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Design, Synthesis and Evaluation of Bouvardin, Deoxybouvardin and RA-I–XIV Pharmacophore Analogs

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Abstract—The synthesis and *in vitro* cytotoxic evaluation of a key set of cycloisodityrosine subunit analogs of deoxybouvardin and RA-VII are detailed and constitute a complete investigation of the natural product pharmacophore. The studies illustrate that the 18-membered ring tetrapeptide potentiation of the cytotoxic activity of cycloisodityrosine is not likely to be due to simple alteration or constraint of the conformation of the 14-membered cycloisodityrosine subunit and that simple derivatization of cycloisodityrosine may not provide the same potentiation.

Bouvardin (**1**, NSC 259968)[1] and deoxybouvardin (**2**),[1] bicyclic hexapeptides isolated from *Rubia cordifolia* represent the initial members of a growing class of potent antitumor antibiotics now including RA-I–RA-XIV,[2–10] Figure 1. The potent antitumor activity of RA-VII (**8**) disclosed in the course of its examination including the demonstration of complete cures in a solid-tumor, colon adenocarcinoma 38, have illustrated the potential efficacy of agents in this series.[11] Bouvardin and RA-VII have been shown to inhibit protein synthesis through eukaryotic 80S ribosomal binding inhibiting both amino acyl–*t*RNA binding and peptidyl–*t*RNA translocation and this is currently regarded as the agent site of action.[12–14] Presently, RA-VII is in Phase 1 clinic trials and offers promise for the treatment of solid tumors.[15]

Initial inspection of the structures **1–2** led to the logical proposal that the biological activity resides in the D-Ala-Ala-NMe-Tyr(OMe)-Ala subunit or that of the related tetrapeptides and that the functional role of the 14-membered *N*-methyl cycloisodityrosine subunit was to restrict the tetrapeptide conformation to its biologically relevant and normally inaccessible conformation.[1,16] However, studies of tetrapeptide and hexapeptide analogs including **19–26** (Figure 2) which lack the cycloisodityrosine subunit but which may adopt the biologically active conformations of **1–13**[16–20] or the full ensemble of limited conformations[19] available to the agents have provided only inactive agents to date. In contrast, the simple derivatives **15–18** of cycloisodityrosine have been found to be potent cytotoxic agents only 10–30x less active than the natural products themselves[20–22] and thus have been shown to constitute the natural products pharmacophore. Moreover, while *N*-methyl cycloisodityrosine *and* cycloisodityrosine both adopt a rigid solution conformation possessing a *trans* N^{10}–C^{11} amide bond,[20,21] **1–2** *and* N^{29}-desmethyl RA-VII (**14**)[20] adopt rigid solution and crystal structure conformations which possess characteristic *cis* N^{29}–C^{30} amide bonds central to the *N*-methyl cycloisodityrosine or cycloisodityrosine subunit. Notable was the observation that even **14** incorporating a secondary N^{29}–C^{30} amide adopts this disfavored *cis* amide conformation.[20] Consequently, the experimental observations have suggested that the functional roles of the agent subunits are reversed from that initially proposed[1] and that it is the tetrapeptide housed within the 18-membered ring that potentiates the inherent biological properties and alters the conformation of cycloisodityrosine.[20–22]

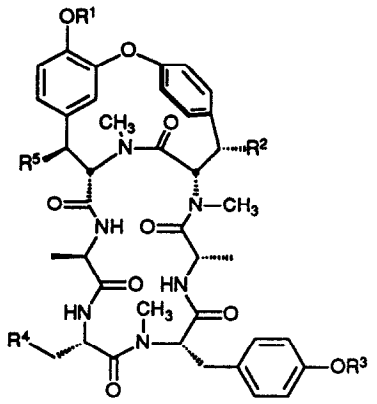
Until recently, efforts to critically examine cycloisodityrosine derivatives have been limited by their synthetic inaccessibility.[16,23–28] Our successful implementation of an effective intramolecular Ullmann reaction as the key macrocyclization reaction for the preparation of 14-membered biaryl ethers[20,21,29,30] and the introduction of modified reaction conditions[31] or techniques[32,33] for minimizing the extent of racemization under the thermal, basic reaction conditions have provided ready access to cycloisodityrosine and related agents.

The preliminary evaluation of key substructures of **1–13** in our laboratories have defined three essential points: (1) the inactivity of **19–26**, tetra- and hexapeptide analogs of **1–2** lacking the intact cycloisodityrosine structure, suggesting that the tetrapeptide plays a passive or potentiating role within the natural products, (2) the simple 14-membered ring biaryl ethers **27–32** were devoid of activity,[22] but (3) the functionalized 14-membered ring cycloisodityrosine derivatives **15–18** were only 10–30 fold less potent than the natural products. Herein, we detail a full study of cycloisodityrosine derivatives conducted with the intent of defining the structural features of **15–18** and, consequently, **1–14** responsible for their biological properties.

Design and Synthesis

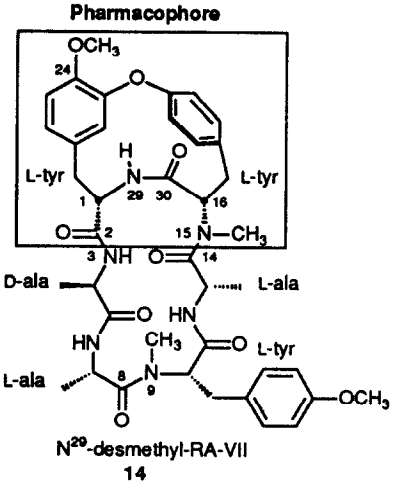
C4 Substituent modifications

Initial efforts to determine the relative importance of the cycloisodityrosine aryl C4 oxygen substituent were conducted with the agents **33–36**. Phenol demethylation

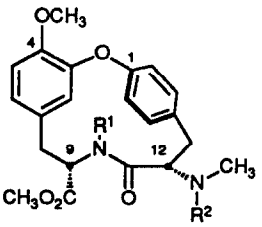


	R^1	R^2	R^3	R^4	R^5	
1	H	OH	Me	H	H	bouvardin
2	H	H	Me	H	H	deoxybouvardin (RA-V)
3	Me	OH	Me	H	H	*O*-Methyl-bouvardin
4	H	H	Me	OH	H	RA-I
5	Me	H	H	H	H	RA-II
6	Me	H	Me	OH	H	RA-III
7	Me	H	Me	H	OH	RA-IV
8	Me	H	Me	H	H	RA-VII
9	Me	H	Me	(OH)Me	H	RA-VIII
10	Me	H	Me	CH_2CO_2H	H	RA-X
11	H	H	Me	CH_2CO_2H	H	RA-XI
12	β-D-glucose	H	Me	H	H	RA-XII
13	β-D-glucose	H	Me	CH_2CO_2H	H	RA-XIII

Pharmacophore

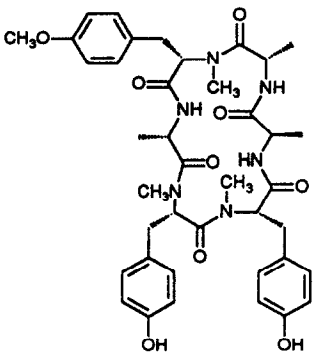


N^{29}-desmethyl-RA-VII
14



	R^1	R^2
15	Me	BOC
16	Me	CBZ
17	H	BOC
18	H	CBZ

Figure 1.



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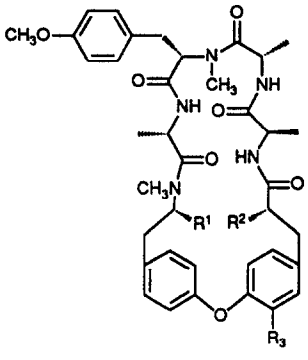
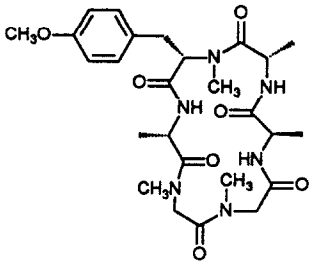


	R^1	R^2	R^3
20	H	H	H
21	CO_2Me	NMeCBZ	OBn
22	CO_2H	NHMe	OH



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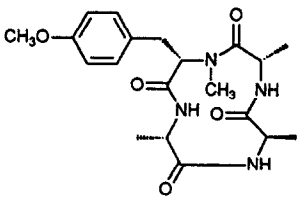

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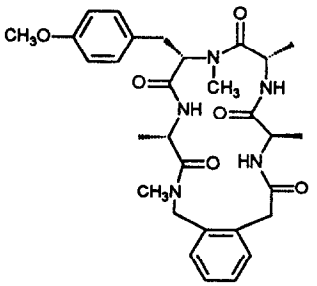

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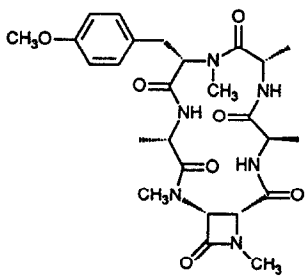

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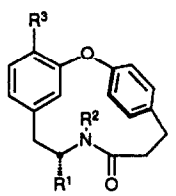
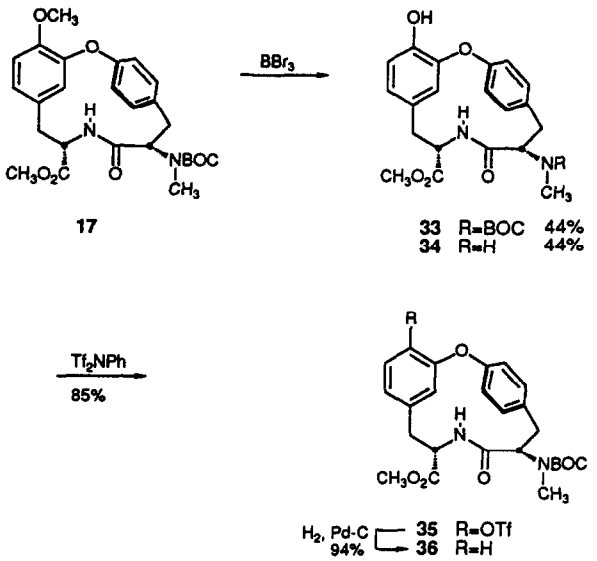


	R^1	R^2	R^3
27	H	H	H
28	H	Me	H
29	H	H	OMe
30	H	Me	OMe
31	CO_2Me	H	OH
32	CO_2Me	H	OMe

Figure 2.

of **17**[20] upon treatment with BBr_3 (1.5 equiv., CH_2Cl_2, -78 to 0 °C, 0.5 h) furnished the phenol **33** (44%) along with the phenol **34** (44%) resulting from additional *N*-BOC deprotection, Scheme I. Conversion of phenol **33** to the corresponding triflate **35** (1.1 equiv. $PhNTf_2$, 1.1 equiv. Et_3N, 25 °C, 8 h, 85%) followed by catalytic hydrogenolysis (H_2, 0.4 wt equiv. 10% Pd–C, CH_3OH, 25 °C, 8 h, 94%) provided **36**.



Scheme I.

C9 Substituent modifications

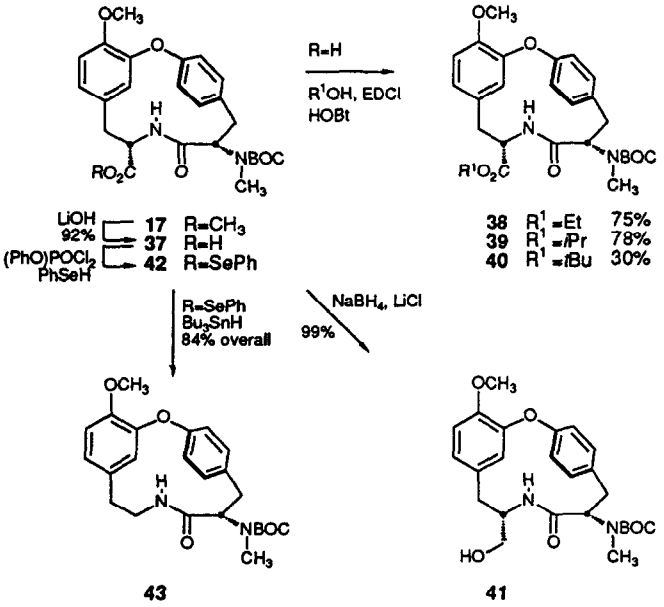
Efforts to determine the relative importance of the C9 carboxylate of cycloisodityrosine were conducted with the agents **37–43**. The methyl ester **17**[20] was converted to the carboxylic acid **37** (3 equiv. LiOH, THF–CH_3OH–H_2O 3:1:1, 0–25 °C, 4 h, 92%), Scheme II. Esterification of the free carboxylic acid with a series of alcohols (1–1.1 equiv. EDCI, 1–1.1 equiv. HOBt, CH_2Cl_2, 25 °C, 18 h) provided the corresponding ethyl (75%), isopropyl (78%), and *t*-butyl (30%) esters **38–40**, respectively. Reduction of the methyl ester **17**[20] ($NaBH_4$–LiCl, EtOH–THF 3:2, 25 °C, 18 h, 99%) provided the primary alcohol **41**. Removal of the C9 carboxylate to provide **43** was accomplished by conversion of the carboxylic acid **37** to the phenylselenoester **42** (2 equiv. $PhOP(O)Cl_2$, 3 equiv. Et_3N, THF, 0 °C, 10 min; 4 equiv. PhSeH,[35] 5 equiv. Et_3N, 0–25 °C, 6 h) followed by Bu_3SnH-mediated reductive decarbonylation[36] (15 equiv. Bu_3SnH, cat AIBN, C_6H_6, reflux, 1 h, 84% overall).

