five times and was then centrifuged at 3000 rpm for 2 min. The slightly cloudy upper phase was removed and centrifuged at 16000 rpm (ca. 31000g) for 45 min. The supernatant (1.6 mL) was separated into small aliquots and kept at -80 °C. Enzyme activity was assayed as described in ref 35. In a final volume of 100 μL, IMPD (4 μL of the above preparation) was measured in 50 mM potassium phosphate buffer, pH 7.4, 10 μM [$^{14}$C]IMP (50 mCi/mM), 0.3 mM NAD, 0.1 M KCl, and 1 mM EDTA. The reactions were stopped after 25 min incubation at 37 °C by addition of 4 μL of 5 N $HClO_4$. After cooling in ice for 15 min, the protein precipitate was removed by centrifugation. The aqueous supernatant was transferred to a new tube and neutralized by mixing with an equal volume of Freon (Du Pont) and trioctylamine (1:1). The aqueous phase was removed, and the nucleotides were analyzed by HPLC, using an Ultrasil AX (10 μm) column over a salt gradient;[36] the amounts of both IMP and XMP were determined by a radioactive flow detector. Three microliters of unlabeled IMP (10 mM) and XMP (10 mM) solutions were added as carriers before HPLC analysis. Compounds were dissolved in DMSO (4 mg/mL); each compound was tested at five different concentrations from 0.02 to 5.0 μg/mL, and $IC_{50}$ values were determined graphically.

**Acknowledgment**. Mycophenolic acid was obtained by fermentation of *Penicillium brevicompactum*, in work undertaken at our request by Dr. Iain M. Campbell, Department of Biological Sciences, University of Pittsburgh.

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# Renin Inhibitors Containing Isosteric Replacements of the Amide Bond Connecting the $P_3$ and $P_2$ Sites

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Renin inhibitors having 13 different isosteres connecting the $P_3$ and $P_2$ positions have been prepared. Synthetic routes and in vitro activity exhibited by these compounds are discussed. The two most potent compounds, **47** and **48**, contained the hydroxyethylene isostere, $\Psi[CHOHCH_2]$, and had $IC_{50}$ values of 61 and 22 nM, respectively.

The success of angiotensin converting enzyme (ACE) inhibitors in the treatment of hypertension[1a,b] has demonstrated that interrupting the biochemical cascade

$$\text{angiotensinogen} \xrightarrow{\text{renin}} \text{angiotensin I} \xrightarrow{\text{ACE}} \text{angiotensin II}$$

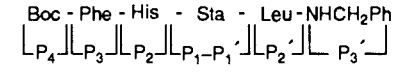
can lead to a lowering of blood pressure. This result has prompted our group and others[2] to seek agents that interrupt this cascade at an earlier stage by inhibition of the action of renin on angiotensinogen.

In recent years increasing interest has been shown in the concept of isosteric replacement of amide bonds in biologically active peptides. Inherent in this concept is the postulate that it might be possible to modify one or more amide bonds in peptides such that conformation and binding are maintained, but enzymatic hydrolysis is prevented. Initial successes utilizing this concept have been reported by Spatola,[3] who used the methylenethio isostere, $\Psi[CH_2S]$, as an amide replacement in enkephalin analogues, and Szelke,[4] who prepared renin inhibitors having both the methyleneamino, $\Psi[CH_2NH]$, and hydroxyethylene, $\Psi[CHOHCH_2]$, isosteres at the scissile Leu-Val amide bond in the 6-13 octapeptide derived from angiotensinogen. Reports from our laboratories have described modified di- and tripeptides derived from the C-terminal portion of oxytocin and vasopressin as possible cognition-activating agents.[5]

**Table I.** Peptide Bond Isosteres Prepared

| | |
|---|---|
| $\Psi[CH{=}CH]$ | $\Psi[COCH_2]$ |
| $\Psi[\overline{CHCHO}]$ | $\Psi[CH_2NH]$ |
| $\Psi[CH_2CH_2]$ | $\Psi[CH_2NOH]$ |
| $\Psi[CHOHCHOH]$ | $\Psi[CH_2S]$ |
| $\Psi[CHOHCH{=}CHCO]$ | $\Psi[CH_2SO]$ |
| $\Psi[CHOHCHOHCHOHCO]$ | $\Psi[CH_2SO_2]$ |
| $\Psi[CHOHCH_2]$ | |

As one aspect of our renin inhibitor strategy, we chose to prepare modified compounds based on the potent renin inhibitor (I) reported by Bock.[6,7]




I

While a number of other groups[4,8a-c,9,10] have described

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(7) Sta is 4(*S*)-amino-3(*S*)-hydroxy-6-methylheptanoic acid.
(8) (a) Thaisrivongs, S.; Pals, D. T.; Harris, D. W.; Kati, W. M.; Turner, S. R. *J. Med. Chem.* **1986**, *29*, 2088. (b) Thaisrivongs, S.; Pals, D. T.; Kroll, L. T.; Turner, S. R.; Han, F. S. *J. Med. Chem.* **1987**, *30*, 976. (c) Smith, C. W.; Saneii, H. H.; Sawyer, T. K.; Pals, D. T.; Scahill, T. A.; Kamdar, B. V.; Lawson, J. A. *J. Med. Chem.* **1988**, *31*, 1377.

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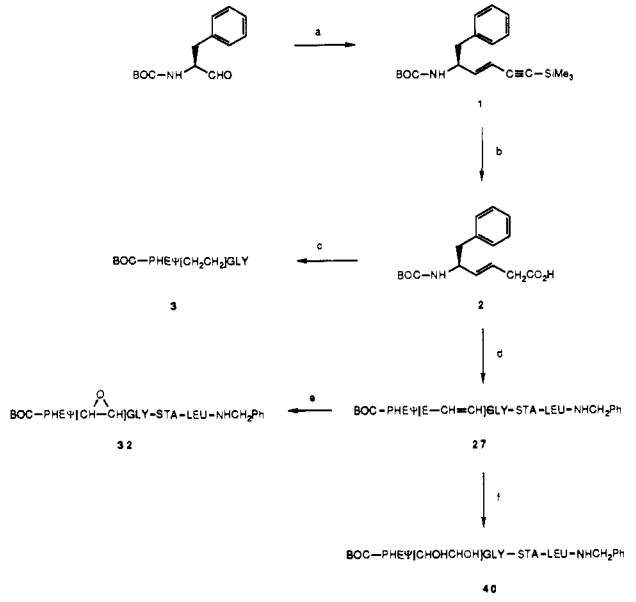
**Scheme I**[a]



[a] (a) $Ph_3PCH_2C{\equiv}CSiMe_3{}^+Br^-$, $n$-$C_4H_9Li$; (b) $(\bigcirc)_2BH$, $H_2O_2$; (c) $H_2$, Pd/C; (d) Sta-Leu-$NHCH_2Ph$, DCC; (e) $m$-chloroperbenzoic acid; (f) $OsO_4$.

isosteric replacements at the $P_1$–$P_1'$ site, only isolated reports have appeared concerning modifications at other sites. TenBrink[11] reported on the use of the $\Psi[CH_2O]$ isostere to connect the $P_4$ and $P_3$ sites in somewhat longer peptides, while Evans[12a,b] described modifying the bond connecting the $P_3$ and $P_2$ sites with isomeric Phe$\Psi$-$[CHOHCH_2]$Phe analogues of I.

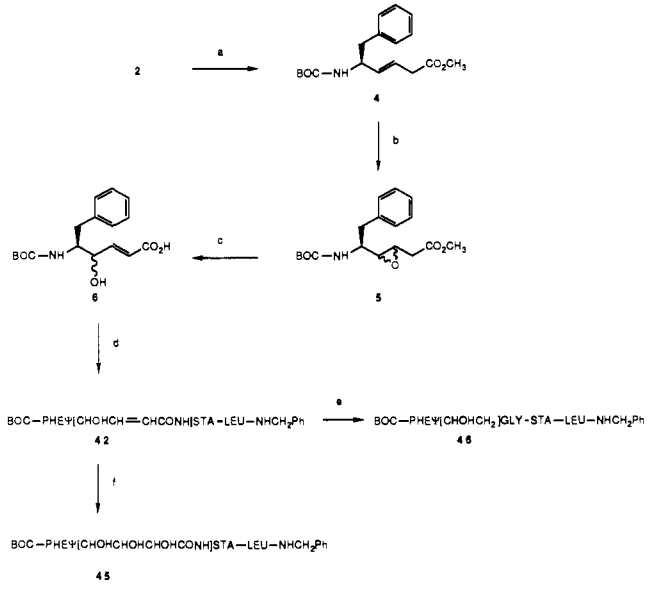
In the present paper we describe the results of replacing the amide linkage connecting the $P_3$ and $P_2$ sites with 13 different isosteres. We hoped that modifying this bond, the only one in the above structure connecting two natural amino acids, would lead to enhanced stability to enzymatic hydrolysis[13] and in turn lead to longer acting, possibly orally active, renin inhibitors.

