Synthesis and Biological Activity of Analogues of the Antimicrotubule Agent *N*,*β*,*β*-Trimethyl-L-phenylalanyl-N^1-[(1*S*,2*E*)-3-carboxy-1-isopropylbut-2-enyl]-N^1,3-dimethyl-L-valinamide (HTI-286)

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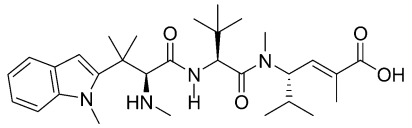
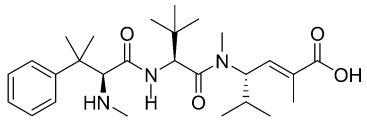
Hemiasterlin (**1**), a tripeptide isolated from marine sponges, induces microtubule depolymerization and mitotic arrest in cells. HTI-286 (**2**), an analogue from an initial study of the hemiasterlins, is presently in clinical trials. In addition to its potent antitumor effects, **2** has the advantage of circumventing the P-glycoprotein-mediated resistance that hampers the efficacy of other antimicrotubule agents such as paclitaxel and vincristine in animal models. This paper describes an in-depth study of the structure−activity relationships of analogues of **2**, their effects on microtubule polymerization, and their in vitro and in vivo anticancer activity. Regions of the molecule necessary for potent activity are identified. Groups tolerant of modification, leading to novel analogues, are reported. Potent analogues identified through in vivo studies in tumor xenograft models include one superior analogue, HTI-042 (**48**).

Modulation of the dynamics of microtubule formation represents a major therapeutic approach for the treatment of cancer.[1] The taxanes and Vinca alkaloids are the two classes of tubulin inhibiting natural products currently in use as anticancer agents. Despite their successes, inherent and acquired resistance by tumors to these agents limits their utility.[2] Hemiasterlin[3] (**1**) is a member of a recently discovered class of natural products whose potent effects on tubulin make them especially attractive as drug targets (Figure 1). These tripeptides contain highly unusual and sterically congested amino acids, which give rise to their stability and in vivo activity. Other peptidic antimicrotubule agents (e.g. dolastatins, cemadotin and cryptophycin), some of which competitively inhibit[4] binding of hemiasterlin to tubulin, have been reported and several are under clinical investigation.[1,5] Clinical trials of compounds interacting with the colchicine domain (e.g. combretastatins) and the taxane site (e.g. epothilones) are also underway.[1] The relative structural simplicity of the hemiasterlins allows for diverse structural manipulation of the molecule via total synthesis. HTI-286[6] (**2**), an analogue from an initial study of the hemiasterlins wherein a phenyl group replaces the indole ring,[7] is presently in clinical trials[8] (Figure 1). In addition to its potent antitumor effects, **2** has the advantage of circumventing the P-glycoprotein-mediated resistance which hampers the efficacy of other antimicrotubule agents such as paclitaxel and vincristine in animal models.[6]

This paper describes an in-depth study of the structure−activity relationships of analogues of **2** in which each region of the molecule was systematically investigated. Each analogue was evaluated in terms of its direct effects on extracellular tubulin polymerization, and its cytotoxic effects both in the absence and presence of expression of P-glycoprotein transporters. In vivo studies in tumor xenograft models identified several potent analogues including one superior analogue.



Hemiasterlin (**1**)



HTI-286 (**2**)

Figure 1.

Synthesis

Several total syntheses of hemiasterlin have been reported in the literature.[9] The synthetic strategy comprises synthesizing the individual amino acids, followed by peptide coupling. This route can also be used for the synthesis of **2** and other analogues (Scheme 1).[7] The most challenging synthesis is that of the A-piece amino acid **3**, necessitating construction of the carbon scaffold and establishing the chirality by means of a chiral auxiliary group.[9a] Alternative A-piece syntheses utilizing $SnCl_4$-mediated ring opening of epoxides by indoles[9b] or asymmetric Strecker synthesis[9c] have appeared in the literature. The chirally pure B-piece (**4**) was commercially available and the CD-piece (**5**) was

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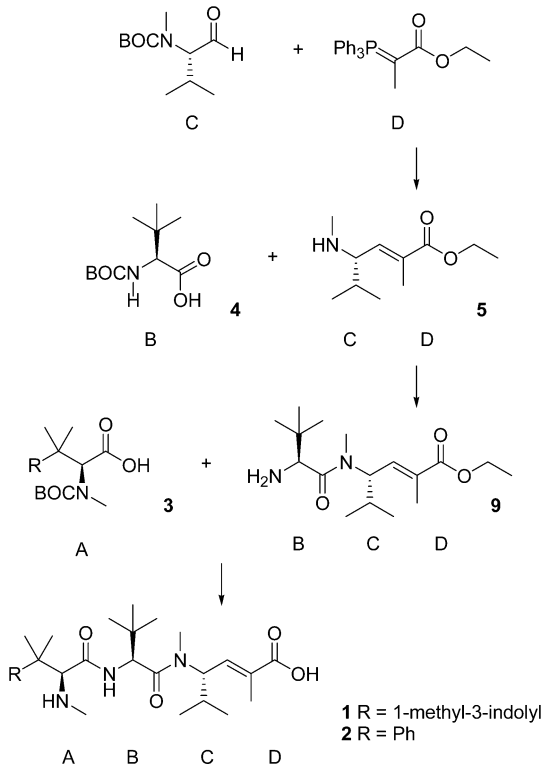
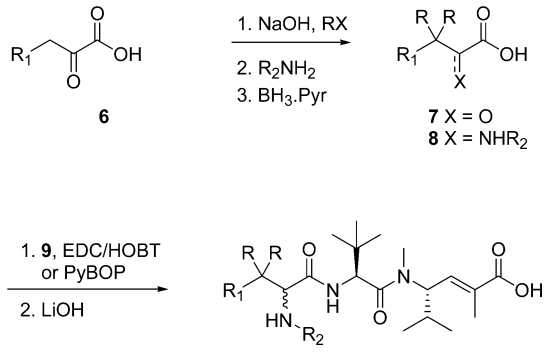
Scheme 1. Synthesis of Tripeptides **1** and **2**



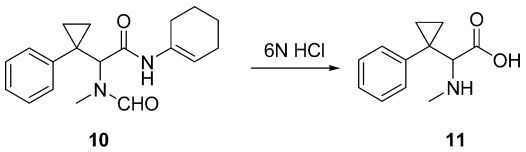
Scheme 2. Pyruvic Acid Route



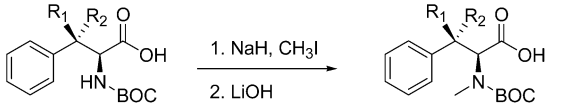
prepared by reduction of the Weinreb amide of D-leucine followed by Wittig reaction of the resultant aldehyde with $Ph_3P{=}C(Me)CO_2Et$.

For A-piece modifications, to efficiently prepare analogues, control of the amino acid stereocenter using chiral auxiliaries was abandoned and the synthesis replaced with a shorter route developed in house[10] utilizing readily available pyruvic acids **6** (Scheme 2). Dimethylation alpha to the ketone occurred upon treatment of the pyruvic acids with iodomethane and sodium hydroxide to give **7**. Reductive amination then gave racemic amino acid **8**. Coupling of **8** to the BCD-piece (**9**),[9a] followed by hydrolysis of the ester, gave the desired analogues as a mixture of two diastereomers (*SSS* and *RSS*) separable by chromatography. The stereochemistry at the methylamine position was determined by NMR. Other substituents could be introduced at the alpha position of the pyruvic acids, including carbocycles, by appropriate choice of alkylating agents. In the case of the cyclopropyl analogue, cyclization was not successful by this route. Instead, the multicomponent Ugi condensation reaction was used,[11] combining 1-phenyl-cyclopropane carbaldehyde,[12] methylamine, 1-isocyanocyclohexene and formic acid to generate intermediate **10**, which upon treatment with 6 N HCl gave the desired amino acid **11** (Scheme 3). The enantiomerically pure A-pieces (**12**), bearing a single methyl substituent at the beta position, were prepared from the corresponding commercially available amino acids **13**, which were *N*-methylated with iodomethane and base and saponified (Scheme 4). Hydrogenation of the aromatic ring in **14** with platinum oxide catalyst gave the cyclohexyl A-piece **15** (Scheme 5). A bromine substituent at the 4-position of the phenyl ring could be introduced by treatment of phenyl pyruvic acid **7** (R = Me, R_1 = Ph) with bromine (Scheme 6). After reductive amination, coupling of the resulting 4-bromophenyl amino acid to **9** and separation of diastereomers by HPLC, the bromine was used in a biaryl coupling reaction to give the biphenyl analogue **16**. Preparation of the adamantanyl containing A-piece **17** was effected by treating alpha-iodo adamantyl acetic acid[13] with methylamine (Scheme 7). Coupling of **17** to **9** followed by hydrolysis and HPLC gave **18**. The A-piece **19** where the methylamine group was incorporated into a thiomorpholine ring reminiscent of milnamide[14] was constructed by methylation of 2,2-dimethyl-thiomorpholine-3-carboxylic acid[15] using formic acid and formalin (Scheme 8). Coupling of **19** to **9** followed by hydrolysis gave **20**.

Scheme 3. Cyclopropyl A-Piece Synthesis

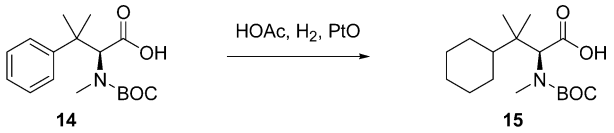


Scheme 4. Monomethyl A-Piece Synthesis



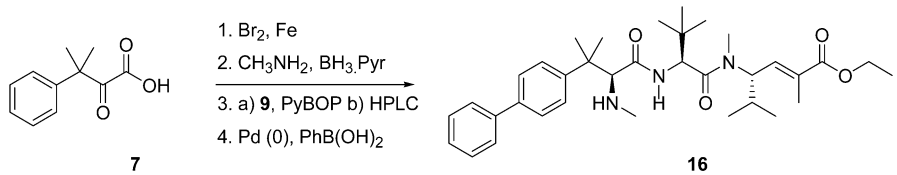
13a $R_1 = CH_3$, $R_2 = H$ **12a** $R_1 = CH_3$, $R_2 = H$
13b $R_1 = H$, $R_2 = CH_3$ **12b** $R_1 = H$, $R_2 = CH_3$

Scheme 5. Preparation of the Cyclohexyl A-Piece **15**

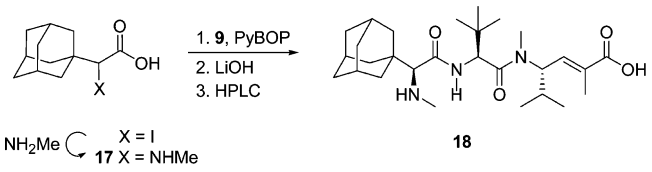


Investigation of the effect of amine substitution in the A-piece on activity was expedited by the steric congestion around the nitrogen in **2**. For alkyl groups larger than methyl, the rate of the second N-alkylation reaction was very slow. This lack of reactivity made possible the preparation of N-monosubstitued derivatives (**21**) by treatment of the unsubstituted nitrogen in **22** with alkyl halides (Scheme 9). A cyclic analogue **23** (i.e. pyrrolidine) could be synthesized by use of dibromopentane as the alkylating agent. Disubstitution could be forced to some extent by treatment of **24**, the ethyl ester of **2,** with excess bromoethanol at elevated temperature leading to analogue **25** (Scheme 9). In contrast, treatment of **24** with 1 equiv of iodomethane readily gave the dialkylated analogue **26** after hydrolysis, as well as starting **24** and its trimethylammonium salt.

