# Novel Renin Inhibitors: Synthesis of Aminostatine and Comparison with Statine-containing Analogues

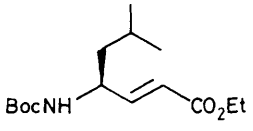
**Richard J. Arrowsmith,[a] Keith Carter,[a] John G. Dann,[b] David E. Davies,[a] C. John Harris,*[a] Judith A. Morton,[a] Philip Lister,[c] John A. Robinson,[c] and David J. Williams[d]**

[a] *Department of Medicinal Chemistry and* [b] *Department of Biochemistry, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, U.K.*
[c] *Department of Chemistry, The University, Southampton SO9 5NH, U.K.*
[d] *Department of Chemistry, Imperial College of Science and Technology, London SW7 2AY, U.K.*

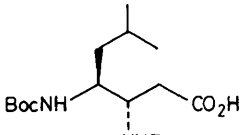
The synthesis of renin inhibitors containing a novel amino-analogue of statine is described and inhibitory potencies are compared with those of statine congeners; the crystal structure of the aminostatine derivative (**6**) is reported.

Statine, (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid, Sta, is the crucial structural unit of the potent aspartyl proteinase inhibitor, pepstatin A.[1] The Sta residue binds to these enzymes as a transition state[2] or combined-substrate[3] mimic, with the 3*S*-hydroxy group causing an entropy-favoured displacement of a water molecule which, in the native enzyme, is bound tightly between the (monoprotonated) carboxy–carboxylate catalytic locus formed by Asp-32 and Asp-215; *epi*-Sta (3*R*-hydroxy) analogues, which presumably do not displace this water molecule, bind around $10^3$ times less strongly. The logical step of incorporating Sta into substrate analogues has led to several potent inhibitors of human renin, an extremely substrate-selective aspartyl proteinase whose role in hypertension renders it an attractive target for inhibition.[4]

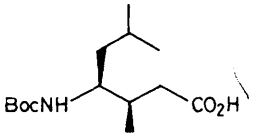
In our search for potent renin inhibitors, we reasoned that replacement of the 3*S*-hydroxy group of Sta by the nearly isosteric ammonium group might result in improved binding through ionic interactions with the carboxy–carboxylate catalytic pair additional to the favoured water displacement. This view was strengthened by recent reports suggesting that, in the native crystal of the related enzyme, penicillopepsin, an ammonium ion rather than a water molecule is bound at the active site.[5] We therefore required derivatives of aminostatine, 3,4-diamino-6-methylheptanoic acid (Asta), suitably protected for conventional incorporation into peptide sequences resembling the renin substrate. The Wittig reaction



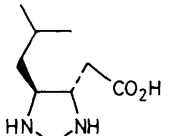
(1)



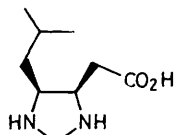
(2)



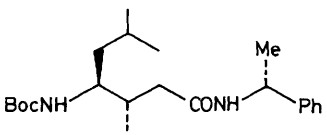
(3)



(4)



(5)



(6)

of *S*-Boc-leucinal[6] {$[\alpha]_D^{22}$ −49.8° (*c* 1, MeOH)} with ethoxycarbonylmethylenetriphenylphosphorane gave the *E*-acrylate (**1**) {81%, $[\alpha]_D^{23}$ −34.5° (*c* 1, MeOH), m.p. 56—8 °C} which underwent conjugate addition with ammonia (saturated ethanolic ammonia, 100 °C, 7 h, 84%) to afford a 1 : 1 mixture of diastereoisomeric amines. N-Protection (benzyl chloroformate) followed by chromatographic separation (silica, 1 : 1 ether–hexane fraction) and ester hydrolysis (pH 12.4, EtOH–$H_2O$) gave the required Boc·Asta(Z)·OH derivatives (**2**) {$R_f$ 0.43,† m.p. 128—130 °C, $[\alpha]_D^{22}$ −46.1° (*c* 0.5, MeOH)} and (**3**) {$R_f$ 0.38,† m.p. 203—5 °C, $[\alpha]_D^{22}$ −3.6° (*c* 0.5, MeOH)}. The stereochemistry of these isomers was determined as follows; deprotection of (**2**) (38% HBr–HOAc) and cyclisation with phosgene (KOH, $H_2O$, $COCl_2$–toluene) gave the cyclic urea (**4**) [45%, m.p. 86—88 °C (decomp.)]; similarly, (**3**) gave (**5**) [44%, m.p. 173—174 °C (decomp.)]. $^1$H N.m.r. difference nuclear Overhauser enhancement (n.O.e.) experiments showed (**4**) to be *trans*- and (**5**) to be *cis*- and thus (**2**) is (3*S*,4*S*) and (**3**) is (3*R*,4*S*). These assignments were confirmed by single-crystal *X*-ray analysis of the (*R*)-α-methylbenzylamide[7] derivative (**6**) of (**2**) as shown in Figure 1.‡