## Chemistry

Table I lists the 13 isosteres discussed in this paper. The preparation of specific compounds containing these isosteres is described in Schemes I–V. The other compounds in Table II were prepared in accordance with these schemes.

While the syntheses of the compounds containing the $\Psi[CH{=}CH]$, $\Psi[CH_2CH_2]$, $\Psi[CH_2NH]$, and $\Psi[CH_2NOH]$ isosteres gave single isomers, the compounds containing

**Scheme II**[a]



[a] (a) CDI, MeOH; (b) $m$-chloroperbenzoic acid; (c) NaOH; (d) Sta-Leu-$NHCH_2Ph$, DCC; (e) $H_2$, Raney Ni; (f) $OsO_4$.

the other isosteres were obtained as mixtures of diastereomers and were tested as such.

Scheme I outlines the routes leading to the double bond, $\Psi[CH{=}CH]$; epoxide, $\Psi[\overline{CHCHO}]$; diol, $\Psi[CHOH$-$CHOH]$; and dimethylene, $\Psi[CH_2CH_2]$, isosteres. Boc-phenylalanal[14] was treated with [1-(trimethylsilyl)-propyn-3-yl] triphenylphosphonium bromide according to the method of Sammes[15] to give 1. Chromatography on silica gel separated the predominant $E$ isomer 1 from the minor $Z$ isomer. Selective hydroboration of the triple bond of 1 and subsequent oxidation gave 2, designated Boc-Phe$\Psi[E$-$CH{=}CH]$Gly. Condensation of 2 with Sta-Leu-$NHCH_2Ph$[12a] using DCC/HOBT gave the modified renin inhibitor 27. Compound 27 served as the precursor to epoxide 32 and diol 40. Conversely, 2 could be reduced to saturated compound 3, designated Boc-Phe$\Psi$-$[CH_2CH_2]$Gly and elaborated with standard peptide chemistry. Compounds 32 and 40 were obtained as mixtures of diastereomers.

Scheme II outlines the route leading to the unsaturated alcohol, $\Psi[CHOHCH{=}CHCO]$, the triol acid, $\Psi[CHOH$-$CHOHCHOHCO]$, and the hydroxyethylene, $\Psi$-$[CHOHCH_2]$, isosteres. Esterification of 2 with CDI/MeOH gave 4 without migration of the double bond. Epoxidation with $m$-chloroperbenzoic acid afforded 5, which when treated with dilute base gave the unsaturated acid 6.[16] NMR spectra showed the double bond to have the $E$ configuration. Condensation with Sta-Leu-$NHCH_2Ph$ under normal conditions gave 42, designated as Boc-Phe$\Psi[CHOHCH{=}CHCO]$Sta-Leu-$NHCH_2Ph$. In turn, 42 could be hydroxylated with $OsO_4$ to afford 45, Boc-Phe$\Psi[CHOHCHOHCHOHCO]$Sta-Leu-$NHCH_2Ph$. Reduction of 42 gave 46, Boc-Phe$\Psi[CHOHCH_2]$Gly-Sta-Leu-$NHCH_2Ph$. Although the three asymmetric centers present in the isosteric portion of 45 might be expected to lead to eight diastereomers, the cis mechanism of $OsO_4$ hydroxylation reduces this number to four.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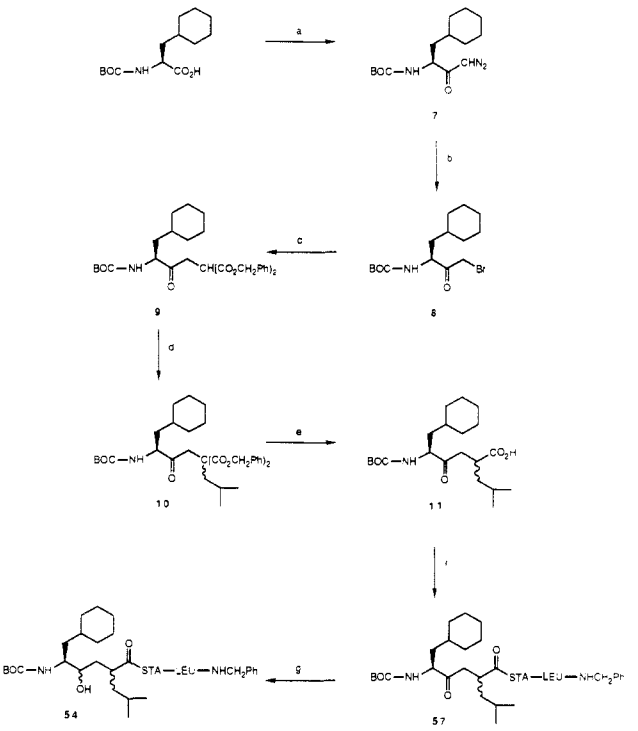
(16) An alternate synthesis of 6 has been described: Hanson, G. J.; Lindberg, T. *J. Org. Chem.* **1985**, *50*, 5399.

**Scheme III**[a]



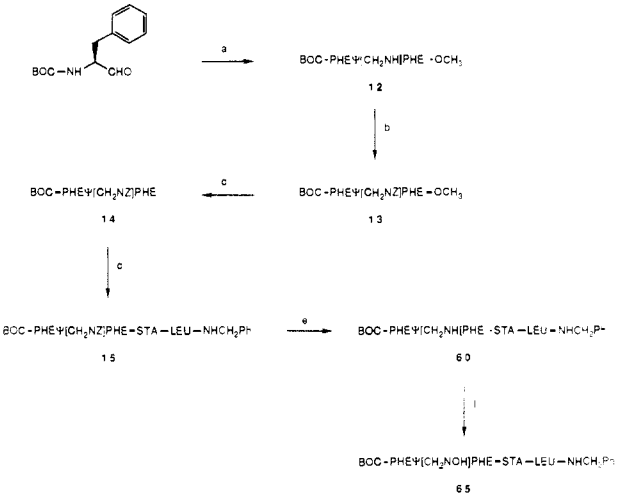
[a] (a) Isobutyl chloroformate, $CH_2N_2$; (b) HBr gas; (c) $CH_2(CO_2CH_2Ph)_2$, NaH, (d) NaH, isobutyl iodide; (e) $H_2$, Pd/C, then reflux in toluene; (f) Sta-Leu-$NHCH_2Ph$, DCC; (g) $NaBH_4$.

An alternate preparation of the hydroxyethylene isostere, $\Psi[CHOHCH_2]$, which proceeds through the ketomethylene isostere, $\Psi[COCH_2]$, is outlined in Scheme III. Formation of the mixed anhydride of Boc-cyclohexylalanine and treatment with diazomethane gave **7**, which afforded **8** on treatment with HBr gas. Condensation of **8** with dibenzyl malonate gave **9**, which was alkylated with isobutyl iodide to give **10**. Hydrogenolysis of the benzyl esters and decarboxylation gave **11**, Boc-CyclohexylalaΨ-[$COCH_2$]Leu. Condensation with Sta-Leu-$NHCH_2Ph$ in the usual manner gave **57**. Reduction of the ketone with $NaBH_4$ gave **54**. In several instances, the diastereomeric ketomethylene isosteres listed in Table I could be separated by chromatography. A similar series of reactions leading to ketomethylene isosteres has recently been described.[17] Another route, giving the $\Psi[CHOHCH_2]$ isostere directly without involving the ketomethylene isostere, has been reported by Evans.[12a]

Preparation of the methyleneamino, $\Psi[CH_2NH]$, and methylenehydroxylamino, $\Psi[CH_2NOH]$, isosteres is outlined in Scheme IV. A reductive amination of Boc-phenylalanal with Phe-$OCH_3$ and $NaBH_4$ in the presence of 3A molecular sieves gave **12**. Protecting the internal amine with Z-Cl provided **13**. Hydrolysis of the methyl ester afforded **14**, which was condensed with Sta-Leu-$NHCH_2Ph$ in the usual manner to give **15**. Removal of the Z group with $H_2$, Pd/C gave renin inhibitor **60**. Oxidation of **60** with *m*-chloroperbenzoic acid gave **65**, a renin inhibitor containing the methylenehydroxylamine isostere.