N10 Substituent modifications

The agents **15–18** available from initial studies[20] permitted two independent assessments of the relative importance of the N10 *N*-methyl amide found in the naturally occurring materials.

C12 Substituent modifications

In initial studies which led to the development of the methodology required for construction of the cycloisodityrosine nucleus, the comparisons of the biological

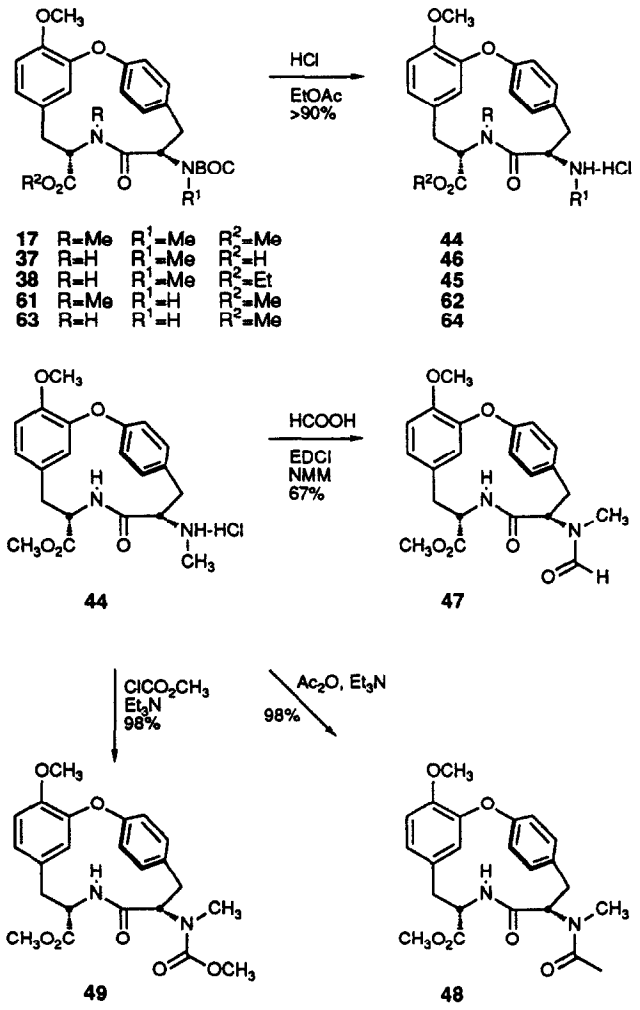


Scheme II.

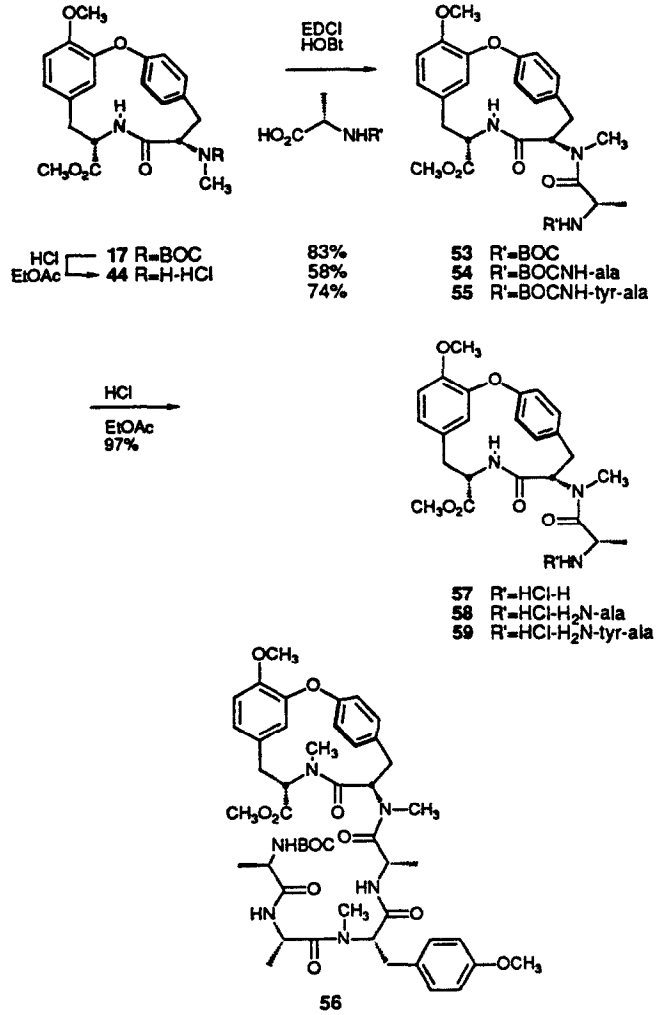
properties of **15–18** (L1210 IC_{50} = 0.03–0.06 μg/mL) versus those of **27–32**[22,29] (L1210 IC_{50} > 100 μg/mL) revealed the essential role of the C12 amine substituent and highlighted the unusual degree of flexibility available for substitution of the C12 amine. Although the amine substituent was required for observation of cytotoxic activity, the BOC and CBZ derivatives **15–18** were nearly indistinguishable. Consequently, a more extensive examination of the C12 amine substitution was undertaken. *N*-BOC deprotection (3M HCl–EtOAc, 25 °C, 50 min, 100%) of parent agent **17**[20] as well as the ethyl ester **38** and the free carboxylic acid **37** provided the C12 *N*-methyl amine hydrochloride salts **44**,[20] **45** and **46**, respectively, Scheme III.

As complements to the BOC and CBZ derivatives **17** and **18**, the *N*-formyl (HCO_2H, EDCI, 67%), *N*-acetyl (Ac_2O, Et_3N, 98%), and *N*-methoxycarbonyl ($ClCO_2Me$, Et_3N, 98%) derivatives **47**, **48** and **49** were prepared from **44** in efforts to more clearly define the role of the *N*-acyl substituent, Scheme III. In addition, the complete series of agents which incorporate the linear amino acid chain of the tetrapeptide linked to the C12 amine were prepared. While **56** was available from past studies,[20] coupling of **44** (3.0 equiv. EDCI, 3.0 equiv. HOBt, 8.0 equiv. $NaHCO_3$, DMF, 25 °C, 18 h) with *N*-BOC-alanine **50** (83%), *N*-BOC-ala-ala-OH **51** (58%), *N*-BOC-tyr-ala-ala-OH **52** (74%) provided **53–55**, respectively, Scheme IV. *N*-BOC deprotection (3M HCl–EtOAc, 25 °C, 50 min, 97%) of **53–55** provided the amine hydrochlorides **57–59**, respectively.

In conjunction with efforts to define the fundamental role of the *N*-methyl substituents of **1–13**, we have recently disclosed the preparations of **61–62**[37] which lacks the C12 *N*-methyl group and **63–64**[33] which lacks both the N10 and C12 *N*-methyl groups thus complementing the agents **15** and **17**. In addition, the comparisons of **62** with **61** and **64** with **63** provide an independent assessment of the role of the C12 *N*-acyl substituent versus the properties of



Scheme III.

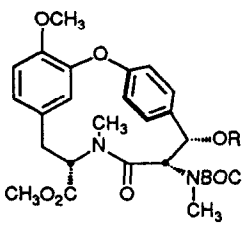


Scheme IV.

the free C12 amine, Scheme III. Similarly, the comparisons of **61** with **63** or **62** with **64** provides an independent assessment of the role of the N10 methyl substituent.

C13 Substituent modifications

Bouvardin (**1**) and deoxybouvardin (**2**) differ structurally only by the presence or absence of a C17 hydroxyl substituent located within the cycloisodityrosine subunit. In conjunction with efforts on the total synthesis of **1**,[38] the cycloisodityrosine derivatives **65** incorporating the C13 hydroxyl substituent were made available for comparison evaluation and an assessment of the role of this C13 substituent.



65a R=H
65b R=TBDMS

Bicyclic analogs

Perhaps the most interesting series of derivatives of cycloisodityrosine examined were **66–74** which constitute bicyclic, conformationally rigid analogs. The agents **66–72** were made available in the course of studies on the total synthesis of (+)-piperazinomycin (**71**),[33] **73** was disclosed in efforts on the preparation of **14**,[20] and **74** was prepared along with **43** in an Ullmann macrocyclization reaction. Especially interesting and important are the agents **66–69** which constitute rigid, bicyclic analogs of cycloisodityrosine adopting a single conformation possessing a characteristic *cis* N^{10}–C^{11} amide effectively mimicing the cycloisodityrosine structure, conformation, and characteristic N^{29}–C^{30} amide bond of **1–2**, Figure 3. Notably, **69** possesses the correct stereochemistry for its secondary amide to overlap with the *cis* N^{29}-C^{30} amide of **1–2**. Although this has been discussed in detail elsewhere,[33] conformational analysis[39–41] of **67** revealed a single low-energy conformation within 12 kcal/mol which possessed a partial or flattened boat diketopiperazine ring. Moreover, this single low-energy conformation of **67** proved consistent with the NOEs observed in the 2D ^{1}H–^{1}H NMR spectrum and was found to correspond precisely to the conformation of the cycloisodityrosine subunit of bouvardin observed in the single-crystal X-ray structure

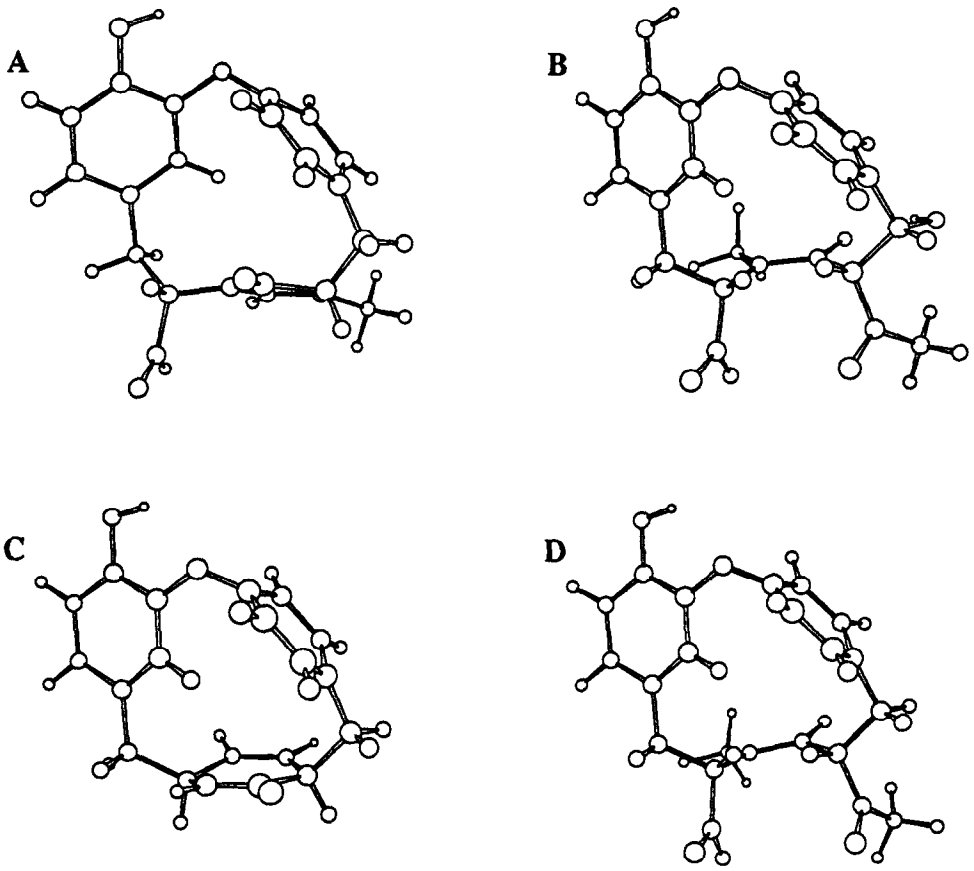
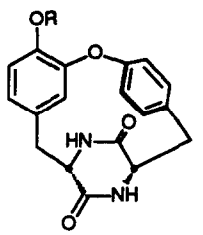


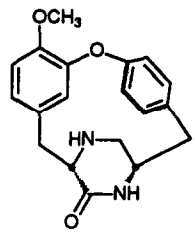
Figure 3. A: OPLSA low energy conformation of **73**; B: 14-membered ring conformation taken from X-ray crystal structure of bouvardin; C: OPLSA low energy conformation of **66**; D: 14-membered ring conformation taken from OPLSA low energy conformation of deoxybouvardin

(RMS = 0.18 Å for all non-hydrogen atoms). This precise adoption of the cycloisodityrosine *cis* amide conformation found within bouvardin (RMS = 0.18 Å) and deoxybouvardin (RMS 0.14 Å) suggested **66–69** may prove especially interesting to examine. Although **66–69** were anticipated to be the most interesting of the agents in this series, the bicyclic imides **72**[33] and **73**[20] unexpectedly proved to be potent derivatives of cycloisodityrosine.

