1,2,4-Triazolo[4,3-*a*]pyrazine Derivatives with Human Renin Inhibitory Activity. 2.[1] Synthesis, Biological Properties and Molecular Modeling of Hydroxyethylene Isostere Derivatives

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A series of inhibitors of human renin have been synthesized, derived from combination of a 2-(8-propyl-6-pyridin-3-yl-1,2,4-triazolo[4,3-*a*]pyrazin-3-yl)-3-pyridin-3-ylpropionic acid moiety **6c** with the hydroxyethylene isostere of the scissile amide bond (2*S*,4*S*,5*S*)-5-amino-6-cyclohexyl-4-hydroxy-2-isopropylhexanoic acid (Cha$\overset{OH}{—}$Val). The more potent members of this series showed good inhibitory activity against partially purified human renin, **7d**, for example, having an IC_{50} of 0.2 nM. Structure–activity relationships for these compounds were consistent with their binding to the S_4–S_2' sites of human renin. Analogues **7e** and **7h–k** with a variety of substituents at the C-terminus all had in vitro IC_{50}s < 1 nM. In contrast with the majority of previously reported inhibitors of similar potency, these compounds contain no natural amino acid fragments. When administered intravenously to anesthetized, sodium-depleted marmosets at doses of 0.3–3.0 mg/kg, compound **7d** caused a marked reduction in mean arterial pressure. Following oral administration at 30 mg/kg in the same animal model, **7d** again elicited a significant fall in mean arterial pressure, accompanied by suppression of plasma renin activity lasting up to 3 h after dosing.

Blockade of the enzyme renin is widely recognized as a potential alternative to inhibition of angiotensin converting enzyme (ACE) for the treatment of hypertension and congestive heart failure, with the possible advantage of fewer side effects due to the high specificity of renin compared with ACE.[2] Although many inhibitors of human renin with high in vitro potency have been described,[3] the majority are peptidic in nature. Consequently, these compounds tend to suffer from all the problems normally linked with peptides, such as poor oral absorption, proteolytic instability, short duration of action, and rapid excretion.[4] As a result, despite the efforts of many research groups during recent years,[3] the target of a long acting, orally effective inhibitor of human renin has yet to be achieved.

In an attempt to overcome the inherent problems associated with peptidic inhibitors, we sought a non-peptidic ligand which might bind in the S_4–S_2 region of human renin. As a consequence of this work, we have already reported[1] inhibitors containing known alkyl alcohol and cyclohexyl statine transition-state mimetics linked to a 2-(8-alkyl-6-aryl-1,2,4-triazolo[4,3-*a*]pyrazin-3-yl)-3-pyridin-3-ylpropionyl moiety. Structure–activity relationships for these series of compounds, for example 1 (Figure 1), were consistent with the substituted heterocyclic propionyl unit spanning the S_4–S_2 sites of the enzyme, and thus acting as a novel, non-peptidic replacement for the substrate amino-acid residues Pro-Phe-His.

The more potent of the inhibitors containing cyclohexyl statine were obtained by addition of known[6] polar P′ end groups. Thus compound 1, with an IC_{50} of 1.7 nM against human kidney renin, displayed in vitro activity comparable to the corresponding inhibitor bearing the acylated dipeptide fragment Boc-Phe-His as the P_4–P_2 residue.[6] On intravenous dosing in anesthetized, sodium-depleted marmosets at 1–3 mg/kg, compound 1 showed good activity and duration of action, but after oral administration at 50 mg/kg no significant effects on blood pressure or plasma renin activity were seen. By extrapolation from pharmacokinetic experiments in rats, the absence of oral activity could be attributed to a total lack of oral absorption.

During the course of this work, oral activity was disclosed for renin inhibitors with the scissile amide bond of the peptide fragment Leu-Val replaced by a hydroxyethylene isostere. U-71038,[7] based on the dipeptide mimetic Leu$\overset{OH}{—}$Val,[5] and CGP 38 560 A,[8] incorporating the analogous mimetic Cha$\overset{OH}{—}$Val,[5] both reduced blood pressure after oral administration in renin-infused rats and sodium-depleted marmosets, respectively, albeit at doses significantly higher than would be predictive of clinically useful activity in man.[9] In seeking orally active inhibitors,

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(2) Foltmann, B.; Pedersen, V. B.; In *Acid Proteases, Structure, Function and Biology*; Tang, J., Ed.; Plenum: New York, 1977; pp 3–22.

(3) Greenlee, W. *J. Pharm. Res.* **1987**, *4*, 364; *Med. Res. Rev.* **1990**, *10*, 173.

(4) Boger, J.; Bennett, C. D.; Payne, L. S.; Ulm, E. H.; Blaine, E. H.; Homnick, C. F.; Schorn, T. W.; Lamont, B. I.; Veber, D. F. *Regul. Peptides* **1985**, *12* (*Supplement 4*), 8.

(5) ACHPA = (3*S*,4*S*)-4-amino-5-cyclohexyl-3-hydroxypentanoic acid; Leu$\overset{OH}{—}$Val = (2*S*,4*S*,5*S*)-5-amino-4-hydroxy-2-isopropyl-7-methyloctanoic acid; Cha$\overset{OH}{—}$Val = (2*S*,4*S*,5*S*)-5-amino-6-cyclohexyl-4-hydroxy-2-isopropylhexanoic acid; Cha$\overset{OH}{—}$(*R*)Val = (2*R*,4*S*,5*S*)-5-amino-6-cyclohexyl-4-hydroxy-2-isopropylhexanoic acid; Cha$\overset{Lac}{—}$Val = γ-lactone derived from Cha$\overset{OH}{—}$Val.

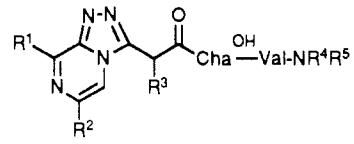
(6) Bock, M. G.; Dipardo, R. M.; Evans, B. E.; Freidinger, R. M.; Rittle, K. E.; Payne, L. S.; Boger, J.; Whitter, W. L.; Lamont, B. I.; Ulm, E. H.; Blaine, E. H.; Schorn, T. W.; Veber, D. F. *J. Med. Chem.* **1988**, *31*, 1918.