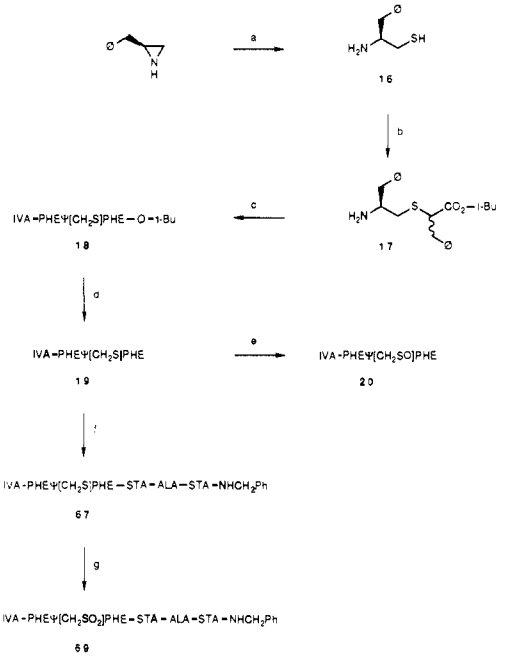
The route to the methylenethio isostere, $\Psi[CH_2S]$, and its oxidized derivatives, $\Psi[CH_2SO]$ and $\Psi[CH_2SO_2]$, is outlined in Scheme V. The aziridine derived from phenylalanine[18] was treated with $H_2S$ to give aminothiol **16**.[19]

**Scheme IV**[a]



[a] (a) Phe-$OCH_3$, 3A molecular sieves, $NaBH_4$; (b) ZCl; (c) NaOH; (d) Sta-Leu-$NHCH_2Ph$, DCC; (e) $H_2$, Pd/C; (f) *m*-chloroperbenzoic acid.

**Scheme V**[a]



[a] (a) $H_2S$; (b) $Br(PhCH_2)CHCO_2$-*t*-Bu; (c) isovaleryl chloride; (d) TFA; (e) $NaIO_4$; (f) Sta-Ala-Sta-$NHCH_2Ph$, DCC; (g) *m*-chloroperbenzoic acid.

Reaction with *tert*-butyl 2-bromo-3-phenylpropionate[20] gave **17**, which was acylated with isovaleryl chloride to give **18**. Removal of the *tert*-butyl ester with TFA afforded **19**, which was condensed with Sta-Ala-Sta-$NHCH_2Ph$ in the usual manner to give **67**. Oxidation with excess *m*-chloroperbenzoic acid gave sulfone **69**. Alternatively, the oxidation could be carried out at an earlier stage. For example, treatment of **19** with $NaIO_4$ provided sulfoxide **20**, which could be elaborated in the usual fashion.

## Results and Discussion

The renin inhibitory activity demonstrated by these compounds is listed in Table II. Compounds **24–26**, which

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**Table II.** In Vitro Renin Inhibitory Activity

| compd | structure | $IC_{50}$, μM | formula[a] |
|---|---|---|---|
| **24** | Boc-Phe-His-Sta-Leu-$NHCH_2Ph$ | 0.0057[b] | $C_{41}H_{59}N_7O_7$ |
| **25** | Boc-Phe-Phe-Sta-Leu-$NHCH_2Ph$ | 0.15 | $C_{44}H_{61}N_5O_7$ |
| **26** | Boc-Phe-Gly-Sta-Leu-$NHCH_2Ph$ | 1.1[c] | $C_{37}H_{55}N_5O_7$ |
| | Double Bond Isosteres | | |
| **27** | Boc-Phe$\Psi$[*E*-CH=CH]Gly-Sta-Leu-$NHCH_2Ph$ | 7.0 | $C_{38}H_{56}N_4O_6 \cdot 0.3H_2O$ |
| **28** | Iva-Phe$\Psi$[*E*-CH=CH]Gly-Sta-Leu-$NHCH_2Ph$ | 13.0 | $C_{38}H_{56}N_4O_5$ |
| **29** | Boc-Phe$\Psi$[*E*-CH=CH]Gly-AHPPA-Leu-$NHCH_2Ph$[d] | 9.2 | $C_{41}H_{54}N_4O_6 \cdot 0.2CHCl_3$[e] |
| **30** | Boc-Phe$\Psi$[*E*-CH=CH]Gly-ACHPA-Leu-$NHCH_2Ph$[f] | 1.3 | $C_{41}H_{60}N_4O_6 \cdot 0.1CHCl_3$ |
| **31** | Boc-Phe$\Psi$[*Z*-CH=CH]Gly-Sta-Leu-$NHCH_2Ph$ | >100 | $C_{38}H_{56}N_4O_6 \cdot 0.1CHCl_3$ |
| | Epoxide Isosteres | | |
| **32** | Boc-Phe$\Psi$[CHCHO]Gly-Sta-Leu-$NHCH_2Ph$ | 1.2 | $C_{38}H_{56}N_4O_7 \cdot 0.15CHCl_3$[g] |
| **33** | Iva-Phe$\Psi$[CHCHO]Gly-Sta-Leu-$NHCH_2Ph$ | 29.0 | $C_{38}N_{56}N_4O_6 \cdot 0.1CHCl_3$[h] |
| **34** | Boc-Phe$\Psi$[CHCHO]Gly-AHPPA-Leu-$NHCH_2Ph$ | 4.4 | $C_{41}H_{54}N_4O_7 \cdot 0.7CH_2Cl_2$ |
| **35** | Boc-Phe$\Psi$[CHCHO]Gly-ACHPA-Leu-$NHCH_2Ph$ | 0.53 | $C_{41}H_{60}N_4O_7 \cdot 0.7CHCl_3$ |
| **36** | Boc-Phe$\Psi$[CHCHO]Gly-ACHPA-Leu-$NHCH_2$-*m*-Ph-$CH_2NH_2$ | 0.32 | $C_{42}H_{63}N_5O_7 \cdot 0.9CHCl_3$ |
| **37** | Boc-Phe$\Psi$[CHCHO]Gly-Sta-Leu-$NHCH_2Ph$ (derived from Z-31) | >100 | $C_{38}H_{56}N_4O_7 \cdot 0.1CHCl_3$[i] |
| | Dimethylene Isosteres | | |
| **38** | Boc-Phe$\Psi$[$CH_2CH_2$]Gly-Sta-Leu-$NHCH_2Ph$ | 23.0 | $C_{38}H_{58}N_4O_6 \cdot 0.2CHCl_3$ |
| **39** | Boc-Phe$\Psi$[$CH_2CH_2$]Gly-ACHPA-Leu-$NHCH_2$-*m*-Ph-$CH_2NH_2$ | 0.63 | $C_{42}H_{65}N_5O_6 \cdot 0.6CHCl_3$ |
| | Diol Isosteres | | |
| **40** | Boc-Phe$\Psi$[CHOHCHOH]Gly-Sta-Leu-$NHCH_2Ph$ | 1.4 | $C_{38}H_{58}N_4O_8 \cdot 0.3CHCl_3$ |
| **41** | Boc-Cyclohexylala$\Psi$[CHOHCHOH]Gly-Sta-Leu-$NHCH_2Ph$ | 6.5 | $C_{38}H_{64}N_4O_8 \cdot 0.25CHCl_3$ |
| | Hydroxy Double Bond Isosteres | | |
| **42** | Boc-Phe$\Psi$[CHOHCH=CHCONH]Sta-Leu-$NHCH_2Ph$ | 2.1 | $C_{38}H_{56}N_4O_7$ |
| **43** | Boc-Phe$\Psi$[CHOHCH=CHCONH]ACHPA-Leu-$NHCH_2Ph$ | 0.09 | $C_{41}H_{60}N_4O_7 \cdot 0.25CHCl_3$ |
| **44** | Boc-Phe$\Psi$[CHOHCH=CHCONH]ACHPA-Leu-$NHCH_2$-*m*-Ph-$CH_2NHZ$ | 0.47 | $C_{50}H_{69}N_5O_9 \cdot 0.45CHCl_3$ |
| | Trihydroxy Isostere | | |
| **45** | Boc-Phe$\Psi$[CHOHCHOHCHOHCONH]Sta-Leu-$NHCH_2Ph$ | 11.0 | $C_{38}H_{58}N_4O_9 \cdot 0.25CHCl_3$ |
| | Hydroxyethylene Isosteres | | |
| **46** | Boc-Phe$\Psi$[$CHOHCH_2$]Gly-Sta-Leu-$NHCH_2Ph$ | 0.64 | $C_{38}H_{58}N_4O_7$ |
| **47** | Boc-Phe$\Psi$[$CHOHCH_2$]Gly-ACHPA-Leu-$NHCH_2Ph$ | 0.061 | $C_{41}H_{62}N_4O_7 \cdot 0.45CHCl_3$ |
| **48** | Boc-Phe$\Psi$[$CHOHCH_2$]Gly-ACHPA-Leu-$NHCH_2$-*m*-Ph-$CH_2NH_2$ | 0.022 | $C_{42}H_{65}N_5O_7 \cdot 0.5CHCl_3$ |
| **49** | Boc-Phe$\Psi$[$CHOHCH_2$]His-Sta-Leu-$NHCH_2Ph$ | 0.78 | $C_{42}H_{62}N_6O_7 \cdot H_2O$ |
| **50** | Z-Phe$\Psi$[$CHOHCH_2$]Phe-Sta-Leu-$NHCH_2Ph$ | 3.7 | $C_{48}H_{62}N_4O_7 \cdot 0.5H_2O$ |
| **51** | Z-Leu$\Psi$[$CHOHCH_2$]Leu-Sta-Leu-$NHCH_2Ph$ | 6.7 | $C_{42}H_{66}N_4O_7 \cdot 0.2CH_2Cl_2$ |
| **52** | Z-Leu$\Psi$[$CHOHCH_2$]Gly-Sta-Leu-$NHCH_2Ph$ | 4.0 | $C_{38}H_{58}N_4O_7 \cdot 0.2CH_2Cl_2$ |
| **53** | Z-Leu$\Psi$[$CHOHCH_2$]Phe-Sta-Leu-$NHCH_2Ph$ | 8.4 | $C_{45}H_{64}N_4O_7$ |
| **54** | Boc-Cyclohexylala$\Psi$[$CHOHCH_2$]Leu-Sta-Leu-$NHCH_2Ph$ | 3.8 | $C_{42}H_{72}N_4O_7$ |
| | Ketomethylene Isosteres | | |
| **55** | Boc-Phe$\Psi$[$COCH_2$]Gly-Sta-Leu-$NHCH_2Ph$ | 21.0 | $C_{38}H_{56}N_4O_7$[j] |
| **56** | Z-Phe$\Psi$[$COCH_2$]Phe-Sta-Leu-$NHCH_2Ph$ | 23.0 | $C_{48}H_{60}N_4O_7$ |
| **57** | Boc-Cyclohexylala$\Psi$[$COCH_2$]Leu-Sta-Leu-$NHCH_2Ph$ | 26.0 | $C_{42}H_{70}N_4O_7$ |
| **58** | Z-Leu$\Psi$[$COCH_2$]Phe-Sta-Leu-$NHCH_2Ph$ | 11.0 | $C_{45}H_{62}N_4O_7 \cdot 0.05CHCl_3$ |
| | Methyleneamino Isosteres | | |
| **59** | Boc-Phe$\Psi$[$CH_2NH$]His-Sta-Leu-$NHCH_2Ph$ | 3.8 | $C_{41}H_{61}N_7O_6 \cdot 0.07CHCl_3$ |
| **60** | Boc-Phe$\Psi$[$CH_2NH$]Phe-Sta-Leu-$NHCH_2Ph$ | 4.2 | $C_{44}H_{63}N_5O_6 \cdot 1.5CH_3OH$[k] |
| **61** | Boc-Phe$\Psi$[$CH_2NH$]His-AHPPA-Leu-$NHCH_2Ph$ | 3.8 | $C_{44}H_{59}N_7O_6 \cdot 0.2CHCl_3$ |
| **62** | Boc-Phe$\Psi$[$CH_2NH$]His-ACHPA-Leu-$NHCH_2Ph$ | 0.2 | $C_{44}H_{65}N_7O_6 \cdot 0.1CH_2Cl_2 \cdot 0.16C_4H_8O_2$[l] |
| **63** | Iva-Val$\Psi$[$CH_2NH$]Val-Sta-Leu-$NHCH_2Ph$ | 15.0 | $C_{38}H_{63}N_5O_5 \cdot 0.2C_2H_4O_2$ |
| **64** | Boc-Cyclohexylala$\Psi$[$CH_2NH$]His-Sta-Leu-$NHCH_2Ph$ | 10.0 | $C_{41}H_{67}N_7O_6 \cdot 0.2CH_2Cl_2$ |
| | Methylenehydroxyamino Isosteres | | |
| **65** | Boc-Phe$\Psi$[$CH_2NOH$]Phe-Sta-Leu-$NHCH_2Ph$ | 18.0 | $C_{44}H_{63}N_5O_7 \cdot 1.0C_4H_8O_2$[m] |
| **66** | Boc-Phe$\Psi$[$CH_2NOH$]His-Sta-Leu-$NHCH_2Ph$ | 6.5 | $C_{41}H_{61}N_7O_7 \cdot 0.4CH_2Cl_2$ |
| | Methylenethio Isosteres | | |
| **67** | Iva-Phe$\Psi$[$CH_2S$]Phe-Sta-Ala-Sta-$NHCH_2Ph$ | 7.4 | $C_{49}H_{71}N_5O_7S$[n] |
| **68** | Iva-Phe$\Psi$[$CH_2SO$]Phe-Sta-Ala-Sta-$NHCH_2Ph$ | 2.0 | $C_{49}H_{71}N_5O_8S \cdot 1.5H_2O$ |
| **69** | Iva-Phe$\Psi$[$CH_2SO_2$]Phe-Sta-Ala-Sta-$NHCH_2Ph$ | 1.5 | $C_{49}H_{71}N_5O_9S \cdot 0.5H_2O$ |
| **70** | Boc-Phe$\Psi$[$CH_2SO$]Phe-Sta-Ala-Sta-$NHCH_2Ph$ | 5.6 | $C_{49}H_{71}N_5O_9S \cdot 1.5H_2O$[o] |
| **71** | Boc-Phe$\Psi$[$CH_2SO$]Phe-Sta-Leu-$NHCH_2Ph$ | 5.4 | $C_{44}H_{62}N_4O_7S \cdot 0.5H_2O$ |
| **72** | Iva-Phe$\Psi$[$CH_2S$]Gly-Sta-Leu-$NHCH_2Ph$ | 18.0 | $C_{37}H_{56}N_4O_5S$ |