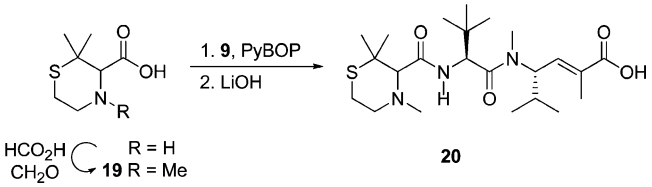
Scheme 6. Biaryl A-Piece Analogue Synthesis



Scheme 7. Adamantyl A-Piece Analogue Synthesis



Scheme 8. Thiazole A-Piece Analogue Synthesis



Replacement of the A-piece methylamine group with nonbasic substituents could be accomplished, using phenylmethylbutyric acid modified with Evan's chiral auxiliary (**27**) (Scheme 10). Deprotonation of **27** with sodium hexamethydisilazide, followed by treatment with iodomethane and removal of the chiral auxiliary with lithium hydroperoxide, gave the desired chiral methyl A-piece (**28**, R = CH_3). Coupling to the BCD-piece (**9**) followed by ester hydrolysis gave a single diastereomer (**29**), as expected. Alternatively, racemic A-pieces could be made through formation of the dianion of phenylmethylbutyric acid, by treatment with 2 equiv of LDA followed by addition of alkyl, allyl or benzyl halide to give **28**, or addition of dimethyl disulfide to give **30**. The sulfone **31** could be prepared by oxidation of **30** with MCPBA. The hydroxy A-piece **32** was prepared by reduction of pyruvic acid ester **33** with sodium borohydride to give **34**, followed by saponification with LiOH. Conversion of the hydroxy ester **34** to the methoxy A-piece **35** was effected by methylation of the hydroxy group with silver oxide and methyl iodide, followed by saponification with LiOH.

Reduction of the olefin was accomplished by treatment of **2** with 10% Pd/C in acetic acid under one atmosphere of hydrogen (Scheme 11). A mixture of two diastereomers was obtained in an 87:13 ratio (**36** and **37**, respectively). The diastereomers were separable by HPLC. The stereochemistry of these isomers was assigned by NMR.

Other modifications of the tripeptide were made according to Scheme 1 by substituting the appropriate amino acid precursor. Esters and amides of **2** were made by treatment of **2** with the appropriate amine or alcohol in the presence of EDC. The *N*,*N*-dimethyl analogue, **38**, was made by treating **39** with NaH and iodomethane followed by saponification and N-BOC deprotection (Scheme 12).

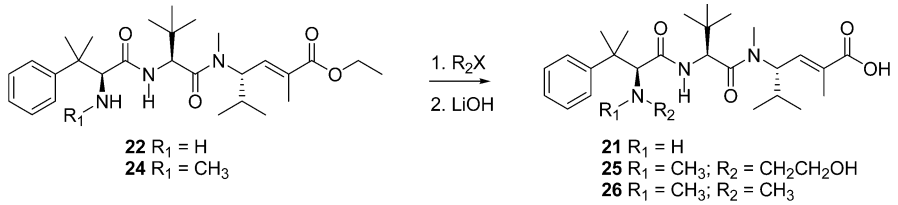
Results and Discussion

Modification of each of the structural components of **2** revealed its respective role in the bioactivity of the tripeptide. Compounds were evaluated for their ability to inhibit extracellular tubulin polymerization at a single concentration (0.3 uM) and for their cytotoxicity in paclitaxel sensitive KB-3-1 cells and in paclitaxel resistant KB-8-5 and KB-V1 cells expressing moderate and high levels of P-glycoprotein, respectively. Certain regions of the molecule could be modified to give analogues retaining full potency while other changes led to less potent analogues. Some sites could not be modified without full loss of biological activity at the concentrations tested. Evaluation of in vivo biological activity identified several analogues with potent antitumor activity including one (**48**) with superior potency.

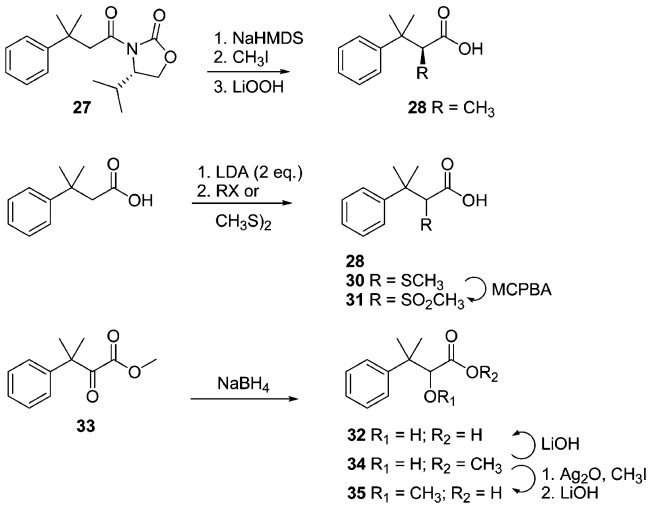
Exploration of the importance of each stereocenter to activity was a goal of high priority (Table 1). Hemiasterlin and **2** contain three chiral centers, one at each of the amino acid subunits (*SSS* configuration). Of the three chiral centers, stereochemistry at the one bearing the methylamine group on the A-piece was least critical to activity. The *RSS* isomer **41** was approximately 28-fold less potent than **2** in the KB-3-1 cells, and it still retained its ability to potently inhibit tubulin polymerization. Approximately 100-fold loss of potency was reported for **41** in MCF-7 cells.[7] A comparable loss of potency was seen in *RSS* isomers isolated from the synthesis of other analogues. The most profound effect on biological activity occurred upon altering the stereochemistry of the carbon bearing the *tert*-butyl group on the B-piece. The resulting *SRS* isomer **42** showed dramatically decreased activity both in cells and in the tubulin polymerization assay. The key importance of this position to activity was also clearly evident when the substituents attached to it were varied (vide infra). Isomerization of the isopropyl group attached to the stereocenter on the C-piece gave the *SSR* analogue **43** which was 2−3 orders of magnitude less potent than **2** in the KB-3-1 cells.

In addition to stereochemistry, investigation of the A-piece included modification of the substituents attached to the quaternary carbon (Tables 2 and 3), variation of the substituents on the nitrogen (Table 4) and replacement of the nitrogen with carbon or heteroatoms (Table 5). While retaining the necessary geminal dimethyl group on the quarternary carbon, the substituent corresponding to the phenyl group in **2** was varied (Table 2). A large reduction in the size of the group at this position, as in the *tert*-butyl analogue **44** (methyl for phenyl replacement), gave a marked decrease both in cellular activity (a comparable decrease was reported in MCF-7 cells[7]) and inhibition of tubulin polymerization. Interestingly, a hydroxy group in the place of phenyl gave analogue **45** that had activity similar to that of the *tert*-butyl analogue **44**, revealing a tolerance for polar groups at this position. An increase in steric bulk and lipophilicity relative to methyl at this position, as in analogue **46** with an *n*-pentyl group, led

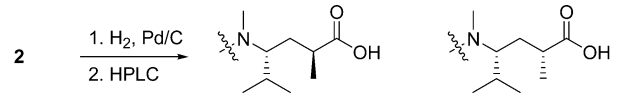
Scheme 9. Synthesis of Amine-Substituted Analogues



Scheme 10. Synthesis of A-Piece Methylamine Replacements



Scheme 11. CD-Piece Olefin Reduction



to regained potency. Other subtituents, both aromatic and alkyl (**47**−**50**), gave potent analogues showing that a minimum degree of lipophilicity and steric bulk at this position is required. Saturation of the aromatic ring gave the cyclohexyl ring analogue **47**, which exhibited an IC_{50} value of 3.9 nM in the KB-3-1 cells. Replacement of the phenyl ring with 4-methoxyphenyl, in turn, gave a potent analogue **48** with improved in vivo properties (vide infra). This region also showed a tolerance for large groups, as seen with the potent biphenyl analogue **49**, indicating interaction with a large binding cavity or a solvent exposed region within tubulin. The tolerance for large R groups on the A-piece has been exploited to prepare a photoaffinity probe with a radiolabeled benzophenone group at this position (Table 2, R = 4-Ph-COPh) whose binding to tubulin was competitively inhibited by **2** and dolastatin-10.[16] Compared with indole-containing hemiasterlin (**1**), **2** showed a lower level of resistance in P-glycoprotein expressing KB-8-5 and KB-V1 cells.[6]

The A-piece geminal dimethyl group is a key component necessary for potent activity (Table 3). Removal of both methyl groups was reported to lead to a loss of potency.[7] Stereospecific removal of either methyl group led to analogues **51** and **52** with a loss of activity of approximately 2 orders of magnitude in the cellular assays. Larger groups at this position, such as diethyl, spirocyclopentyl or spirocyclohexyl led to analogues **53**, **54** and **55** with reduced activity, suggesting limited space in the tubulin binding pocket. Consistent with this analysis the analogue containing the smaller spirocyclopropyl ring (**56**), showed excellent inhibition of tubulin polymerization (99% at 0.3 uM). However, the cytotoxicity of **56** fell between that of the spirocyclopentyl analogue (**54**) and the diethyl analogue (**53**). Connecting the substituents on the quaternary carbon into a tricyclic adamantyl ring system led to analogue **18** with an IC_{50} = 603 nM in the KB-3-1 cells.

Exploration of substitution on the nitrogen in the A-piece revealed that a degree of variation was tolerated (Table 4). The *N*-ethyl analogue **57** and the *N*,*N*-dimethyl analogue **26** were equipotent with **2** in the cellular assays. Larger groups, such as in the benzyl analogue **58** or the cyclic analogue **59**, led to a decrease of activity, indicating a small binding pocket at this position. Incorporation of polar groups such as an alcohol, acid or amide led to analogues **25**, and **60**−**62** with decreased activity. Removal of the methyl group led to analogue **63**, with a dramatic decrease of cellular cytotoxicity at the doses tested. N-Acylation led to compound **64**, with 3 orders of magnitude decrease in activity. Decreased activity was also seen in many of the N-BOC intermediates tested, and was reported[7] for *N*-acylhemiasterlin. Incorporation of the nitrogen into a thiomorpholine ring, reminiscent of the structurally related natural product milnamide,[14] was tolerated and led to an active, albeit less potent analogue **20** with an IC_{50} = 427 nM in the KB-3-1 cells.

The basic nitrogen in the A-piece was found to be critical for potent cellular activity (Table 5). Replacement of the methylamine group by alkyl, allyl or benzyl groups (compounds **65**−**68**) led to a 2−3 order of magnitude decrease in KB cell activity. However, these analogues were still inhibitors of tubulin polymerization. Analogues **69**−**72** where the nitrogen was replaced by other heteroatoms showed similar effects. A methoxy subtituent gave analogue **69**, with activity comparable to that of analogue **66** bearing an ethyl group. In contrast, a hydroxy group led to analogue **70** with no cellular activity and a considerable loss in its ability to inhibit tubulin polymerization at the concentrations tested. A thiomethyl group or methylsulfonyl group gave analogues **71** and **72**, respectively, which were comparable in activity to that of analogues with alkyl group replacements. Lack of a substituent at this position gave analogue **73** with no activity at the concentration tested. These results, in combination with the nitrogen acylation described above, reveal that a basic nitrogen in the A-piece is a requirement for potent activity. Several compounds in Table 5 were tested as mixtures of the SSS and RSS isomers.

