Pergamon

Bioorganic & Medicinal Chemistry, Vol. 2, No. 9, pp. 939-947, 1994

0968-0896/94 $7.00 + .00

0968-0896(94)00113-8

Synthesis and Biological Activity of Ras Farnesyl Protein Transferase Inhibitors. Tetrapeptide Analogs with Amino Methyl and Carbon Linkages†

John S. Wai,*[a] Dona L. Bamberger,[a] Thorsten E. Fisher,[a] Samuel L. Graham,[a] Robert L. Smith,[a] Jackson B. Gibbs,[b] Scott D. Mosser,[b] Allen I. Oliff,[b] David L. Pompliano,[b] Elaine Rands[b] and Nancy E. Kohl[b]

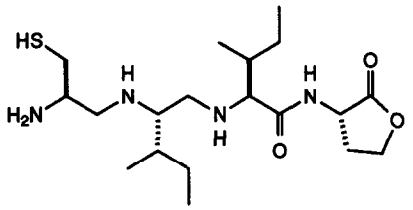
[a]*Department of Medicinal Chemistry,* [b]*Department of Cancer Research, Merck Research Laboratories, West Point, PA 19486, U.S.A.*

Abstract—Replacement of the central amino methylene linkage of C[ψCH_2NH]A[ψCH_2NH]AX tetrapeptide inhibitors with carbon tethers led to compounds with potency in the nanomolar range. Some of the more potent olefinic compounds inhibit Ras processing in intact v-*ras* transformed NIH 3T3 cells with IC_{50} values in the 0.1 to 1 μM range, and inhibit selectively the anchorage-independent growth of H-*ras* transformed Rat1 cells at 10 μM.

Introduction

Many biologically active substances are peptidal in nature. However, the direct use of exogenously administered peptides to achieve a desired biological response (i.e., as drugs) is usually ineffective. Poor solubility, poor membrane permeability and susceptibility to proteolysis all conspire to limit the activity of many peptides in living systems. Nevertheless, peptides frequently provide logical starting points for the design of new molecules with useful biological activities. They contain, albeit in cryptic form, most of the information needed to design stable molecules able to mimic or antagonize the function of an endogenous peptide. Among the strategies that have evolved for obtaining useful peptide analogs, replacement of amide bonds with isosteric units has sometimes proven useful.[1] In this paper, we describe inhibitors of the enzyme farnesyl protein transferase (FPTase). These were developed from a tetrapeptide lead and contain two peptide bond substitutions, a reduced peptide linkage and an olefinic dipeptide isostere. These modifications provided potent enzyme inhibitors that were then rendered membrane permeable by a prodrug strategy.

Our interest in inhibiting FPTase stems from its involvement in the post-translational processing of the protein product of the Ras oncogene.[2] The Ras protein is a critical part of the path linking stimulation of cell surface growth factor receptors to cellular proliferation.[3] Mutations in the *ras* gene are known to lead to abnormal growth and are involved in a significant fraction of human tumors.[4] Farnesylation of Ras has been shown to be obligatory for the expression of its transforming potential.[5] Recently, several groups have shown that pharmacological inhibition of Ras farnesylation can selectively suppress the transformed phenotype of *ras*-transformed cells grown in



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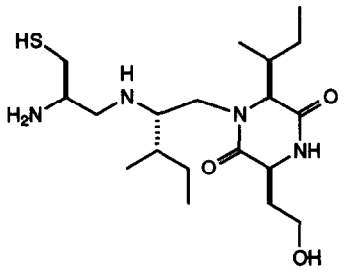

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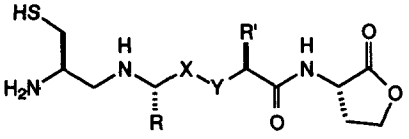
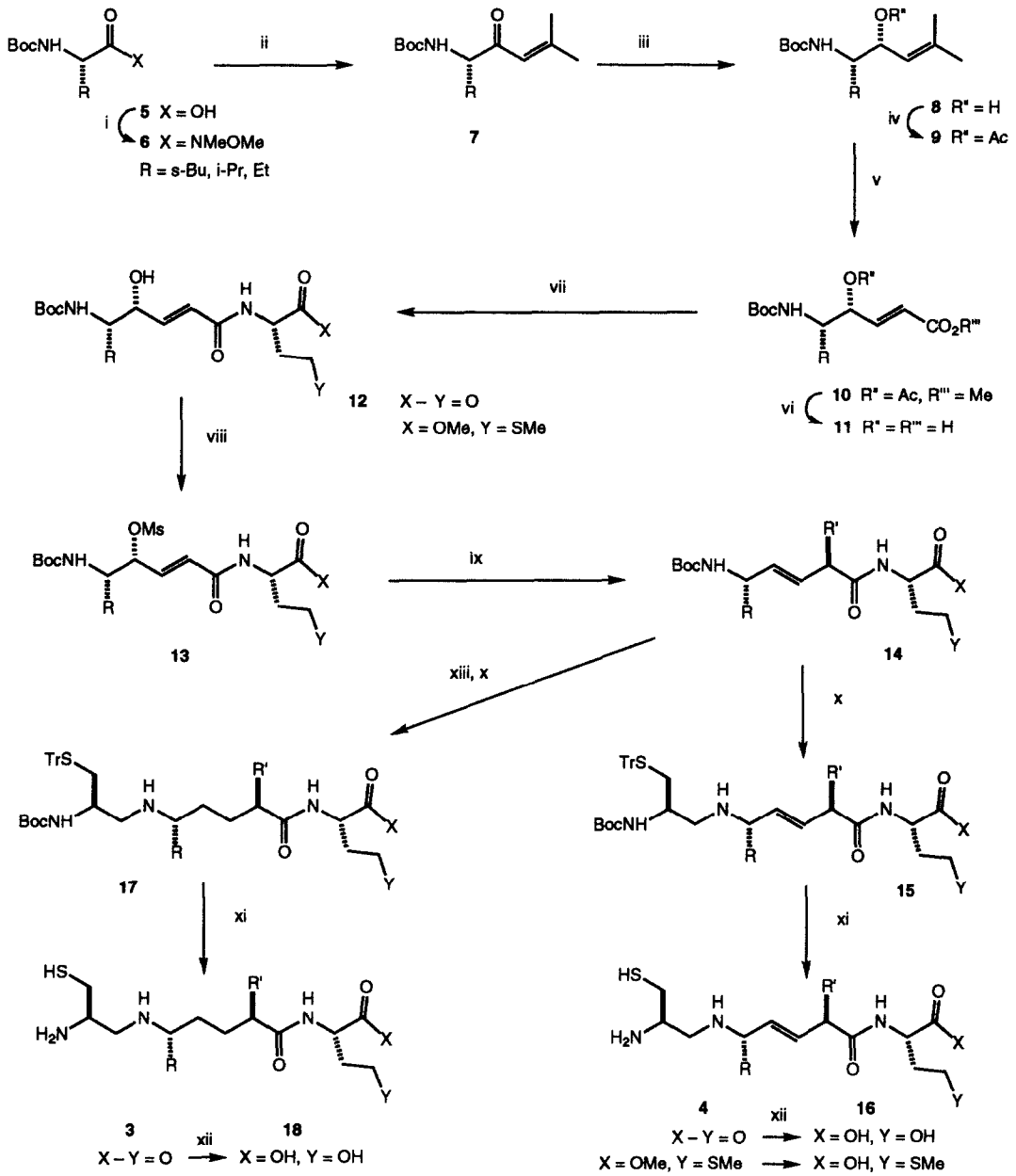