The agents **72–74** adopt a single rigid conformation in solution and a conformational analysis[39–41] of **73** revealed a single low-energy conformation within 5 kcal/mol available to agent. This conformation, which proved consistent with the coupling constants observed in the ^1H NMR spectrum (C9/C12–H; experimental: 1.7, 12.0; 3.8, 5.2 versus calculated: 1.7, 11.5; 2.3, 4.1) and NOE crosspeaks observed in the 2D ^1H–^1H NMR spectrum,[39] proved distinct from the *cis* amide conformation of the cycloisodityrosine subunit of **1–14** (RMS = 1.38 Å for non-hydrogen ring atoms), the low energy conformation of **66** (RMS = 1.29 Å for non-hydrogen atoms) and the low energy conformation of **17** which possesses a *trans* N^{10}–C^{11} amide (RMS = 0.35 Å for non-hydrogen atoms). Characteristic of these distinctions, the C9–H/C12–H distance with **73** was found to be 4.62 Å versus 1.98 Å, 3.66 Å, and 4.22 Å for **1** (X-ray), **66** and **17**, respectively. Of the agents examined, **73** most closely approximates the *trans* amide conformation of **17** although the biaryl ether conformation and presentation for each of the agents is very similar, Figure 3.



66 R=CH_3
67 R=H
68 R=$COCH_3$



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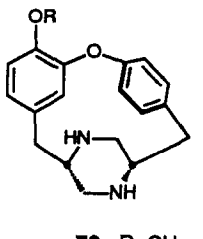
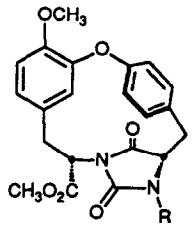
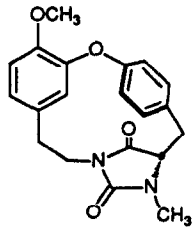


70 R=CH_3
71 R=H (piperazinomycin)



72 R=H
73 R=CH_3



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Biological Evaluations and Discussion

Each of the agents were evaluated for *in vitro* L1210 cytotoxic activity for direct comparison with past efforts and followed a well established protocol which has been described[42] in detail elsewhere. Table 1 summarizes the results of our preceding efforts. Bouvardin (**1**) and *O*-methyl bouvardin (**3**) as well as deoxybouvardin (**2**) and RA-VII (**8**) each exhibit the same cytotoxic potency (IC_{50} = 0.008 and 0.002 μg/mL, L1210, respectively) indicating that in the intact natural products the C24 aryl methyl ether versus phenol and the presence or absence of the C17 hydroxyl group do not significantly alter the potency of the agents. Removal of the N^{29} methyl group within the key N^{29}–C^{30} *cis* amide of **8** with the agent **14** led to a small (2x) increase in cytotoxic potency clearly indicating that the tertiary N^{29}–C^{30} *N*-methyl amide was not essential for observation of the biological activity.

Table 1.

Agent	IC_{50} (L1210, μg/mL)	Rel IC_{50} (L1210)
1	0.008	7.5
2	0.002	30.
3	0.008	7.5
8	0.002	30.
14	0.001	60.
15	0.03	2.0
16	0.04	1.5
17	0.06	1.0
18	0.05	1.2
19-26	>10	<0.006
27-32	>100	<0.0006

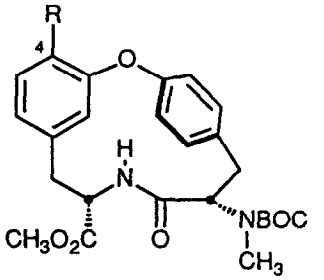
In addition, the cyclic tetra- and hexapeptide analogs **19–26** lacking the intact 14-membered cycloisodityrosine subunit proved inactive. Notably, *O-seco*-deoxybouvardin (**19**) lacking only the 14-membered ring biaryl ether linkage and **20–22** lacking only the transannular N^{29}–C^{30} amide bond central to the 14-membered cycloisodityrosine subunit proved inactive highlighting its essential role. Consistent with these observations, the parent 18-membered cyclic hexapeptide **23**, the cyclic hexapeptide analog **25** bearing a simple N^{29}–C^{30} *cis* amide replacement, and the 12-membered cyclic tetrapeptide **24** also proved inactive. Especially interesting was the inactive β-lactam analog **26** which accurately serves to restrict the accessible conformations of the tetrapeptide to the full ensemble of conformations available to **1–2** but which lacks the key cycloisodityrosine subunit. The inactivity of **26** may well represent a demonstration that the tetrapeptide subunit of **1–2** even when constrained to the natural product accessible conformations is insufficient for observation of biological activity.

In addition, the comparison of **15–18** versus **27–32** illustrated that potent cytotoxic activity may be observed with the fully functionalized 14-membered cycloisodityrosine derivatives, that the presence of the C12 amine substituent was essential to the properties of the agent but that a large degree of flexibility for the C12 amine substitution was well tolerated, and that the tertiary N10 *N*-methyl amide was not essential for observation of the biological activity. Finally, derivatives of RNH-tyr-tyr-OCH_3 or RNMe-tyr-NMe-tyr-OCH_3 lacking the biaryl ether linkage do not display cytotoxic activity.

C4 Substituent modifications

A comparison of the relative cytotoxic potency of **17** versus **33** illustrates the slight potentiation that is achieved (1.8x) with the presence of the free phenol versus the aryl methyl ether, Table 2. However, removal of the C4 oxygen substituent with **36** provided an agent equivalent or slightly more potent (1.2x) than **17** illustrating that the C4 oxygen substituent does not play an essential role. The same trend (OH > OCH_3) was observed in the comparison of **66** with **67**, Table 9, and in their limited comparison with **68** (inactive) further suggest that *O*-acyl substituents diminish the cytotoxic properties of the agents.

Table 2.



R=OH > H ≥ OCH_3

Agent	R	Rel IC_{50} (L1210)
17	OCH_3	1.0
33	OH	1.8
36	H	1.2

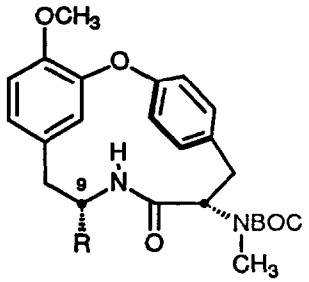
C9 Substituent modifications

The comparisons of the relative cytotoxic potency of **17** versus **37–43** proved revealing, Table 3. Removal of the C9 methyl ester with the agent **43** provided an agent that was less potent than **17** (0.3x) illustrating that the C9 methyl ester or carboxylate is playing an important but not essential role. Consistent with this observation, little significant variation in biological activity was observed within the series of esters **38–40** (ethyl, isopropyl, *t*-butyl) versus **17** (methyl) although the parent methyl ester **17** proved to be the most potent of the series. Nonetheless, this substituent possesses the capabilities to strongly potentiate the biological activity of the cycloisodityrosine derivatives. The C9 free carboxylic acid **37** proved inactive, while the C9 hydroxymethyl derivative was found to be 2.4x more potent than **17**.

N10 Substituent modifications

Several independent comparisons are now available for the assessment of the relative importance of the N10 methyl substituent of *N*-methyl cycloisodityrosine. In initial studies, this comparison was made with the agents **15** versus **17** and **16** versus **18**, Table 4. In these comparisons, and in the additional subsequent comparisons summarized in Table 4, the tertiary amide N10 methyl derivatives proved to be equipotent or slightly more potent than the unsubstituted or secondary N10 amide derivatives indicating that the N10 *N*-methyl substituent is not essential to the properties of **15** or **1–2** and is not

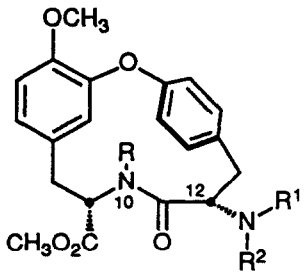
Table 3.



R=CH_2OH > CO_2CH_3 > H, CO_2R >> CO_2H

Agent	R	Rel IC_{50} (L1210)
17	CO_2CH_3	1.0
37	CO_2H	<0.1 (inactive)
38	CO_2Et	0.4
39	CO_2iPr	0.8
40	CO_2tBu	0.3
41	CH_2OH	2.4
43	H	0.3

Table 4.



R=Me ≥ H 1-2x

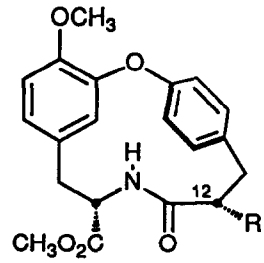
Agent	R	R^1/R^2	Rel IC_{50} (L1210)
15	Me	Me/BOC	2.0
17	H	Me/BOC	1.0
16	Me	Me/CBZ	1.5 (1.0)
18	H	Me/CBZ	1.2 (0.8)
61	Me	H/BOC	1.8 (1.0)
63	H	H/BOC	1.8 (1.0)
62	Me	H-HCl	1.5 (1.0)
64	H	H-HCl	1.5 (1.0)

contributing strongly to the potentiation of the agents properties.

C12 Substituent modifications

The most prominent structural feature of the cyclo-isodityrosine derivatives defined in the investigations is the essential role that the C12 amine substituent plays. This was first disclosed in the comparisons of **15–18** with **27–32**, Table 1, and is clearest in the direct comparisons of **17** with **32**, Table 5, or in the comparison of **33** with **31**. Removal of the C12 substituent results in a loss of cytotoxic activity. Although this substituent is required for activity, a great deal of flexibility in the nature of this substituent is tolerated. The relative potency of the agents is almost invariant with the nature of the C12 *N*-acyl substituent as illustrated by the observation that the BOC

Table 5.



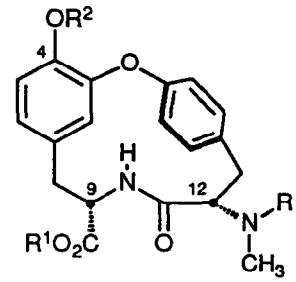
RNCOR > RNH-HCl >> H
HNCOR > MeNCOR
HNH-HCl > MeNH-HCl

Agent	R	Rel IC_{50} (L1210)
17	MeNBOC	1.0
32	H	<0.006(inactive)
18	MeNCBZ	1.2
47	MeNCHO	1.0
48	$MeNCOCH_3$	1.2
49	$MeNCO_2CH_3$	0.2
44	MeNH-HCl	0.3
63	HNBOC	1.8 (1.0)
64	HNH-HCl	1.5 (0.8)

(**17**), CBZ (**18**), formyl (**47**), and acetyl (**48**) derivatives are nearly indistinguishable.

In addition, removal of the *N*-acyl substituent to provide the corresponding *N*-methyl amine **44** tested as its hydrochloride salt resulted in slight reduction in the cytotoxic potency of the agent. This trend was examined further with the comparisons of **33** with **34**, **38** with **45**, and **37** with **46**, Table 6. In general, the free amine hydrochloride salts proved less potent than the corresponding *N*-BOC derivatives but the distinctions were found to be surprisingly small. The comparison of **34** with **44** provided an additional verification that the C4 free phenol derivatives are more potent than the C4 methyl ethers and the examination of **46** provided an independent verification that C9 carboxylic acids are inactive.

Table 6.



R=MeNBOC ≥ MeNH-HCl

Agent	R^1	R^2	R	Rel IC_{50} (L1210)
17	Me	Me	BOC	1.0
44	Me	Me	H-HCl	0.3
33	Me	H	BOC	1.8 (1.0)
34	Me	H	H-HCl	1.6 (0.9)
38	Et	Me	BOC	0.4 (1.0)
45	Et	Me	H-HCl	0.5 (1.3)
37	H	Me	BOC	inactive
46	H	Me	H-HCl	inactive

Examination of the primary amine versus *N*-methyl secondary amine derivatives with the comparisons of **17** versus **63** and **44** versus **64** revealed that the removal of the C12 *N*-methyl substituent results in a general but small increase in cytotoxic potency, Table 5.

A similar trend was observed with the more elaborate *N*-acyl derivatives **53–56** and their corresponding free amine hydrochloride salts **57–59**, Table 7. Little change in the cytotoxic potency of the agents was observed with the sequential addition of the tetrapeptide amino acids to the C12 *N*-methyl amino group.

Table 7.

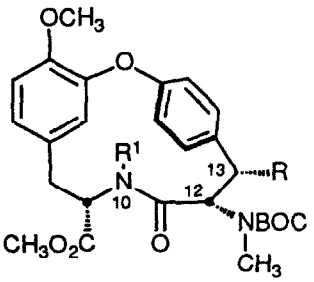
Agent	Rel IC_{50} (L1210)
17	1.0
53	0.6
54	0.4
55	1.3
56	1.5[a]
57	1.1
58	0.5
59	0.5

[a]The corresponding N10 *N*-methyl derivative displays a rel. IC_{50} (L1210) potency of 1.2 and the corresponding bouvardin derivative possessing a N10 *N*-methyl and C13 hydroxy substituent displays a rel. IC_{50} (L1210) potency of 1.6.

C13 Substituent modifications

The introduction of the 13(*S*)-hydroxy group into *N*-methyl cycloisodityrosine (**15**) resulted in an increase in cytotoxic potency (1.6–1.7x).

Table 8.