The nature of the B-piece substituent was critical to activity (Table 6). As seen previously (vide supra), isomerization at this position led to profoundly de-

Scheme 12. Synthesis of *N,N*-Dimethyl Analogue **38**

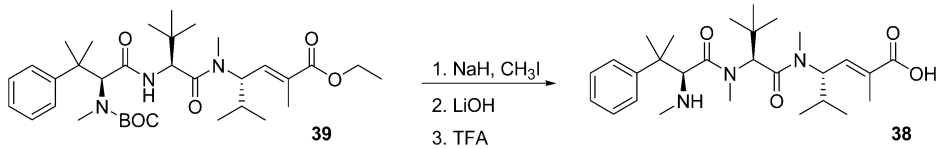
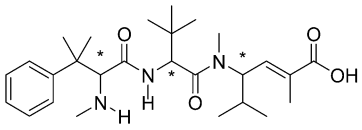


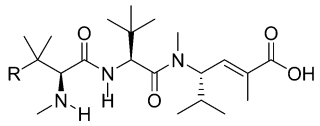
Table 1. Isomers of **2**



Cmpd	Isomer	Tubulin[a]	KB-3-1[b]	KB-8-5[b]	KB-V1[b]
2	SSS	88	0.96 ± 0.5	2.3 ± 1.2	77.4 ± 44
41	RSS	91	28 ± 6	39 ± 16	879 ± 521
42	SRS	1	>3000	>3000	>3000
43	SSR	0	605	1654	>3000

[a] % inhibition of tubulin polymerization at 0.3 μM; **2** was run as a standard. Over 10 runs its standard deviation was ± 6%. [b] Average IC_{50} (nM) ± SEM in cells. Where no SEM is shown, the value is <10% of the average.

Table 2. Replacement of the A-Piece Phenyl Group

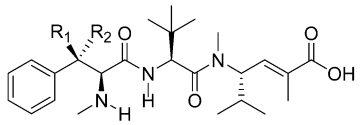


Cmpd	R	Tubulin[a]	KB-3-1[b]	KB-8-5[b]	KB-V1[b]
1	indole	-	0.32 ± 0.1	1 ± 0.5	76 ± 14
2	Ph	88	0.96 ± 0.5	2.3 ± 1.2	77.4 ± 44
44	CH_3	43	114 ± 57	180 ± 45	>3000
45	OH	40	151	293	>3000
46	n-pentyl	72	9	19	513
47	cyclohexyl	93	3.9 ± 0.2	5.7 ± 0.1	155 ± 4
48	4-anisolyl	91	1.4 ± 0.5	4.1 ± 1.8	143 ± 77
49	4-Ph-Ph	86	3.2 ± 1.5	6.8 ± 1.9	177 ± 48
50	$PhCH_2$	87	3	6	219

[a] % inhibition of tubulin polymerization at 0.3 μM; **2** was run as a standard. Over 10 runs its standard deviation was ± 6%. [b] Average IC_{50} (nM) ± SEM in cells. Where no SEM is shown, the value is <10% of the average.

creased activity for the SRS isomer (**42**). Variation of this substituent revealed that activity was related to the degree of branching at the beta carbon of the B-piece amino acid. Thus, while analogue **74** having a methyl substituent had an IC_{50} of 381 nM in the KB-3-1 cells, analogues **75** and **76** having an ethyl or *n*-propyl substituent, respectively, showed a dramatic increase in potency. Addition of a second alkyl group at the beta carbon (i.e. isopropyl substituent) gave compound **77**, with an additional increase in potency. An illustration of the dramatic effect of branching at the beta carbon on activity was the 50-fold greater potency of the

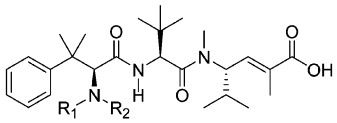
Table 3. A-Piece Geminal Dimethyl Group Replacement



Cmpd	R_1	R_2	tubulin[a]	KB-3-1[b]	KB-8-5[b]	KB-V1[b]
18			66	603	646	>3000
51	CH_3	H	77	438	584	>3000
52	H	CH_3	84	207	414	>3000
53	Et	Et	86	8.5	18	479
54	-$(CH_2)_4$-		77	59 ± 7	63 ± 0.1	2665 ± 153
55	-$(CH_2)_5$-		5	204	363	>3000
56	-$(CH_2)_2$-		99	27	57	1664

[a] % inhibition of tubulin polymerization at 0.3 μM; **2** was run as a standard. Over 10 runs its standard deviation was ±6%. [b] Average IC_{50} (nM) ± SEM in cells. Where no SEM is shown, the value is <10% of the average.

Table 4. A-Piece Amine Substitution

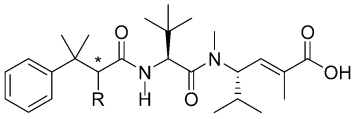


Cmpd	R_1	R_2	tubulin[a]	KB-3-1[b]	KB-8-5[b]	KB-V1[b]
20	-	-	95	427	550	>3000
25	CH_2CH_2OH	Me	72	45	62	>3000
26	Me	Me	93	1.6 ± 0.4	3.7 ± 1.9	116 ± 46
57	Et	H	96	1.6	2.4	114
58	Bn	H	55	1794	1875	>3000
59	-$(CH_2)_4$-		83	75	158	>3000
60	CH_2CH_2OH	H	90	183	616	>3000
61	CH_2CO_2H	H	35	>3000	>3000	>3000
62	$CH_2CONHCH_3$	H	0	>3000	>3000	>3000
63	H	H	49	>3000	>3000	>3000
64	-$COCH_2NHMe$	H	4	1398	2116	>3000

[a] % inhibition of tubulin polymerization at 0.3 μM; **2** was run as a standard. Over 10 runs its standard deviation was ± 6%. [b] Average IC_{50} (nM) ± SEM in cells. Where no SEM is shown, the value is <10% of the average.

dimethyl-4-methoxybenzyl analogue **79** relative to the unmethylated benzyl analogue **78**. The tolerance for a large aromatic or substituted aromatic group at this position points to a large binding cavity or solvent

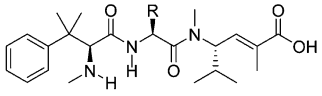
Table 5. A-Piece Methylamine Replacement



Cmpd	R	*	Tubulin[a]	KB-3-1[b]	KB-8-5[b]	KB-V1[b]
65	Me	S	94	561 ± 37	639 ± 64	>3000
66	Et	R/S	54	912	1202	5248
67	allyl	R/S	63	1148	1202	>6000
68	Bn	R/S	46	1691 ± 155	2245 ± 253	>6000
69	OMe	S	54	1416	1229	>3000
70	OH	S	21	>3000	>3000	>3000
71	SMe	R/S	79	691	1148	>6000
72	SO_2Me	R/S	80	1750 ± 240	2106 ± 447	>3000
73	H	-	21	>3000	>3000	>3000

[a] % inhibition of tubulin polymerization at 0.3 μM; **2** was run as a standard. Over 10 runs its standard deviation was ± 6%. [b] Average IC_{50} (nM) ± SEM in cells. Where no SEM is shown, the value is <10% of the average.

Table 6. Variation of B-Piece Substituents



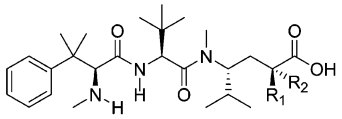
Cmpd	R	Tubulin[a]	KB-3-1[b]	KB-8-5[b]	KB-V1[b]
74[c]	Me	8	381 ± 223	902 ± 684	>3000
75	Et	73	48 ± 30	70 ± 38	1062 ± 502
76	n-Pr	51	15	19	575
77	i-Pr	78	5.2 ± 0.9	11 ± 5	288
78	CH_2Ph	40	1318	2398	>3000
79	$CH_2(CH_3)_2$Ph-4-OMe	56	27 ± 2	56 ± 1	1966 ± 172
80	$C(CH_3)_2SCH_3$	76	2.7 ± 0.7	7.1 ±2.0	303 ± 63

[a] % inhibition of tubulin polymerization at 0.3 μM; **2** was run as a standard. Over 10 runs its standard deviation was ±6%. [b] Average IC_{50} (nM) ± SEM in cells. Where no SEM is shown, the value is <10% of the average. [c] Purity ~ 79%. Contains ~20% of a diastereomer.

exposure at this site. The SAR information was used to design and synthesize an analogue with a radiolabeled benzophenone photoprobe (Table 6, R = 4-PhCOPh) at this position whose binding to tubulin was competitively inhibited by **2** and dolastatin-10.[16] Enlargement of the *tert*-butyl group via incorporation of the amino acid penicillamine as the B-piece gave analogue **80**, comparable in potency to **2** both in vitro and in vivo (vide supra). The correlation of branching with potency at this position is consistent with the reduction of conformational space accessible to the central portion of the peptide by bulkier substituents.[17] This rigidity preorganizes the molecule into a conformation necessary for tubulin binding.

The importance of the olefin in the potent antimicrotubule effects of the hemiasterlins and **2** was a significant mechanistic question. Conjugation of the olefin to the carboxylic acid group makes it an electrophile and potentially reactive with cysteine or lysine residues in tubulin. Previous results showed a major loss of potency upon reduction of the olefin in hemiasterlin, using catalytic reduction conditions.[7] For **2**, loss of potency was also seen (Table 7), however, analysis of the reduction mixture of **2** revealed that one isomer was predominant (87:13 mixture). Separation of the isomers by HPLC revealed that the major isomer **36** was weakly active, while the minor isomer **37** was very potent. This result supports the view that the olefin serves not as a reactive group, but as a center of conformational rigidity, presenting the carboxylic acid group in the proper orientation for binding to tubulin. Presumably, the potent reduced isomer **37** has a low energy conformation similar to that of **2**. The stereochemistry of the isomers was established by NMR.

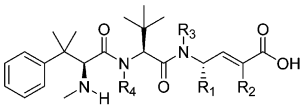
Table 7. CD-Piece Olefin Reduction



Cmpd	R_1	R_2	Tubulin[a]	KB-3-1[b]	KB-8-5[b]	KB-V1[b]
36:37[c]	CH_3/H	CH_3/H	74	48	69	>3000
36	CH_3	H	48	891	537	>3000
37	H	CH_3	99	8.5 ± 2.3	19 ± 1	1316 ± 102

[a] % inhibition of tubulin polymerization at 0.3 μM; **2** was run as a standard. Over 10 runs its standard deviation was ±6%. [b] Average IC_{50} (nM) ± SEM in cells. Where no SEM is shown, the value is <10% of the average. [c] 87:13 mixture.

Table 8. Amide Mitrogen and CD-Piece Variations

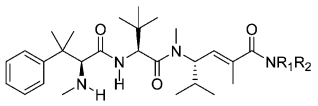


Cmpd	R_1	R_2	R_3	R_4	tubulin[a]	KB-3-1[b]	KB-8-5[b]	KB-V1[b]
38	i-Pr	Me	Me	Me	4	>3000	>3000	>3000
81	i-Pr	Et	Me	H	83	5.4 ± 0.5	14 ± 3	513 ± 134
82	n-Bu	Me	Me	H	52	36 ± 5	78 ± 17	>3000
83	Bn	Me	Me	H	44	479	1318	>3000
84	i-Pr	Me	H	H	79	43 ± 16	130 ± 48	>3000
85	i-Pr	Me	Et	H	11	109	197	>3000

[a] % inhibition of tubulin polymerization at 0.3 μM; **2** was run as a standard. Over 10 runs its standard deviation was ±6%. [b] Average IC_{50} (nM) ± SEM in cells. Where no SEM is shown, the value is <10% of the average.

