.09 mmol, 3 equiv). After 72 h at 25 °C, the reaction mixture was concentrated, EtOAc was added (10 mL), and the mixture was washed with water (2 × 2 mL) and saturated aqueous NaCl (1 × 2 mL), dried ($MgSO_4$), and concentrated in vacuo. Chromatography (Florisil, 1 × 10 cm, 30% tetrahydrofuran–hexane eluant) afforded **9** (7.7 mg, 18.3 mg theoretical, 42%) as a white solid: mp 178–179 °C; $[\alpha]^{21}_D$ −7.7° (*c* 0.55, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz, ppm) major conformation 7.21–7.18 (d, 1 H, *J* = 8.3 Hz, Ala-NH), 7.06 (two d, 2 H, *J* = 7.9 Hz, Tyr-δ), 6.84 (d, 2 H, *J* = 8.3 Hz, Tyr-ε), 5.48 (d, 1 H, *J* = 5.1 Hz, H16), 4.91 (d, 1 H, *J* = 7.3 Hz, Ala-NH), 4.80 (m, 1 H, Ala-α), 4.75 (m, 1 H, Ala-α), 4.55 (m, 1 H, Ala-NH), 4.25 (d, 1 H, *J* = 5.1 Hz, H1), 4.19 (m, 1 H, Ala-α), 3.78 (s, 3 H, OCH_3), 3.61 (dd, 1 H, *J* = 10.0, 6.7 Hz, Tyr-α), 3.21 (m, 2 H, Tyr-β), 2.95 (s, 3 H, Tyr-NCH_3), 2.92 (s, 3 H, NCH_3), 2.87 (s, 3 H, NCH_3), 1.34 (d, 3 H, *J* = 6.7 Hz, Ala-β), 1.28 (d, 3 H, *J* = 6.0 Hz, Ala-β), 1.26 (d, 3 H, *J* = 6.4 Hz, Ala-β); ^{13}C NMR ($CDCl_3$, 125 MHz, ppm) 17.0 (Ala-β), 17.6 (Ala-β), 18.1 (Ala-β), 28.1 (NMe), 29.3 (NMe), 33.3 (NMe), 34.2 (Tyr-β), 43.3 (Ala-α), 45.9 (Ala-α), 49.2 (Ala-α), 55.4 (OMe), 60.3 (C16), 61.8 (C1), 66.1 (Tyr-α), 114.4 (Tyr-ε), 128.5 (Tyr-γ), 130.2 (Tyr-δ), 158.7 (Tyr-ξ), 163.8, 166.7, 168.1, 172.1, 172.3, 173.0; IR (KBr) ν_{max} 1763 (β-lactam), 1702, 1686, 1656, 1650, 1561 cm^{-1}; CIHRMS (isobutane), *m/e* 545.2713 ($C_{26}H_{37}N_6O_7$ requires 545.2724).

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Supplementary Material Available: ^1H NMR or ^{13}C NMR spectra of **15**, **18–23**, and **25** (9 pages). Ordering information is given on any current masthead page.