(7) (a) Thaisrivongs, S.; Pals, D. T.; Harris, D. W.; Kati, W. M.; Turner, S. R. *J. Med. Chem.* **1986**, *29*, 2088. (b) Pals, D. T.; Thaisrivongs, S.; Lawson, J. A.; Kati, W. M.; Turner, S. R.; DeGraaf, G. L.; Harris, D. W.; Johnson, G. A. *Hypertension* **1986**, *8*, 1105.

(8) Buhlmeyer, P.; Caselli, A.; Fuhrer, W.; Goschke, R.; Rasetti, V.; Rueger, H.; Stanton, J. L.; Criscione, L.; Wood, J. M. *J. Med. Chem.* **1988**, *31*, 1839.

0022-2623/90/1833-2335$02.50/0

Table I. Characterization and in Vitro Human Renin Inhibition of Compounds **7a–n**



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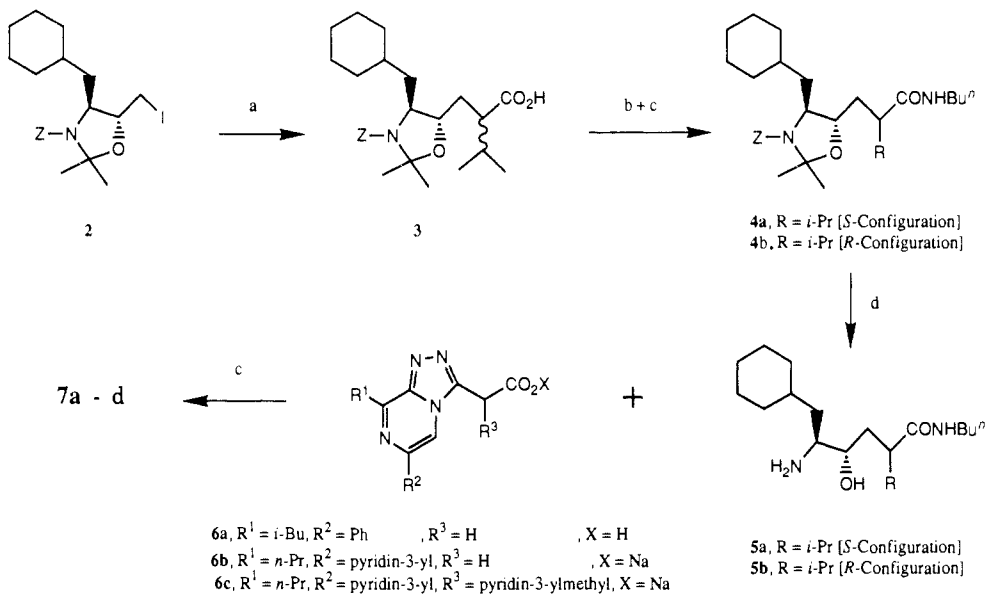
no.	R^1	R^2	R^3[a]	R^4	R^5	mp, °C	formula[b]	human renin, IC_{50}, nM[c]
a	*i*-Bu	Ph	H	*n*-Bu	H	205–209	$C_{36}H_{54}N_6O_3 \cdot 0.5H_2O$	30
b	*n*-Pr	pyridin-3-yl	H	*n*-Bu	H	246–248	$C_{34}H_{51}N_7O_3$	2.7
c[d]	*n*-Pr	pyridin-3-yl	H	*n*-Bu	H	99–102	$C_{34}H_{51}N_7O_3 \cdot H_2O$	26
d	*n*-Pr	pyridin-3-yl	pyridin-3-ylmethyl	*n*-Bu	H	134–137	$C_{40}H_{56}N_8O_3 \cdot H_2O$	0.2
e	*n*-Pr	pyridin-3-yl	pyridin-3-ylmethyl (more polar epimer)	$(CH_2)_2N(CH_3)_2$	H	118–120	$C_{40}H_{57}N_9O_3 \cdot 1.5H_2O$	0.3
f	*n*-Pr	pyridin-3-yl	pyridin-3-ylmethyl (less polar epimer)	$(CH_2)_2N(CH_3)_2$	H	130–134	$C_{40}H_{57}N_9O_3 \cdot H_2O$[e]	8.6
g	*n*-Pr	pyridin-3-yl	pyridin-3-ylmethyl	$(CH_2)_2NH_2$	H	123–128	$C_{38}H_{53}N_9O_3 \cdot 2.5H_2O$[f]	78
h	*n*-Pr	pyridin-3-yl	pyridin-3-ylmethyl	*N*-Morpholino ethyl	H	122–126	$C_{42}H_{54}N_9O_4 \cdot H_2O$	0.4
i	*n*-Pr	pyridin-3-yl	pyridin-3-ylmethyl	*N*-Piperazino ethyl	H	113–117	$C_{42}H_{60}N_{10}O_3 \cdot 2H_2O$	0.6
j	*n*-Pr	pyridin-3-yl	pyridin-3-ylmethyl	pyridin-2-ylmethyl	H	114–118	$C_{42}H_{53}N_9O_3 \cdot 2H_2O$[g]	0.6
k	*n*-Pr	pyridin-3-yl	pyridin-3-ylmethyl	$(CH_2)_4OH$	H	164–170	$C_{40}H_{56}N_8O_4 \cdot H_2O$	0.3
l	*n*-Pr	pyridin-3-yl	pyridin-3-ylmethyl	*n*-Bu	CH_3	100–103	$C_{41}H_{58}N_8O_4 \cdot H_2O$	41
m	*n*-Pr	pyridin-3-yl	pyridin-3-ylmethyl	$(CH_2)_2N(CH_3)_2$	CH_3	97–99	$C_{41}H_{59}N_9O_3 \cdot H_2O$	212
n	*n*-Pr	pyridin-3-yl	pyridin-3-ylmethyl	$(CH_2CH_2)_2O$		108–110	$C_{40}H_{54}N_8O_4 \cdot H_2O$	64

[a] Mixture of diastereoisomers at center bearing substituent R^3 unless otherwise stated. [b] Analyses for C, H, N were correct within ±0.4% unless otherwise stated. [c] Test details are described in ref 1. [d] Contains Cha$\overline{\text{OH}}$(*R*)Val.[5] [e] C, N; H: calcd, 8.1; found 7.6. [f] C, N; H: calcd, 8.0; found 7.5. [g] C, H; N: calcd, 16.4; found 15.7.

Scheme I[a]



[a] (a) *i*-PrCH(Li)CO_2Li/HMPA/THF; (b) *n*-BuNH_2; (c) HOBT/HCl·$Me_2N(CH_2)_3N$=C=NEt/DMF; (d) NH_4O_2CH/Pd/C/EtOH/H_2O.