R=OH > H

Agent	R^1	R	Rel IC_{50} (L1210)
17	H	H	1.0
15	Me	H	2.0 (1.0)
65a	Me	OH	3.3 (1.6)
65b	Me	$OSitBuMe_2$	2.5

Bicyclic analogs

Perhaps the most interesting series of cycloisodityrosine derivatives were the bicyclic analogs **66–73**, Table 9. The agents **66–69** mimic precisely the structure, conformation, and critical N^{10}–C^{11} *cis* amide of cycloisodityrosine as it is incorporated into **1–2**, Figure 3. The agent **66** proved to be only slightly more potent than **17** and, consistent with past observations, the removal of the methyl ether of **66** to provide **67** resulted in an increase in cytotoxic potency. Conversion of the free phenol to the acetate **68** resulted in the loss of cytotoxic activity indicating that C4 *O*-acyl groups are not well tolerated. The agent **69** proved to be only slightly less potent than **66** or **17** and the structure and results are analogous to the observation made in the comparisons of C12 *N*-acyl versus C12 free amine cycloisodityrosine derivatives. Even **70** and **71**, lacking the carbonyl of the central amide were found to possess cytotoxic activity albeit at a reduced level. The evaluations of **72–74** also proved surprising. Even though the three agents possess conformations of the 14-membered ring distinctly different from that of **17**, the cycloisodityrosine subunit of **1–14**, or **66** they maintained comparable cytotoxic activity and removal of the methyl ester of **73**, in contrast to the **17/43** comparison, led to a modest increase in cytotoxic potency. Consequently, the studies illustrate that the 18-membered ring tetrapeptide potentiation of the cytotoxic activity of **17** is not likely to be due to simple alteration or constraint of the conformation of the 14-membered cycloisodityrosine subunit.

Table 9.

Agent	Rel IC_{50} (L1210)
17	1.0
66	1.1
67	1.6
68	inactive
69	0.6
70	0.6
71	0.5
72	1.0
73	0.5
74	1.2

Conclusions

The C4 substituent of cycloisodityrosine is not essential for observation of cytotoxic activity ($H \geq OCH_3$) but can strongly influence potency ($OH > H \geq OCH_3 >> OAc$). Similarly, the C9 substituent is not essential but may significantly potentiate the cytotoxic activity of the agents ($CH_2OH > CO_2CH_3 > CO_2R \geq H >> CO_2H$). In general, *N*-methylation of the central N^{10}–C^{11} amide leads to slightly enhanced cytotoxic potency and an unusual degree of flexibility is tolerated with substitution of the C12 amine although its presence is essential for observation of cytotoxic activity. Finally, introduction of the bouvardin 13(*S*)-hydroxy group into **15** also enhanced the potency of the agent although it is not essential for observation of activity. Finally, the constrained analogs **66–67** and **69** proved essentially equipotent with **17** indicating that, while they are not inactive, their constraint to the cycloisodityrosine conformation found in **1–14** is not sufficient to further potentiate the activity of the agents to the point of being comparable to that of the natural products. Further studies on the structural origin of the biological properties of **1–14** and related agents[43] are in progress and will be reported in due course.

Experimental

Methyl 12(S)-[N-[(1,1-dimethylethoxy)carbonyl]-N-methylamino]-4-hydroxy-11-oxo-10-aza-2-oxatricyclo-[12.2.2.1^{3,7}]nonadeca-3,5,7(19),14,16,17-hexaen-9(S)-carboxylate (33) and methyl 4-hydroxy-12(S)-(N-methylamino)-11-oxo-10-aza-2-oxatricyclo[12.2.2.1^{3,7}]nonadeca-3,5,7(19),14,16,17-hexaen-9(S)-carboxylate (34)

A solution of **17**[20] (10.1 mg, 0.021 mmol) in anhydrous CH_2Cl_2 (0.1 mL) was cooled to -78 °C and treated with BBr_3 (1.0M in CH_2Cl_2, 31.3 μL, 0.0313 mmol, 1.5 equiv.) and the reaction mixture was allowed to warm gradually to 0 °C (0.5 h). The mixture quenched with the addition of saturated aqueous $NaHCO_3$ (5.0 mL) and extracted with EtOAc (4 x 5.0 mL). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Flash chromatography (SiO_2, 1.0 x 10.0 cm, 0–5% EtOH–EtOAc) afforded **33** (4.3 mg, 9.7 mg theoretical, 44%) and **34** (3.4 mg, 9.8 mg theoretical, 44%). For **33**: white solid, m.p. 148–151 °C; $[\alpha]_D^{25}$ -6.1 (c 0.11, $CHCl_3$); ^1H NMR ($CDCl_3$, 250 MHz) δ 7.22 (dd, 1H, obscured by $CHCl_3$, C15–H), 7.13 (br d, 1H, *J* = 8.3 Hz, C18–H), 6.96 (m, 2H, C16–H and C17–H), 6.80 (d, 1H, *J* = 8.3 Hz, C5–H), 6.54 (br d, 1H, *J* = 8.3 Hz, C6–H), 4.86 (br s, 1H, C19–H), 4.18 (m, 1H, C12–H), 3.81 (m, 1H, C9–H), 3.75 (s, 3H, $COOCH_3$), 3.56 (t, 1H, *J* = 11.7 Hz, C13–H_αH), 3.00 (br s, 3H, NCH_3), 2.7–3.0 (m, 2H, C13–H_βH and C8–H_αH), 2.65 (dd, 1H, *J* = 11.2, 16.7 Hz, C8–H_βH), 1.34 (s, 9H, $NCOOC(CH_3)_3$); IR (neat) ν_{max} 3409, 2956, 2923, 2856, 1729, 1658, 1598, 1467, 1452, 1261, 1165, 1100, 1034, 798 cm^{-1}; FABHRMS (NBA) m/e 471.2117 (M^+ + H, $C_{25}H_{30}N_2O_7$ requires 471.2131).

For **34**: white solid, m.p. 108–110 °C; $[\alpha]_D^{25}$ -6.8 (c 0.11, $CHCl_3$); ^1H NMR ($CDCl_3$, 250 MHz) δ 7.76 (d, 1H, *J* = 8.3 Hz, NH), 7.43 (dd, 1H, *J* = 2.2, 8.4 Hz, C15–H), 7.29 (dd, 1H, *J* = 2.2, 8.2 Hz, C18–H), 7.12 (m, 2H, NH and C16–H), 6.92 (br d, 1H, *J* = 8.2 Hz, C17–H), 6.67 (d, 1H, *J* = 8.0 Hz, C5–H), 6.50 (br d, 1H, *J* = 8.0 Hz, C6–H), 5.04 (br s, 1H, C19–H), 4.99 (m, 1H, C12–H), 3.88 (m, 1H, C9–H), 3.67 (s, 3H, $COOCH_3$), 3.1–3.3 (m, 2H, $ArCH_2$), 2.6–2.8 (m, 2H, $ArCH_2$), 2.36 (d, 3H, *J* = 4.8 Hz, NCH_3); IR (neat) ν_{max} 3344, 3282, 2954, 2923, 2851, 1744, 1657, 1595, 1518, 1498, 1441, 1282, 1216, 1159, 1113, 1098, 882, 836, 805 cm^{-1}; FABHRMS (NBA-CsI) m/e 503.0594 (M^+ + Cs, $C_{20}H_{22}N_2O_5$ requires 503.0583).

Methyl 12(S)-[N-[(1,1-dimethylethoxy)carbonyl]-N-methylamino]-11-oxo-10-aza-2-oxatricyclo[12.2.2.1^{3,7}]-nonadeca-3,5,7(19),14,16,17-hexaen-9(S)-carboxylate (36)

A solution of **33** (2.1 mg, 0.0045 mmol) in anhydrous CH_2Cl_2 (45 μL) was treated with Et_3N (0.5 mg, 0.7 μL, 0.0049 mmol, 1.1 equiv.) and Tf_2NPh (1.8 mg, 0.0049 mmol, 1.1 equiv.) overnight (8 h, 25 °C) before the mixture was concentrated *in vacuo*. Flash chromatography (SiO_2, 0.5 x 5.0 cm, 0–40% EtOAc–hexane) afforded **35** (2.3 mg, 2.7 mg theoretical, 85%). A solution of **35** (2.3 mg, 0.0038 mmol) in CH_3OH (0.3 mL) was treated with 10% Pd–C (1 mg) and $NaHCO_3$ (3 mg, 0.036 mmol, 10 equiv.) under H_2 until complete conversion by TLC (8 h). The mixture was filtered through Celite and concentrated *in vacuo*. Flash chromatography (SiO_2, 0.5 x 5.0 cm, 0–30% EtOAc–hexane) afforded **36** (1.6 mg, 1.7 mg theoretical, 94%): white solid, m.p. 136–139 °C; $[\alpha]_D^{25}$ -5.2 (c 0.04, $CHCl_3$); ^1H NMR ($CDCl_3$, 250 MHz) δ 7.41 (br m, 3H, ArH), 7.17 (br m, 2H, ArH), 6.96 (br m, 2H, ArH), 6.63 (br d, 1H, *J* = 8.3 Hz, C6–H), 5.10 (br m, 1H, C12–H), 4.93 (br s, 1H, C19–H), 4.30 (br m, 1H, C9–H), 3.73 (s, 3H, $COOCH_3$), 3.57 (br d, 1H, *J* = 11.9 Hz, C13–H_αH), 3.00 (s, 3H, NCH_3), 2.9–3.0 (m, 2H, C13–H_βH and C8–H_αH), 2.63 (dd, 1H, *J* = 10.3, 16.4 Hz, C8–H_βH), 1.55 (s, 9H, $NCOOC(CH_3)_3$); IR (neat) ν_{max} 3354, 2955, 2923, 2853, 1732, 1652, 1604, 1588, 1504, 1446, 1376, 1260, 1226, 1164, 1096, 1031, 967, 884, 836, 754 cm^{-1}; FABHRMS (NBA-NaI) m/e 455.2189 (M^+ + H, $C_{25}H_{30}N_2O_6$ requires 455.2182).

12(S)-[N-[(1,1-Dimethylethoxy)carbonyl]-N-methylamino]-4-methoxy-11-oxo-10-aza-2-oxatricyclo[12.2.2.1^{3,7}]-nonadeca-3,5,7(19),14,16,17-hexaen-9(S)-carboxylic acid (37)

A solution of **17**[20] (38.5 mg, 0.080 mmol) in $THF/CH_3OH/H_2O$ (3:1:1, 0.8 mL) was treated with $LiOH–H_2O$ (10 mg, 0.24 mmol, 3 equiv.) at 0 °C and the mixture was allowed to warm gradually to 25 °C (4 h). The reaction mixture was quenched with the addition of 5% aqueous HCl (2.0 mL) and the mixture was extracted with EtOAc (4 x 2.0 mL). The combined organic extracts were washed (3 x 1.5 mL) each with H_2O and saturated aqueous NaCl, dried (Na_2SO_4), filtered and concentrated *in vacuo* followed by thorough drying of the product under high vacuum to afford **37** (35.5 mg, 37.4 mg theoretical, 92%): white foam, m.p. 213–215 °C; $[\alpha]_D^{25}$ -68 (c 0.05, $CHCl_3$); ^1H NMR ($CDCl_3$, 250 MHz) δ 9.75 (br s, 1H, COOH), 7.38 (br d, 1H, *J* = 8.3 Hz, C15–H), 7.25 (dd, 1H, *J* = 2.2, 8.3 Hz, C18–H), 7.09 (dd, 1H, *J* = 2.3, 8.3 Hz, C16–H), 6.95 (dd, 1H, *J* = 2.3, 8.3 Hz, C17–H), 6.75 (d, 1H, *J* = 8.2 Hz, C5–H), 6.66 (br d, 1H, NH), 6.58 (dd, 1H, *J* = 2.0, 8.2 Hz, C6–H), 5.09 (br s, 1H, C19–H), 4.59 (dd, 1H, *J* = 2.0, 12.1 Hz, C12–H), 4.15 (t, 1H, *J* = 9.2 Hz, C9–H), 3.92 (s, 3H, $ArOCH_3$), 3.25 (t, 1H, *J* = 11.9 Hz, C13–H_αH), 3.00 (s, 3H, NCH_3), 2.85–3.00 (m, 2H, C13–H_βH and C8–H_αH), 2.74 (m, 1H, C8–H_βH), 1.44 (s, 9H, $NCOOC(CH_3)_3$); IR (neat) ν_{max} 3327, 2966, 2928, 1722, 1662, 1514, 1481, 1444, 1393, 1365, 1263, 1222, 1147, 1027, 800 cm^{-1}; FABHRMS (NBA-CsI) m/e 603.1116 (M^+ + Cs, $C_{25}H_{30}N_2O_7$ requires 603.1107).