Variation of the substituent on the olefin showed that **2**, bearing a methyl group, was 5-fold more potent than the ethyl analogue **81** (Table 8). The allylic position was tolerant of larger groups, with the *n*-butyl group, **82**, giving a 23-fold decrease in activity. However, the benzyl group gave analogue **83** with a further decrease in activity. Exploration of the amide linkages revealed that changes on the B−C amide nitrogen could be tolerated with removal of the methyl group, or its replacement with the larger ethyl group, giving rise to active, albeit substantially less potent compounds **84**

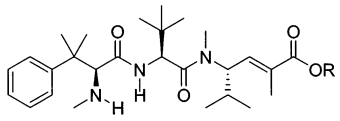
Table 9. D-Piece Amides



Cmpd	R_1	R_2	tubulin[a]	KB-3-1[b]	KB-8-5[b]	KB-V1[b]
86	H	H	72	65 ± 15	162 ± 24	>3000
87	Me	H	78	68 ± 4	129 ± 47	1590 ± 8
88	CH_2CH_2Ph	H	72	163	226	1768
89	CH_2-p-PhPh	H	11	667	1675	>3000
90	$-(CH_2)_2O(CH_2)_2-$		69	42 ± 7	75 ± 16	1189 ± 235
91	$-(CH_2)_2NBn(CH_2)_2-$		29	15 ± 4	22 ± 4	366 ± 127
92	$-(CH_2)_2NMe(CH_2)_2-$		69	33 ± 1	60 ± 1	2326 ± 36
93	OH	H	75	39 ±1	227 ± 37	>3000

[a] % inhibition of tubulin polymerization at 0.3 μM; **2** was run as a standard. Over 10 runs its standard deviation was ±6%. [b] Average IC_{50} (nM) ± SEM in cells. Where no SEM is shown, the value is <10% of the average.

Table 10. D-Piece Esters



Cmpd	R	tubulin[a]	KB-3-1[b]	KB-8-5[b]	KB-V1[b]
24	Et	97	34 ± 9	499 ± 104	1870 ± 275
94	n-octyl	26	150	1634	>3000
95	CH_2PhOCH_2Ph	55	136	846	>3000
96	CH_2-2-thienyl	19	4.2 ± 0.9	196 ± 35	2347 ± 653

[a] % inhibition of tubulin polymerization at 0.3 μM; **2** was run as a standard. Over 10 runs its standard deviation was ± 6%. [b] Average IC_{50} (nM) ± SEM in cells. Where no SEM is shown, the value is <10% of the average.

and **85**, respectively. Methylation of the A−B amide nitrogen, on the other hand, led to loss of potency (**38**), presumably as a result of its effect on the required conformation of the molecule.

Conversion of the D-piece carboxylic acid group to amides **86**−**92** led to substantial retention of tubulin binding and cellular activity (Table 9). Acyclic and cyclic amides had comparable activity, however, in the acyclic series potency decreased with larger groups on nitrogen (**88** and **89**). In the cyclic analogues a benzylic group on the piperazine nitrogen led to the most potent amide **91**, with a 2-fold greater cellular potency than that of the corresponding *N*-methyl analogue, **92**. The hydroxamic acid analogue, **93**, showed activity similar to that of the amides.

The ethyl ester of **2** (**24**) showed good activity in the cellular assay (Table 10). Longer alkyl groups such as *n*-octyl and CH_2Ph-4-CH_2OBn led to less potent analogues (**94** and **95**, respectively). The 2-thienylmethyl ester analogue **96** gave excellent activity in the KB-3-1 cells but with a disproportionate decrease in activity in the P-glycoprotein expressing KB-8-5 and KB-V1 cells relative to other analogues, suggesting that it is a better substrate for this transporter.

Table 11. K_D Values

Cmpd	K_D (uM)[a]	Tubulin[b]	KB-3-1[c]
2	0.26	88	0.96 ± 0.5
24	4.0	97	34 ± 9
25	1.0	93	1.6 ± 0.4
37	4.0	99	8.5 ± 2.3
41	d	91	28 ± 6
47	1.2	93	3.9 ± 0.2
48	0.7	91	1.4 ± 0.5
63	e	49	>3000
65	e	94	561 ± 37
80	0.7	76	2.7 ± 0.7

[a] standard error < 10% for **2**, **25**, **47**, **48**, **80** and 30−35% for **24** and **37**. [b] % inhibition of tubulin polymerization at 0.3 μM; **2** was run as standard. Over 10 runs its standard deviation was ±6%. [c] Average IC_{50} (nM) ± SEM in cells. [d] Weak binding, nonsaturable. [e] No evidence for binding.

Tubulin Binding Affinity (K_D). Binding affinities (K_D) to tubulin were obtained for representative compounds bearing structural changes in each portion of the molecule (Table 11). Low micromolar or submicromolar binding constants were seen for compounds with very potent (<5 nM) cellular cytotoxicity, independent of the position of the structural modification. Two compounds, with modifications of the methylamine group (i.e. NH_2, **63**, and CH_3, **65**), which had led to a profound decrease of cellular cytotoxicity, also showed no evidence for binding. The ethyl ester of **2** (**24**) had a K_D value of 4 uM, as did the saturated analogue **37**, both somewhat higher than K_D values of analogues with more potent cellular cytotoxicity. The *RSS* isomer **41** showed evidence for binding, however the binding was nonsaturable within the concentration range of 0 to 20 uM of **41**, consistent with weak binding.

Activity in Resistant Cell Lines. Compound **2** and its analogues are highly effective at inhibiting the growth of tumors (both in vitro and in vivo) including those that are resistant to paclitaxel and vincristine due to expression of P-glycoprotein. In the cellular screening assays, across the cell lines KB-3-1, KB-8-5 and KB-V1, where expression of P-glycoprotein varies from none, to moderate to very high, respectively,[18] the cytotoxicity of the analogues decreases according to the levels of cellular P-glycoprotein. Certain analogues, representing modifications in various representative portions of the tripeptide were tested in other cell lines (Table 12). The Lox melanoma and KM20 cells, with no P-glycoprotein expression, gave IC_{50} values comparable to the KB-3-1 cell values. The NCI-H1299, HCT-15 and S1 cells, which express P-glycoprotein, in general gave IC_{50} values higher than the non-P-glycoprotein expressing cells. The 4-methoxyphenyl analogue **48** was assayed in a panel of cells representing different tumor types and levels of P-glycoprotein expression and showed potency comparable to that of **2**.[6] Both compounds performed dramatically better than paclitaxel in the resistant cell lines.

In Vivo. Select compounds that met the criteria of good cellular potency (KB-3-1 cells) and activity in the

Table 12. Cytotoxicity in Other Cell Lines[a]

Cmpd	KB-3-1	Lox	KM20	NCI-H1299	HCT-15	S1
paclitaxel	3.9 ± 1.8	17.3 ± 5.6	8.9 ± 1.9	37.7 ± 12.6	438 ± 248	92 ± 100
2	0.96 ± 0.5	1.4 ± 0.6	1.8 ± 0.6	6.8 ± 6.1	4.2 ± 2.5	3.7 ± 2
24	34 ± 9	13.9 ± 4.4		100	575	
41	28 ± 6	37 ± 10		79 ± 21	58 ± 8	
48	1.4 ± 0.5	1.1 ± 0.2		10	6.9	7.3
65	561 ± 37	1380		1072	1202	
79	27 ± 2	10		83	132	
80	2.7 ± 0.7	3	6	15.8 ± 6.7	18.6	
81	5.4 ± 0.5	6.3 ± 0.7	6.6	19.3 ± 5.3	20.8	
82	36 ± 5		53 ± 1	268 ± 78		
84	43 ± 16	23		54	204	
87	68 ± 15			90	25.5 ± 10	657 ± 68

[a] Average IC_{50} (nM) ± SEM in cells. Where no SEM is shown, the value is <10% of the average.

Table 13. Effect on Lox Melanoma Xenografts in Athymic Mice

Cmpd	MED[a]	MTD[a]	KB-3-1[b]
2[c]	0.2	1.6	0.96 ± 0.5
24	0.4	1-3	34 ± 9
26	1	5-10	1.6 ± 0.4
47[d]	1	10-20	3.9 ± 0.2
48[c]	0.1	1.6	1.4 ± 0.5
77	0.7	>3	5.2 ± 0.9
80	0.5	3-10	2.7 ± 0.7
81	2	3-10	5.4 ± 0.5
90	3	10-25	42 ± 0.7
92	3	15	33 ± 1

[a] mg/kg on days 1, 5, 9, i.v. dosing ($p < 0.5$). [b] Average IC_{50} (nM) ± SEM in cells. [c] Also active in HCT 15, DLD1 and MX1W xenografts. [d] Also active in HCT15 and MX1W xenografts.

resistant cell lines (KB-8-5 and KB-V1) were evaluated in a Lox melanoma human tumor xenograft model in athymic mice (Table 13). This model is very responsive to taxanes.[18] Both the minimum effective dose (MED) and the maximum tolerated dose (MTD) were determined (compounds administered i.v. on a 1, 5, 9 day schedule, $p < 0.5$),[6] and taken together they were used to measure the therapeutic window (MTD/MED) of the compounds. Several compounds were also tested in xenograft models using cell lines expressing moderate to high levels of P-glycoprotein transporters (HCT-15, DLD1 and MX1W). The ethyl ester of **2** (**24**) showed excellent in vivo activity in the Lox xenograft model. The MED (0.4 mpk) was 2-fold less than **2**, despite the 40-fold decrease in cellular activity. The conversion of ester **24** to **2** in vivo by plasma esterases could be a cause of the potent in vivo activity. Qualitative in vitro plasma stability studies of **24** in CD-1 mouse plasma revealed low levels of **2**, which increased over time suggesting a prodrug effect on the observed in vivo potency. The amides **90** and **92** displayed potent antitumor activity in the Lox xenograft model with MED's = 3 mpk. Their in vivo potency was approximately 10-fold less than that of **2**, but in line with their higher cellular IC_{50} values. Other structural modifications such as a cyclohexyl ring in the A-piece (**47**), a thiomethyl (**80**) or isopropyl group (**77**) in the B-piece, an ethyl group on the olefin (**81**) or dimethylation of the basic amine (**26**) led to potent compounds with comparable therapeutic windows. One compound, the 4-methoxyphenyl analogue (**48**) of **2**, showed superior in vivo activity (Figure 2). The MED of 0.1 mpk for **48** in the Lox xenograft model was the most potent of those tested, and together with an MTD of 1.6 mpk, **48** had the widest therapeutic window (16×). At day 25−28, the relative tumor growth (RTG) at 1 mpk, iv dose of **48** was 0.17 ± 0.11 (**2** RTG = 3.2 ± 3.6). Therefore, on average, tumor regression occurred with **48** but not **2** at this time point. In addition, **48**, when administered at the maximum tolerated dose, displayed cures (no tumor detected) in many animals in the Lox melanoma model. Compound **48** was also active in vivo using cell lines expressing P-glycoprotein (HCT-15, DLD1 and MX1W) that are not responsive to taxanes given at the MTD.