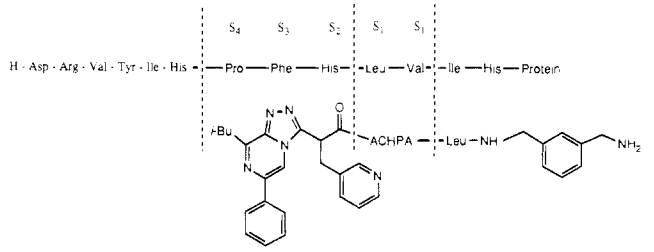


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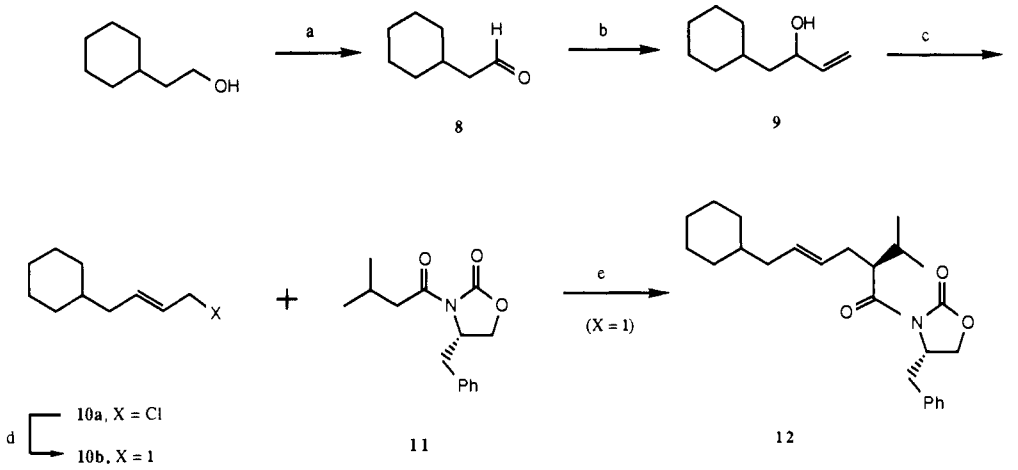
Figure 1. Human angiotensinogen and the structure of inhibitor 1 (see ref 5 for definition of ACHPA).

we therefore chose to prepare compounds with our non-peptidic P_4–P_2 replacement linked to the dipeptide mimetic Cha$\overline{\text{OH}}$Val. We hypothesized that high molecular weight could be a key factor in the lack of oral absorption of compounds such as **1** (MW 829), and were encouraged that this approach might permit significant reduction of this parameter. The synthesis and biological evaluation of the resulting non-peptidic inhibitors are presented here, together with details of their proposed binding mode to a molecular model of human renin.

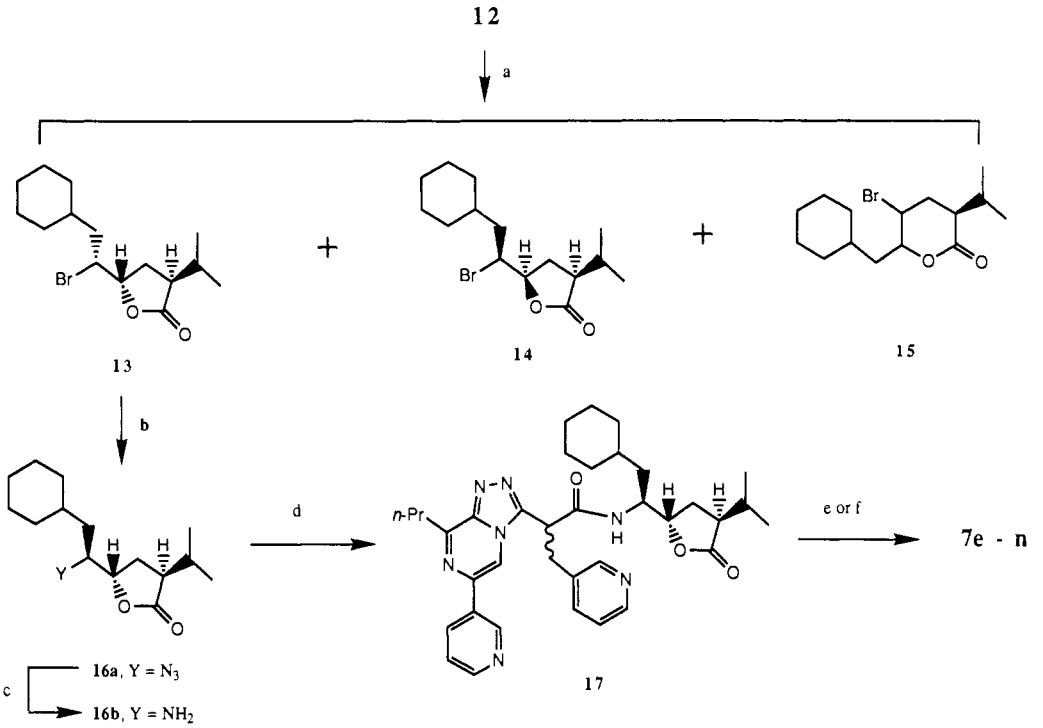
(9) Pharmacological evaluation of CGS 38 563 A in normal volunteers has recently been reported, indicating oral bioavailability < 1%; (a) Nussberger, J.; Delabays, A.; DeGasparo, M.; Cumin, F.; Waeber, B.; Brunner, H. R.; Menard, J. *Hypertension* **1989**, *13*, 948. (b) DeGasparo, M.; Cumin, F.; Nussberger, J.; Guyenne, T. T.; Wood, J. M.; Menard, J. *Br. J. Clin. Pharmacol.* **1989**, *27*, 587.

Scheme II[a]



[a] (a) $P_2O_5/Et_3N/DMSO/CH_2Cl_2/0$–20 °C; (b) CH_2=CHMgBr/THF/−15 °C; (c) $SOCl_2/Et_2O$; (d) NaI/Me_2CO; (e) LDA/THF/0 °C.

Scheme III[a]



[a] (a) NBS/H_2O/DME/0–20 °C; (b) NaN_3/DMPU; (c) H_2/Pd/C/EtOH; (d) **6c**/HOBT/HCl·$Me_2N(CH_2)_3N$=C=NEt/DMF; (e) R^4NH_2/Heat; (f) $R^4R^5NH/Me_2AlCl/CH_2Cl_2$/Reflux.