X – Y = CH_2CH_2 3

X – Y = E-CH=CH 4

†In the memory of our colleague, Dr Ta-Jyh Lee.



Scheme I. i. $ClCO_2i$-Bu, MeONHMe; ii. $Me_2C{=}CHMgBr$; iii. $NaBH_4$; iv. Ac_2O; v. O_3, Me_2S; $Ph_3P{=}CHCO_2Me$; vi. LiOH; vii. EDC, HOBt, homoserine lactone or methionine methyl ester; viii. MsCl; ix. R'MgCuCNCl·BF_3; x. HCl; $NaCNBH_3$, L-N-Boc-S-tritylcysteine aldehyde; xi. TFA, Et_3SiH; xii. NaOH; xiii. H_2, 5 % Pt/C.

soft agar or in monolayer culture,[6] however, the inhibitors reported to date are limited in potency.

FPTase catalyzes the alkylation of a protein thiol by farnesyl pyrophosphate.[7] The enzyme is specific for cysteine residues contained in a C-terminal tetrapeptide signal sequence usually referred to as a CAAX box. In this sequence, two aliphatic amino acids (A) follow the reactive cysteine and the C-terminal residue X is usually serine or methionine. Furthermore, the X residue is key in distinguishing whether a protein is a substrate for FPTase or the closely related enzyme geranylgeranyl protein transferase (GGPTase).[8] The latter enzyme prefers leucine as the C-terminal residue X. Using a CAAX motif as a starting point, our group designed the FPTase inhibitor **1**.[6c] Compound **1** is a good inhibitor of FPTase (K_i = 20 nM) and inhibits farnesylation of the Ras protein in cultured cells with an IC_{50} of 100 μM. However, the concentration of **1** required to inhibit the transformed phenotype of Ras-transformed cells (inhibition of anchorage independent growth in soft agar) was too high (1 mM) to be clinically useful. A partial explanation of the poor activity of **1** was its intrinsic chemical lability. At neutral pH, **1** rapidly cyclized to the diketopiperazine **2**, which is a poor inhibitor of FPTase. Among several options considered to block this mode of reactivity, replacement of the central aminomethyl linker of **1** with a two carbon tether, such as alkane isostere **3** and the *E*-alkene compound **4**, were studied. This strategy proved successful, affording compounds with potency in the low nanomolar range. Of

the two classes of carbon isosteres, the olefinic compounds were significantly more active as FPTase inhibitors. Furthermore, some of these olefinic compounds were active in cell culture at concentrations 100-fold below that required for expression of activity by **1**.

Chemistry

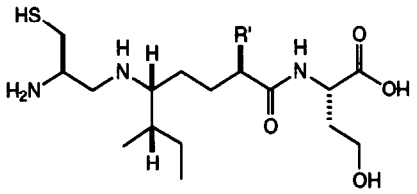
The general synthetic route to this series of compounds is illustrated in Scheme I, adapting chemistry developed by Ibuka and coworkers[9] for the preparation of mono-*E*-olefin tripeptide isosteres, *N*-BocAla-[ψ*E*-CH=CH]-Val-Phe methyl ester. Two key modifications were incorporated: (a) diastereoselective reduction of enone **7** to the *anti* vinyl alcohol **8** reversing the selectivity for *syn* vinyl alcohol in Ibuka's sequence, and (b) *anti*-S_N2' cuprate displacement reaction on the amide **13** instead of the ester **10**. The latter modification is crucial to avoid racemization of the newly created chiral center during ester hydrolysis and amide coupling, and does not seem to affect the regio- and diastereoselectivity of the cuprate reaction. For the alkane analogs, an additional catalytic hydrogenation step was incorporated.