Ethyl 12(S)-[N-[(1,1-dimethylethoxy)carbonyl]-N-methylamino]-4-methoxy-11-oxo-10-aza-2-oxatricyclo-[12.2.2.1^{3,7}]nonadeca-3,5,7(19),14,16,17-hexaen-9(S)-carboxylate (38)

A solution of **37** (14 mg, 0.030 mmol), EDCI–HCl (5.8 mg, 0.030 mmol, 1.0 equiv.), $HOBt–H_2O$ (2.3 mg, 0.030 mmol, 1.0 equiv.), and EtOH (1.4 mg, 1.8 μL, 0.030

mmol, 1.0 equiv.) in CH_2Cl_2 (0.15 mL) was stirred at 25 °C (18 h). The reaction mixture was quenched by the addition of H_2O (1.0 mL) and extracted with CH_2Cl_2 (4 x 1.0 mL). The organic phase was washed with 5% aqueous HCl (3 x 1.0 mL), saturated aqueous $NaHCO_3$ (3 x 2.0 mL), H_2O (3 x 1.0 mL) and saturated aqueous NaCl (3 x 1.0 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Flash chromatography (SiO_2, 1.0 x 8.0 cm, 30% EtOAc–hexane) afforded **38** (11.1 mg, 14.8 mg theoretical, 75%): pale yellow solid, m.p. 150–152 °C; $[\alpha]_D^{25}$ -5 0 (c 0.16, CH_3OH); ^1H NMR ($CDCl_3$, 250 MHz) δ 7.42 (br d, 1H, *J* = 8.4 Hz, C15–H), 7.26 (dd, 1H, *J* = 2.2, 8.3 Hz, C18–H), 7.08 (dd, 1H, *J* = 2.3, 8.4 Hz, C16–H), 6.95 (br d, 1H, *J* = 8.3 Hz, C17–H), 6.75 (d, 1H, *J* = 8.3 Hz, C5–H), 6.58 (dd, 1H, *J* = 1.9, 8.3 Hz, C6–H), 5.81 (d, 1H, *J* = 8.3 Hz, NH), 5.10 (br s, 1H, C19–H), 4.57 (br d, 1H, *J* = 8.8 Hz, C12–H), 4.16 (t, 1H, *J* = 7.4 Hz, C9–H), 4.10 (q, 2H, *J* = 7.1 Hz, $COOCH_2$), 3.92 (s, 3H, OCH_3), 3.25 (t, 1H, *J* = 11.9 Hz, C13–H_αH), 2.97 (s, 3H, NCH_3), 2.60–2.95 (m, 3H, C13-H_βH and C8–H_2), 1.49 (s, 9H, $NCOOC(CH_3)_3$), 1.18 (t, 3H, *J* = 7.1 Hz, CH_2CH_3); IR (neat) ν_{max} 3354, 2965, 2924, 2852, 1736, 1700, 1680, 1667, 1514, 1443, 1367, 1262, 1221, 1142, 1131, 1026 cm^{-1}; FABHRMS (NBA-CsI) m/e 631.1429 (M^+ + Cs, $C_{27}H_{34}N_2O_7$ requires 631.1420).

iso*Propyl 12(S)-[N-[(1,1-dimethylethoxy)carbonyl]-N-methylamino]-4-methoxy-11-oxo-10-aza-2-oxatricyclo-[12.2.2.1^{3,7}]nonadeca-3,5,7(19),14,16,17-hexaen-9(S)-carboxylate (39)*

A solution of **37** (10.1 mg, 0.022 mmol), EDCI–HCl (4.6 mg, 0.024 mmol, 1.1 equiv.), HOBt–H_2O (3.3 mg, 0.024 mmol, 1.1 equiv.), and *i*PrOH (1.4 mg, 2.0 μL, 0.024 mmol, 1.1 equiv.) in CH_2Cl_2 (0.2 mL) was stirred at 25 °C (18 h). The reaction mixture was quenched by the addition of H_2O (1.0, mL) and extracted with CH_2Cl_2 (4 x 1.0 mL). The organic phase was washed with 5% aqueous HCl (3 x 1.0 mL), saturated aqueous $NaHCO_3$ (3 x 2.0 mL), H_2O (3 x 1.0 mL) and saturated aqueous NaCl (3 x 1.0 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Flash chromatography (SiO_2, 1.0 x 8.0 cm, 30% EtOAc–hexane) afforded **39** (8.6 mg, 11.0 mg theoretical, 78%) as a pale yellow solid: m.p. 154–157 °C; $[\alpha]_D^{25}$ -4.7 (c 0.12, CH_3OH); ^1H NMR ($CDCl_3$, 250 MHz) δ 7.43 (br d, 1H, *J* = 8.4 Hz, C15–H), 7.27 (dd, 1H, *J* = 2.2, 8.4 Hz, C18–H), 7.07 (dd, 1H, *J* = 2.3, 8.4 Hz, C16–H), 6.97 (dd, 1H, *J* = 2.3, 8.3 Hz, C17–H), 6.75 (d, 1H, *J* = 8.2 Hz, C5–H), 6.59 (dd, 1H, *J* = 2.2, 8.2 Hz, C6–H), 5.75 (br d, 1H, NH), 5.09 (br s, 1H, C19–H), 4.94 (app pentet, 1H, *J* = 6.2 Hz, $OCH(CH_3)_2$), 4.59 (d, 1H, *J* = 8.3 Hz, C12–H), 4.11 (t, 1H, *J* = 9.1 Hz, C9–H), 3.92 (s, 3H, OCH_3), 3.25 (t, 1H, *J* = 11.9 Hz, C13–H_αH), 2.98 (s, 3H, NCH_3), 2.68–2.95 (m, 2H, C13–H_βH and C8–H_αH), 2.63 (dd, 1H, *J* = 11.4, 17.7 Hz, C8–H_βH), 1.49 (s, 9H, $NCOOC(CH_3)_3$), 1.16 (d, 3H, *J* = 6.4 Hz, $CHCH_3$), 1.14 (d, 3H, *J* = 6.2 Hz, $CHCH_3$); IR (neat) ν_{max} 3357, 2980, 2934, 1729, 1700, 1680, 1666, 1586, 1517, 1500, 1443, 1392, 1367, 1333, 1282, 1217, 1145, 1130, 1105, 1030 cm^{-1}; FABHRMS (NBA-CsI) m/e 645.1560 (M^+ + Cs, $C_{28}H_{36}N_2O_7$ requires 645.1577).

tert*Butyl 12(S)-[N-[(1,1-dimethylethoxy)carbonyl]-N-methylamino]-4-methoxy-11-oxo-10-aza-2-oxatricyclo-[12.2.2.1^{3,7}]nonadeca-3,5,7(19),14,16,17-hexaen-9(S)-carboxylate (40)*

A solution of **37** (14.0 mg, 0.032 mmol), EDCI–HCl (6.8 mg, 0.035 mmol, 1.1 equiv.), HOBt–H_2O (4.8 mg, 0.035 mmol, 1.1 equiv.), and *t*BuOH (2.6 mg, 3.3 μL, 0.035 mmol, 1.1 equiv.) in CH_2Cl_2 (0.15 mL) was stirred at 25 °C (18 h). The reaction mixture was quenched by the addition of H_2O (1.0 mL) and extracted with CH_2Cl_2 (4 x 1.0 mL). The organic phase was washed with 5% aqueous HCl (3 x 1.0 mL), saturated aqueous $NaHCO_3$ (3 x 2.0 mL), H_2O (3 x 1.0 mL) and saturated aqueous NaCl (3 x 1.0 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Flash chromatography (SiO_2, 1.0 x 8.0 cm, 30% EtOAc–hexane) afforded **40** (4.8 mg, 16.7 mg theoretical, 30%) as a pale yellow solid and recovered starting acid **37**. For **40**: m.p. 156–158 °C; $[\alpha]_D^{25}$ -3.2 (c 0.065, CH_3OH); ^1H NMR ($CDCl_3$, 250 MHz) δ 7.44 (br d, 1H, *J* = 8.3 Hz, C15–H), 7.26 (dd, 1H, *J* = 2.2, 8.3 Hz, C18–H), 7.12 (dd, 1H, *J* = 2.3, 8.4 Hz, C16–H), 6.96 (br d, 1H, *J* = 8.3 Hz, C17–H), 6.74 (d, 1H, *J* = 8.3 Hz, C5–H), 6.60 (dd, 1H, *J* = 1.8, 8.3 Hz, C6–H), 6.18 (br d, 1H, NH), 5.02 (d, 1H, *J* = 1.8 Hz, C19–H), 4.51 (m, 1H, C12–H), 4.19 (m, 1H, C9–H), 3.92 (s, 3H, OCH_3), 3.42 (dd, 1H, *J* = 3.5, 16.5 Hz, C8–H_αH), 3.29 (t, 1H, *J* = 11.9 Hz, C13–H_αH), 3.00 (s, 3H, NCH_3), 2.70–2.90 (m, 2H, C13–H_βH and C8–H_βH), 1.42 and 1.40 (two s, 9H, $NCOOC(CH_3)_3$), 1.30 (s, 9H, $C(CH_3)_3$); IR (neat) ν_{max} 3356, 2961, 2925, 2854, 1731, 1693, 1668, 1515, 1504, 1454, 1361, 1261, 1075, 1021, 801 cm^{-1}; FABHRMS; (NBA-NaI) m/e 527.2768 (M^+ + H, $C_{29}H_{38}N_2O_7$ requires 527.2757).

Methyl 12(S)-[N-[(1,1-dimethylethoxy)carbonyl]-N-methylamino]-9(S)-hydroxymethyl-4-methoxy-11-oxo-10-aza-2-oxatricyclo[12.2.2.1^{3,7}]nonadeca-3,5,7(19),14,16,17-hexaene (41)

A solution of **17**[20] (5.8 mg, 0.012 mmol) in EtOH–THF (3:2, 0.12 mL) was treated with $NaBH_4$ (1.4 mg, 0.036 mmol, 3.0 equiv.) and LiCl (1.6 mg, 0.036 mmol, 3.0 equiv.) at 25 °C (18 h). The reaction mixture was quenched by the addition of acetone (2.0 mL) and concentrated *in vacuo*. The residue was dissolved in 4.0 mL EtOAc–H_2O (1:1), partitioned and the aqueous phase was extracted with EtOAc (3 x 2.0 mL). The combined organic extracts were washed (3 x 2.0 mL) each with H_2O and saturated aqueous NaCl, dried ($MgSO_4$), filtered and concentrated *in vacuo*. Flash chromatography (SiO_2, 1.0 x 5.0 cm, 50% EtOAc–hexane) afforded **41** (5.6 mg, 5.64 mg theoretical, 99%): white solid, m.p. 210–212 °C; $[\alpha]_D^{25}$ -4.2 (c 0.04, CH_3OH); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.38 (br d, 1H, *J* = 8.3 Hz, C15–H), 7.28 (dd, 1H, *J* = 2.3, 8.3 Hz, C18–H), 7.20 (dd, 1H, *J* = 2.4, 8.4 Hz, C16–H), 6.96 (dd, 1H, *J* = 2.4, 8.3 Hz, C17–H), 6.73 (d, 1H, *J* = 8.2 Hz, C5–H), 6.55 (dd, 1H, *J* = 1.8, 8.2 Hz, C6–H), 5.67 (d, 1H, *J* = 8.3

Hz, NH), 5.16 (br s, 1H, C19–H), 4.26 (dd, 1H, J = 4.3, 12.2 Hz, C12–H), 3.92 (s, 3H, OCH_3), 3.66 (m, 1H, C*H*HOH), 3.61 (dd, 1H, J = 4.3, 10.8 Hz, C*H*HOH), 3.36 (m, 1H, C9–H), 3.25 (t, 1H, J = 12.0 Hz, C13–H_αH), 3.03 (s, 3H, NCH_3), 3.00 (br d, 1H, J = 12.0 Hz, C13–H_βH), 2.64 (m, 1H, C8–H_βH), 2.50 (br d, 1H, J = 14.9 Hz, C8–H_αH), 1.44 (s, 9H, $NCOOC(CH_3)_3$); IR (neat) ν_{max} 3355, 2929, 1660, 1586, 1515, 1504, 1442, 1367, 1335, 1260, 1220, 1147, 1130, 1029, 972 cm^{-1}; FABHRMS (NBA-CsI) m/e 589.1296 (M^+ + Cs, $C_{25}H_{32}N_2O_6$ requires 589.1315).

*12(S)-[N-[(1,1-Dimethylethoxy)carbonyl]-N-methylamino]-4-methoxy-11-oxo-10-aza-2-oxatricyclo-[12.2.2.1^{3,7}]nonadeca-3,5,7(19),14,16,17-hexaene (**43**)*

A solution of **37** (4.1 mg, 0.0087 mmol) in THF (120 µL) was treated with Et_3N (2.7 mg, 3.6 µL, 0.026 mmol, 3 equiv.), $PhOP(O)Cl_2$ (3.9 mg, 2.8 µL, 0.017 mmol, 2 equiv.), and was followed by treatment with Et_3N (4.5 mg, 6.2 µL, 0.044 mmol, 5 equiv.) and PhSeH[35] (6.1 mg, 4.1 µL, 0.035 mmol, 4 equiv.) at 0 °C (10 min) and the mixture was allowed to warm to 25 °C (6 h). The reaction mixture was quenched with the addition of H_2O (3.0 mL) and extracted with EtOAc (4 x 3.0 mL). The organic phase was washed with saturated aqueous $NaHCO_3$ (3 x 2.0 mL), H_2O (3 x 1.0 mL) and saturated aqueous NaCl (3 x 1.0 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Flash chromatography (SiO_2, 1.0 x 8.0 cm, 30% EtOAc–hexane) afforded **42** which was carried directly into the next reaction.