In conclusion, investigation of the SAR of analogues of **2** revealed the importance of several key components to the activity of the molecule. The critical role of branching in the B-piece was established and its role in the conformational rigidity of the molecule determined. The CD-piece olefin was shown to act not as a group reactive with tubulin, but as a structural element of the molecule conferring rigidity. The necessity of a basic group in the A-piece, as evidenced by decrease of activity upon replacement with alkyl groups or other

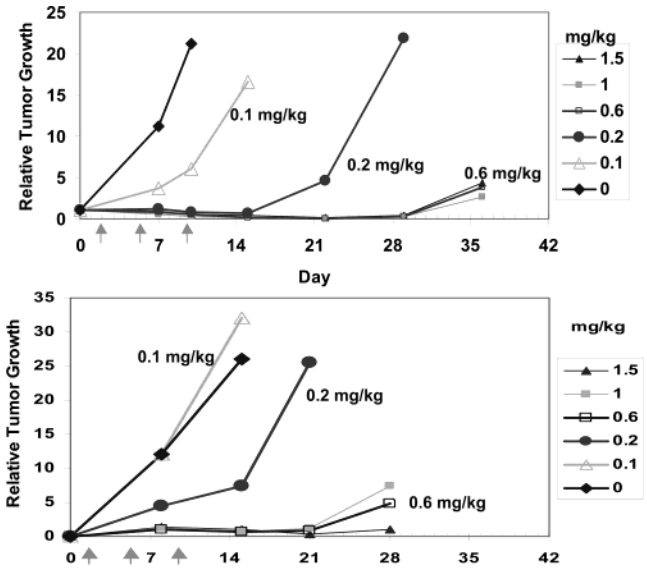


Figure 2. Compounds **48** (top) and **2** (bottom) inhibit in vivo growth of Lox melanoma tumors in athymic mice.

heteroatoms, was clearly demonstrated. Replacement of the aromatic ring with other groups of sufficient steric bulk and lipophilicity was possible. Many of the analogues described are potent cytotoxic agents, active against a variety of tumors, both in vitro and in vivo. Importantly, they also overcame resistance to taxanes and Vinca alkaloids associated with P-glycoprotein expression. One analogue (**48**) demonstrated superior in vivo activity.

Experimental Section

Chemistry. ^{1}H NMR spectra were determined with a Bruker DPX-300 spectrometer at 300 MHz. Chemical shifts (δ) are reported in parts per million relative to residual chloroform (7.26 ppm), TMS (0 ppm), or dimethyl sulfoxide (2.49 ppm) as an internal reference with coupling constants (J) reported in Hertz (Hz). The peak shapes are denoted as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Electrospray (ES) mass spectra were recorded in positive or negative mode on a Micromass Platform spectrometer. Electron impact and high-resolution mass spectra were obtained on a Finnigen MAT-90 spectrometer. Combustion analyses were obtained using Perkin-Elmer Series II 2400 CHNS/O analyzer. Chromatographic purifications were performed by flash chromatography using EM Science 0.040−0.063 mm silica gel. Thin-layer chromatography (TLC) was performed on EM Science silica gel 60 250 mm plates. Unless otherwise noted preparative reverse phase HPLC was run on a Phenomenex Prodigy 5 μM ODS column using a gradient solvent system (Solvent A: 0.01% TFA/water, Solvent B: CH_3CN). The terms "concentrated" and "evaporated" refer to removal of solvents using a rotary evaporator at water aspirator pressure with a bath temperature equal to or less than 60 °C. Unless otherwise noted, reagents were obtained from commercial sources and were used without further purification. Pyruvic acids were either commercially available or were prepared according to literature methods via the corresponding azlactones or hydantoins.[19]

Biology. The tubulin polymerization assay, cellular cytotoxicity assays and in vivo xenograft assays were performed as described previously.[6] The cell lines used in the cytotoxicity assays and the xenograft assays were obtained as described previously.[6] K_D values were determined as described in the literature.[20]

General Procedure 1: Preparation of α,α-Dimethylpyruvic Acids (7). To a solution of a pyruvic acid in THF (ca. 0.1 mol/100 mL) at 0 °C under a nitrogen atmosphere was added CH_3I (2.5−3.0 mol equivalent) and 5 N aq NaOH (3−3.5 mol equiv). The resulting mixture was allowed to warm to room temperature. After 15−48 h, the volatiles were removed under reduced pressure and the resulting aqueous solution was extracted with EtOAc. The aqueous phase was cooled to 0 °C, acidified with 10% aq HCl to pH 1.0, and extracted with EtOAc (three times). The combined organic extracts were washed with brine, dried over Na_2SO_4 or $MgSO_4$, filtered and concentrated in vacuo. The product thus obtained was recrystallized or used without further purification.

General Procedure 2: Preparation of Racemic A-Pieces (8) from α,α-Dimethylpyruvic Acids (7). To a solution of α,α-dimethylpyruvic acid **7** in anhydrous THF (0.1 mol/100 mL) was added methylamine (2.0−2.5 mol equiv, 2 M solution in anhydrous THF) at room temperature, under a nitrogen atmosphere (during this procedure, the formation of a thick solid may be observed). The resulting mixture was stirred for 1−2 h, and a solution of borane-pyridine complex (1.2 mol equiv, 8 M solution) was introduced. The mixture was then heated at 45−55 °C (bath temperature) for 2.0−2.5 h and cooled to room temperature. CH_3OH (ca 30 mL/0.1 mol) was added to the mixture with stirring, and the volatiles were removed in vacuo. The resulting syrupy residue was triturated with THF. The product precipitated upon cooling with an ice−water bath. The solid was collected by filtration and dried under high vacuum.

General Procedure 3: Coupling of A-Pieces and the BCD-Piece (9). To a cooled (0 °C, ice bath) solution of an A-piece (1.1 mmol) and benzotriazole-1-yl-oxy-tris-pyrrolidinophosphonium hexafluorophosphate (PyBOP) (1.1 mmol) in DMF (3−5 mL), under a nitrogen atmosphere, was added diisopropylethylamine (1.0 mmol). To the resulting solution was added **9** (1.0 mmol) in DMF (3 mL). After stirring at 0 °C for 5−10 min, the cooling bath was removed, and the resulting reaction mixture was stirred at room temperature for 15−20 h. The mixture was diluted with water, and the aqueous layer extracted with EtOAc (three times). The combined extracts were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo.

Alternatively, to a cooled (0 °C, ice bath) solution of A-piece (1.1 mmol), HOBT (1.1 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimine hydrochloride (EDC) (1.2 mmol) in anhydrous DMF (3−5 mL) under a nitrogen atmosphere was added *N*-methylmorpholine (1.4 mmol) via syringe. After stirring for 15 min at 0 °C, the cooling bath was removed, and the resulting mixture was stirred for 2−24 h. The solution was cooled to 0 °C (ice water bath), and to this mixture was added a solution of **9** (1.0 mmol) in anhydrous DMF (3 mL). The cooling bath was removed, and the resulting mixture was stirred for 15−36 h at room temperature under a nitrogen atmosphere. The mixture was diluted with water, and the aqueous layer extracted with EtOAc (three times). The combined extracts were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo.

General Procedure 4: Ester Hydrolysis. The ester (1 mmol) was dissolved in CH_3OH (24 mL) and cooled to 0 °C (ice−water bath). To this solution were added water (8 mL) and 1 M aq LiOH (8 mmol). The cooling bath was removed, and the resulting mixture was stirred at room temperature for 15 h. CH_3OH was removed in vacuo, and the residual aqueous mixture was cooled with an ice−water bath, and acidified to pH 5.5−6.0 with aq 1 N citric acid. The precipitate was collect by filtration, and the solid was washed with cold water and dried in vacuo (alternatively, the product could be purified by reverse phase HPLC).

General Procedure 5: D-Piece Amide Formation. To a solution of **2** (0.1 mmol) in CH_3CN or DMF (5.0 mL) at 25 °C was added HOBT (0.12 mmol) and EDC (1.33 mmol). After 2−18 h, an amine (0.4 mmol) was added. After 1−18 h, the reaction mixture was concentrated in vacuo and purified by reverse phase HPLC.

General Procedure 6: Formation of D-Piece Esters. A mixture of **2** (1.0 equiv), EDC (1.2 equiv), an alcohol (1.2 equiv) and DMAP (0.2 equiv) in anhydrous CH_2Cl_2 (1 mmol/

20 mL) was stirred under a nitrogen atmosphere, at room temperature, for 20 h. The solvent was removed, the residue was taken up in CH_3OH, and the product was purified by reverse phase HPLC.

3-Methyl-3-(4-bromophenyl)-2-oxobutanoic Acid (7; R = CH_3, R_1 = 4-BrPh). To a solution of 3-methyl-3-phenyl-2-oxobutanoic acid[21] (1.0 g, 5.2 mmol) in CCl_4 (3.5 mL) were added bromine (0.86 g, 5.4 mmol) and iron powder (0.007 g). The resulting mixture was heated at reflux overnight. The deep red reaction mixture was washed with cold 10% aq HCl and then water. The organic phase was then extracted with 2.5 M aq NaOH (40 mL). The basic extracts were then acidified to pH 1.0 with 12 N aq HCl. A dark red liquid oiled out, which was isolated by extraction with EtOAc. The organic extracts were washed with brine, dried over Na_2SO_4, decanted, and concentrated under reduced pressure to afford a straw-colored semisolid (1.1 g, 79%). MS, *m/z*: 269.1, 271.1 $(M - H)^-$. Anal. ($C_{11}H_{11}BrO_3$).

4-Bromo-*N*,*β*,*β*-trimethylphenylalanine (8; R = CH_3, R_1 = 4-BrPh, R_2 = CH_3). According to General Procedure 2,3-methyl-3-(4-bromophenyl)-2-oxobutanoic acid (**7**; R = CH_3, R_1 = 4-BrPh) (1.0 g, 3.7 mmol), THF (8 mL), methylamine (4.1 mL, 2 M solution in THF) and borane−pyridine (0.45 mL, 8 M) gave the title compound as a white powder (0.32 g, 49%). MS, *m/z*: 284.2, 286.2 $(M - H)^-$. Anal. ($C_{12}H_{16}BrNO_2$).

(Methylamino)(1-phenylcyclopropyl)acetic Acid (11). A mixture of 1-phenyl-cyclopropanecarbaldehyde[12] (1.46 g, 10.0 mmol), methylamine (6.25 mL of 2 M solution in CH_3OH, 12.5 mmol) and CH_3OH (7 mL) was stirred for 15 min. Then 1-isocyano-cyclohexene (1.07 g, 10.0 mmol) and formic acid (600 mg, 96%, 12.5 mmol) were added. After 18 h the reaction mixture was concentrated in vacuo and triturated with 10:1 hexane/EtOAc, filtered and dried to give *N*-cyclohex-1-en-1-yl-2-[formyl(methyl)amino]-2-(1-phenylcyclopropyl)acetamide **10** as a white solid (1.23 g). MS, *m/z*: 313.2 $(M + H)^+$. Compound **10** (400 mg, 1.23 mmol) was refluxed with aq 6 N HCL (3 mL) for 1 h. The mixture was concentrated in vacuo and the residue purified by HPLC to give the title compound as a white solid (224 mg). MS, *m/z*: 220.1 $(M + H)^+$.