Treatment of L-*N*-Boc amino acid **5** with isobutyl chloroformate and *N*,*O*-dimethylhydroxylamine provided the corresponding amide **6**, which reacted with 2-methylpropenyl magnesium bromide to give enone **7** (Scheme I). Diastereoselective reduction of **7** with sodium borohydride provided the *anti* vinyl alcohol **8** in greater than 12:1 selectivity for both the L-isoleucine and L-valine derivatives.[10] Selectivity dropped to 4:1 for the reduction of the enone derived from L-*N*-Boc-aminobutyric acid, but a single recrystallization of the mixture from *n*-hexane afforded the pure *anti* vinyl alcohol. The relative stereochemistry of the alcohols was established by conversion of the mixture to the corresponding oxazolidinones by treatment with sodium hydride in DMF. For example, for the oxazolidinones derived from L-isoleucine, the allylic proton of the major component resonates at δ 5.27 and exhibits a coupling constant of 7.6 Hz, while the corresponding proton of the minor component resonates at δ 4.94 and exhibits a coupling constant of 6.1 Hz. These spectroscopic data indicate that the major product possesses the required *anti* relative stereochemistry and are consistent with related oxazolidinones.[9,11] The vinyl alcohol **8** was successively treated with acetic anhydride, ozone and (carbomethoxymethylene)-triphenylphosphorane to give γ-acetoxy α,β-enoate (**10**). Base hydrolysis afforded the corresponding γ-hydroxy α,β-enoic acid, which was coupled either with L-homoserine lactone or L-methionine methyl ester. The coupled product was converted to the corresponding mesylate **13**. Treatment of **13** with an organomagnesiocuprate activated with boron trifluoride–ether complex afforded a single compound. The stereochemistry of the newly formed chiral center was assumed to be as depicted according to Ibuka's working model.[9] The Boc group was cleaved from the coupling product **14** with hydrogen chloride, and the resulting amine–HCl salt was reductively alkylated with *N*-Boc-*S*-trityl-cysteine aldehyde to yield tetrapeptide ester **15**. Reaction of the latter material with trifluoroacetic acid and triethylsilane in methylene chloride afforded pure lactone or ester **4** after HPLC purification. The acid **16** was liberated by treatment with aqueous sodium hydroxide prior to assay for inhibitory activity against FPTase.

Results and Discussion

Analogs presented in this paper focused on modification of four major structural features of the tetrapeptide lead **1**: replacement of the central aminomethyl linker of **1** with an

Table 1.



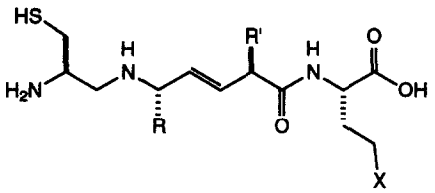
Entry	R'	FPTase Inhibition[1] IC_{50} (nM)	GGPTase Inhibition[2] IC_{50} (nM)	Substrate for FPTase[3]
18a	n-Pr	29	15,000	N
18b	i-Pr	470	NT	N
18c	Bn	40	16,000	N

[1]enzyme assay using 1 nM of human recombinant FTPase (see reference 6c).
[2]enzyme assay using bovine GGTPase (see reference 6c); NT, not being tested.
[3]analogs were evaluated to see whether they are substrate for FTPase; N indicates that the analog is not a substrate (see reference 6c).

ethylene; conformational constraint of the carbon isostere in the form of a *trans* olefin; variations of both the side chains of the central olefinic dipeptide isostere, and variations of the C-terminal amino acid. Compounds were initially screened for their ability to inhibit recombinant human FPTase. Many of these compounds were evaluated for their selectivity with respect to inhibition of the closely related GGPTase (isolated from bovine brain). Several compounds were also tested to determine whether they were substrates for farnesylation.

Replacement of the central aminomethyl linker of **1** with an ethylene led to alkane isostere analogs **18**. Compounds **18a** and **18c** maintained good FPTase potency as compared to **1** (IC_{50} 18 nM) while the isopropyl analog **18b** was >25-fold less active (Table 1). Compounds **18a** and **18c** did not inhibit the enzyme GGPTase. None of these compounds was a substrate for FPTase. In contrast to **1**, the homoserine lactone in this series was chemically stable at neutral pH.

Table 2.



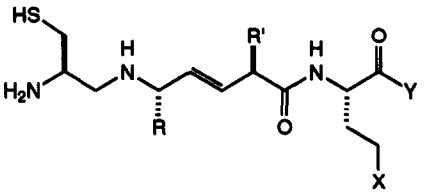
Entry	R	R'	X	FPTase Inhibition[1] IC_{50} (nM)	GGPTase Inhibition[2] IC_{50} (nM)	Substrate for FPTase[3]
16a	s-Bu[4]	Me	OH	45	NT	NT
16b	s-Bu	Et	OH	6.6	3,200	N
16c	s-Bu	n-Pr	OH	4	1,900	N
16d	s-Bu	n-Bu	OH	1.5	400	N
16e	s-Bu	i-Pr	OH	3.5	3,300	N
16f	s-Bu	c-Pentyl	OH	14	1,000	N
16g	s-Bu	c-Hexyl	OH	12	1,400	NT
16h	s-Bu	t-Bu	OH	11	1,200	N
16i	s-Bu	s-Bu[5]	OH	10	110	N
16j	s-Bu	Bn	OH	6	7,400	NT
16k	i-Pr	i-Pr	OH	5	600	N
16l	i-Pr	Bn	OH	7	1,500	NT
16m	Et	n-Pr	OH	40	3,700	NT
16n	s-Bu	n-Bu	SMe	2.5	28	N
16o	s-Bu	i-Pr	SMe	20	NT	NT
16p	s-Bu	Bn	SMe	1.9	100	NT
16q	i-Pr	i-Pr	SMe	4.5	700	NT

[1]enzyme assay using 1 nM of human recombinant FTPase (see reference 6c).
[2]enzyme assay using Bovine GGTPase (see reference 6c).
[3]analogs were evaluated to see whether they are substrate for FTPase; N (not a substrate), NT (not being tested) (see reference 6c).
[4]analogs were prepared starting with natural L-isoleucine.
[5]a 1:1 mixture of diastereomers.