A solution of **42** (4.7 mg) in C_6H_6 (1 mL) was treated with Bu_3SnH (35 µL, 0.13 mmol, 15 equiv.) and AIBN (0.5 mg) and the solution was warmed at reflux (1 h) before being cooled and concentrated *in vacuo*. Flash chromatography (SiO_2, 1.0 x 6.0 cm, 25% EtOAc–hexane) afforded **43** (3.1 mg, 3.7 mg theoretical, 84%) as a viscous colorless oil: $[\alpha]_D^{25}$ -3.2 (c 0.055, CH_3OH); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.41 (br d, 1H, J = 8.4 Hz, C15–H), 7.29 (dd, 1H, J = 2.2, 8.3 Hz, C18–H), 7.07 (dd, 1H, J = 2.4, 8.4 Hz, C16–H), 7.01 (br d, 1H, J = 8.3 Hz, C17–H), 6.73 (d, 1H, J = 8.2 Hz, C5–H), 6.58 (br d, 1H, J = 8.2 Hz, C6–H), 5.37 (d, 1H, J = 7.3 Hz, NH), 5.06 (d, 1H, C19–H), 4.43 (dd, 1H, J = 3.8, 12.0 Hz, C12–H), 3.92 (s, 3H, OCH_3), 3.36 (m, 1H, C9–H_βH), 3.27 (t, 1H, J = 12.0 Hz, C13–H_αH), 3.13 (br m, 1H, J = 14.6 Hz, C9–H_αH), 3.03 (s, 3H, NCH_3), 2.93 (dd, 1H, J = 4.3, 11.4 Hz, C13–H_βH), 2.60 (br s, 2H, C8–H_2), 1.45 (s, 9H, $NCOOC(CH_3)_3$); IR (neat) ν_{max} 3287, 2974, 2929, 1682, 1652, 1585, 1516, 1504, 1443, 1367, 1335, 1265, 1215, 1210, 1145, 1130, 1030, 905, 882, 834, 731 cm^{-1}; FABHRMS (NBA) m/e 427.2230 (M^+ + H, $C_{24}H_{30}N_2O_5$ requires 427.2233).

*Ethyl 4-methoxy-12(S)-(N-methylamino)-11-oxo-10-aza-2-oxatricyclo[12.2.2.1^{3,7}]nonadeca-3,5,7(19),14,16,17-hexaen-9(S)-carboxylate (**45**)*

A solution of **38** (5.1 mg, 0.011 mmol) in 1.5 mL 3.0 M HCl–EtOAc was stirred at 25 °C (50 min). The reaction mixture was concentrated *in vacuo* and the residue was triturated with anhydrous Et_2O (3 x 1.0 mL). Drying the product under vacuum afforded **45** (3.9 mg, 4.0 mg theoretical, 99%): $[\alpha]_D^{25}$ +57.5 (c 0.04, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 7.72 (d, 1H, J = 5.2 Hz, NH), 7.51 (dd, 1H, J = 2.2, 8.4 Hz, C15–H), 7.20 (dd, 1H, J = 2.3, 8.4 Hz, C18–H), 7.18 (dd, 1H, J = 2.4, 8.3 Hz, C16–H), 6.98 (dd, 1H, J = 2.4, 8.3 Hz, C17–H), 6.87 (d, 1H, J = 8.2 Hz, C5–H), 6.67 (dd, 1H, J = 2.2, 8.2 Hz, C6–H), 5.09 (d, 1H, J = 1.7 Hz, C19–H), 4.14 (q, 2H, J = 7.1 Hz, $COOCH_2$), 3.89 (m, 1H, obscured by $ArOCH_3$, C12–H), 3.89 (s, 3H, OCH_3), 3.81 (dd, 1H, J = 5.3, 11.7 Hz, C9–H), 2.98 (t, 1H, J = 11.8 Hz, C13–H_αH), 2.86 (dd, 1H, J = 1.6, 16.8 Hz, C8–H_αH), 2.77 (m, 1H, obscured by NCH_3, C13–H_βH), 2.76 (s, 3H, NCH_3), 2.64 (d, 1H, J = 7.7 Hz, C8–H_βH), 1.25 (t, 3H, J = 7.1 Hz, CH_2CH_3); IR (neat) ν_{max} 3405, 2925, 2923, 2856, 1738, 1679, 1654, 1586, 1549, 1517, 1467, 1437, 1263, 1221, 1200, 1130, 1096, 1021 cm^{-1}; FABHRMS (NBA-CsI) m/e 531.0899 (M^+ + Cs, $C_{22}H_{26}N_2O_5$ requires 581.0896).

*4-Methoxy-12(S)-(N-methylamino)-11-oxo-10-aza-2-oxatricyclo[12.2.2.1^{3,7}]nonadeca-3,5,7(19),14,16,17-hexaen-9(S)-carboxylic acid (**46**)*

A solution of **37** (5.1 mg, 0.011 mmol) in 1.5 mL 3.0 M HCl–EtOAc was stirred at 25 °C (50 min). The reaction mixture was concentrated *in vacuo* and the residue was triturated with anhydrous Et_2O (3 x 1.0 mL). Drying the product under vacuum afforded **46** (3.9 mg, 4.0 mg theoretical, 99%) as a white solid: m.p. 265–270 °C (dec); $[\alpha]_D^{25}$ +40 (c 0.03, $CHCl_3$); ^1H NMR (CD_3OD, 400 MHz) δ 8.91 (d, 1H, J = 6.7 Hz, NH), 7.72 (d, 1H, J = 8.4 Hz, NH), 7.51 (dd, 1H, J = 2.3, 8.3 Hz, C15–H), 7.21 (dd, 1H, J = 2.3, 8.3 Hz, C18–H), 7.18 (dd, 1H, J = 2.5, 8.4 Hz, C16–H), 7.02 (d, 1H, J = 8.4 Hz, NH), 6.97 (dd, 1H, J = 2.5, 8.3 Hz, C17–H), 6.87 (d, 1H, J = 8.3 Hz, C5–H), 6.68 (dd, 1H, J = 1.8, 8.2 Hz, C6–H), 5.11 (d, 1H, J = 1.8 Hz, C19–H), 3.89 (s, 3H, OCH_3), 3.88 (m, 1H, obscured by OCH_3, C9–H), 3.80 (dd, 1H, J = 5.3, 11.7 Hz, C12–H), 3.43 (dd, 1H, J = 5.3, 11.7 Hz, C13–H_βH), 2.99 (t, 1H, J = 11.7 Hz, C13–H_αH), 2.92 (br d, 1H, J = 15.5 Hz, C8–H_αH), 2.77 (s, 3H, NCH_3), 2.64 (m, 1H, C8–H_βH); IR (neat) ν_{max} 3376, 3025, 2966, 2933, 1712, 1679, 1586, 1517, 1463, 1441, 1350, 1264, 1224, 1205, 1129, 1021 cm^{-1}; FABHRMS (NBA-CsI) m/e 503.0562 (M^+ + Cs, $C_{20}H_{22}N_2O_5$ requires 503.0583).

*Methyl 12(S)-(N-formyl-N-methylamino)-4-methoxy-11-oxo-10-aza-2-oxatricyclo[12.2.2.1^{3,7}]nonadeca-3,5,7(19),14,16,17-hexaen-9(S)-carboxylate (**47**)*

A solution of **17**[20] (5.3 mg, 0.019 mmol) in 1.5 mL 3.0 M HCl–EtOAc was stirred at 25 °C (50 min). The reaction mixture was concentrated *in vacuo*, the residue was triturated with anydrous Et_2O (3 x 1.0 mL) and dried under vacuum. The amine hydrochloride salt **44** was added to a premixed solution of formic acid (2.3 mg, 0.044 mmol, 2.4 equiv.) and EDCI–HCl (4.3 mg, 0.022 mmol, 1.2

equiv.) in CH_2Cl_2 (0.15 mL) at 0 °C (15 min) and was followed by the addition of *N*-methylmorpholine (2.3 mg, 0.022 mmol, 1.2 equiv.) and the mixture was stirred for 48 h (25 °C). The reaction mixture was quenched by the addition of 5% aqueous HCl (2.0 mL), extracted with CH_2Cl_2 (4 x 2.0 mL), washed with saturated aqueous $NaHCO_3$ (3 x 2.0 mL), 5% aqueous HCl (3 x 2.0 mL), H_2O (3 x 2.0 mL) and saturated aqueous NaCl (3 x 2.0 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. Flash chromatography (SiO_2, 1.0 x 5.0 cm, 30% EtOAc–hexane) afforded **47** (3.0 mg, 4.5 mg theoretical, 67%) as a white solid: m.p. 85–87 °C; $[\alpha]_D^{25}$ -79 (c 0.025, $CHCl_3$); ^1H NMR ($CDCl_3$, 250 MHz) δ 8.08 (s, 1H, CHO), 7.43 (dd, 1H, *J* = 1.8, 8.5 Hz, C15–H), 7.25 (dd, 1H, *J* = 2.0, 8.2 Hz, C18–H), 7.11 (dd, 1H, *J* = 2.2, 8.4 Hz, C16–H), 6.97 (dd, 1H, *J* = 2.3, 8.3 Hz, C17–H), 6.75 (d, 1H, *J* = 8.2 Hz, C5–H), 6.59 (d, 1H, *J* = 8.1 Hz, C6–H), 5.96 (d, 1H, *J* = 8.6 Hz, NH), 5.11 (br s, 1H, C19–H), 4.77 (dd, 1H, *J* = 4.3, 12.1 Hz, C12–H), 4.16 (t, 1H, *J* = 9.1 Hz, C9–H), 3.92 (s, 3H, $ArOCH_3$), 3.65 (s, 3H, $COOCH_3$), 3.30 (t, 1H, *J* = 11.9 Hz, C13–H_αH), 3.10 (s, 3H, NCH_3), 2.8–3.0 (m, 2H, $ArCH_2$), 2.65 (dd, 1H, *J* = 10.8, 16.5 Hz, C8–H_βH); IR (neat) ν_{max} 3300, 2926, 1741, 1661, 1588, 1518, 1440, 1263, 1212, 1124, 1078, 1022, 780 cm^{-1}; FABHRMS (NBA-NaI) m/e 413.1713 (M^+ + Na, $C_{22}H_{24}N_2O_6$ requires 413.1725).

Methyl 12(S)-(N-acetyl-N-methylamino)-4-methoxy-11-oxo-10-aza-2-oxatricyclo[12.2.2.1^{3,7}]nonadeca-3,5,7(19),14,16,17-hexaen-9(S)-carboxylate (48)

A solution of **17**[20] (5.1 mg, 0.011 mmol) in 1.5 mL 3.0 M HCl–EtOAc was stirred at 25 °C (50 min). The reaction mixture was concentrated *in vacuo*, the residue was triturated with anhydrous Et_2O (3 x 1.0 mL) and dried under vacuum. The amine hydrochloride salt **44** in anhydrous CH_2Cl_2 (0.1 mL) was treated with Et_3N (2.7 mg, 0.026 mmol, 2.5 equiv.) and Ac_2O (1.6 mg, 0.016 mmol, 1.5 equiv.) and the mixture was stirred for 12 h (25 °C). The reaction mixture was concentrated *in vacuo*. Flash chromatography (SiO_2, 1.0 x 5.0 cm, 35% EtOAc–hexane) afforded **48** (4.1 mg, 4.2 mg theoretical, 98%) as a white solid: m.p. 195–198 °C; $[\alpha]_D^{25}$ -78 (c 0.075, $CHCl_3$); ^1H NMR ($CDCl_3$, 250 MHz) δ 7.40 (br d, 1H, *J* = 8.1 Hz, C15–H), 7.27 (dd, 1H, *J* = 1.6, 8.4 Hz, C18–H), 7.09 (dd, 1H, *J* = 2.1, 8.1 Hz, C16–H), 6.96 (dd, 1H, *J* = 2.3, 8.4 Hz, C17–H), 6.75 (d, 1H, *J* = 8.2 Hz, C5–H), 6.58 (dd, 1H, *J* = 1.6, 8.1 Hz, C6–H), 6.01 (d, 1H, *J* = 8.2 Hz, NH), 5.13 (d, 1H, *J* = 1.7 Hz, C19–H), 5.01 (br d, 1H, *J* = 10.3 Hz, C12–H), 4.17 (t, 1H, *J* = 9.3 Hz, C9–H), 3.92 (s, 3H, $ArOCH_3$), 3.65 (s, 3H, $COOCH_3$), 3.30 (t, 1H, *J* = 11.9 Hz, C13–H_αH), 3.10 (s, 3H, NCH_3), 2.8–3.0 (m, 2H, $ArCH_2$), 2.65 (dd, 1H, *J* = 10.8, 16.5 Hz, C8–H_βH), 2.13 (s, 3H, $COCH_3$); IR (neat) ν_{max} 3271, 2956, 2925, 1751, 1627, 1516, 1436, 1407, 1263, 1222, 1129, 1096, 1022, 888, 832, 800, 749 cm^{-1}; FABHRMS (NBA) m/e 427.1888 (M^+ + H, $C_{23}H_{26}N_2O_6$ requires 427.1869).