Chemistry

The compounds **7a–n** prepared during the course of this program are listed in Table I. With efficient routes in hand to the carboxylic acids corresponding to the P_4–P_2 replacement,[1] the key synthetic problem became synthesis of the dipeptide mimetic Cha$\overset{OH}{—}$Val. It is well established[8,10] that for effective binding of inhibitors the *S* configuration is required at each of the three stereocenters present in this type of isostere. Initially for preparation of compounds **7a–d** a modification of published work[8] was adopted as shown in Scheme I. Like the majority of syntheses of hydroxyethylene isosteres,[11] this approach suffers from shortcomings in stereochemical control, and in order to obtain the wider range of analogs **7e–n**, we developed[12] the more efficient route outlined in Schemes II and III.

Displacement of chiral iodide **2**, readily obtained[8] in three steps from Z-cyclohexylalaninal, with the dianion of

(10) Evans, B. E.; Rittle, K. E.; Ulm, E. H.; Veber, D. F.; Springer, J. P.; Poe, M. In Peptides, Structures and Function. *Proceedings of the Eighth American Peptide Symposium*; Hruby, V. J., Rich, D. H., Eds.; Pierce Chemical Co.: Rockford, IL, 1983; pp 579–582.

(11) (a) Herold, P.; Duthaler, R.; Rihs, G.; Angst, C. *J. Org. Chem.* **1989**, *54*, 1178 and references therein. (b) Wuts, P. G. M.; Putt, S. R.; Ritter, A. R. *J. Org. Chem.* **1988**, *53*, 4503. (c) Chakravarty, P. K.; DeLaslo, S. E.; Sarnella, C. S.; Springer, J. P.; Schuda, P. F. *Tetrahedron Lett.* **1989**, *30*, 415. (d) Shiozaki, M.; Hata, T.; Furukawa, Y. *Tetrahedron Lett.* **1989**, *30*, 3669.

(12) Preliminary communication: Bradbury, R. H.; Revill, J. M.; Rivett, J. E.; Waterson, D. *Tetrahedron Lett.* **1989**, *30*, 3845.

isovaleric acid gave a 1:1 mixture of diastereoisomeric carboxylic acids **3** (Scheme I). For characterization purposes the diastereoisomers of **3**[8] were separated, but on a preparative scale it was more convenient to couple crude **3** with *n*-butylamine and then to separate the desired diastereoisomeric amides **4a**[8] and **4b**. In our hands, use of dianion chemistry[13] proved more reliable than the reported[8] sequence involving reaction of **2** with the enolate of methyl isovalerate and subsequent ester hydrolysis. Deprotection of **4a** and **4b** gave amino alcohols **5a**[8] and **5b**, which were then converted to **7a–d** by carbodiimide coupling with carboxylic acids **6a–c**,[1] by using either the free acid or the corresponding sodium salt.

Participation of chiral acyloxazolidinone **12** in a stereoselective bromolactonization reaction, giving **13**, is the key step in the route summarized in Schemes II and III.[12] A related approach has recently been reported[11a] involving halolactonization of the corresponding *N,N*-dimethyl amide. The precursor **12** was prepared (Scheme II) in 73% yield by alkylation of the lithium enolate of acyloxazolidinone **11**,[14] derived from (*S*)-(−)-4-benzyloxazolidinone, with the allyl iodide **10b**, readily obtained on a large scale in four steps from 2-cyclohexylethanol. As expected[15] greater than 99% diastereoselectivity was seen for induction of the required *S* stereochemistry at the newly formed asymmetric center. Treatment of **12** with *N*-bromosuccinimide in aqueous dimethoxyethane gave (Scheme III) directly in 67% yield after chromatography an 82:18 mixture of trans γ-lactone **13**,[11a] and a regioisomeric δ-lactone **15**,[11a] of undetermined configuration, from which the desired γ-lactone **13**, enriched to ca. 95% purity, could be obtained by a single recrystallization from hexane. By analysis of appropriate chromatographic fractions, formation of a minor amount of the diastereoisomeric *cis*-γ-lactone **14**[11a] could also be inferred. Bromolactonization of substrate **12** thus shows regio- and stereoselectivity similar to that reported previously for α-substituted γ,δ-unsaturated amides.[11a,16]

Bromolactone **13** was readily converted to aminolactone **16b**[11d] corresponding to the dipeptide isostere Cha$\overset{OH}{—}$Val in two high yielding steps via azidolactone **16a**.[11a] As detailed in our preliminary communication,[12] an X-ray crystal structure determination confirmed the relative stereochemistry expected for **16b**. Intermediate **17**, which proved a versatile precursor for C-terminal variation of the dipeptide isostere, was obtained as a mixture of diastereoisomers by carbodiimide-mediated acylation of **16b** with **6c**. On heating with excess *n*-butylamine, **17** underwent clean opening of the lactone ring to give compound **7d**, identical in all respects with material obtained by the previous route. Similarly, reaction of **17** with the appropriate primary amines provided **7e–k**. For the preparation of **7l–n** lactone cleavage by secondary amines was effected in the presence of dimethylaluminum chloride.[17] Except for **7e** and **7f**, compounds **7d–n** were all obtained as diastereoisomeric mixtures at the asymmetric center α to the 1,2,4-triazolo[4,3-*a*]pyrazine ring.

(13) (a) Pfeffer, P. E.; Silbert, L. S.; Chirinko, J. M. *J. Org. Chem.* **1972**, *37*, 451. (b) Petragnani, N.; Yonashiro, M. *Synthesis* **1982**, 521.
(14) Evans, D. A.; Britton, T. C.; Darrow, R. L.; Dellaria, J. F. *Tetrahedron* **1988**, *44*, 5525.
(15) Evans, D. A. *Aldrichimica Acta* **1982**, *15*, 23.
(16) Tamaru, Y.; Mizuzani, M.; Furukawa, Y.; Kawamura, S.; Yoshida, Z.; Yanagi, K.; Minobe, S. *J. Am. Chem. Soc.* **1984**, *106*, 1079.
(17) Barrett, A. G. M.; Dhanak, D. *Tetrahedron Lett.* **1987**, *28*, 3327.