Conformational constraint of the carbon isosteres in the form of *trans* olefins led to analogs **16** and resulted in a 7–100 fold improvement in potency (**18a–c**, Table 1 vs **16 c, e** and **j**, Table 2). The effect of varying the R' side chain, corresponding to the third residue of the CAAX sequence, was then investigated. An ethyl, *n*-propyl, *n*-butyl, isopropyl, *s*-butyl, or benzyl side chain provided the most active inhibitors (**16c–e, 16i** and **16j**) of FPTase, followed by the cyclopentyl, cyclohexyl, or *t*-butyl side chain analogs (**16f–h**, Table 2). When the side chain was truncated to a methyl group (**16a**), a significant decrease in potency was observed. As such, it appears that the R' side chain is required for intrinsic potency and is not very sensitive to branching. Changing the side chain R, corresponding to the second amino acid of the CAAX box, in **16e** and **16j** from a secondary butyl group to an isopropyl group did not lead to any change in potency (**16k** and **16l**, Table 2). However, further truncation to an ethyl group led to a significant loss in potency (**16c** vs **16m**). In common with the alkane isostere compounds, the olefinic inhibitors were selective for FPTase versus GGPTase, and none of them was a substrate of the enzyme. Substituting the C-terminal homoserine of selected analogs from the compounds studied above with methionine led to equally potent inhibitors (**16n–16q**, Table 2) in the enzyme assay. However, a general decrease in selectivity for FPTase versus GGPTase was observed.

Selected olefin isosteres in the form of the lactone or ester were evaluated for their effects in cell culture. The cytotoxicity of the compounds was assessed by uptake and transformation of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) by viable cells.[12] The cytotoxic endpoint, shown in Table 3, is the highest

Table 3.



Entry	R	R'	X,Y	FPTase Inhibition[1] IC_{50} (nM)	Cytotoxic endpoint[2] (nM)	Ras Processing Inhibition in intact cells[3] (μM)
4c	s-Bu	n-Pr	O	4	>1,000	+/- (1)
4d	s-Bu	n-Bu	O	1.5	>1,000	+/- (1)
4e	s-Bu	i-Pr	O	3.5	>1,000	++ (2.5)
4j	s-Bu	Bn	O	6	NT	++ (5-10)
4k	i-Pr	i-Pr	O	5	>250	++ (1)
4l	i-Pr	Bn	O	7	>100	+ (1)
4n	s-Bu	n-Bu	SMe, OMe	2.5	50	++ (1)
4o	s-Bu	i-Pr	SMe, OMe	20	100	++ (0.25)
4p	s-Bu	Bn	SMe, OMe	1.9	25	++ (1)
4q	i-Pr	i-Pr	SMe, OMe	4.5	>100	++ (0.25)
4r	s-Bu	i-Pr	SMe, Oi-Pr	20	25	++ (0.1)
4s	i-Pr	i-Pr	SMe, Oi-Pr	4.5	50	++ (0.1)

[1]enzyme assay using 1 nM of human recombinant FTPase (see reference 6c); IC_{50} values were obtained with the acids.
[2]highest compound concentration tolerated by NIH 3T3 cells in a 48 hour assay[12]; NT, not being tested.
[3]ras protein processing assay in v-*ras* transformed NIH 3T3 intact cells. +/- indicated 10–20 % inhibition; + about 30 %; ++ about 50 %. In parentheses are the concentrations required to achieve the degree of inhibition indicated (see references 6c).

compound concentration tolerated by NIH 3T3 cells in a 48 h assay. The analogs were then evaluated for inhibition of Ras processing in intact NIH 3T3 cells transformed by v-*ras* at compound concentrations lower than the cytotoxic endpoint. Briefly, the cells were incubated in the presence of the lactone or ester form of the inhibitors for 24 h and were labeled with [^{35}S]methionine during the final 20 h. Ras was immunoprecipitated from detergent lysates of cells. Farnesylated and unprocessed Ras were resolved by SDS-polyacrylamide gel electrophoresis and detected by fluorography.[6c] The homoserine lactones (**4c–e**, **4j–l**, Table 3) significantly inhibited Ras processing between 1 and 10 μM, representing a 100–10 fold improvement as compared to **1** (IC_{50} ~100 μM). The methionine methyl ester analogs (**4n–4q**, Table 3) were somewhat more cytotoxic than the homoserine lactones. However, they exhibited enhanced potency against Ras farnesylation. Further improvement in inhibiting Ras processing was observed by replacing the methionine methyl ester moiety with methionine isopropyl ester (**4o** vs **4r**, **4q** vs **4s**, Table 3). These compounds inhibited Ras processing in cell culture with an IC_{50} of 0.1 μM, a ~1000 fold enhancement compared to **1** (IC_{50} ~100 μM)[6c] evaluated in the same assay.

Immortalized cells, such as NIH 3T3 and Rat1 cells, grow only in an anchorage-dependent culture environment, and do not grow in soft agar. However, after they were transfected with oncogenes, such as H-*ras* and v-*raf*, they acquired the ability to grow in an anchorage-independent manner, forming large colonies of cells in soft agar. The ability of the methionine esters **4o**, **4q**, **4r** and **4s** (Table 3) to reverse this aspect of the phenotype was evaluated. At a concentration of 10 μM, each of these FPTase inhibitors prevented colony formation of H-*ras* transformed Rat1 cells in soft agar. The effect is dose dependent with normal colony formation observed at concentrations of 1 μM or less. Furthermore, all four inhibitors did not inhibit growth of Ras independent v-*raf* transformed Rat1 cells at 10 μM. These results suggest that the growth inhibition observed with H-*ras* transformed cells is due to inhibition of Ras farnesylation, and is not due to nonspecific growth inhibition or general cytotoxicity. In the same assay, 1000 μM of compound **1** was required to inhibit growth of H-*ras* transformed Rat1 cells in soft agar.[6c]

Conclusion

Replacement of the central amide bond of the CAAX motif in tetrapeptide inhibitors of Ras FPTase with carbon isosteres affords compounds with potency in the low nanomolar range. Of the two classes of carbon isosteres, the olefinic compounds are significantly more active as FPTase inhibitors than the alkane isosteres. These inhibitors are not substrates for the enzyme and are selective for FPTase versus the closely related GGPTase. Some of the more potent olefinic compounds inhibit Ras processing in intact v-*ras* transformed NIH 3T3 cells with IC_{50} values in the 0.1–1 μM range, and inhibit selectively the anchorage-independent growth of H-*ras* transformed Rat1 cells at 10 μM.