Methyl 12(S)-[(N-methoxycarbonyl)-N-methylamino]-4-methoxy-11-oxo-10-aza-2-oxatricyclo[12.2.2.1^{3,7}]nonadeca-3,5,7(19),14,16,17-hexaen-9(S)-carboxylate (49)

A solution of **17**[20] (6.3 mg, 0.013 mmol) in 1.5 mL 3.0 M HCl–EtOAc was stirred at 25 °C (50 min). The reaction mixture was concentrated *in vacuo*, the residue was triturated with anhydrous Et_2O (3 x 1.0 mL) and dried under vacuum. The amine hydrochloride salt **44** in CH_2Cl_2 (0.15 mL) was treated with Et_3N (3.3 mg, 0.033 mmol, 2.5 equiv.) and methyl chloroformate (1.8 mg, 0.020 mmol, 1.5 equiv.) and the mixture was stirred for 8 h (25 °C). The reaction mixture was quenched by the addition of saturated aqueous NH_4Cl (2.0 mL), extracted with CH_2Cl_2 (4 x 2.0 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. Flash chromatography (SiO_2, 1.0 x 5.0 cm, 60% EtOAc–hexane) afforded **49** (5.7 mg, 5.8 mg theoretical, 98%) as a pale yellow solid: m.p. 180–182 °C; $[\alpha]_D^{25}$ -57 (c 0.12, $CHCl_3$); ^1H NMR ($CDCl_3$, 250 MHz) δ 7.41 (br d, 1H, *J* = 8.3 Hz, C15–H), 7.25 (dd, 1H, *J* = 2.2, 8.4 Hz, C18–H), 7.09 (dd, 1H, *J* = 2.4, 8.3 Hz, C16–H), 6.96 (dd, 1H, *J* = 2.2, 8.3 Hz, C17–H), 6.74 (d, 1H, *J* = 8.2 Hz, C5–H), 6.58 (dd, 1H, *J* = 1.8, 8.2 Hz, C6–H), 5.87 (d, 1H, *J* = 8.6 Hz, NH), 5.11 (d, 1H, *J* = 1.8 Hz, C19–H), 4.58 (br d, 1H, *J* = 11.3 Hz, C12–H), 4.17 (t, 1H, *J* = 9.1 Hz, C9–H), 3.92 (s, 3H, $ArOCH_3$), 3.74 (s, 3H, $NCOOCH_3$), 3.64 (s, 3H, $COOCH_3$), 3.26 (t, 1H, *J* = 12.0 Hz, C13–H_αH), 3.01 (s, 3H, NCH_3), 2.8–3.0 (m, 2H, $ArCH_2$), 2.68 (dd, 1H, *J* = 11.1, 16.6 Hz, C8–H_βH); IR (neat) ν_{max} 3346, 2952, 1744, 1723, 1688, 1677, 1588, 1515, 1441, 1366, 1262, 1226, 1130, 1096, 1030, 884, 839, 793 cm^{-1}; FABHRMS (NBA–CsI) m/e 575.0813 (M^+ + Cs, $C_{23}H_{26}N_2O_7$ requires 575.0794).

Methyl 12(S)-[N-(N-BOC-alanyl)-N-methylamino]-4-methoxy-11-oxo-10-aza-2-oxatricyclo[12.2.2.1^{3,7}]-nonadeca-3,5,7(19),14,16,17-hexaen-9(S)-carboxylate (53)

A solution of **17**[20] (9.6 mg, 0.020 mmol) in 1.5 mL 3.0 M HCl–EtOAc was stirred at 25 °C (50 min). The reaction mixture was concentrated *in vacuo*, the residue was triturated with anhydrous Et_2O (3 x 1.0 mL) and dried under vacuum. A solution of the amine hydrochloride salt **44** (7.3 mg, 0.020 mmol), EDCI–HCl (11.5 mg, 0.059 mmol, 3 equiv.), HOBt–H_2O (8.2 mg, 0.059 mmol, 3 equiv.), $NaHCO_3$ (13.3 mg, 0.16 mmol, 8 equiv.), and *N*-BOC-alanine (**50**, 3.9 mg, 0.020 mmol, 1 equiv.) in DMF (67 μL) was stirred at 25 °C (18 h). The reaction mixture was quenched by the addition of H_2O (1.0 mL) and extracted with EtOAc (4 x 1.0 mL). The organic phase was washed with 5% aqueous HCl (3 x 1.0 mL), saturated aqueous $NaHCO_3$ (3 x 2.0 mL), H_2O (3 x 1.0 mL), saturated aqueous NaCl (3 x 1.0 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Flash chromatography (SiO_2, 1.0 x 8.0 cm, 60% EtOAc–hexane) afforded **53** (9.1 mg, 11.0 mg theoretical, 83%) as a pale yellow foam: m.p. 133–135 °C; $[\alpha]_D^{25}$ -47 (c 0.075, $CHCl_3$); ^1H NMR

($CDCl_3$, 250 MHz) δ 7.42 (dd, 1H, J = 2.0, 8.3 Hz, C15–H), 7.25 (dd, 1H, J = 2.1, 8.3 Hz, C18–H), 7.10 (dd, 1H, J = 2.3, 8.3 Hz, C16–H), 6.95 (dd, 1H, J = 2.4, 8.3 Hz, C17–H), 6.74 (d, 1H, J = 8.2 Hz, C5–H), 6.57 (dd, 1H, J = 2.0, 8.2 Hz, C6–H), 6.08 (d, 1H, J = 8.6 Hz, NH), 5.12 (d, 1H, J = 1.9 Hz, C19–H), 5.00 (dd, 1H, J = 4.4, 11.3 Hz, C12–H), 4.65 (pentet, 1H, J = 6.9 Hz, ala$^{\alpha}$–H), 4.15 (t, 1H, J = 9.7 Hz, C9–H), 3.92 (s, 3H, $ArOCH_3$), 3.64 (s, 3H, $COOCH_3$), 3.25 (m, 1H, C13–H_{α}H), 3.13 (s, 3H, NCH_3), 2.8–3.1 (m, 2H, C13–H_{β}H and C8–H_{α}H), 2.64 (dd, 1H, J = 10.9, 16.5 Hz, C8–H_{α}H), 1.40 (three s, 9H, $COOC(CH_3)_3$), 1.30 (d, 3H, J = 6.9 Hz, ala$^{\beta}$–CH_3); IR (neat) ν_{max} 3300, 2948, 2931, 1745, 1677, 1638, 1630, 1545, 1502, 1442, 1369, 1263, 1223, 1203, 1161, 1130, 1025, 885, 837, 793 cm^{-1}; FABHRMS (NBA-CsI) m/e 688.1644 (M^+ + Cs, $C_{29}H_{37}N_3O_8$ requires 688.1635).

Methyl 12(S)-[N-(N-BOC-ala-alanyl)-N-methylamino]-4-methoxy-11-oxo-10-aza-2-oxatricyclo[12.2.2.1^{3,7}]nonadeca-3,5,7(19),14,16,17-hexaen-9(S)-carboxylate (54)

A solution of **17**[20] (14.1 mg, 0.029 mmol) in 1.5 mL 3.0 M HCl–EtOAc was stirred at 25 °C (50 min). The reaction mixture was concentrated *in vacuo*, the residue was triturated with anhydrous Et_2O (3 x 1.0 mL) and dried under vacuum. A solution of the amine hydrochloride salt **44** (10.7 mg, 0.029 mmol), EDCI–HCl (17.0 mg, 0.087 mmol, 3 equiv.), HOBt–H_2O (12.0 mg, 0.087 mmol, 3 equiv.), $NaHCO_3$ (20.0 mg, 0.23 mmol, 8 equiv.), and *N*-BOC-ala-alanine (**51**, 8.1 mg, 0.029 mmol, 1 equiv.) in DMF (100 μL) was stirred at 25 °C (18 h). The reaction mixture was quenched by the addition of H_2O (1.0 mL) and extracted with EtOAc (4 x 1.0 mL). The organic phase was washed with 5% aqueous HCl (3 x 1.0 mL), saturated aqueous $NaHCO_3$ (3 x 2.0 mL), H_2O (3 x 1.0 mL), saturated aqueous NaCl (3 x 1.0 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Flash chromatography (SiO_2, 1.0 x 8.0 cm, 60% EtOAc–hexane) afforded **54** (10.5 g, 18.2 mg theoretical, 58%) as a pale yellow foam: m.p. 143–145 °C; $[\alpha]_D^{25}$ -32 (c 0.08, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.72 (d, 1H, J = 9.5 Hz, NH), 7.42 (dd, 1H, J = 2.2, 8.3 Hz, C15–H), 7.26 (dd, 1H, J = 2.1, 8.4 Hz, C18–H), 7.09 (dd, 1H, J = 2.3, 8.3 Hz, C16–H), 6.95 (dd, 1H, J = 2.4, 8.3 Hz, C17–H), 6.76 (d, 1H, J = 8.3 Hz, C5–H), 6.58 (dd, 1H, J = 1.8, 8.2 Hz, C6–H), 6.20 (d, 1H, J = 8.5 Hz, NH), 5.13 (d, 1H, J = 1.8 Hz, C19–H), 5.03 (dd, 1H, J = 4.4, 12.2 Hz, C12–H), 5.01 (m, 1H, obscured by C12–H, ala$^{\alpha}$–H), 4.52 (br m, 1H, ala$^{\alpha}$-H), 4.14 (t, 1H, J = 7.3 Hz, C9–H), 3.91 (s, 3H, $ArOCH_3$), 3.64 (s, 3H, $COOCH_3$), 3.25 (t, 1H, J = 11.7 Hz, C13–H_{α}H), 3.13 (s, 3H, NCH_3), 2.8–3.2 (m, 2H, C13–H_{β}H and C8–H_{α}H), 2.67 (dd, 1H, J = 5.4, 16.5 Hz, C8–H_{β}H), 1.43 (s, 9H, $COOC(CH_3)_3$), 1.36 (d, 3H, J = 7.2 Hz, ala$^{\beta}$–CH_3), 1.32 (d, 3H, J = 7.1 Hz, ala$^{\beta}$–CH_3); IR (neat) ν_{max} 3300, 2960, 2924, 2848, 1738, 1708, 1678, 1663, 1642, 1631, 1515, 1443, 1363, 1262, 1226, 1206, 1165, 1130, 1023, 794, cm^{-1}; FABHRMS (NBA-CsI) m/e 759.1990 (M^+ + Cs, $C_{32}H_{42}N_4O_9$ requires 759.2006).

Methyl 12(S)-[N-(N-BOC-tyr-ala-alanyl)-N-methylamino]-4-methoxy-11-oxo-10-aza-2-oxatricyclo[12.2.2.1^{3,7}]-nonadeca-3,5,7(19),14,16,17-hexaen-9(S)-carboxylate (55)

A solution of **17**[20] (12.4 mg, 0.026 mmol) in 1.5 mL 3.0 M HCl–EtOAc was stirred at 25 °C (50 min). The reaction mixture was concentrated *in vacuo*, the residue was triturated with anhydrous Et_2O (3 x 1.0 mL) and dried under vacuum. A solution of the amine hydrochloride salt **44** (9.8 mg, 0.026 mmol), EDCI–HCl (14.9 mg, 0.077 mmol, 3 equiv.), HOBt–H_2O (10.6 mg, 0.077 mmol, 3 equiv.), $NaHCO_3$ (17.2 mg, 0.20 mmol, 8 equiv.), and *N*-BOC-tyr-ala-alanine (**52**, 10.8 mg, 0.026 mmol, 1 equiv.) in DMF (85 μL) was stirred at 25 °C (18 h). The reaction mixture was quenched by the addition of H_2O (1.0 mL) and extracted with EtOAc (4 x 1.0 mL). The organic phase was washed with 5% aqueous HCl (3 x 1.0 mL), saturated aqueous $NaHCO_3$ (3 x 2.0 mL), H_2O (3 x 1.0 mL), saturated aqueous NaCl (3 x 1.0 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Flash chromatography (SiO_2, 1.0 x 8.0 cm, EtOAc) afforded **55** (14.8 mg, 20.2 mg theoretical, 74%) as a white foam: m.p. 167–170 °C; $[\alpha]_D^{25}$ -36 (c 0.07, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.39 (br d, 1H, J = 8.2 Hz, C15–H), 7.26 (dd, 1H, J = 2.2, 8.3 Hz, C18–H), 7.10 (dd, 1H, J = 2.3, 8.3 Hz, C16–H), 6.96 (br m, 3H, C17–H and tyr$^{3\delta}$–H), 6.70 (br m, 3H, C5–H and tyr$^{3\varepsilon}$–H), 6.56 (dd, 1H, J = 1.8, 8.3 Hz, C6–H), 6.25 (d, 1H, J = 8.3 Hz, NH), 5.12 (br s, 1H, C19–H), 5.00 (dd, 1H, J = 4.2, 12.3 Hz, C12–H), 4.79 (m, 1H, ala$^{\alpha}$–H or tyr$^{\alpha}$–H) 4.42 (br m, 1H, ala$^{\alpha}$–H or tyr$^{\alpha}$–H), 4.29 (m, 1H, ala$^{\alpha}$–H or tyr$^{\alpha}$–H), 4.13 (t, 1H, J = 7.3 Hz, C9–H), 3.92 (s, 3H, $ArOCH_3$), 3.64 (s, 3H, $COOCH_3$), 3.25 (t, 1H, J = 12.0 Hz, C13–H_{α}H), 2.8–3.2 (m, 4H, tyr$^{\beta}$–H, C13–H_{β}H and C8–H_{α}H), 2.93 (s, 3H, NCH_3), 2.67 (dd, 1H, J = 4.4, 17 Hz, C8–H_{β}H), 1.38 and 1.35 (two s, 9H, $COOC(CH_3)_3$), 1.38 (d, 3H, J = 7.3 Hz, ala$^{\beta}$–CH_3), 1.22 (d, 3H, J = 6.8 Hz, ala$^{\beta}$–CH_3); IR (neat) ν_{max} 3318, 2960, 2933, 1712, 1692, 1549, 1515, 1497, 1451, 1369, 1261, 1226, 1164, 1128, 1097, 1026, 805, 754 cm^{-1}; FABHRMS (NBA-CsI) m/e 922.2643 (M^+ + Cs, $C_{41}H_{51}N_5O_{11}$ requires 922.2639).