In Vitro Renin Inhibition

The reported[8] inhibitors based on the dipeptide mimetic Cha$\overset{OH}{—}$Val incorporated an *n*-butylamide at the C-terminus, and the initial compounds **7a–d** (Table I) therefore included this C-terminal group. In addition, our previous structure–activity investigations[1] had shown either a 6-phenyl or a 6-pyridin-3-yl substituted 1,2,4-triazolo[4,3-*a*]pyrazine to be optimum for renin inhibitory potency. Taking these data together, we first prepared compound **7a**, which was ca. 30-fold more potent than the equivalent cyclohexyl statine analogue (data not shown), the increased binding being presumably due to occupation of the P_1' site by the *S*-isopropyl substituent. Similarly to our previous alkyl alcohol derived inhibitors,[1] replacement of the 6-substituent by pyridin-3-yl (**7b**) provided a further 10-fold enhancement in activity. With this analogue we had achieved a significant decrease in molecular weight compared with compounds of similar affinity from our earlier work (e.g. MW 605 for **7b** vs 829 for **1**). In accord with the proposed binding mode for these inhibitors, the *R*-isopropyl analogue **7c** was significantly less potent. Like the earlier series,[1] substitution of the methylene group α to the 1,2,4-triazolo[4,3-*a*]pyrazine heterocycle by pyridin-3-ylmethyl (**7d**) resulted in a further gain in activity, consistent with occupation of the S_2 site by the α-substituent. In vitro, compound **7d** is approximately equiactive with inhibitors such as CGP 38 560 A,[8] but, in contrast, **7d** contains no natural amino acid residues.

As described later, **7d** has poor oral bioavailability, which might be improved by modifying the physical properties of the inhibitor. Compounds **7e–k** were prepared to probe the effect of polar groups at the C-terminus of the dipeptide mimetic on in vitro activity, physical properties, and oral bioavailability. Incorporation of tertiary amine (**7e**), morpholine (**7h**), piperazine (**7i**), pyridine (**7j**), and alcohol (**7k**) functionality all provided potent compounds without compromising intrinsic affinity. However, the primary amine **7g** analogous to **7e** showed significantly reduced potency. In terms of enzyme interactions, these hydrophilic groups could either be occupying the S_2' site or protruding beyond the active site cleft into solvent.

Compound **7e** was ca. 30-fold more potent than **7f**, the corresponding less polar diastereoisomer at the asymmetric center α to the 1,2,4-triazolo[4,3-*a*]pyrazine heterocycle. In common with our previous series of inhibitors,[1] we hypothesize that the absolute configuration at this asymmetric center must be *S* as in the natural substrate, although again we have no definitive proof of this assignment. By analogy we assume the activity of **7d** and **7g–n** to reside mainly with the corresponding *S* diastereoisomers.

Finally, alkylation of the C-terminal amide nitrogen atom (**7l–n**) typically resulted in >100-fold loss of activity, presumably reflecting removal of a favorable interaction with a hydrogen bond acceptor in the enzyme.

Molecular Modeling Studies

The possible binding modes of these novel renin inhibitors were explored, utilizing the coordinates for a human renin model[18,19] which was based on the structure of another aspartyl proteinase, endothiapepsin. Figure 2 shows a stereopair diagram of the binding-site region of this model complexed with compound **7a**. The proposed binding mode is derived from comparison of published

(18) Sibanda, B. L.; Blundell, T. L.; Hobart, P. M.; Fogliano, M.; Bindra, J. S.; Dominy, B. W.; Chirgwin, J. M. *FEBS Lett.* **1984**, *174*, 102.
(19) Coordinates kindly supplied by Professor T. L. Blundell.

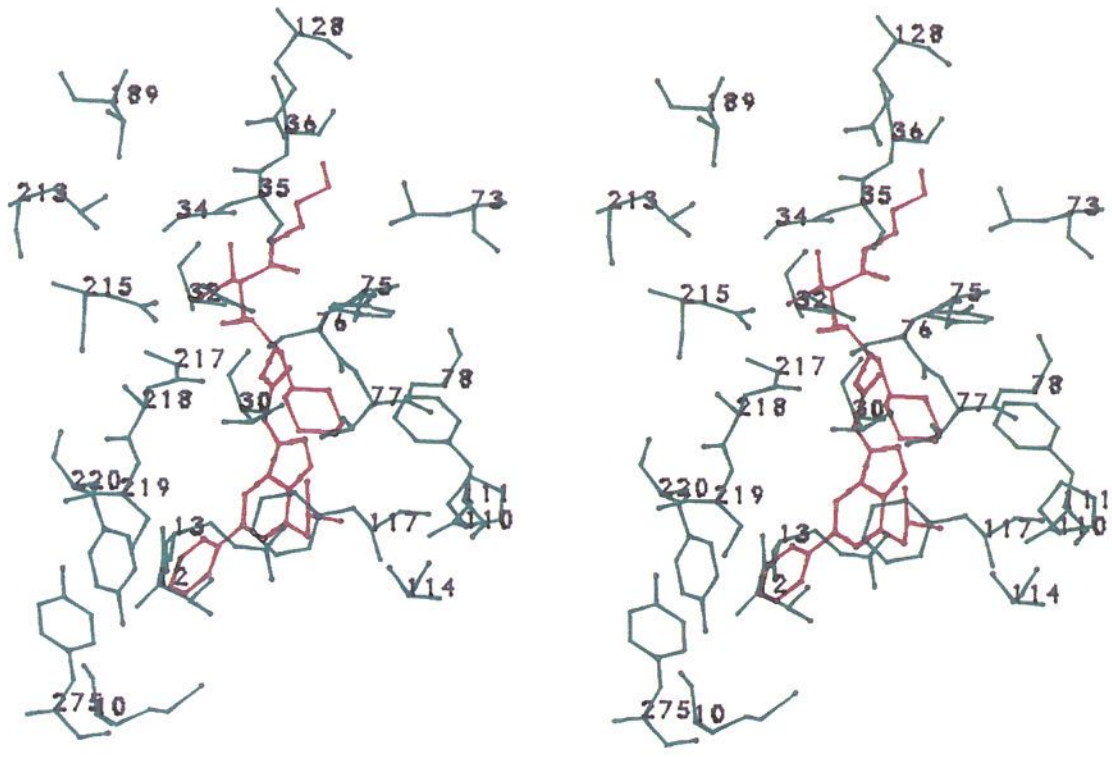


Figure 2. Stereopair diagram of proposed binding site region of human renin model (colored green) complexed with inhibitor **7a** (colored red).

aspartyl proteinase/inhibitor complexes[20-22] and of the complex[19] between endothiapepsin and the peptide inhibitor, H142, which led to the conclusion that certain key intermolecular interactions should be satisfied around the active aspartates. These were, first, the interaction of the hydroxyl group with Asp 32 and Asp 215 (pepsin numbering); second, the P_2–P_1 amide carbonyl function with the main chain NHs of residues 76 and 77 in the "flap"; and third, the NH of the same amide with the main chain carbonyl of Gly 217. In addition, the cyclohexyl group was allowed to relax into the S_1 site. With these interactions as anchor points, the two torsion angles prior to the P_2–P_1 amide group would then position the 1,2,4-triazolo[4,3-*a*]pyrazine moiety.