Experimental

General methods

L-*N*-Boc-Isoleucine, valine and aminobutyric acid were purchased from Bachem, California; L-homoserine lactone hydrogen chloride and L-methionine methyl ester hydrogen chloride were obtained from Sigma. All the other reagents, including all the Grignard reagents, were purchased from Aldrich. Copper (I) cyanide was used as recieved. Tetrahydrofuran was freshly distilled from sodium benzophenone ketyl under an atmosphere of argon. ^{1}H NMR spectra were recorded on a Varian Unity 300 spectrometer. Silica gel 60 (E. Merck) was used for column chromatography.

Procedure for preparation of E-*olefin isostere tetrapeptide analogs*

All the olefin isostere analogs were prepared as depicted in Scheme I, and are exemplified by the preparation of 5(*S*)-[2(*R*)-amino-3-mercaptopropylamino]-6(*S*)-methyl-2(*R*)-*n*-propyl-3,4-*E*-octenyl-homoserine (**16c**) described in the following.

4(S)-N-tert-(Butyloxy)carbonylamino-3(S)-7-dimethyl-6,7-octen-5-one (7). To a cold (0 °C) solution of *N-t*-(butoxy)-carbonyl-L-isoleucine hemihydrate (6.01 g, 25 mmol) in ethyl acetate (90 mL), *N*-methyl morpholine (2.75 mL, 25 mmol) and isobutyl chloroformate (3.25 mL, 25.1 mmol) were added successively. The resultant white suspension was stirred at 0 °C for 15 min, treated with *N,O*-dimethylhydroxylamine hydrochloride (2.52 g, 25.8 mmol) and *N*-methyl morpholine (2.75 mL, 25 mmol), and then stirred at room temp. overnight. The resultant mixture was washed successively with water, 10 % aqueous citric acid, brine and was dried over anhydrous magnesium sulfate, filtered and concentrated. The residual oil was chromatographed on silica gel eluting with 30 % ethyl acetate in hexane. Collection and concentration of appropriate fractions provided 5.0 g (73 %) of the corresponding amide **6**. ^{1}H NMR ($CDCl_3$) δ 5.17 (br d, J = 9.8 Hz, 1 H), 4.62 (ddd, J = 7.3, 7.3, 2.4 Hz, 1 H), 3.78 (s, 3 H), 3.22 (s, 3H), 0.92 (d, J = 6.6 Hz, 3H), 0.89 (t, J = 7.3 Hz, 3H). A 1 L three neck round bottom flask was charged with magnesium turnings (44 g, 1.8 mol) and flame-dried under a steady stream of dry argon. The turnings were activated by stirring under an atmosphere of argon for an additional 3–4 h at room temp. THF (450 mL), 2-methylpropenyl bromide (50 g, 0.37 mol), and a crystal of iodine were added. The mixture was warmed gently with a mantle until slight reflux occurred. Without removing the mantle heating was discontinued, and the mixture was stirred overnight under an atmosphere of argon. The resultant Grignard reagent was used directly. To a cold (–50 °C) solution of *N-tert*-(butyloxy)carbonyl-isoleucine *N,O*-dimethylhydroxylamide (**6**, 17.2 g, 63 mmol) in THF (400 mL), the above Grignard reagent in THF (prepared from 50 g of 2-methylpropenyl bromide) was added over a period of 20 min and with the temperature of the reacting solution maintained below –40 °C. The mixture was then allowed to warm up slowly to

room temp. The resultant solution was diluted with diethyl ether, treated with 10 % aqueous citric acid, washed with brine, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The residual oil was chromatographed on silica gel eluting with 7 % ethyl acetate in hexane. Collection and concentration of appropriate fractions provided 12.6 g (74 %) of ketone **7**. ^{1}H NMR ($CDCl_3$) δ 6.13 (s, 1 H), 5.26 (br d, J = 7 Hz, 1 H), 4.28 (dd, J = 8.5, 4.1 Hz, 1 H), 2.17 (d, J = 1.2 Hz, 3 H), 1.93 (d, J = 1.2 Hz, 3 H), 1.43 (s, 9 H), 0.95 (d, J = 6.8 Hz, 3H), 0.87 (t, J = 7.3 Hz, 3H).

4(S)-N-tert-(Butyloxy)carbonylamino-5(R)-acetoxy-3(S)-7-dimethyl-6,7-octene (9). To a cold (0 °C) solution of 4(*S*)-*N-tert*-(butyloxy) carbonylamino-3(*S*)-7-dimethyl-6,7-octen-5-one (**7**, 12.57 g, 46.7 mmol) in methanol (200 mL), sodium borohydride was added portion wise until reaction was complete as monitored by TLC on silica gel eluting with 20 % ethyl acetate in hexane. The resultant mixture was concentrated *in vacuo*. The residue was suspended in diethyl ether, washed successively with 1 M aqueous hydrochloric acid and brine, dried over magnesium sulfate, filtered and concentrated *in vacuo* to provide the corresponding alcohol **8** (11.93 g). ^{1}H NMR ($CDCl_3$) δ 5.21 (br d, J = 9 Hz, 1H), 4.43 (m, 2 H), 3.61 (m, 1 H), 1.99 (br s, 1 H), 1.75 (s, 3H), 1.73 (s, 3H), 1.45 (s, 9H), 0.92 (d, J = 6.8 Hz, 3H), 0.90 (t, J = 7.1 Hz, 3 H). Without further purification, the crude alcohol, 4-*N,N*-dimethylaminopyridine (0.132 g), and pyridine (17 mL) were dissolved in dichloromethane (48 mL), cooled to 0 °C and treated with acetic anhydride (18.8 mL, 199 mmol). The resultant mixture was stirred at room temp. for 2 h and concentrated *in vacuo*. The residual oil was chromatographed on silica gel eluting with 20 % ethyl acetate in hexane. Collection and concentration of appropriate fractions provided 10.7 g (73 %) of the acetate **9** as a white solid. ^{1}H NMR ($CDCl_3$) δ 5.57 (dd, J = 8.5, 5.9 Hz, 1 H), 5.15 (br d, J = 9.5 Hz, 1H), 4.36 (br d, J = 11 Hz, 1 H), 3.75 (m, 1 H), 2.02 (s, 3 H), 1.76 (s, 3H), 1.75 (s, 3H), 1.43 (s, 9H), 0.93 (d, J = 6.8 Hz, 3H), 0.87 (t, J = 7.3 Hz, 3 H).