[a] Analyses for C, H, N were within ±0.4% except as noted. [b] Reference 6 gives $IC_{50}$ as 0.026 μM. [c] $IC_{50}$ determined with monkey plasma. [d] AHPPA is 4(*S*)-amino-3(*S*)-hydroxy-5-phenylpentanoic acid. [e] C: calcd, 68.46; found, 68.04. [f] ACHPA is 4(*S*)-amino-3(*S*)-hydroxy-5-cyclohexylpentanoic acid. [g] N: calcd, 8.02; found 8.44. [h] H: calcd, 8.36; found 7.89. [i] N: calcd, 8.09; found 8.63. [j] N: calcd, 8.23; found 8.77. [k] H: calcd, 8.63; found 8.13. [l] C: calcd, 66.29; found 65.85. [m] N: calcd, 8.12; found 8.59. [n] C: calcd, 67.33; found 66.91. [o] H: calcd, 7.99; found 7.53.

Table III. Percent Parent Compound Remaining following Incubation with Chymotrypsin for 3 h

| compound | % remaining | compound | % remaining |
|---|---|---|---|
| **25** | 60 | **47** | 89 |
| **30** | 91 | **55** | 94 |
| **32** | 92 | **59** | 100 |
| **39** | 100 | **65** | 99 |
| **40** | 97 | **69** | 96 |
| **43** | 87 | **71** | 94 |
| **45** | 97 | **72** | 89 |

do not contain isosteres, provide standards for comparison with the isostere-containing compounds. Compound **26**, having a Gly as the $P_2$ substituent, provides a comparison for many compounds that, because of synthetic considerations, also have a Gly equivalent in this position.

Overall, none of the isostere-containing compounds matched the high potency shown by **24**. The three direct analogues (**49**, **59**, and **66**) having an isostere connecting a Phe-His grouping were considerably less potent than **24**.

The direct analogues of **25**, compounds **60**, **65**, and **71**, where an isostere connects a Phe-Phe grouping, had activities 28–120-fold less than that of the standard. A 7-fold drop in potency for an isostere-containing compound was also observed by Evans[12b] when he compared the hog renin $IC_{50}$ value of **25** with that of the corresponding Phe$\Psi$[CHOHCH$_2$]Phe analogue.

When compared with **26**, which has a Gly in $P_2$, several of the direct analogues (**32**, **40**, **42**, and **46**) showed comparable potencies, with the range varying from 1.9-fold less potent to 1.7-fold more potent. The most potent direct analog of **26**, compound **46**, contains the $\Psi$[CHOHCH$_2$] isostere. Indeed, when this isostere was combined with the potency-enhancing ACHPA group[21] in the $P_1$-$P_1'$ position, the highly potent **47** ($IC_{50}$ = 0.061 $\mu$M) was obtained. Preparation of an analogue containing an amide derived from *m*-xylenediamine[22] gave an even more potent derivative, **48** ($IC_{50}$ = 0.022 $\mu$M). These latter two analogues do approach the activity of the highly potent standard **24**, being 11-fold and 4-fold less potent, respectively.