A subset of the atoms in the human renin model was used in this study. These atoms defined 43 residues in the active site. Hydrogens were added to all heteroatoms in this subset. Carbon atoms in the protein were treated as united atoms where appropriate. Kollman's charges[23] were used for the protein and an in-house polarized Del Re method[24] was used for calculating charges for the inhibitor. A simple force field (Lennard-Jones, Coulombic, 10–12 hydrogen bond) was used to describe the interactions. To explore the space available to the 1,2,4-triazolo[4,3-*a*]-pyrazine group, the two torsions indicated above were driven through 60° increments, keeping the amide fixed. At each point, certain torsion angles in both the inhibitor molecule and the protein were optimized in the presence of the protein active site. These angles were those being driven, the four torsions of the isobutyl group in the inhibitor, and the two torsion angles for the side chains of both Ser 76 and Thr 77 in the protein. There was no rigid body motion of the inhibitor relative to the protein. The optimizations were performed with use of an in-house program, SCANOPT,[25] on a VAX 11/785.

This approach yielded a single binding mode. The energy of the ligand conformation in isolation was not at a minimum, and small manual adjustments of some ligand torsion angles together with a slight rigid body motion were performed which produced an improved interaction. In the resulting complex (Figure 2), it was encouraging that the powerful hydrogen bond accepting nitrogens of the fused triazole ring had found an interaction with the side chain of Thr 77. Also of note was the orientation of the pendant 6-phenyl group towards the hydrophobic S_4 pocket consisting of Met 10, Tyr 220, and Tyr 275. We believed that a suitable analogue might be able to pick up a hydrogen bond interaction with the side chain of Thr 12, and, as mentioned earlier, compound **7b** with a 6-pyridin-3-yl substituent instead of the phenyl group yielded improved activity. In this model the role of the 8-alkyl substituent was less well-defined, the indication being that it is directed towards the S_3 site. However, by analogy with published[19-22] X-ray structures of complexes between aspartyl proteases and peptidic inhibitors, the model did reinforce the suggestion that substitution at the methylene adjacent to the P_2–P_1 amide might lead to occupancy of the S_2 site. As discussed previously, comparison of the activities of compounds **7b** and **7d** where a pyridin-3-ylmethyl substitution was made at this position reveals a significant increase in affinity.

The model was also used for rationalizing other structure activity data. For example, by comparing compounds **7d** and **7l** the 200-fold decrease in binding that is observed when the hydrogen atom of the C-terminal amide is replaced by a methyl group can be directly attributed to the loss of a critical hydrogen bond interaction with the main chain carbonyl of Gly 34, which is thought to play a key role in aligning the substrate during the catalytic cycle.

Pharmacological Evaluation

All of the compounds except **7c** and **7f** listed in Table I were evaluated for hypotensive efficacy in anesthetized, sodium-depleted marmosets. Typical responses for the more potent analogues following a bolus intravenous administration of 3 mg/kg are illustrated in Figure 3 for **7b**, **7d**, and **7e**, using a supra-maximal dose of captopril as an

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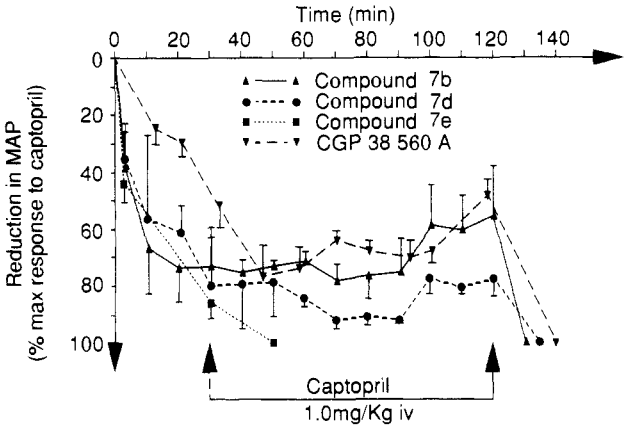

Figure 3. Effects of compounds **7b**, **7d**, **7e**, and CGP 38 560 A after intravenous dosing at 3 mg/kg to anesthetized, sodium-depleted marmosets. Effects on mean arterial pressure (MAP) are expressed as a percentage of the maximum response to captopril 1 mg/kg, dosed intravenously 30 min after compound **7e** and 120 min after compounds **7b**,**d**, and CGP 38 560 A. Mean ± SE values are shown (n = 3).

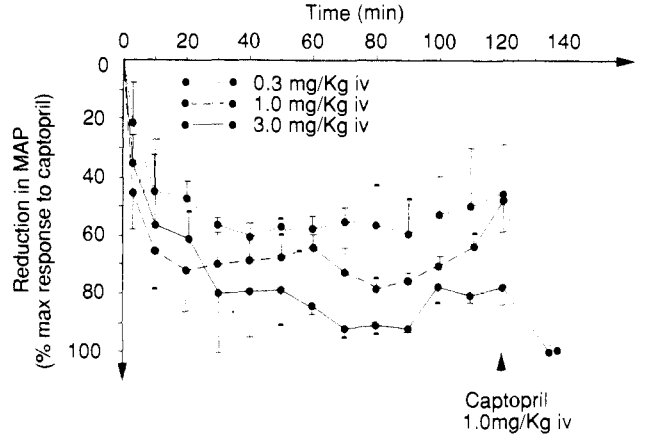

Figure 4. Effects of compound **7d** after intravenous dosing to anesthetized, sodium-depleted marmosets. Effects on mean arterial pressure (MAP) are expressed as a percentage of the maximum response to captopril 1 mg/kg, dosed intravenously 120 min after administration of the compound. Mean ± SE values are shown (n = 3).

internal standard. The fall in mean arterial pressure (MAP) produced by captopril in these experiments was 34.4 ± 4.6 mm. For comparison the hypotensive activity in this model of the recently described[8] CGP 38 560 A is also included. All three compounds **7b**, **7d**, and **7e** caused significant falls in MAP of similar magnitude to that produced by CGP 38 560 A, and in each case the effect was maintained for the duration of the experiment. Compound **7d** was also studied at lower doses as shown in Figure 4, and even at 0.3 mg/kg a marked lowering of MAP was still seen.