Methyl 5(S)-N-tert-(butyloxy)carbonylamino-4(R)-acetoxy-6(S)-methyl-2,3-E-octenoate (10). To a cold (–78 °C) solution of 4(*S*)-*N-tert*-(butyloxy)carbonylamino-5(*R*)-acetoxy-3(*S*)-7-dimethyl-6,7-octene (**9**, 6.5 g, 20.7 mmol) in dichloromethane (100 mL), a steady stream of ozone was bubbled through until a blue color persisted. The mixture was stirred for an additional 5 min and purged with argon to remove excess ozone. Then dimethyl sulfide (15 mL) was added and the reaction mixture was allowed to warm to room temp. The resultant mixture was cooled back to –78 °C, and (carbomethoxymethylene)triphenylphosphorane (15.3 g, 45.7 mmol) was added. The mixture was stirred at room temp. overnight and concentrated on to silica gel (20 g). The resultant solid was loaded on to a column of silica gel saturated with 15 % ethyl acetate in hexane, and the column eluted with the same solvent mixture. Collection and concentration of appropriate fractions provided 6.5 g (91 %) of the octenoate **10**. ^{1}H NMR ($CDCl_3$) δ 6.87 (dd, J = 15.9, 5.4 Hz, 1 H), 6.07 (dd, J = 15.9, 1.7 Hz, 1 H), 5.57 (br t, J = 4 Hz, 1 H), 4.32 (br d, J = 10.5 Hz, 1H), 3.81 (br m, 1 H), 3.76 (s, 3 H), 2.08 (s, 3 H), 1.43 (s, 9H), 1.01 (d, J = 6.6 Hz, 3H), 0.88 (t, J = 7.3 Hz, 3 H).

5(S)-N-tert-(Butyloxy)carbonylamino-4(R)-hydroxy-6(S)-methyl-2,3-E-octenoic acid (11). To a solution of methyl 5(*S*)-*N-tert*-(butyloxy) carbonylamino-4(*R*)-acetoxy-6(*S*)-methyl-2,3-*E*-octenoate (**10**, 1 g, 2.9 mmol) in tetrahydrofuran (2 mL), a saturated solution of lithium hydroxide (0.5 g, 12 mmol) in methanol–water, 3:1 v/v, was added. The mixture was then brought to homogeneity by addition of a minimum amount of methanol–water, 3:1 v/v, mixture, and stirred at room temp. for 2 days. The resultant solution was acidified with aqueous hydrochloride to pH 5 and concentrated *in vacuo*. The residue was subjected to column chromatography on silica gel eluting with 20 % methanol in chloroform. Collection and concentration of appropriate fraction provides 0.71 g (87 %) of the corresponding hydroxyacid **11**. ^{1}H NMR ($CDCl_3$) δ 7.02 (dd, J = 15.4, 4.2 Hz, 1 H), 6.07 (dd, J = 15.4, 1.2 Hz, 1 H), 4.57 (br s, 1 H), 4.51 (br d, J = 9 Hz, 1H), 3.71 (br m, 1 H), 1.44 (s, 9H), 1.01 (d, J = 6.6 Hz, 3H), 0.91 (t, J = 7.1 Hz, 3 H).

5(S)-N-tert-(butyloxy) carbonylamino-4(R)-hydroxy-6(S)-methyl-2,3-E-octenyl homoserine lactone (12). To a solution of 5(*S*)-*N-tert*-(butyloxy)carbonylamino-4(*R*)-hydroxy-6(*S*)-methyl-2,3-*E*-octenoic acid (**11**, 0.71 g, 2.5 mmol) in dimethylformamide (10 mL), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.58 g, 3 mmol), 1-hydroxybenzotriazole hydrate (0.4 g, 3 mmol), L-homoserine lactone hydrochloride (0.52, 3.7 mmol), and diisopropylethylamine (0.66 mL, 3.7 mmol) were added. The resultant mixture was stirred at room temp. overnight, and concentrated *in vacuo*. The residue was diluted with ethyl acetate, and the organic solution washed successively with water, 10 % aqueous citric acid, brine, dried over magnesium sulfate, filtered and concentrated. The residue was then subjected to column chromatography on silica gel eluting with 5 % methanol in chloroform. Collection and concentration of appropriate fractions provided 0.76 g (82 %) of the coupled product **12**. ^{1}H NMR (CD_3OD) δ 6.83 (dd, J = 15.4, 5.4 Hz, 1 H), 6.36 (br d, J = 10.3 Hz, 1H), 6.16 (dd, J = 15.4, 1.7 Hz, 1 H), 4.67 (dd, J = 11, 9 Hz, 1 H), 4.67 (ddd, J = 8.8, 8.8, 1.8 Hz, 1 H), 4.35–4.23 (m, 2 H), 3.55–3.45 (m, 1 H), 2.62–2.52 (m, 1 H), 2.38–2.20 (m, 1 H), 1.79–1.65 (m, 1 H), 1.65–1.52 (m, 1 H), 1.42 (s, 9H), 0.93 (d, J = 6.8 Hz, 3H), 0.91 (t, J = 7.3 Hz, 3 H).