In addition to testing the effect of isosteres connecting the $P_3$ and $P_2$ sites on potency, another aspect of this research was to test the effect of these isosteric replacements on stability to enzymatic hydrolysis. Table III shows the results of incubation with the digestive enzyme chymotrypsin. A representative of each of the 13 isosteric types discussed here was tested, and all showed enhanced stability when compared to peptide model **25**. With **25**, metabolic products were observed which increased with time. There was no evidence of metabolites being formed with any of the other compounds, nor with the blank treatment of **25**. Thus the addition of an isostere to replace the amide bond connecting the $P_3$ and $P_2$ sites, the only site connecting two natural amino acids, appears to add a measure of stability to enzymatic hydrolysis.

Finally, it was of interest to see if this increased stability manifested itself in oral activity. Compound **47** was tested orally in two high-renin, salt-depleted, normotensive Cynomolgus monkeys. At an oral dose of 25 mg/kg, negligible blood pressure lowering was observed when compared with the effect of vehicle (7.5% DMA/30% Tween 80/62.5% $H_2O$). This lack of response could be due to the low bioavailability by the oral route that is characteristic of this and many other renin inhibitors.

## Experimental Section

The NMR spectra were recorded on a Varian EM-390, Varian XL-200, or a IBM WP100SY instrument. The FAB-MS was determined on a VG analytical 7070E/HF mass spectrometer in a thioglycerol matrix using xenon as the target gas. Rotations were recorded on a Perkin-Elmer Model 142 polarimeter. TLC was done on precoated plates (silica gel 60F 254, Merck). Silica gel chromatography was done with Kieselgel 60 (70–230 mesh or 230–400 mesh for flash).

All compounds were purified by chromatography on silica gel and were usually obtained as solid foams that often retained solvent, even on prolonged drying under vacuum. Intermediates and the compounds of Table II all showed the correct molecular ion in the FAB mass spectrum. The NMR was consistent with the assigned structures.

**(S)-[1-(Phenylmethyl)-5-(trimethylsilyl)-3-hexen-5-ynyl]carbamic Acid, 1,1-Dimethylethyl Ester (1).** A suspension of 36.6 g (0.081 mol) of [1-(trimethylsilyl)propyn-3-yl]-triphenylphosphonium bromide[15] in 420 mL of THF and under $N_2$ was cooled to −80 °C and treated slowly via a syringe with 31 mL (0.081 mol) of *n*-butyllithium (2.6 M in hexane). After stirring at −80 °C for 1 h, the solution was treated dropwise with a solution of 20.1 g (0.081 mol) of Boc-phenylalanal in 420 mL of THF. After 1 h at −80 °C, the mixture was allowed to stir at room temperature overnight. The solvent was then removed under reduced pressure and the residue was triturated several times with $Et_2O$ to remove triphenylphosphine oxide. The combined $Et_2O$ phases were washed with saturated NaCl and dried over $MgSO_4$. Removal of the $Et_2O$ under reduced pressure left 29.1 g of the crude product as a brown oil. Chromatography on silica gel, eluting with $CH_2Cl_2$, gave 11.93 g (43%) of the *E* isomer as an oil which solidified on standing. NMR showed $J$ = 16 Hz for the coupling constant between the vinyl protons, confirming this as the *E* isomer.

Continued elution from the column gave 1.65 g (6%) of the *Z* isomer. NMR showed $J$ = 11.2 Hz for the coupling constant between the vinyl protons, confirming this as the *Z* isomer.

**Boc-Phe$\Psi$[*E*-CH=CH]Gly (2).** A THF solution of 122 mL (0.122 mmol) of a 1 M solution of $BH_3$ was cooled in ice and treated dropwise with 24.7 mL (0.243 mol) of cyclohexene in 260 mL of THF. After 1 h at 0 °C, the suspension was treated dropwise with a solution of 11.93 g (0.035 mol) of **1** in 45 mL of THF and then kept at 0 °C for 1 h. This was then treated dropwise with 46 mL of MeOH, 63 mL of 2 N NaOH, and then with 41 mL of 30% $H_2O_2$, and the temperature was kept below 18 °C. After stirring for 1 h at room temperature, the solution was poured into $H_2O$ containing 46 mL of 2 N NaOH. The basic solution was extracted three times with $Et_2O$, the pH was adjusted to 2.2, and this in turn was extracted three times with $Et_2O$. The combined $Et_2O$ extracts were washed with saturated NaCl and dried over $MgSO_4$. Removal of the $Et_2O$ under reduced pressure left 9.93 g (93.7%) of **2** as an oil. The crude material was used directly in the following step.

**Boc-Phe$\Psi$[*E*-CH=CH]Gly-Sta-Leu-NHCH$_2$Ph (27).** A solution of 400 mg (0.97 mmol) of Sta-Leu-NHCH$_2$Ph·HCl, 295 mg (0.97 mmol) of **2**, and 131 mg (0.97 mmol) of HOBT in 25 mL of DMF was cooled in ice and 0.14 mL (0.97 mmol) of $Et_3N$ was added, followed by a solution of 202 mg (0.97 mmol) of DCC in 5 mL of DMF. After 0.5 h at 0 °C, the mixture was allowed to stir at room temperature overnight. The solvent was removed under reduced pressure and the residue was taken up in EtOAc. The precipitated *N,N'*-dicyclohexylurea was filtered off and the filtrate was washed with 1 N HCl, saturated $NaHCO_3$, and saturated NaCl. After drying over $MgSO_4$ and removal of the solvent under reduced pressure, there was obtained 640 mg of crude **27**. Chromatography on silica gel, eluting with $CHCl_3$/MeOH (97/3), gave 430 mg of **27** as a white foam.

**Boc-Phe$\Psi$[CHCHO]Gly-Sta-Leu-NHCH$_2$Ph (32).** A solution of 870 mg (1.3 mmol) of **27** in 20 mL of $CH_2Cl_2$ was treated with 409 mg (2.0 mmol) of *m*-chloroperbenzoic acid and allowed

(21) Boger, J.; Payne, L. S.; Perlow, D. S.; Lohr, N. S.; Poe, M.; Blaine, E. H.; Ulm, E. H.; Schorn, T. W.; LaMont, B. I.; Lin, T. Y.; Kawai, M.; Rich, D. H.; Veber, D. F. *J. Med. Chem.* **1985**, *28*, 1779.

(22) Boger, J.; Payne, L. S.; Perlow, D. S.; Poe, M.; Blaine, E. H.; Ulm, E. H.; Schorn, T. W.; LaMont, B. I.; Bock, M. G.; Friedinger, R. M.; Evans, B. E.; Veber, D. F. In *Peptides. Structure and Function*, Proceedings of the Ninth American Peptide Symposium; Deber, C. M., Hruby, V. J., Kopple, K. D., Eds.; Pierce Chemical Co.: Rockford, IL, 1985; pp 747–750.

to stir at room temperature for 3 days. The solution was diluted with $CH_2Cl_2$ and washed with 10% $Na_2SO_3$, $H_2O$, saturated $NaHCO_3$, and saturated NaCl. Drying over $MgSO_4$ and removal of the solvent under reduced pressure gave 800 mg of crude **32**. Chromatography on silica gel, eluting with $CHCl_3$/MeOH (97/3), gave 620 mg of **32**.

**Boc-PheΨ[CHOHCHOH]Gly-Sta-Leu-NHCH$_2$Ph (40)**. A solution of 500 mg (0.75 mmol) of **27** in 10 mL of dioxane was treated with 11 mL (0.9 mmol) of a 2% solution of $OsO_4$ in dioxane and allowed to stir at room temperature for 3 days. The dark solution was saturated with $H_2S$ gas and filtered. After removal of the solvent under reduced pressure, the residue was chromatographed on silica gel, eluting with $CHCl_3$/MeOH (95/5). There was obtained 280 mg of **40** as a white foam.

**Boc-PheΨ[CH$_2$CH$_2$]Gly (3)**. A solution of 1.11 g (3.6 mmol) of **2** in 100 mL of 2-propanol was treated with 0.1 g of 10% Pd/C and reduced with hydrogen at 20 °C, 51 psi. The solution was filtered to remove the catalyst and the solvent was removed under reduced pressure. The residue was recrystallized from MeOH/$H_2O$ to give 0.45 g of **3**: mp 102–105 °C, $[\alpha]^{23}_D$ +1.4° (c 0.58, MeOH). Anal. H, N; C: calcd, 66.42; found, 67.07.