When administered orally in the same animal model at a dose of 30 mg/kg, **7d** elicited a fall in MAP comparable to that produced by CGP 38 560 A (Figure 5). The significance of this result was confirmed by the suppression of plasma renin activity (PRA) (Figure 6) observed during the 3-h time course of the experiment, a similar inhibition of PRA having been reported[8] for CGP 38 560 A. Compound **7d** thus demonstrates oral activity in the marmoset, but from a crude comparison of oral and intravenous efficacy, the oral bioavailability of **7d** is likely to be below that needed for a clinically useful agent. By extrapolation from a measured value for an appropriately substituted 1,2,4-triazolo[4,3-a]pyrazine fragment, **7d** has a calculated

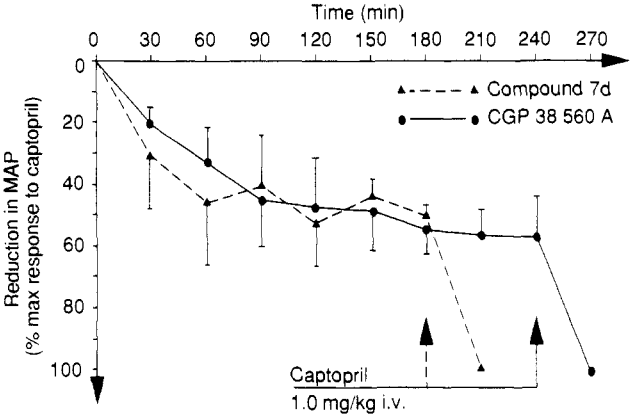

Figure 5. Effects of compound **7d** and CGP 38 560 A after oral dosing at 30 mg/kg to anesthetized, sodium-depleted marmosets. Effects on mean arterial pressure (MAP) are expressed as a percentage of the maximum response to captopril 1 mg/kg, dosed intravenously 180 min after compound **7d** and 240 min after CGP 38 560 A. Mean ± SE values are shown (n = 3).

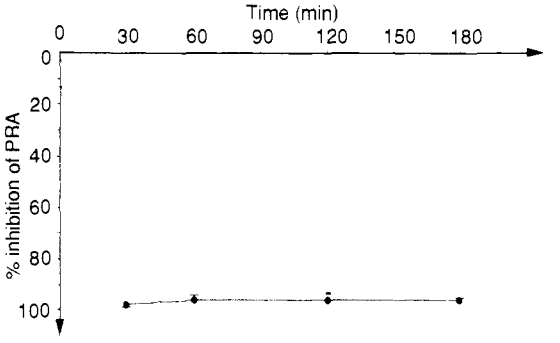

Figure 6. Effects of compound **7d** after oral dosing at 30 mg/kg to anesthetized, sodium-depleted marmosets. Effects on plasma renin activity (PRA) are expressed as a percentage of starting values. Mean ± SE values are shown (n = 3).

log D greater than 6 (octanol/water). Consequently, the compound has poor aqueous solubility (ca. 15 μg/mL at pH 7.4), which is unlikely to be optimal for oral absorption.

In order to investigate the effects on oral bioavailability of varying the physical properties of the inhibitors, the more hydrophilic analogues listed in Table I were evaluated. While most of these compounds retained good activity on intravenous dosing, no advance over **7d** was seen following oral administration at 30 mg/kg (data not shown). As discussed in our previous paper,[1] we again believe these results to reflect poor oral absorption, rather than metabolic instability or rapid biliary clearance. Despite a modest reduction in molecular weight compared with our earlier compounds,[1] for example 696 for **7d** versus 829 for **1**, we suspect this parameter to remain a key factor mitigating against oral absorption. The synthesis and pharmacology of analogues with lower molecular weight, and compounds containing alternative bicyclic heterocycles in place of the 1,2,4-triazolo[4,3-a]pyrazine moiety, will be reported in subsequent publications.

Summary

This paper describes novel inhibitors of human renin with in vitro IC_{50}s approaching 0.1 nM, derived from combination of an appropriately substituted 1,2,4-triazolo[4,3-a]pyrazin-3-ylpropionic acid with a hydroxyethylene isostere of the scissile amide bond. Structure–activity relationships for this series of compounds are consistent with the substituted 1,2,4-triazolo[4,3-a]-pyrazin-3-ylpropionyl moiety acting as a non-peptidic re-

placement for the P_4–P_2 residues of the substrate. Analogues with a wide variety of substituents at the C-terminus of the hydroxyethylene isostere were found to maintain in vitro activity. In contrast with the majority of previously reported inhibitors of similar potency, these compounds contain no natural amino-acid fragments. When administered intravenously to anesthetized, sodium-depleted marmosets low doses of the compounds caused marked reductions in mean arterial pressure. Oral activity was also seen in the same animal model at doses of 30 mg/kg.

Experimental Section

All operations were carried out at ambient temperature unless otherwise stated. Tetrahydrofuran (THF) and ether were dried by distillation from calcium hydride. CH_2Cl_2 was dried by distillation from P_2O_5. All evaporations were carried out at below 50 °C by using a Buchi rotary evaporator. Flash chromatography was performed on silica (Merck Kieselgel: Art. 9385). Melting points were taken on a Buchi apparatus with use of glass capillary tubes and are uncorrected. ^{1}H NMR spectra were recorded on Bruker WM200, WM250, or WM400 instruments and are reported as δ values (parts per million) relative to Me_4Si as an internal standard. Chemical-ionization mass spectra (CIMS) were recorded on a VG 12-12 quadrapole or a VG 70-250 SE spectrometer. Positive fast-atom bombardment mass spectra (FABMS) were determined on a VG ZAB 2-SE or a VG modified AE1/Kratos MS9 spectrometer. IR spectra were determined on a Perkin-Elmer 1420 spectrophotometer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. The experimental procedures for the biological tests have been described previously.[1]