5(S)-N-tert-(Butyloxy) carbonylamino-4(R)-(methylsulfonyl)oxy-6(S)-methyl-2,3-E-octenyl homoserine lactone (13). To a cold (–20 °C) solution of 5(*S*)-*N-tert*-(butyloxy)-carbonylamino-4(*R*)-hydroxy-6(*S*)-methyl-2,3-*E*-octenyl homoserine lactone (**12**, 0.35 g, 0.94 mmol) in a mixture of dichloromethane (6 mL) and pyridine (3 mL), methanesulfonyl chloride (0.4 mL) was added. The resultant mixture was kept at 0 °C overnight, and concentrated *in vacuo*. The residue was diluted with dichloromethane, washed successively with sat. sodium bicarbonate and brine. The organic phase was dried over magnesium sulfate, filtered and concentrated. The residue was subjected to column chromatography on silica gel eluting with a mixture of ethyl acetate and hexane, 8:2 v/v. Collection and concentration of appropriate fractions

provided 0.3 g (71 %) of the mesylate **13**, which is stable for storage at –10 °C. ^{1}H NMR (CD_3OD) δ 6.78 (dd, *J* = 15.4, 6.3 Hz, 1 H), 6.30 (dd, *J* = 15.4, 1.2 Hz, 1 H), 5.31 (ddd, *J* = 6.4, 6.4, 1.5 Hz, 1 H), 4.69 (dd, *J* = 11.2, 9.3 Hz, 1 H), 4.46 (ddd, *J* = 9, 9, 1.7 Hz, 1 H), 4.57–4.26 (m, 1 H), 3.78 (t, *J*= 6.1 Hz, 1 H), 3.10 (s, 3 H), 2.65–2.50 (m, 1 H), 2.38–2.20 (m, 1 H), 1.70–1.50 (m, 2 H), 1.42 (s, 9H), 0.99 (d, *J* = 6.6 Hz, 3H), 0.90 (t, *J* = 7.3 Hz, 3 H).

*5(S)-*N*-tert-(Butyloxy)carbonylamino-6(S)-methyl-2(*R*)-n-propyl-3,4-E-octenylhomoserine lactone (**14**).* To a cold (–78 °C) suspension of copper(I) cyanide (0.28 g, 3.1 mmol) in tetrahydrofuran (30 mL, freshly distilled from sodium benzophenone ketyl), a solution of *n*-propylmagnesium chloride (1.47 mL, 2.0 M, 2.9 mmol) in diethyl ether was added. The mixture was stirred at –10 °C until a homogeneous solution was formed. Once a solution was formed, it was cooled to –78 °C, boron-trifluoride etherate (0.36 mL, 2.9 mmol) was added, and the resulting mixture was stirred at –78 °C for 5 min. A solution of 5(*S*)-*N-tert*-(butyloxy)carbonylamino-4(*R*)-(methylsulfonyl)-oxy-6(*S*)-methyl-2,3-*E*-octenyl homoserine lactone (**13**, 0.33 g, 0.74 mmol) in tetrahydrofuran (25 mL) was added dropwise to the above mixture. The resultant solution was stirred at –78 °C for 2 h, quenched with sat. aqueous ammonium chloride (pH 8), diluted with diethyl ether. The organic solution was washed with brine, dried over magnesium sulfate, filtered and concentrated. The residue was chromatographed on silica gel eluting with 50 % ethyl acetate in hexane. Collection and concentration of appropriate fractions provided 0.26 g (93 %) of the 3,4-*E*-octenyl-homoserine (**14**). ^{1}H NMR ($CDCl_3$) δ 6.63 (br s, 1H), 5.56 (d, *J* = 14 Hz, 1 H), 5.50 (d, *J* = 14 Hz, 1 H), 4.60 (br s, 1 H), 4.56–4.42 (m, 2 H), 4.32–4.21 (m, 1 H), 4.00 (br s, 1 H), 3.95 (br m, 1 H), 3.72 (br m, 1 H), 2.21 (br pentet, *J* = 11 Hz, 1 H), 1.85 (br m, 1 H), 1.43 (s, 9 H), 0.92 (t, *J* = 7.1 Hz), 0.90 (t, *J* = 7.1 Hz), 0.87 (d, *J* = 6.6 Hz).

*5(S)-[2(R)-*N*-tert-(Butyloxy)carbonylamino-3-S-triphenylmethylmercaptopropylamino]-6(S)-methyl-2(R)-n-propyl-3,4-E-octenyl-homoserineXlactone (**15**).* To a cold (0 °C) solution of 5(*S*)-*N-tert*-(butyloxy)carbonylamino-6(*S*)-methyl-2(*R*)-*n*-propyl-3,4-*E*-octenyl-homoserine (**14**, 0.26 g, 0.68 mmol) in a mixture of ethyl acetate (30 mL) and dichloromethane (30 mL), a steady stream of anhydrous hydrogen chloride gas was bubbled through for a period of 20 min. The mixture was capped and stirred for an additional 30 min at 0 °C. The resultant solution was then purged with a stream of argon, concentrated *in vacuo* to provide the corresponding hydrochloride salt (0.24 g). To a mixture of the amine–HCl salt (0.24 g), *N-tert*-(butyloxy)carbonyl-*S*-triphenylmethyl-L-cysteine aldehyde[13] (0.57 g, 1.64 mmol), molecular sieves (3 Å, powder) and methanol (5 mL), (pH adjusted to 6 by addition of diisopropylethylamine at room temp.), sodium cyanoborohydride (62 mg, 1 mmol) was added and stirred at room temp. overnight. The resultant slurry was filtered and concentrated. The residue was diluted with ethyl acetate, washed with brine, dried over magnesium sulfate, filtered, and concentrated *in vacuo*. The residue was chromatographed on silica gel eluting with 1.5 % methanol in chloroform to afford 283 mg (24 %) of the coupled product **15**. ^{1}H NMR (CD_3OD) δ 7.40–7.18 (m, 15 H), 5.51 (dd, *J* = 15.4, 8.6 Hz, 1 H), 5.30 (dd, *J* = 15.4, 8.8 Hz, 1 H), 4.59 (dd, *J* = 11, 9 Hz, 1 H), 4.40 (td, *J* = 8.8, 5.6 Hz, 1 H), 4.30–4.21 (m, 1 H), 3.72–3.58 (br m, 1H), 2.92 (q, *J* = 7.3 Hz, 1 H), 2.74 (dd, *J* = 11, 9 Hz, 1 H), 2.61 (dd, *J* = 12.2, 5.1 Hz, 1 H), 2.55–2.44 (m, 1 H), 2.34–2.15 (m, 4 H), 1.43 (s, 9H), 0.86 (t, *J* = 7.1 Hz, 3 H), 0.84 (t, *J* = 6.3 Hz, 3 H), 0.79 (d, *J* = 6.6 Hz, 3 H).