**Boc-PheΨ[*E*-CH=CH]Gly-OCH$_3$ (4)**. A solution of 10.9 g (0.035 mol) of **2** in 110 mL of THF was cooled in ice and treated with 6.4 g (0.039 mol) of 1,1′-carbonyldiimidazole and stirred for 0.5 h. The solution was diluted with 300 mL, of MeOH and stirred for 3 h. The solvent was removed under reduced pressure and the residue was taken up in EtOAc and washed with 1 N HCl, $H_2O$, saturated $NaHCO_3$, and saturated NaCl. Drying over $MgSO_4$ and removal of the solvent under reduced pressure gave 8.05 g of crude product. This was chromatographed on silica gel, eluting with $CHCl_3$ to give 7.3 g (64%) of **4** as a pale yellow oil: $[\alpha]^{23}_D$ −52° (c 1.43, MeOH). Anal. C, H, N.

**Boc-PheΨ[CHCHO]Gly-OCH$_3$ (5)**. A solution of 1.0 g (3.1 mmol) of **4** in 10 mL of $CH_2Cl_2$ was treated with 1.0 g (4.7 mmol) of *m*-chloroperbenzoic acid and stirred at room temperature for 3 days. Some benzoic acid was filtered off and the filtrate was washed with 10% $Na_2SO_3$, 1 N HCl, saturated $NaHCO_3$, and saturated NaCl. Drying over $MgSO_4$ and removal of the solvent under reduced pressure gave 1.05 g (100%) of **5** as an oil.

**(2*E*,4*R*,*S*,5*S*)-5-[[(1,1-Dimethylethoxy)carbonyl]-amino]-4-hydroxy-6-phenyl-2-hexenoic Acid (6)**. A solution of 2.17 g (6.5 mmol) of **5** in 20 mL of MeOH was treated with 14 mL (14 mmol) of 1 N NaOH and the solution was stirred at room temperature for 2 h. The solvent was removed under reduced pressure and the residue was taken up in $H_2O$ and washed with $Et_2O$. The aqueous phase was brought to pH 2.1 and extracted twice with $Et_2O$. The $Et_2O$ was washed with saturated NaCl and dried over $MgSO_4$, and the solvent was removed under reduced pressure, leaving 1.7 g of crude **6**. This was chromatographed on silica gel, eluting with $CHCl_3$/MeOH (95/5), to give 1.1 g of **6** as an oil which solidified. NMR showed $J$ = 16.2 Hz for the coupling constant between the vinyl protons, confirming that this is the *E* isomer. Anal. C, H, N.

**Boc-PheΨ[CHOHCH=CHCONH]Sta-Leu-NHCH$_2$Ph (42)**. A solution of 323 mg (1.0 mmol) of **6**, 416 mg (1.0 mmol) of Sta-Leu-$NHCH_2Ph$·HCl, and 136 mg (1.0 mmol) of HOBT in 15 mL of DMF was cooled in ice and 0.14 mL (1.0 mmol) of $Et_3N$ was added followed by a solution of 210 mg (1.0 mmol) of DCC in 5 mL of DMF. The solution was kept at 0 °C for 0.5 h and then at room temperature overnight. The solvent was removed under reduced pressure and the residue was taken up in EtOAc. The precipitated *N*,*N*′-dicyclohexylurea was filtered off and the filtrate was washed with 1 N HCl, saturated $NaHCO_3$, and saturated NaCl. Drying over $MgSO_4$ and removal of the solvent under reduced pressure gave 630 mg of crude **42**. Chromatography on silica gel, eluting with $CHCl_3$/MeOH (95/5), gave 360 mg of a white solid. Recrystallization from MeOH/$H_2O$ gave 325 mg of **42**, mp 207–210 °C.

**Boc-PheΨ[CHOHCHOHCHOHCONH]Sta-Leu-NHCH$_2$Ph (45)**. A solution of 620 mg (0.91 mmol) of **42** in 20 mL of THF was treated with 12 mL (0.94 mmol) of a 2% solution of $OsO_4$ in dioxane and allowed to stir at room temperature for 4 days. The dark solution was saturated with $H_2S$ gas and filtered. Removal of the solvent under reduced pressure gave 660 mg of crude **45**. Chromatography on silica gel, eluting with $CHCl_3$/MeOH (95/5), gave 430 mg of **45**.

**Boc-PheΨ[CHOHCH$_2$]Gly-Sta-Leu-NHCH$_2$Ph (46)**. A solution of 754 mg (1.1 mmol) of **42** in 75 mL of MeOH and containing a small amount of Raney Ni catalyst was reduced with hydrogen at 24 °C, 50 psi. The material was filtered and the filtrate was concentrated under reduced pressure. The residue was taken up in $CH_2Cl_2$ and the solvent was again removed under reduced pressure to give 694 mg of **46**.

**(*S*)-[1-(Cyclohexylmethyl)-3-diazo-2-oxopropyl]carbamic Acid, 1,1-Dimethylethyl Ester (7)**. A solution of 20.0 g (0.073 mol) of Boc-cyclohexylalanine in 200 mL of EtOAc was cooled to −20 °C and 8.9 mL (0.073 mol) of 1-methylpiperidine was added, followed by the dropwise addition of 9.56 mL (0.073 mol) of isobutyl chloroformate. The mixture was stirred for 10 min and then filtered under $N_2$ into a cold flask. Diazomethane in $Et_2O$ was added in excess and the mixture was allowed to stand at 2 °C overnight. $N_2$ was bubbled through the solution to remove excess diazomethane, and the solution was washed with $H_2O$, saturated $NaHCO_3$, and saturated NaCl. After drying over $Na_2SO_4$ and removal of the solvent under reduced pressure, the residue was recrystallized from hexane to give 13.0 g (60.3%) of product: mp 93–94 °C; $[\alpha]^{23}_D$ −60.8° (c 1.0, EtOH). Anal. C, H, N.

**(*S*)-[3-Bromo-1-(cyclohexylmethyl)-2-oxopropyl]carbamic Acid, 1,1-Dimethylethyl Ester (8)**. A solution of 10.0 g (0.034 mol) of **7** in 300 mL of $Et_2O$ was cooled to −20 °C and HBr gas was bubbled in. The mixture was then washed with 1 N citric acid, saturated $NaHCO_3$, and saturated NaCl. After drying over $Na_2SO_4$ and removal of the solvent under reduced pressure, the residue was recrystallized from hexane to give 10.2 g (86.4%) of product: mp 89–90 °C; $[\alpha]^{23}_D$ −61.6° (c 1.29, EtOH). Anal. C, H, N.

**2-[4-Cyclohexyl-3-[[(1,1-dimethylethoxy)carbonyl]-amino]-2-oxobutyl]propanedioic Acid, Bis(phenylmethyl) Ester (9)**. A suspension of 0.83 g (20.8 mmol) of NaH (60% in mineral oil) in hexane was washed free of mineral oil and then suspended in 30 mL of THF. A solution of 4.9 g (17.2 mmol) of dibenzyl malonate in 40 mL of THF was added slowly, and the solution was stirred for 1 h and then cooled to 0 °C. A solution of 6.0 g (17.2 mmol) of **8** in 20 mL of THF was then added and the mixture was stirred at 0 °C for 0.5 h and then at room temperature for 1 h. The mixture was diluted with $Et_2O$ and washed with 1 N citric acid, saturated $NaHCO_3$, then saturated NaCl. After drying over $Na_2SO_4$ and removal of the solvent under reduced pressure, the residue was chromatographed on silica gel, eluting with hexane/$Et_2O$ (3/1). There was obtained 9.0 g (94.8%) of the product as an oil. Anal. C, H, N.

**2-[4-Cyclohexyl-3-[[(1,1-dimethylethoxy)carbonyl]-amino]-2-oxobutyl]-2-(2-methylpropyl)propanedioic Acid, Bis(phenylmethyl) Ester (10)**. A suspension of 0.37 g (9.3 mmol) of NaH (60% in mineral oil) in hexane was washed free of mineral oil and then suspended in 20 mL of DMSO. To this suspension was added 5.1 g (9.2 mmol) of **9** and the mixture was stirred for 1 h. The mixture was then treated with 2.1 mL (18.2 mmol) of isobutyl iodide and stirred for 24 h. The mixture was diluted with EtOAc and washed with 1 N citric acid, saturated $NaHCO_3$, and saturated NaCl. After drying over $Na_2SO_4$ and removal of the solvent under reduced pressure, the residue was chromatographed on silica gel, eluting with hexane/$Et_2O$ (85/15). The appropriate fractions were combined with the aid of $CH_2Cl_2$ to give 5.0 g (89%) of the product: $[\alpha]^{23}_D$ −24.5° (c 1.12, EtOH). Anal. C, H, N.