The octapeptide substrate and a modified substrate[8] sequence were chosen for initial comparison of Asta and Sta inhibitors (Table 1).§ Compounds (**7**) and (**8**) were prepared using standard polyacrylamide-based solid-phase methods, whereas (**9**)—(**12**) were prepared in solution using the acyl azide fragment-coupling procedure. The two fragment peptides, Boc·Phe·His·$NHNH_2$ and H·Sta/Asta·Leu·ABP, were obtained using conventional carbodi-imide and mixed-anhydride coupling methods respectively. As we expected, it was found that the Asta-based inhibitors have the advantage of greater aqueous solubility compared with the analogous Sta-based inhibitors.

These data reveal that, as anticipated, 3*S*-stereochemistry is preferred for both Asta and Sta inhibitors, suggesting similar overall binding modes.¶ In each case, the (*S*,*S*)-Asta analogue is of similar potency to its (*S*,*S*)-Sta congener whereas, in the less potent diastereoisomers, (*S*,*R*)-Asta confers much higher potency than (*S*,*R*)-Sta. It is known[10] that des-3-hydroxystatine and (*S*,*R*)-Sta analogues have similar potency, showing that the 3*R*-hydroxy group contributes little to binding. On this basis, we suggest that the increased binding of (*S*,*R*)-Asta compared with (*S*,*R*)-Sta is a consequence of the introduction of an additional ionic interaction as conceived in our original proposal. Similar increases in binding through the introduction of additional ionic interactions have been reported for penicillopepsin[3] and dihydrofolate reductase[11] inhibitors. The lack of increased binding for the (*S*,*S*)-Asta analogues over (*S*,*S*)-Sta congeners reflects that, unlike the (*S*,*R*) case, the ammonium interaction presumably replaces a *strong* hydroxy interaction. We speculate that the more favourable ionic interaction is balanced by the larger energy requirements for partial desolvation of the ammonium group as it displaces water and binds to the active site.

We conclude that replacement of the Leu-10 residue of renin substrate sequences with (*S*,*S*)-Asta results in novel, potent inhibitors of human renin. The potency of these inhibitors is similar to that of the corresponding (*S*,*S*)-Sta compounds while (*S*,*R*)-Asta-containing inhibitors have substantially greater potency than their (*S*,*R*)-Sta counterparts. The introduction of additional ionic binding to the catalytic functionality may have further benefit in the design of aspartyl proteinase inhibitors.

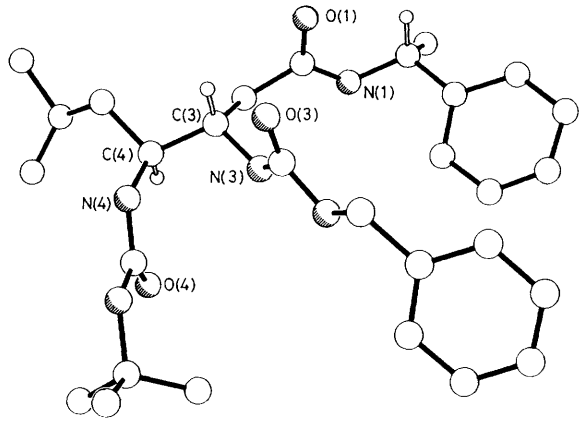



**Figure 1.** The molecular structure of (**6**). There are two discrete orientations (rotated by ~40° to each other) for the α-methylbenzylamide phenyl ring. All three amide groups are intermolecularly hydrogen-bonded to their lattice-translated counterparts: N(1) ··· O(1′) 2.97 Å, H(1) ··· O(1′) 2.01 Å, N–H ··· O angle 166°; N(4) ··· O(4′) 2.95 Å, H(4) ··· O(4′) 2.01 Å, N–H ··· O angle 160°; N(3) ··· O(3′) 2.98 Å, H(3) ··· O(3′) 2.06 Å, N–H ··· O angle 157°.

**Table 1.** Inhibition of human renin by aminostatine and statine-containing substrate analogues.

| | Inhibitor | $IC_{50}$, $10^{-6}$M |
|---|---|---|
| (**7**) | H·His·Pro·Phe·His·(*S*,*S*)Asta·Val·Ile·Phe·OH | 0.06 |
| *cf.* | Iva·His·Pro·Phe·His·(*S*,*S*)Sta·Leu·Phe·$NH_2$ | 0.03 (Ref. 9) |
| (**8**) | H·His·Pro·Phe·His·(*S*,*R*)Asta·Val·Ile·Phe·OH | 0.71 |
| *cf.* | Iva·His·Pro·Phe·His·(*S*,*R*)Sta·Leu·Phe·$NH_2$ | 14.0 (Ref. 9) |
| (**9**) | Boc·Phe·His·(*S*,*S*)Asta·Leu·ABP | 0.36 |
| (**10**) | Boc·Phe·His·(*S*,*S*)Sta·Leu·ABP | 0.19 |
| (**11**) | Boc·Phe·His·(*S*,*R*)Asta·Leu·ABP | 1.20 |
| (**12**) | Boc·Phe·His·(*S*,*R*)Sta·Leu·ABP | 43.0 |

† Silica, 8 : 1 chloroform–methanol.