*5(S)-[2(R)-Amino-3-mercaptopropylamino]-6(S)-methyl-2(R)-n-propyl-3,4-E-octenylhomoserine lactone (**4**).* To a solution of 5(*S*)-[2(*R*)-*N-tert*-(butyloxy)carbonylamino-3-*S*-triphenylmethylmercaptopropylamino]-6(*S*)-methyl-2-(*R*)-methyl-3,4-*E*-octenylhomoserine lactone (245 mg, 0.34 mmol) in a mixture of dichloromethane (6 mL) and trifluoroacetic acid (3 mL) at room temp., triethylsilane (217 μL, 1.36 mmol) was added. The resultant solution was stirred at room temp. for 2 h, and concentrated *in vacuo*. The residue was dissolved in a mixture of 0.1 % aqueous trifluoroacetic acid (5 mL) and hexane (2 mL). The aqueous layer was washed four more times with hexane, stirred under reduced pressure to remove residual hexane, and lyophilized overnight. The residue was subjected to HPLC purification on a C-18 reverse phase column. Appropriate fractions were collected and lyophilized overnight to provide 193 mg of an analytical pure sample of the homoserine lactone as a white solid. ^{1}H NMR (CD_3OD) δ 8.53 (br d, *J* = 8 Hz, 1 H), 6.04 (dd, *J* = 15.4, 8.6 Hz, 1 H), 5.60 (ddd, *J* = 15.4, 8.8, 1.5 Hz, 1 H), 4.51–4.39 (m, 2 H), 4.35–4.25 (m, 1H), 3.75–3.62 (m, 2H), 3.45 (dd, *J* = 14.3, 4.6 Hz, 1 H), 3.19 (dd, *J* = 14.3, 4.6 Hz, 1 H), 2.92 (q, *J* = 7.3 Hz, 1 H), 2.74 (dd, *J* = 11, 9 Hz, 1 H), 2.61 (dd, *J* = 13, 7 Hz, 1 H), 3.15 (br q, *J* = 6.5 Hz, 1 H), 2.98–2.83 (m, 1 H), 2.57–2.45 (m, 1 H), 2.45–2.29 (m, 1 H), 1.99–1.86 (br m, 1 H), 1.86–1.72 (m, 1 H), 1.64–1.50 (m, 1 H), 1.45–1.32 (m, 2 H), 1.28–1.14 (m, 1 H), 1.00 (d, *J* = 6.6 Hz, 3H), 0.96 (t, *J* = 7.1 Hz, 3 H), 0.95 (t, *J* = 7.1 Hz, 3 H). Anal. calcd for $C_{19}H_{35}O_3N_3S \cdot 2.6\ CF_3COOH$: C, 42.62; H, 5.58; N, 6.16. Found: C, 42.55; H, 5.68; N, 6.15.

*5(S)-[2(R)-Amino-3-mercaptopropylamino]-6(S)-methyl-2(R)-n-propyl-3,4-E-octenyl-homoserine (**16**).* To a solution of 5(*S*)-[2(*R*)-amino-3-mercaptopropylamino]-6(*S*)-methyl-2(*R*)-*n*-propyl-3,4-*E*-octenyl-homoserine (**4**, 4.32 mg, 6.33 μmol) in methanol (50 μL), an aqueous solution of sodium hydroxide (25.3 μL, 1.00 M) was added. After standing at room temp. for 1 h, the solution was diluted with methanol to 10 mM. HPLC analysis of an aliquot of the mixture after treatment with dithiothreitol confirmed complete hydrolysis of the lactone.

Procedure for preparation of alkane isostere tetrapeptide analogs

The three alkane isostere analogs were prepared as depicted in Scheme I incorporating an additional catalytic hydrogenation step after the S_N2' cuprate displacement reaction. The remainder of the sequence is exactly the same as described above for the preparation of 5(*S*)-[2(*R*)-amino-3-mercaptopropylamino]-6(*S*)-methyl-2(*R*)-*n*-propyl-3,4-*E*-octenyl-homoserine (**16c**).

5(S)-N-tert-(Butyloxy)carbonylamino-6(S)-methyl-2(R)-n-propyl-octanyl-homoserine lactone (17)

A solution of 5(*S*)-*N-tert*-(Butyloxy)carbonylamino-6(*S*)-methyl-2(*R*)-*n*-propyl-3,4-*E*-octenyl-homoserine lactone (**14**, 200 mg) in ethyl acetate (10 mL) was stirred under an atmosphere of hydrogen in the presence of 5 % palladium on charcoal (20 mg) overnight. The resultant mixture was filtered, and the filtrate concentrated to provide quantitatively the hydrogenated product **17**. ^{1}H NMR ($CDCl_3$) δ 6.62 (br d, J = 5.6 Hz, 1H), 4.56–4.25 (m, 4 H), 4.28 (br m, 1 H), 2.32 (br m, 1 H), 1.43 (s, 9 H), 0.93–0.86 (m, 9 H).

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(*Received* 6 *May* 1994)