Methyl (*βS*)-*N*-(*tert*-butoxycarbonyl)-*N*,*β*-dimethyl-L-phenylalaninate (12b). A solution of (*βS*)-*N*-(*tert*-butoxycarbonyl)-*β*-methyl-L-phenylalanine (200 mg, 0.716 mmol) in DMF at 25 °C was treated with NaH (60% in mineral oil, 60 mg, 1.5 mmol). After 1 h iodomethane (0.13 mL, 2.15 mmol) was added. After 24 h EtOAc and 1 N aq citric acid was added. The organic phase was washed with water (5×) and dried over $MgSO_4$. Concentration in vacuo gave the title compound as a colorless oil (230 mg). HRMS (ESI) calcd for $C_{17}H_{25}NO_4$: 308.18620 $(M + H)^+$; found 308.18577.

Adamantan-1-yl-methylamino-acetic acid (17). Adamantan-1-yl-iodo-acetic acid[13] (7.86 g, 24.55 mmol) was treated with 2 M methylamine (100 mL, 200 mmol) in THF and heated at reflux for 18 h. Concentration in vacuo followed by addition of water and acidification to pH 6 with aq HCl gave the title compound as a white solid (2.16 g). MS, *m/z*: 224.2 $(M + H)^+$.

(2*S*)-2,3-Dimethyl-3-phenylbutanoic Acid (28, R = CH_3). A solution of **27** (0.23 g, 0.79 mmol) in anhydrous THF (3 mL) under a nitrogen atmosphere was cooled to −78 °C. Sodium hexamethylsilazide (1.0 M in THF, 0.95 mL, 0.95 mmol) was added dropwise via syringe. After 30 min at −78 °C, iodomethane (0.25 mL, 4.0 mmol) in THF (1 mL) was added dropwise. The reaction mixture was allowed to warm to room temperature. After 18 h the reaction mixture was treated with water (10 mL) and EtOAc (10 mL). The aqueous phase was extracted 3× with EtOAc. The combined extracts were washed with saturated aq $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated under reduced pressure to afford an amorphous solid. The crude material was purified by flash chromatography (EtOAc/hexanes) to afford (4*S*)-3-[(2*S*)-2,3-dimethyl-3-phenylbutanoyl]-4-isopropyl-1,3-oxazolidin-2-one as a crystalline solid (0.17 g, 71%). MS, *m/z*: 303.9 $(M + H)^+$. To a solution of this compound (0.13 g, 0.43 mmol) in THF (6 mL) and water (2 mL) at 0 °C was added 30% aq hydrogen peroxide (2.4 mL). After 2 min, LiOH monohydrate (36 mg) was added. The reaction mixture was allowed to warm to room temperature. After 18 h the reaction mixture was cooled to 0 °C and aq sodium sulfite solution (1.5 M, 1.3 mL) was added. After stirring for 1 h at room temperature, the reaction mixture was concentrated under reduced pressure. The residue was extracted twice with CH_2Cl_2, acidified to pH 1 with 10% aq HCl, then extracted 3× with EtOAc. The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give the title compound as a white crystalline solid (60 mg, 72%). Mp 69−72 °C MS, *m/z*: 191.0 $(M - H)^-$.

(4*S*)-4-Isopropyl-3-(3-methyl-3-phenylbutanoyl)-1,3-oxazolidin-2-one (27). To a solution of 3,3-dimethyl-3-phenyl propionic acid (2.1 g, 12 mmol) in anhydrous THF (24 mL) under nitrogen was added triethylamine (2.0 mL). The mixture was cooled to −78 °C and pivaloyl chloride (1.5 mL, 13 mmol) was added dropwise. The reaction mixture was held for 15 min at − 78 °C and was then warmed to 0 °C with an ice−water bath. In a separate flask, a solution of (*S*)-4-isopropyl-2-oxazolidinone (1.5 g, 12 mmol) in anhydrous THF (27 mL) was prepared under inert atmosphere and cooled to − 35 °C. A small amount of triphenylmethane (<5 mg) was added as an indicator of deprotonation. *n*-Butyllithium (1.6 M solution in hexanes, 7.7 mL, 12 mmol) was added dropwise via syringe. After 30 min at 0 °C, the flask containing the mixed anhydride was recooled to −78 °C in a dry ice/acetone bath. The solution of the lithium anion of the oxazolidinone was added to the mixed anhydride solution via cannula. The reaction mixture was stirred at −78 °C for 1 h, and at 0 °C for 1 h, and allowed to warm to room temperature. After 18 h, water (∼ 15 mL) was added. After 20 min the aq phase was extracted 3× with Et_2O. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure to afford a pale yellow oil (3.6 g). The crude material was purified by flash chromatography (EtOAc/hexanes) to afford the title compound as a clear, colorless oil (1.5 g, 44%). MS, *m/z*: 290.0 $(M + H)^+$.

2-Benzyl-3-methyl-3-phenylbutanoic Acid (28, R = Bn). In accordance with a modified literature procedure[22] a solution of 3,3-dimethyl-3-phenyl propionic acid (0.22 g, 1.2 mmol) in THF (2 mL) under nitrogen was cooled to − 40 °C. Lithium diisopropylamide (2.0 M in THF, 1.4 mL, 2.8 mmol) was added dropwise via syringe, followed by 1,3-dimethyl-3,4,5,6-tetrahydro-2(1 *H*)-pyrimidinone (DMPU, 0.16 mL, 1.3 mmol). The reaction mixture was then allowed to warm to room temperature and to stir for 30 min before being recooled to 0 °C (ice water bath). Benzyl bromide (0.15 mL, 1.3 mmol) was then added via syringe, and the reaction mixture was allowed to warm to room temperature. After 18 h, the reaction was quenched by the addition of 10% aq HCl and extracted 3× with EtOAc. The combined extracts were washed twice with 10% aq HCl and once with saturated NaCl solution, dried over anhydrous sodium sulfite, and concentrated under reduced pressure. Flash chromatography (silica gel, ether/hexanes) gave the title compound as a straw-colored gum (70%). MS, *m/z*: 266.9 $(M - H)^-$.

2-Ethyl-3-methyl-3-phenylbutanoic Acid (28, R = Et). By the method described for **28** (R = Bn), 3,3-dimethyl-3-phenyl propionic acid (0.47 g, 2.6 mmol), lithium diisopropylamide (2.0 M, 2.9 mL, 5.8 mmol), DMPU (0.35 mL, 2.9 mmol) and ethyl iodide (0.31 mL, 3.9 mmol) gave, after recrystallization from hexanes, the title compound as a white solid (0.16 g, 30%). MS, *m/z*: 205.0 $(M - H)^-$.

2-Allyl-3-methyl-3-phenylbutanoic Acid (28, R = allyl). By the method described for **28** (R = Bn), 3,3-dimethyl-3-phenyl propionic acid (0.50 g, 2.8 mmol), lithium diisopropylamide (2.0 M, 3.1 mL, 6.2 mmol), DMPU (0.37 mL, 4.2 mmol) and allyl iodide (0.15 mL, 1.3 mmol) gave, after flash chromatography (silica gel, hexane/CH_2Cl_2/EtOAc), the title compound as a clear blond oil (0.39 g, 64%). MS, *m/z*: 217.0 $(M - H)^-$.

3-Methyl-2-(methylsulfanyl)-3-phenylbutanoic Acid (30). By the method described for **28 (R = Bn)** 3,3-dimethyl-3-phenyl propionic acid (0.50 g, 2.8 mmol), lithium diisopropyl-

amide (2.0 M, 3.1 mL, 6.2 mmol), DMPU (0.37 mL, 3.1 mmol) and dimethyl disulfide (0.38 mL, 4.2 mmol) gave, after flash chromatography (silica gel, hexanes/CH_2Cl_2/CH_3OH), the title compound as a tan solid (0.54 g, 86%). MS, *m/z*: 222.9 $(M - H)^-$.

3-Methyl-2-(methylsulfonyl)-3-phenylbutanoic Acid (31). A solution of **30** (0.28 g, 1.2 mmol) in CH_2Cl_2 (10 mL) was cooled to 0 °C and treated with peracetic acid (32% aqueous solution, 0.76 mL, 3.6 mmol). The reaction mixture was allowed to warm to room temperature. After 3 h, additional peracetic acid (0.76 mL) was added. After 18 h, the reaction mixture was concentrated under reduced pressure and 1.5 M aq sodium sulfite solution (20 mL) was added. The reaction mixture was extracted 3× with EtOAc. The combined organic extracts were washed with 10% aq HCl and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give the title compound as a white powder (0.29 g, 94%). MS, *m/z*: 255.0 $(M - H)^-$.

2-Hydroxy-3-methyl-3-phenylbutanoic Acid (32). To a solution of 3-phenyl-3-methyl-2-oxobutanoate (9.0 g, 47 mmol) in Et_2O (40 mL) and CH_3OH (60 mL) at 0 °C was added trimethylsilyl diazomethane (2.0 M solution in hexanes, 35 mL, 70 mmol) dropwise. The solvents were removed under reduced pressure and the residue was taken up in Et_2O/hexanes (1:1) and washed with 2% aq HCl, saturated aq $NaHCO_3$, and brine. The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give methyl 3-phenyl-3-methyl-2-oxobutanoate (**33**) as a straw-colored liquid (8.5 g, 88%). MS, *m/z*: 206.9 $(M + H)^+$. According to a literature procedure[23] a solution of **33** (2.5 g, 12 mmol) in CH_3OH (144 mL) was cooled to 0 °C. Sodium borohydride (0.21 g, 5.5 mmol) was added in three portions over 3 min. After 10 min, the reaction mixture was allowed to warm to room temperature. After 30 min, the reaction mixture was acidified with 10% aq HCl until pH = 6 and then extracted 3× with Et_2O. The combined organic extracts were washed with water and brine, dried over anhydrous sodium sulfite, and concentrated under reduced pressure. Flash chromatography (silica gel, hexanes/EtOAc) gave methyl 2-hydroxy-3-methyl-3-phenylbutanoate (**34**) (80%) as a clear, colorless oil. MS, *m/z*: 209.1 $(M + H)^+$. According to General Procedure 4, **34** (0.27 g, 1.3 mmol), CH_3OH (6 mL), THF (6 mL), water (3 mL) and LiOH monohydrate (0.12 g, 2.9 mmol) were heated at 60−65 °C for 16 h to give the title compound as a solid (0.25 g, 100%). MS, *m/z*: 193.0 $(M - H)^-$.

2-Methoxy-3-methyl-3-phenylbutanoic Acid (35). To a solution of **34** (0.50 g, 2.4 mmol) under nitrogen in diethyl ether (11 mL) was added silver(I) oxide (4.3 g) and iodomethane (8.7 mL). The reaction mixture was heated at 40 °C. After 26 h, the reaction mixture was filtered through a plug of Celite. The filtrate was concentrated in vacuo and purified by chromatography (silica gel, hexanes/EtOAc) to give methyl 2-methoxy-3-methyl-3-phenylbutanoate as a clear, colorless liquid (68% yield). MS, *m/z*: 222.9 $(M + H)^+$. According to General Procedure 4, this compound (0.49 g, 2.2 mmol), CH_3OH (10 mL), THF (10 mL), water (5 mL) and LiOH monohydrate (0.18 g, 4.4 mmol) were heated at 60 °C for 16 h to give **35** as a crystalline solid (0.46 g, 100%). MS, *m/z*: 207.0 $(M - H)^-$.