‡ *Crystal data:* (**6**), $C_{29}H_{41}N_3O_5$, $M$ = 511.7, triclinic, space group $P\bar{1}$, $a$ = 5.045(1), $b$ = 11.769(5), $c$ = 13.479(4) Å, $\alpha$ = 108.02(3), $\beta$ = 98.23(2), $\gamma$ = 96.57(3)°, $U$ = 742 Å$^3$, $Z$ = 1, $D_c$ = 1.15 g cm$^{-3}$. Data were measured on a Nicolet R3m diffractometer with Cu-$K_\alpha$ radiation (graphite monochromator) using ω-scans. The structure was solved by direct methods and refined anisotropically. The amide hydrogen atoms were refined isotropically; all other hydrogen atoms were placed in calculated positions. Refinement converged to give $R$ = 0.054, $R_w$ = 0.064 for 1835 independent observed reflections [θ <58°, $|F_o| > 3\sigma(|F_o|)$]. Atomic co-ordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1, 1986.

§ Structures were confirmed by microanalysis, 200 or 360 MHz $^1$H n.m.r. and fast atom bombardment mass spectroscopy and amino acid analysis. Human renal renin was purified and inhibitors assayed as described in ref. 12; under these conditions, $IC_{50} \approx K_i$ for competitive inhibition. ABP represents the amide derived from 4-amino-1-benzylpiperidine. As there is a large discrepancy between the observed and reported[8] inhibitory potency of (**10**), lack of histidine racemisation during the coupling sequences was confirmed, in this case, by digestion with L-amino acid oxidase after peptide hydrolysis. Iva = isovaleryl.

¶ Detailed structure–activity relationships, to be published elsewhere, support this conclusion.

We thank Dr. D. Farrant for the n.O.e. studies, and Dr. C. R. Beddell, Dr. G. W. Hardy, and Mr. D. Simpkin for valuable advice and assistance.

*Received, 30th January 1986; Com. 131*

## References

1 H. Umezawa, T. Aoyagi, H. Morishima, M. Matsuzaki, M. Hamada, and T. Takeuchi, *J. Antibiotics*, 1970, **23**, 259.
2 M. P. Marciniszyn, J. A. Hartsuck, and J. J. N. Tang, *J. Biol. Chem.*, 1976, **251**, 7088; G. R. Marshall, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, 1976, **35**, 2494.
3 D. H. Rich, *J. Med. Chem.*, 1985, **28**, 263.
4 E. Haber, *J. Hypertension*, 1984, **2**, 223, and references cited therein.
5 M. N. G. James and A. R. Sielecki, *Biochemistry*, 1985, **24**, 3701.
6 J. A. Fehrentz and B. Castro, *Synthesis*, 1983, 676.
7 D. M. Jones, J. Sueiras-Diaz, M. Szelke, B. Leckie, and S. Beattie, 9th U.S. Peptide Symposium, Toronto, 1985.
8 Eur. Pat. Appl. No. 82111359.6; B. E. Evans, K. E. Rittle, M. G. Bock, C. D. Bennett, R. M. DiPardo, J. Boger, M. Poe, E. H. Ulm, B. I. LaMont, E. H. Blaine, G. M. Fanelli, I. I. Stabilito, and D. F. Veber, *J. Med. Chem.*, 1985, **28**, 1756.
9 D. F. Veber, 608th Meeting of the Biochemical Society, University of Keele, April, 1984.
10 D. H. Rich, M. S. Berncatowicz, N. S. Agarwal, M. Kawai, F. G. Salituro, and P. G. Schmidt, *Biochemistry*, 1985, **24**, 3165.
11 L. F. Kuyper, B. Roth, D. P. Baccanari, R. Ferone, C. R. Beddell, J. N. Champness, D. K. Stammers, J. G. Dann, F. E. A. Norrington, D. J. Baker, and P. J. Goodford, *J. Med. Chem.*, 1982, **25**, 1120.
12 J. G. Dann, D. K. Stammers, C. J. Harris, R. J. Arrowsmith, D. E. Davies, G. W. Hardy, and J. A. Morton, *Biophys. Biochem. Res. Commun.*, 1986, **134**, 71.