Methyl 12(S)-[N-alanyl-N-methylamino]-4-methoxy-11-oxo-10-aza-2-oxatricyclo[12.2.2.1^{3,7}]nonadeca-3,5,7(19),14,16,17-hexaen-9(S)-carboxylate (57)

A solution of **53** (4.5 mg, 0.0081 mmol) in 1.5 mL 3.0 M HCl–EtOAc was stirred at 25 °C (50 min). The reaction mixture was concentrated *in vacuo*. The residue was triturated with anhydrous Et_2O (3 x 1.0 mL) and dried under vacuum to afford **57** (3.6 mg, 3.7 mg theoretical, 97%) as a white solid: m.p. 180–185 °C; $[\alpha]_D^{25}$ -51 (c 0.09, CH_3OH); ^{1}H NMR (CD_3OD, 250 MHz) δ 7.53 (br d, 1H, J = 8.3 Hz, C15–H), 7.24 (m, 1H, C18–H), 7.16 (m, 1H, C16–H), 6.91 (m, 1H, obscured by C5–H, C17–H), 6.87 (br d, 1H, J = 8.2 Hz, C5–H), 6.63 (br d, 1H, J = 8.2 Hz, C6–H), 5.13 (d, 1H, J = 1.8 Hz, C19–H), 5.11 (dd, 1H, J = 2, 11.7 Hz, C12–H), 4.80 (m, 1H, obscured by H_2O, ala$^{\alpha}$–H), 3.91 (s, 3H, $ArOCH_3$), 3.91 (m, 1H,

obscured by $ArOCH_3$, C9–H), 3.67 (s, 3H, $COOCH_3$), 3.35 (s, 3H, NCH_3), 3.0–3.3 (m, 3H, C13–H_2 and C8–H_αH), 2.79 (br d, 1H, J = 11.8 Hz, C8–H_βH), 1.30 (d, 3H, J = 7.2 Hz, ala$^\beta$–CH_3); IR (neat) ν_{max} 3374, 2932, 1744, 1658, 1631, 1516, 1502, 1441, 1263, 1224, 1206, 1130, 1025, 886, 836, 798 cm^{-1}; FABHRMS (NBA-CsI) m/e 588.1111 (M^+ + Cs, $C_{24}H_{29}N_3O_6$ requires 588.1111).

Methyl 12(S)-[N-(ala-alanyl)-N-methylamino]-4-methoxy-11-oxo-10-aza-2-oxatricyclo[12.2.2.1^{3,7}]nonadeca-3,5,7(19),14,16,17-hexaen-9(S)-carboxylate (58)

A solution of **54** (4.2 mg, 0.0067 mmol) in 1.5 mL 3.0 M HCl–EtOAc was stirred at 25 °C (50 min). The reaction mixture was concentrated *in vacuo* and the residue was triturated with anhydrous Et_2O (3 x 1.0 mL), dried under vacuum to afford **58** (3.4 mg, 3.5 mg theoretical, 97%): white solid, m.p. 190–192 °C (dec); $[\alpha]_D^{25}$ -30 (c 0.08, CH_3OH); ^{1}H NMR (CD_3OD, 250 MHz) δ 7.51 (br d, 1H, C16–H), 7.23 (dd, 1H, J = 2.3, 8.3 Hz, C18–H), 7.13 (dd, 1H, J = 2.2, 8.3 Hz, C16–H) 6.89 (dd, 1H, J = 2.2, 8.3 Hz, C17–H), 6.84 (d, 1H, J = 8.3 Hz, C5–H), 6.62 (dd, 1H, J = 1.8, 8.3 Hz, C6–H), 5.13 (br s, 1H, C19–H), 5.12 (m, 1H, obscured by C19–H, C12–H), 4.80 (m, 1H, obscured by C12–H, ala$^\alpha$–H), 4.18 (m, 1H, C9–H), 3.92 (m, 1H, ala$^\alpha$-H), 3.91 (s, 3H, $ArOCH_3$), 3.65 (s, 3H, $COOCH_3$), 3.18 (s, 3H, NCH_3), 3.16 (m, 2H, $ArCH_2$), 2.96 (br d, 1H, J = 11.8 Hz, ArCH), 2.66 (br d, 1H, J = 7.4 Hz. ArCH), 1.3–1.5 (several d, 6H, ala$^\beta$–CH_3); IR (neat) ν_{max} 3319, 2950, 2928, 1726, 1690, 1660, 1630, 1550, 1514, 1442, 1262, 1226, 1129 cm^{-1}; FABHRMS (NBA-CsI) m/e 659.1511 (M^+ + Cs, $C_{27}H_{34}N_4O_7$ requires 659.1482).

Methyl 12(S)-[N-methyl-N-(tyr-ala-alanyl)amino]-4-methoxy-11-oxo-10-aza-2-oxatricyclo[12.2.2.1^{3,7}]nonadeca-3,5,7(19),14,16,17-hexaen-9(S)-carboxylate (59)

A solution of **55** (7.3 mg, 0.0092 mmol) in 1.5 mL 3.0 M HCl–EtOAc was stirred at 25 °C (50 min). The reaction mixture was concentrated *in vacuo*, the residue was triturated with anhydrous Et_2O (3 x 1.0 mL), and dried under vacuum to afford **59** (6.5 mg, 6.7 mg theoretical, 97%) as a white solid: m.p. 285–290 °C (dec); $[\alpha]_D^{25}$ -9.1 (c 0.055, CH_3OH); ^{1}H NMR (CD_3OD, 250 MHz) δ 7.53 (br d, 1H, J = 8.3 Hz, C15–H), 7.24 (br d, 1H, J = 8.3 Hz, C18–H), 7.14 (m, 1H, C16–H), 6.90 (m, 4H, C5–H, C17–H and tyr$^{3\delta}$–H), 6.60 (br m, 3H, C6–H and tyr$^{3\varepsilon}$–H), 5.16 (d, 1H, J = 1.8 Hz, C19–H), 5.14 (dd, 1H, J = 4, 12 Hz, C12–H), 4.80 (m, 2H, obscured by H_2O, ala$^\alpha$–H or tyr$^\alpha$–H), 4.34 (m, 2H, ala$^\alpha$–H or tyr$^\alpha$–H and C9–H), 3.89 (s, 3H, $ArOCH_3$), 3.65 (s, 3H, $COOCH_3$), 3.49 (m 1H, C13–H_αH), 3.10 (two s, 3H, NCH_3), 2.8–3.2 (m, 5H, tyr$^\beta$–H, C13–H_βH, C8–H), 1.30 (br m, 6H, ala$^\beta$–CH_3); IR (neat) ν_{max} 3388, 2823, 1712, 1660, 1650, 1632, 1590, 1556, 1538, 1515, 1446, 1392, 1313, 1262, 1128, 1087, 1021, 831 cm^{-1}; FABHRMS (NBA-CsI) m/e 821.2024 (M^+ + Cs, $C_{36}H_{43}N_5O_9$ requires 821.2037).

13(S)-4-Methoxy-21-oxo-10,12-diaza-2-oxatetracyclo-[13.2.2.1^{3,7}.1^{10,13}]heniecosa-3,5,7(20),15,17,18-hexaene (74)

A solution of *N*-[(1,1-dimethylethoxy)carbonyl]-2-(3-hydroxy-4-methoxyphenyl)ethylamine[29] (67.7 mg, 0.25 mmol) in 1.5 mL 3.0 M HCl–EtOAc was stirred at 25 °C (50 min). The reaction mixture was concentrated *in vacuo*, the residue was triturated with anhydrous Et_2O (3 x 1.0 mL) and dried under vacuum. The crude amine hydrochloride salt (50.0 mg, 0.25 mmol), EDCI–HCl (58.0 mg, 0.295 mmol, 1.2 equiv.), HOBt–H_2O (40.3 mg, 0.295 mmol, 1.2 equiv.), and 4-iodo-*N*-methyl-*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanine[20] (99.6 mg, 0.25 mmol, 1 equiv.) was stirred at 25 °C (8 h). The reaction mixture was quenched by the addition of 5% aqueous HCl (10 mL) and extracted with EtOAc (4 x 10 mL). The organic phase was washed with saturated aqueous $NaHCO_3$ (3 x 25 mL), 5% aqueous HCl (3 x 25 mL), H_2O (3 x 25 mL), and saturated aqueous NaCl (3 x 25 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Flash chromatography (SiO_2, 3 x 12 cm, 15–35% EtOAc–hexane) afforded the amide (110.4 mg, 136.2 mg theoretical, 81%) as a white solid: m.p. 138–140 °C; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.57 (d, 2H, J = 8.2 Hz, Ar C3– and C5–H), 6.96 (m, 2H, ArH), 6.73 (d, 2H, J = 8.2 Hz, Ar C2– and C6–H), 6.57 (m, 1H, ArH), 6.11 (br s, 1H, OH), 5.86 and 5.63 (br m, 1H, NH), 4.84 and 4.62 (t, 1H, J = 6.7 Hz, C*H*NCH_3), 3.83 (s, 3H, OCH_3), 3.20–3.55 (br m, 4H, $ArCH_2$ and CH_2N), 2.70–2.90 (br m, 2H, $ArCH_2$), 2.64 (s, 3H, NCH_3), 1.38, 1.26 and 1.24 (three s, 9H, $NCOOC(CH_3)_3$).

A solution of the amide (49.5 mg, 0.089 mmol) in 1.0 mL of anhydrous collidine was added dropwise to a suspension of NaH (60% oil dispersion in mineral oil, 7.9 mg, 0.196 mmol, 2.2 equiv.) in 1.0 mL of dry collidine under Ar at 0 °C and the solution was allowed to stir for 10 min. The solution was treated with CuBr–SMe_2 (188.0 mg, 0.893 mmol, 10 equiv.) and was allowed to stir at 25 °C for 50 min before the mixture was diluted with anhydrous degassed collidine to 0.004 M (30 mL total) and warmed at 130 °C (bath) for 9 h. The cooled reaction mixture was concentrated *in vacuo*. The resulting residue was dissolved in EtOAc (20 mL) and saturated aqueous NH_4Cl (20 mL), partitioned and the aqueous phase was extracted with EtOAc (4 x 20 mL). The combined organic extracts were washed with 5% aqueous HCl (3 x 25 mL), H_2O (3 x 25 mL) and saturated aqueous NaCl (3 x 25 mL), dried ($MgSO_4$), and concentrated *in vacuo*. Flash chromatography (SiO_2, 1.0 x 7.0 cm, 0–30% EtOAc–hexane) afforded **43** (7.6 mg, 38.0 mg theoretical, 20%), the acyclic hydantoin, recovered starting material (3.2 mg, 49.5 mg theoretical, 6.5%), and **74** (3.7 mg, 31.4 mg theoretical, 12%) as a pale yellow oil. For **74**: $[\alpha]_D^{25}$ +31 (c 0.05, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.25 (dd, 1H, J = 2.2, 8.2 Hz, C16–H), 7.12 (dd, 1H, J = 2.2, 8.3 Hz, C19–H), 7.01 (dd, 1H, J = 2.2, 8.2 Hz, C17–H), 6.96

(dd, 1H, J = 2.2, 8.3 Hz, C18–H), 6.72 (d, 1H, J = 8.2 Hz, C5–H), 6.54 (dd, 1H, J = 2.0, 8.2 Hz, C6–H), 4.72 (d, 1H, J = 2.0 Hz, C20–H), 4.06 (t, 1H, J = 3.3 Hz, C13–H), 3.90 (s, 3H, OCH_3), 3.78 (m, 2H, C9–H_2), 3.27 (ddd, 1H, J = 1.6, 12.5, 15.9 Hz, C8–H_βH), 3.21 (ddd, 2H, J = 3.1, 14.2, 14.2 Hz, C14–H_2), 3.08 (s, 3H, NCH_3), 2.44 (ddd, 1H, J = 1.2, 4.4, 15.9 Hz, C8–H_αH); IR (neat) ν_{max} 2930, 1766, 1704, 1585, 1516, 1504, 1455, 1408, 1222, 1129, 1022, 890, 806, 757 cm^{-1}; FABHRMS (NBA-NaI) *m/e* 375.1320 (M^+ + Na, $C_{20}H_{20}N_2O_4$ requires 375.1321).

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39. Global and close low-lying minima (≤ 12 kcal/mol) were located in a conformational search with use directed Monte Carlo sampling and subsequent minimization of conformations generated by random variations (0–180°) in 8 of the 10 available torsional angles[40] excluding those originating in the phenyl rings (MacroModel,[41] Batchmin Version 3.5a, OPLSA and AMBER force fields, MCMM = 1000, MCSS = 2, 12 kcal/mol window). The global minimum for **67** and **73** were located 290 and 263 (OPLSA) times, respectively. The 2D ^1H–^1H NOESY NMR spectrum ($CDCl_3$, 400 MHz) of **73** displayed the following diagnostic NOE crosspeaks: C16–H/C17–H, C16–H/C13–H, C16–H/C14–H_β, C19–H/C18–H, C19–H/C14–H_α, C17–H/C20–H, C18–H/C20–H, C5–H/C6–H, C5–H/C4–OCH_3, C6–H/C8–H_β, C20–H/C9–H, C20–H/C8–H_α, C14–H/C13–H, C9–H/C8–H_α, C8–H_α/C8–H_β.

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