***N,β,β*-Trimethyl-L-phenylalanyl-N^1-[(1*R*,3*S*)-3-carboxy-1-isopropylbutyl]-N^1,3-dimethyl-L-valinamide (36) and *N,β,β*-Trimethyl-L-phenylalanyl-N^1-[(1*R*,3*R*)-3-carboxy-1-isopropylbutyl]-N^1,3-dimethyl-L-valinamide (37).** A solution of **2** (200 mg) in acetic acid (10 mL) was treated with 10% Pd/C under 1 atm of hydrogen for 18 h. Concentration in vacuo, filtration through diatomaceous earth with 1:1 CH_3OH:water (0.02% TFA) gave an 87:13 mixture of isomers as determined by analytical HPLC. Separation by preparative reverse phase HPLC (1:1 CH_3OH:water (0.02% TFA) gave the TFA salt of the major isomer (**36**) (first off of HPLC column) as a white powder (56 mg). HRMS (ESI) calcd for $C_{27}H_{45}N_3O_4$: 476.34885 $(M + H)^+$; found 476.34796. Anal. ($C_{27}H_{45}N_3O_4$. 1.5 TFA. 1.5 H_2O) HPLC gave the TFA salt of the minor isomer (**37**) (7 mg). MS, *m/z*: 476.5 $(M + H)^+$. HPLC = 98.85%.

4-Phenyl-*N,β,β*-trimethyl-L-phenylalanyl-N^1-[(1*S*,2*E*)-3-carboxy-1-isopropyl-2-butenyl]-N^1,3-dimethyl-L-valinamide (49). According to General Procedure 3, 4-bromo-*N,β,β*-trimethylphenylalanine (**8**; R = CH_3, R_1 = 4-BrPh, R_2 = CH_3) (0.29 g, 1.5 mmol), PyBOP (0.57 g, 1.1 mmol), diisopropylethylamine (0.35 mL, 2.0 mmol) and **9** (HCl salt) (0.38 g, 1.1 mmol) in CH_2Cl_2 (10 mL) gave after purification by preparative reverse-phase HPLC, the TFA salt of 4-bromo-*N,β,β*-trimethylphenylalanyl-N^1-[(1S,2E)-4-ethoxy-1-isopropyl-3-methyl-4-oxo-2-butenyl]-N^1,3-dimethyl-L-valinamide. MS, *m/z*: 580.6, 582.6 $(M + H)^+$. calcd for Anal. ($C_{29}H_{46}BrN_3O_4$·1.6TFA·CH_3CN) To a solution of this material (0.25 g, 0.43 mmol) in ethylene glycol dimethyl ether (9 mL) and water (4 mL) was added phenylboronic acid (0.10 g, 0.86 mmol), Na_2CO_3 (0.14 g, 1.3 mmol), and (tetrakis)triphenylphosphine palladium (0.05 mg, 0.04). The reaction mixture was heated at reflux for 18 h and then allowed to cool to room temperature. Volatiles were evaporated under reduced pressure, and the residue was partitioned between ether and water. The aqueous phase was extracted 3× with ether. The combined organic extracts were washed with water then brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to afford a reddish-brown semisolid (0.20 g) which was purified by preparative reverse-phase HPLC to give the TFA salt of **16** (60 mg). MS, *m/z*: 578.5 $(M + H)^+$. According to General Procedure 4, **16** (0.06 g, 0.10 mmol) and LiOH (0.30 mmol) in THF/CH_3OH/water (0.5 mL) gave the TFA salt of the title compound (0.020 g, 26%) after purification by preparative reverse-phase HPLC. MS, *m/z*: 550.4 $(M + H)^+$. Anal. ($C_{33}H_{47}N_3O_4$·3TFA·$2H_2O$).

***N*-Ethyl-*β,β*-dimethyl-L-phenylalanyl-N^1-[(1*S*,2*E*)-3-carboxy-1-isopropylbut-2-enyl]-N^1,3-dimethyl-L-valinamide (57).** Treatment of **22** (143 mg, 0.273 mmol) in DMF (1.5 mL) with iodoethane (0.025 mL, 0.30 mmol) and diisopropylethylamine (100 uL, 0.57 mmol) for 6 h, followed by HPLC gave a solid (32 mg). This material (28 mg) was treated as described in General Procedure 4 to give, after HPLC, the TFA salt of **57** as a white solid (20 mg). MS, *m/z*: 488.3 $(M + H)^+$.

***N*-(2-hydroxyethyl)-*N,β,β*-trimethyl-L-phenylalanyl-N^1-[(1*S*,2*E*)-3-carboxy-1-isopropylbut-2-enyl]-N^1,3-dimethyl-L-valinamide (25).** To **24** (300 mg, 0.56 mmol) in DMF (2 mL) were added 2-bromoethanol (0.04 mL, 0.56 mmol) and diisopropylethylamine (0.195 mL, 1.12 mmol). After 18 h, tetrabutylammonium iodide (10 mg) was added. After 4 h 2-bromoethanol (0.1 mL) was added. After 18 h, the reaction mixture was heated at 70 °C and additional 2-bromoethanol and diisopropylethylamine were added. The reaction mixture was purified by reverse phase HPLC. As described in General Procedure 4, the resulting material (50 mg, 0.0758 mmol) was treated with LiOH (0.23 mmol) for 18 h. Purification by HPLC gave the TFA salt of **25** as a white powder (33 mg). MS, *m/z*: 518.4 $(M + H)^+$. Anal. ($C_{29}H_{47}N_3O_5$·1.5TFA·$0.2H_2O$).

***N,N,β,β*-Tetramethyl-L-phenylalanyl-N^1-[(1*S*,2*E*)-3-carboxy-1-isopropyl-2-butenyl]-N^1,3-dimethyl-L-valinamide (26).** To **24** (200 mg, 0.372 mmol) in DMF (3 mL) were added iodomethane (29 uL, 0.44 mmol) and diisopropylethylamine (200 uL, 1.12 mmol). After 30 min, the reaction mixture was purified by HPLC to give the desired dimethylamine (99 mg). As described in General Procedure 4, this material was treated with LiOH (0.47 mmol) in CH_3OH/THF/water for 18 h. Purification by HPLC gave the TFA salt of **26** as a white powder (20 mg). HRMS (ESI) calcd for $C_{28}H_{45}N_3O_4$: 488.34885 $(M + H)^+$; found 488.3412.

***β,β*-Dimethyl-L-phenylalanyl-N^1-[(1*S*,2*E*)-3-carboxy-1-isopropylbut-2-enyl]-N^1,3-dimethyl-L-valinamide (63).** Commercially available (S)-*N*-BOC-2-amino-3-methyl-3-phenyl-butyric acid was converted to the HCl salt of **22** by the use of General Procedure 3 followed by treatment with HCl in dioxane to remove the BOC protecting group. Treatment of the resulting material according to General Procedure 4 gave the HCl salt of **63**. Anal. ($C_{26}H_{41}N_3O_4$·1.5HCl·$0.2CH_3OH$).

(2*E*,4*S*)-2,5-Dimethyl-4-(methyl{3-methyl-*N*-[(2,2,4-trimethylthiomorpholin-3-yl)carbonyl]-L-valyl}amino)hex-2-enoic Acid (20). A mixture of 2,2-dimethyl-thiomorpholine-

3-carboxylic acid[15] (3.51 g, 20 mmol), formic acid (5.12 g of 90%) and formalin (4.5 mL of 37%) was heated at reflux for 8 h. The reaction mixture was cooled, treated with 4 N aq HCl (10 mL) and concentrated in vacuo. Purification by HPLC gave 2,2,4-trimethyl-thiomorpholine-3-carboxylic acid. According to General Procedure 3, this compound (388 mg, 2.05 mmol), PyBOP (1.07 g, 2.05 mmol), **9** (0.640 g, 2.05 mmol) and diisopropylethylamine (0.8 mL, 4.4 mmol) gave the coupled compound (314 mg, 0.65 mmol), which was treated according to General Procedure 4 with LiOH (3.3 mmol) to provide, after HPLC, the TFA salt of **20**. MS, *m/z*: 456.3 $(M + H)^+$. Anal. ($C_{23}H_{41}N_3O_4S \cdot 1.25TFA \cdot 1.25H_2O$).

(2*E*,4*S*)-4-[{*N*-[(2*S*)-2-(1-adamantyl)-2-(methylamino)-ethanoyl]-3-methyl-L-valyl}(methyl)amino]-2,5-dimethyl-2-hexenoic Acid (18). According to General Procedures 3 and 4, **17** and **9** gave, after purification by HPLC, the TFA salt of **18** as a white solid. MS, *m/z*: 490.2 $(M + H)^+$. Anal. ($C_{28}H_{47}N_3O_4 \cdot 1.25TFA \cdot 0.5H_2O$).

(*E*,4*S*)-4-[((2*S*)-2-{[(2*S*)-2,3-Dimethyl-3-phenylbutanoyl]-amino}-3,3-dimethylbutanoyl)(methyl)amino]-2,5-dimethyl-2-hexenoic Acid (65). Following General Procedures 3 and 4 (using **28**, R = CH_3, in General Procedure 3) gave **65** as a white solid. MS, *m/z*: 459.0 $(M + H)^+$.

***N*,*β*,*β*-Trimethyl-L-phenylalanyl-*N*^{1}-[(1*S*,2*E*)-3-carboxy-1-isopropyl-2-butenyl]-*N*^{1},*N*^{2},3-trimethyl-L-valinamide (38).** To a solution of **39**[7,9a] (300 mg, 0.5 mmol) and iodomethane (570 mg, 4.0 mmol) in DMF (20 mL) at 0 °C was added NaH (60 mg, 1.5 mmol). After 3−4 h, aqueous workup gave, after silica gel chromatography, the *N*-methylated intermediate (174 mg). This material (100 mg, 0.16 mmol) was treated according to General Procedure 4 to give the corresponding acid (50 mg), which was then treated with TFA (0.065 mL) in CH_2Cl_2 for 12 h to remove the BOC group. HPLC gave the TFA salt of **38** (15 mg).

(*E*,4*S*)-4-[((2*S*)-3,3-dimethyl-2-{[(2S)-3-methyl-2-(methylamino)-3-phenylbutanoyl]amino}butanoyl)(methyl)-amino]-2,5-dimethyl-2-hexenamide (86). As described in General Procedure 5, **2** (50 mg, 0.106 mmol) in CH_3CN (5 mL) was treated with HOBT (17 mg, 0.127 mmol) and EDC (27 mg, 1.41 mmol). After 2 h 2.0 M ammonia in CH_3OH was added (0.2 mL, 0.42 mmol). Purification by preparative reverse phase HPLC gave the TFA salt of **86** as a white powder (13 mg). MS, *m/z*: 473.4 $(M + H)^+$. Anal. ($C_{27}H_{44}N_4O_3 \cdot 1.5$ TFA$\cdot 2H_2O$).

(*E*,4*S*)-4-[((2*S*)-3,3-Dimethyl-2-{[(2S)-3-methyl-2-(methylamino)-3-phenylbutanoyl]amino}butanoyl)(methyl)-amino]-*N*,2,5-trimethyl-2-hexenamide (87). As described in General Procedure 5, **2** (30 mg, 0.064 mmol) in CH_3CN (5 mL) was treated with HOBT (10.2 mg, 0.076 mmol) and EDC (16.2 mg, 0.85 mmol). After 2 h, methylamine (0.021 mL, 0.25 mmol) was added. Purification by preparative reverse phase HPLC gave the TFA salt of **87** as a white powder (20 mg). MS, *m/z*: 487.6 $(M + H)^+$.