**Boc-cyclohexylalaΨ[COCH$_2$]Leu (11)**. A solution of 6.3 g (0.01 mol) of **10** in 70 mL of MeOH was treated with 0.5 g of 20% Pd/C and stirred in a hydrogen atmosphere for 3 h. The mixture was filtered and the solvent was removed under reduced pressure. The residue was taken up in 50 mL of toluene and heated at reflux for 2 h. The solvent was removed under reduced pressure and the residue was recrystallized from hexane to give 2.5 g (65%) of product: mp 107–110 °C. The material was used directly in the next reaction.

**Boc-cyclohexylalaΨ[COCH$_2$]Leu-Sta-Leu-NHCH$_2$Ph (57)**. A solution of 400 mg (1.04 mmol) of **11**, 430 mg (1.04 mmol) of Sta-Leu-$NHCH_2Ph$·HCl, and 140 mg (1.04 mmol) of HOBT in 20 mL of DMF was cooled in ice and treated with 0.15 mL (1.1 mmol) of $Et_3N$, followed by 220 mg (1.06 mmol) of DCC. The

mixture was stirred at room temperature overnight and then filtered. The filtrate was diluted with EtOAc and washed with $H_2O$, saturated $NaHCO_3$, and saturated NaCl. After drying over $Na_2SO_4$, the solvent was removed under reduced pressure and the residue was chromatographed on silica gel, eluting with EtOAc/hexane (1/1). There was obtained 680 mg (88%) of the product as a white solid.

**Boc-cyclohexylalaΨ[CHOHCH$_2$]Leu-Sta-Leu-NHCH$_2$Ph (54).** A solution of 600 mg (0.8 mmol) of **57** in 20 mL of EtOH was cooled to 0 °C and 100 mg (2.6 mmol) of $NaBH_4$ was added. The mixture was allowed to warm to room temperature over 2 h and then was treated with 50% HOAc, and the solvent was evaporated under reduced pressure. The residue was taken up in EtOAc and washed with 10% $Na_2CO_3$ solution and then saturated NaCl. After drying over $Na_2SO_4$, the solvent was removed under reduced pressure and the residue was chromatographed on silica gel, eluting with $CH_2Cl_2$ and then EtOAc. There was obtained 600 mg (99.7%) of product.

**Boc-PheΨ[CH$_2$NH]Phe-OCH$_3$ (12).** To a solution of 4.5 g (0.021 mol) of Phe-$OCH_3$·HCl in 100 mL of $CH_2Cl_2$ at 5 °C was added 100 mL of 2 N $Na_2CO_3$. The mixture was shaken, and the $CH_2Cl_2$ layer was separated, dried over $MgSO_4$, and evaporated to an oil. The oil was taken up in 100 mL of toluene/$CH_2Cl_2$ (3/1), and 5.0 g (0.02 mol) of Boc-phenylalanal was added, together with 30 g of activated 3A molecular sieves. The mixture was stirred for 5 h, cooled in an ice bath, and 800 mg (0.021 mol) of $NaBH_4$ in 25 mL of MeOH was added. After 0.5 h, 2 N citric acid was added until the solution was acidic. The mixture was filtered and the solvent was removed under reduced pressure. The residue was taken up in EtOAc, dried over $MgSO_4$, and evaporated under reduced pressure to an oil. Chromatography on silica gel, eluting with EtOAc, gave 6.0 g (72.7%) of the product as an oil. Anal. C, H, N.

**Boc-PheΨ[CH$_2$NZ]Phe-OCH$_3$ (13).** A solution of 6.0 g (14.5 mmol) of **12** in 75 mL of THF and 20 mL of $H_2O$ was adjusted to pH 10 with 1 N $Na_2CO_3$. This was then treated with 2.6 g (15.3 mmol) of benzyl chloroformate and the pH was maintained at 10 by the addition of 1 N $Na_2CO_3$. After 3 h, the THF was removed under reduced pressure and the oily precipitate was taken up in EtOAc. The EtOAc was dried over $MgSO_4$ and the solvent was removed under reduced pressure, leaving 7.0 g (87%) of the product as a pale yellow oil: $[\alpha]^{25}_D$ −58.3° (c 1.0, MeOH). Anal. H, N; C: calcd, 70.21; found, 69.78.

**Boc-PheΨ[CH$_2$NZ]Phe (14).** A solution of 3.0 g (5.5 mmol) of **13** in 25 mL of MeOH was treated with 10 mL (20 mmol) of 2 N NaOH. The solution was kept at 4 °C for 12 h, then acidified with 2 N citric acid. The mixture was extracted with EtOAc, and the EtOAc was dried over $MgSO_4$ and evaporated under reduced pressure to an oil. This was chromatographed on silica gel, eluting with EtOAc and yielding 2.0 g (68%) of the product as an oil. Anal. C, H, N.

**Boc-PheΨ[CH$_2$NZ]Phe-Sta-Leu-NHCH$_2$Ph (15).** To a solution of 500 mg (1.2 mmol) of Sta-Leu-$NHCH_2Ph$·HCl in 20 mL of DMF was added 0.2 mL (1.4 mmol) of $Et_3N$ and the solution, was then treated with 630 mg (1.2 mmol) of 14, 180 mg (1.2 mmol) of HOBT, and 250 mg (1.2 mmol) of DCC. After 24 h, the mixture was filtered and the solvent was removed under reduced pressure. The residue was taken up in EtOAc and washed with $H_2O$, 1 N citric acid, $H_2O$, and 1 N $Na_2CO_3$. Drying over $MgSO_4$ and removal of the solvent under reduced pressure left an oil which was chromatographed on silica gel, eluting with $CHCl_3$/MeOH (9/1). There was obtained 1.0 g (96.4%) of the product as an oil. Anal. C, H, N.

**Boc-PheΨ[CH$_2$NH]Phe-Sta-Leu-NHCH$_2$Ph (60).** A solution of 1.0 g (1.1 mmol) of **15** in 20 mL of MeOH was treated with 0.1 g of 20% Pd/C, and $H_2$ gas was bubbled through the solution for 1 h. The catalyst was removed by filtration and the solvent was removed under reduced pressure, leaving 0.5 g (60%) of the product as a white solid.

**Boc-PheΨ[CH$_2$NOH]Phe-Sta-Leu-NHCH$_2$Ph (65).** A solution of 0.5 g (0.7 mmol) of **60** in 20 mL of $CH_2Cl_2$ was cooled to 0 °C and 0.14 g (0.7 mmol) of *m*-chloroperbenzoic acid was added and the solution was allowed to stir at room temperature for 4 h. The solvent was evaporated and the residue was taken up in EtOAc and washed with 10% NaOH and then saturated NaCl. After drying over $Na_2SO_4$, the solvent was removed under reduced pressure and the residue was chromatographed on silica gel, eluting with EtOAc/hexane (1/1). There was obtained 0.2 g (35.2%) of the product.

**(*S*)-2-Amino-3-phenyl-1-propanethiol (16).** A solution of 9.0 g of $H_2S$ in 250 mL of EtOH was cooled to −78 °C and was treated dropwise with 9.33 g (0.07 mol) of (*S*)-2-benzylaziridine.[18] The solution was allowed to warm to room temperature and the solvent was removed under reduced pressure. There was obtained 11.7 g (100%) of the product as a hygroscopic solid: $[\alpha]^{23}_D$ +97.9° (*c* 0.79, MeOH). Anal. C, H, N.

**PheΨ[CH$_2$S]Phe-*O*-*t*-Bu (17).** A solution of 3.0 g (17.9 mmol) of **16** in 300 mL of liquid $NH_3$ was treated dropwise with 5.11 g (17.9 mmol) of (*R,S*)-*tert*-butyl 2-bromo-3-phenylpropionate. After stirring overnight while allowing the $NH_3$ to evaporate, the residue was taken up in $Et_2O$, washed with 10% $Na_2CO_3$ solution, and dried over $MgSO_4$, and the solvent was removed under reduced pressure, leaving an oil. Chromatography on silica gel, eluting with $CH_2Cl_2$/MeOH (97/3) gave 5.43 g (82%) of the product as a light yellow oil. $[\alpha]^{23}_D$ +29.3° (c 0.58, MeOH). Anal. C, H, N.

**Iva-PheΨ[CH$_2$S]Phe-*O*-*t*-Bu (18).** A solution of 2.5 g (6.7 mmol) of **17** in 50 mL of $CH_2Cl_2$ was treated with 2.0 mL (14.3 mmol) of $Et_3N$ and cooled to 0 °C. This was then treated with 0.82 mL (6.7 mmol) of isovaleryl chloride and the solution left stirring overnight. The solution was washed with 10% $Na_2CO_3$, 10% citric acid, and $H_2O$. Drying over $MgSO_4$ and removal of the solvent under reduced pressure left an oil. Chromatography on silica gel, eluting with $CH_2Cl_2$ gave 1.81 g (59%) of the product as an oil: $[\alpha]^{23}_D$ +35.7° (c 0.54, MeOH). Anal. C, N, H: calcd, 8.26; found, 7.77.