(*E*,4*S*)-4-[((2*S*)-3,3-Dimethyl-2-{[(2S)-3-methyl-2-(methylamino)-3-phenylbutanoyl]amino}butanoyl)(methyl)-amino]-*N*-hydroxy-2,5-dimethyl-2-hexenamide (93). Following General Procedure 5, **2** (75 mg) and 50% aq hydroxylamine (0.38 mL) were coupled to give, after HPLC, the TFA salt of **93** as a white powder (30 mg). MS, *m/z*: 489.0 $(M + H)^+$. Anal. ($C_{27}H_{44}N_4O_4 \cdot 1.5$ TFA$\cdot 1.5H_2O$).

***N*, *β*,*β*-Trimethyl-L-phenylalanyl-*N*^{1}-[(1*S*,2*E*)-1-isopropyl-3-methyl-4-(octyloxy)-4-oxobut-2-enyl]-*N*^{1},3-dimethyl-L-valinamide (94).** Following General Procedure 6, **2** (287 mg, 0.607 mmol) and 1-octanol (81 mg, 0.622 mmol) gave, after HPLC, the TFA salt of **94** as a white gum (247 mg). MS, *m/z*: 586.5 $(M + H)^+$. Anal. ($C_{35}H_{59}N_3O_4 \cdot 1.5$ TFA$\cdot 1.5H_2O$).

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Supporting Information Available: ^{1}H NMR spectral data, elemental analyses, analytical HPLC purity determinations, experimental assignment of the stereochemistry of **36, 37** and the *RSS* and *SSS* diasteromers by ^{1}H NMR, experimentals for **24**, **41**−**43**, **45**−**48**, **50**−**56**, **58**−**62**, **64**, **66**−**85**, **88**−**92**, **95**, **96**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Rowinsky, E. K.; Tolcher, A. W. Antimicrotubule agents. In *Cancer Principles and Practice*; Devita, V. T., Jr., Hellman, S., Rosenberger, S. A., Eds.; Philadelphia: Lippincott Williams and Wilkins, 2001, Vol. 6, pp 431−452. (b) von Angerer, E. New inhibitors of tubulin polymerization *Exp. Opin. Ther. Pat.* **1999**, *9*, 1069−1081. (c) Jordan, M. A.; Wilson, L. Microtubules as a target for anticancer drugs. *Nat. Rev.: Cancer* **2004**, *4*, 253−265.

(2) Gottesman, M. M. Mechanisms of cancer drug resistance. *Annu. Rev. Med.* **2002**, *53*, 615−627.

(3) (a) Talpir, R.; Benayahu, Y.; Kashman, Y.; Pannell, L.; Schleyer, M. Hemiasterlin and Geodiamolide TA; Two new cytotoxic peptides from the marine sponge Hemiasterella minor (Kirkpatrick). *Tetrahedron Lett.* **1994**, *35*, 4453−4456. (b) Coleman, J. E.; de Silva, E. D.; Kong, F.; Andersen, R. J.; Allen, T. M. Cytotoxic peptides from the marine sponge Cymbastela sp. *Tetrahedron* **1995**, *51*, 10653−10662.

(4) Bai, R.; Durso, N. A.; Sackett, D. L.; Hamel, L. Interactions of the sponge-derived antimitotic tripeptide hemiasterlin with tubulin: comparison with dolastatin 10 and cryptophycin 1. *Biochemistry* **1999**, *38*, 14302−14310.

(5) (a) Kavallaris, M.; Verrills, N. M.; Hill, B. T. Anticancer therapy with novel tubulin interacting drugs. *Drug Resist. Update* **2001**, *4*, 392−401. (b) Smyth, J.; Boneterre, M. E.; Schellens, J.; Calvert, H.; Greim, G.; Wanders J.; Hanauske, A. Activity of the dolastatin analogue, LU103793, in malignant melanoma. *Ann. Oncol.* **2001**, *12*, 509−511. (c) Shih, C.; Teicher, B. A. Cryptophycins: a novel class of potent antimitotic antitumor depsipeptides. *Curr. Pharm. Design* **2001**, *7*, 1259−1276. (d) Pettit, G. R. The dolastatins. *Prog. Chem. Org. Nat. Prod.* **1997**, *70*, 1−79.

(6) Loganzo, F.; Discafani, C. M.; Annable, T.; Beyer, C.; Musto, S.; Hari, M.; Tan, X.; Hardy, C.; Hernandez, R.; Baxter, M.; Singanallore, T.; Khafizova, G.; Poruchynsky, M. S.; Fojo, T.; Nieman, J. A.; Ayral-Kaloustian, S.; Zask, A.; Andersen, R. J.; Greenberger, L. M. HTI-286, a synthetic analogue of the tripeptide hemiasterlin, is a potent antimicrotubule agent that circumvents P-glycoprotein-mediated resistance in vitro and in vivo. *Cancer Res.* **2003**, *63*, 1838−1845.

(7) Nieman, J. A.; Coleman, J. E.; Wallace, D. J.; Piers, E.; Lim, L. Y.; Roberge, M.; Andersen, R. J. Synthesis and antimitotic/cytotoxic activity of hemiasterlin analogues. *J. Nat. Prod.* **2003**, *66*, 183−199.

(8) Ratain, M. J.; Undevia, S.; Janisch, L.; Roman, S.; Mayer, P.; Buckwalter, M.; Foss, D.; Hamilton, B. L.; Fischer, J.; Bukowski, R. M. Phase 1 and pharmacological study of HTI-286, a novel antimicrotubule agent: Correlation of neutropenia with time above a threshold serum concentration. *Proc. Am. Soc. Clin. Oncol.* **2003**, *22*, 129.

(9) (a) Andersen, R. J.; Coleman, J. E. Total synthesis of (−)-hemiasterlin, a structurally novel tripeptide that exhibits potent cytotoxic activity. *Tetrahedron Lett.* **1997**, *38*, 317−320. (b) Reddy, R.; Jaquith, J. B.; Neelagiri, V. R.; Saleh-Hanna, S.; Durst, T. Asymmetric synthesis of the highly methylated tryptophan portion of the hemiasterlin tripeptides. *Org. Lett.* **2002**, *4*, 695−697 (c) Vedejs, E.; Kongkittingam, C. A total synthesis of (−)-hemiasterlin using N−Bts Methodology. *J. Org. Chem.* **2001**, *66*, 7355−7364.

(10) Wu, Y.; Megati, S.; Gletsos, C.; Kendall, J.; Wilk, B.; Padmanathan, T.; Panolil, R. Unpublished results.

(11) Hulme, C.; Gore, V. Multicomponent reactions: emerging chemistry in drug discovery from xylocain to crixivan. *Curr. Med. Chem.* **2003**, *10*, 51−80.

(12) Kuentzel, H.; Wolf, H.; Schaffner, K. Photochemical reactions. 63. Photodecarbonylation of α-aryl aldehydes. *Helv. Chim. Acta* **1971**, *54*, 868−96.

(13) Krasutskii, P. A.; Semenova, I. G.; Novikova, M. I.; Yurchenko, A. G.; Tikhonov, V. P.; Belikov, V. M.; Belokon, Yu. N. α-Amino acids of the adamantane series. I. Synthesis and resolution of 1-adamantylglycine and *β*-(1-adamantyl)alanine. *J. Org. Chem. USSR (Engl. Trans.)* **1985**, 1327−1332.

(14) Crews, P.; Farias, J. J.; Ermich, R.; Keifer, P. A. Milnamide A, an unusual cytotoxic tripeptide from the marine sponge Auletta cf. constricta. *J. Org. Chem.* **1994**, *59*, 2932−2934.
(15) 1,4-Thiazanecarboxylic acid derivatives and their use. BE 893025, 1982.
(16) (a) Kaplan, J. A.; Nunes, M.; Ayral-Kaloustian, S.; Krishnamurthy, G.; Loganzo, F.; Greenberger, L. M.; Minnick, A.; May, M.; Zask, A. *Natl. Med. Chem. Symp.* **2002**, abstract 49. (b) Nunes, M.; Kaplan, J.; Wooters, J.; Minnick, A. A.; May, M. K.; Shi, C.; Musto, S.; Beyer, B.; Krishnamurthy, G.; Qiu, Y.; Loganzo, F.; Ayral-Kaloustian, S.; Zask, A.; Greenberger, L. M. A photoaffinity analog of the tripeptide hemiasterlin labels alpha-tubulin within residues 314−338. *Proc. Am. Assoc. Cancer Res.* **2004**, *45*, 2483. (c) Hari, M.; Nunes, M.; Zask, A.; Kaplan, J.; Wooters, J.; Minnick, A. A.; May, M. K.; Musto, S.; Beyer, C.; Krishnamurthy, G.; Qiu, Y.; Loganzo, F.; Ayral-Kaloustian, S.; Greenberger, L. M. Hemiasterlin analogs exclusively label α-tubulin at the interdimer region and specifically block subtilisin digestion of α-tubulin. *Proc. Am. Assoc. Cancer Res.* **2004**, *45*, 5441.
(17) Ramnarayan, K.; Chan, M. F.; Balaji, V. N.; Profeta, S., Jr.; Rao, S. N. Conformational studies on model dipeptides of Gly, L-Ala and their C^{α}-substituted analogs. *Int. J. Pept. Protein Res.* **1995**, *45*, 366−76.
(18) Sampath, D.; Discafani, C. M.; Loganzo, F.; Beyer, C.; Liu, H.; Tan, X.; Musto, S.; Annable, T.; Gallagher, P.; Rios, C.; Greenberger, L. M. MAC-321, a novel taxane with greater efficacy than paclitaxel and docetaxel in vitro and in vivo. *Mol. Cancer Ther.* **2003**, *2*, 873−884.
(19) Meiwes, J.; Schudok, M.; Kretzschmar, G. Asymmetric synthesis of L-thienylalanines. *Tetrahedron: Asymmetry* **1997**, *8*, 527−536. Audia, J. E.; Evrard, D. A.; Murdoch, G. R.; Droste, J. J.; Nissen, J. S.; Schenck, K. W.; Fludzinski, P.; Lucaites, V. L.; Nelson, D. L.; Cohen, M. L. Potent, Selective Tetrahydro-β-carboline Antagonists of the Serotonin 2B (5HT2B) Contractile Receptor in the Rat Stomach Fundus. *J. Med. Chem.* **1996**, *39*, 2773−2780.
(20) Krishnamurthy, G., Cheng, W., Lo, M.-C., Aulabaugh, A., Razinkov, V., Ding, W., Loganzo, F., Zask, A., and Ellestad, G. Biophysical characterization of the interactions of HTI-286 with tubulin heterodimer and microtubules. *Biochem.* **2003**, *42*, 13484−13495.
(21) Dagli, D. J.; Gorski, R. A.; Wemple, J. Synthesis and rearrangement of glycidic thiol esters. Migratory aptitudes. *J. Org. Chem.* **1975**, *40*, 1741−5.
(22) Pfeffer, P. E.; Silbert, L. S.; Chirinko, J. M. alpha-Anions of carboxylic acids. II. Formation and alkylation of alpha-metalated aliphatic acids. *J. Org. Chem.* **1972**, *37*, 451−458.
(23) Ferguson, C. G.; Money, T.; Pontillo, J.; Whitelaw, P. D. M.; Wong, M. K. C. A remarkable multiple rearrangement process in the bromination of *endo*-3-bromo-4-methylcamphor: Intermediates for triterpenoid synthesis. *Tetrahedron*. **1996**, *52*, 14661−14627.

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