**Iva-PheΨ[CH$_2$S]Phe (19).** A solution of 1.56 g (3.4 mmol) of **18** in 10 mL of trifluoroacetic acid was left standing overnight. The solvent was removed under reduced pressure and the residue was taken up in $Et_2O$. This was extracted with 10% $Na_2CO_3$ and the basic solution was washed with $Et_2O$. The solution was acidified with citric acid and extracted with EtOAc. Drying over $MgSO_4$ and removal of the solvent under reduced pressure left an oil. Chromatography on silica gel, eluting with $CH_2Cl_2$/MeOH (97/3), gave 0.63 g (57%) of the product as an oil. Anal. C, H, N.

**Iva-PheΨ[CH$_2$S]Phe-Sta-Ala-Sta-NHCH$_2$Ph (67).** A solution of 0.49 g (1.2 mmol) of **19**, 0.68 g (1.4 mmol) of Sta-Ala-Sta-$NHCH_2Ph$, 0.17 g (1.3 mmol) of HOBT, and 0.5 mL (3.6 mmol) of $Et_3N$ in 80 mL of a 5/3 mixture of $CH_2Cl_2$/DMF was cooled in ice and 0.27 g (1.3 mmol) of DCC was added. The solution was allowed to warm to room temperature overnight. The solvent was removed under reduced pressure and the residue was taken up in $CH_2Cl_2$. The *N,N'*-dicyclohexylurea was filtered off and the filtrate was washed with 10% $Na_2CO_3$ and then saturated NaCl. After drying over $MgSO_4$ and removal of the solvent under reduced pressure, the residue was chromatographed on silica gel, eluting with $CH_2Cl_2$/MeOH (95/5). There was obtained 0.63 g (59%) of the product as a white solid.

**Iva-PheΨ[CH$_2$SO$_2$]Phe-Sta-Ala-Sta-NHCH$_2$Ph (69).** A solution of 0.4 g (0.5 mmol) of **67** in 50 mL of $CHCl_3$ was treated with 0.4 g (2.0 mmol) of *m*-chloroperbenzoic acid and allowed to stir at room temperature overnight. The solution was diluted with $CHCl_3$ and washed with 10% $NaHSO_3$ and 10% $Na_2CO_3$. After drying over $MgSO_4$ and removal of the solvent under reduced pressure, the residue was chromatographed on silica gel, eluting with $CH_2Cl_2$/MeOH (95/5). There was obtained 0.18 g (44%) of the product as a white solid.

**Iva-PheΨ[CH$_2$SO]Phe (20).** A solution of 0.49 g (1.2 mmol) of **19** in 20 mL of MeOH was treated with 11 mL (5.5 mmol) of 0.5 M $NaIO_4$ and stirred at room temperature for 2 h. The solution was filtered and the solvent was removed under reduced pressure. The residue was taken up in EtOAc, washed with $Na_2SO_3$ solution, and dried over $MgSO_4$. Removal of the solvent under reduced pressure left 0.36 g (70.6%) of the product as a white solid. Anal. C, H, N.

**Biological Methods.** $IC_{50}$ values were determined by using a standard radioimmunoassay for angiotensin I (New England Nuclear, angiotensin I [$^{125}$I] radioimmunoassay kit). In this assay, human plasma containing native renin and angiotensinogen was incubated for 2 h at 37 °C. Generation of angiotensin I was linear during this incubation period. The plasma for this assay was obtained from normal volunteers. Plasma renin activity ranged

from 0.68 to 4.04 ng/AI per mL/h.

Test compounds dissolved in DMSO were added to the incubation mixture. At the concentration employed, DMSO inhibits the generation of angiotensin I by <10%. All values are reported as percent of the vehicle (DMSO) control response. The amount of angiotensin I measured was corrected for endogenous angiotensin I in the plasma.

The $IC_{50}$ values were obtained by plotting three or more inhibitor concentrations on semilog paper and estimating the concentration producing 50% inhibition.

**Chymotrypsin Stability Studies.** Stock solutions of the renin inhibitors in methanol (1 mg/mL) were prepared. A 20-μL aliquots of this solution was then added to 3 mL of 0.03 M sodium phosphate buffer/0.1 M NaCl, pH 6.9, containing 10 μg/mL bovine chymotrypsin (Sigma C-4129) and the mixture was incubated at 37 °C. At 0, 15, 45, 90, and 180 min, 0.4 mL was removed and diluted with acetonitrile. A blank in which the buffer/chymotrypsin solution was heated in boiling $H_2O$ for 30 min to inactivate the enzyme prior to addition of the renin inhibitor was also run for each inhibitor. A 100-μL aliquot of the incubation mixture was analyzed by injection onto an Alltech (C-8 5 μm Econosil 250 mm × 4.6 mm) column equilibrated with 65% acetonitrile/35% 0.1% TEA, pH 3.2. A Waters Lambda-Max LC Spectrophotometer at 214 nm was used for detection and the Spectra Physics SP4270 integrator was used for quantitation. The results are expressed as percent parent remaining (chymotrypsin treatment - blank) following incubation for 3 h.

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# Synthesis, Antiretrovirus Effects, and Phosphorylation Kinetics of 3′-Isocyano-3′-deoxythymidine and 3′-Isocyano-2′,3′-dideoxyuridine

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The silylated AzddThd **5** and AzddUrd **6** prepared from 2,3′-anhydronucleoside derivatives **3** and **4** were transformed to formamides **7** and **8** by using the sequence $RN_3 \rightarrow RN{=}P(C_6H_5) \rightarrow RNHCHO$. Formamides **7** and **8** were dehydrated to the protected 3′-isocyano derivatives **9** and **10**; deblocking gave **11** and **12**. Neither 3′-isocyano-3′-deoxythymidine (**11**) nor 3′-isocyano-2′,3′-dideoxyuridine (**12**) showed anti-HIV activity at noncytotoxic concentrations. ddThd derivative **11** was considerably more toxic to MT-4 cells than ddUrd derivative **12**; it also had a much greater affinity ($K_i$) for MT-4 cell dThd kinase than ddUrd derivative **12**. Both compounds appear to be linear mixed-type inhibitors of MT-4 cell dThd kinase.

Since the discovery of 3′-azido-3′-deoxythymidine (AZT) as an antiretroviral agent,[1] a number of structurally related nucleoside analogues have been synthesized and evaluated for their antiretroviral properties (for a review see refs 2 and 3). Recently, analogous compounds containing the electronically comparable cyano,[4b,5-7] ethinyl,[8] thiocyano,[4a,b] and isothiocyano group[4b] instead of the azido group have been synthesized.

To our knowledge, the 3′-isocyano-substituted derivatives of 3′-deoxythymidine and 2′,3′-dideoxyuridine have not been reported yet.[22] The most important difference between the chemical properties of the azido and the isocyano groups is the electrophilicity of the first and the nucleophilicity of the second.

We have recently described direct transformation of the azido group to the formamido group,[9] thus avoiding the disadvantages of the sequence $RN_3 \rightarrow RNH_2 \rightarrow RNHCHO$. With respect to the easily practicable transformation of the azido to the isocyano group,[9] we applied this functionality interchange to the protected 3′-azido-3′-deoxythymidine (AzddThd, AZT) and 3′-azido-2′,3′-dideoxyuridine (AzddUrd).

The individual steps are summarized in Scheme I. In the first step, 5′-*O*-*tert*-butyldimethylsilyl)thymidine (**1**) and the corresponding 2′-deoxyuridine derivative **2** were treated with triphenylphosphine-diethyl azodicarboxylate[10] to give cyclonucleoside derivatives **3** and **4**, respectively. By a nucleophilic opening reaction with sodium azide in DMF-$H_2O$ (9:1, v/v),[11] these compounds were transformed to the protected AzddThd derivative **5** and AzddUrd derivative **6**. Then the P-N ylides obtained by Staudinger reaction were treated with acetic formic anhydride[12] to give the intermediates **5a** and **6a**. Enols **5b** and **6b**, which were in equilibrium with imino acetates **5a** and **6a**, were transformed to the formamides **7** and **8**. The yields were about 90%. Dehydration to isocyano derivatives **9** and **10** was achieved according to the procedure of Ugi.[13] Finally, the 5′-*O*-*tert*-butyldimethylsilyl group was removed by tetrabutylammonium fluoride.[14]

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