***N*-Z-*N*,*O*-isopropylidene-Cha$\overset{OH}{—}$Val-NH$(CH_2)_3CH_3$ (4a) and *N*-Z-*N*,*O*-isopropylidene-Cha$\overset{OH}{—}$Val(*R*)Val-NH$(CH_2)_3CH_3$ (4b).** A 1.6 M solution of butyllithium in hexane (45 mL, 72.0 mmol) was added to a stirred solution of diisopropylamine (7.27 g, 72.0 mmol) in dry THF (60 mL) at 0 °C under an atmosphere of argon. The temperature was maintained at 0 °C for 0.5 h, and then a solution of isovaleric acid (3.67 g, 36.0 mmol) in THF (30 mL) was added. The solution was heated at 35 °C for 30 min and then cooled to ambient temperature. Hexamethylphosphoramide (HMPA) (6.44 g, 36.0 mmol) was added, followed by a solution of **2**[8] (5.65 g, 12.0 mmol) in THF (30 mL). The solution was stirred for 2 h and then added to saturated NH_4Cl (300 mL). The mixture was extracted with EtOAc (3 × 100 mL), and the combined extracts were washed with water (100 mL) and saturated brine (100 mL) and then dried ($MgSO_4$). The solvent was removed by evaporation to give crude carboxylic acid **3** (5.29 g, 99%), as a clear oil, which was used without further purification.

Crude **3** (5.29 g, 11.9 mmol) was dissolved in *N*,*N*-dimethylformamide (DMF) (50 mL) containing triethylamine (1.20 g, 11.9 mmol). *n*-Butylamine (0.87 g, 11.9 mmol), 1-hydroxybenzotriazole hydrate (HOBT) (1.61 g, 11.9 mmol), and 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC) (2.28 g, 11.9 mmol) were added, and the solution was allowed to stand overnight. The solvent was removed in vacuo and the residue partitioned between EtOAc (100 mL) and water (100 mL). The organic layer was separated and washed with saturated $NaHCO_3$ (100 mL), followed by water (100 mL) and saturated brine (100 mL). The organic phase was dried ($MgSO_4$), and the solvent was removed by evaporation. The residue was purified by flash chromatography, eluting with EtOAc/hexane (1:4 v/v), to give initially **4a** (1.37 g, 23% from **2**) as a clear oil with spectral data in agreement with the published values.[8] Further elution of the chromatography column provided **4b** (1.51 g, 25% from **2**), as an oil which solidified on standing: mp 94–96 °C (from hexane); ^{1}H NMR ($CDCl_3$) 0.9 (m, 9 H), 1.0–2.1 (complex m, 27 H), 3.25 (m, 2 H), 3.75 (m, 1 H), 3.9 (m, 1 H), 5.1 (dd, 2 H), 5.4 (br, 1 H), 7.4 (m, 5 H); CIMS *m*/*e* 501 $(M + H)^+$, 485, 443. Anal. ($C_{30}H_{48}N_2O_4$) C, H, N.

Cha$\overset{OH}{—}$Val-$(CH_2)_3CH_3$ (5a). Ammonium formate (250 mg, 4.0 mmol) was added to a mixture of **4a** (500 mg, 1.0 mmol) and 10% Pd/C catalyst (250 mg) in water/EtOH (1:9 v/v; 25 mL). The mixture was stirred for 2 h, and then the catalyst was removed by filtration through Celite and washed with EtOH (25 mL) and water (25 mL). The combined filtrate and washings were allowed to stand for 0.5 h, and then the solution was evaporated. The residue was dissolved in $CHCl_3$ (50 mL), and the solution was dried ($MgSO_4$). Evaporation gave **5a** (320 mg, 98%) as a foam with spectral data in agreement with the published values.[8]

Cha$\overset{OH}{—}$(R)Val-NH$(CH_2)_3CH_3$ (5b). Transfer hydrogenation of **4b** (300 mg, 0.60 mmol) by the method described for preparation of **5a** gave **5b** (165 mg, 85%) as a foam, which was used without further purification: ^{1}H NMR ($CDCl_3$) 0.8–1.9 (complex m, 29 H), 2.1 (m, 1 H), 3.0 (m, 2 H), 3.4 (m, 1 H), 3.5 (m, 1 H), 5.1 (br, 2 H), 6.6 (br s, 1 H), 8.5 (br s, 1 H); FABMS *m*/*e* 327 $(M + H)^+$.

Cyclohexylacetaldehyde (8). Phosphorus pentoxide (255.6 g, 1.8 mol) was added portionwise (caution exotherm) to an efficiently stirred solution of 2-cyclohexylethanol (139.3 g, 1.0 mol) and dimethyl sulfoxide (156 g, 2.0 mol) in CH_2Cl_2 (1.75 L). The resulting slurry was stirred for 1 h and then cooled to 0 °C. Triethylamine (353.5 g, 3.5 mol) was added dropwise, and the resulting clear solution was stirred at ambient temperature for 0.5 h. The solution was added to ice-cold 1 M H_2SO_4 (2 L). The organic layer was separated, washed with water (1 L) and saturated brine (1 L), and then dried ($MgSO_4$). The solvent was removed by evaporation, and the residue was vacuum distilled to provide **8** (107.7 g, 85%), as a colorless oil: bp 59–60 °C (10 mmHg); ^{1}H NMR ($CDCl_3$) 0.9–1.8 (complex m, 10 H), 1.9 (m, 1 H), 2.3 (dd, 2 H), 9.7 (t, 1 H).

1-Cyclohexyl-3-buten-2-ol (9) was prepared from **8** as described previously.[11a]

(*E*)-1-Chloro-4-cyclohexyl-2-butene (10a). Thionyl chloride (81.3 g, 0.68 mol) was added dropwise over 1 h to a stirred solution of **9** (50 g, 0.32 mol) in dry ether (1 L). The solution was allowed to stand overnight and then water (750 mL) was added, and the mixture was stirred for 1 h. The organic phase was separated, washed with water (500 mL) and saturated brine (2 × 250 mL), and then dried ($MgSO_4$). The solvent was removed by evaporation, and the residue was vacuum distilled to give **10a** (42.2 g, 76%), as a clear liquid: bp 64–68 °C (0.5 mmHg); ^{1}H NMR ($CDCl_3$) 0.8–1.8 (complex m, 11 H), 2.0 (t, 2 H), 4.0 (d, 2 H), 5.5–5.9 (complex m, 2 H). Anal. ($C_{10}H_{15}Cl$) C, H, Cl.

(*E*)-4-Cyclohexyl-1-iodo-2-butene (10b). **10a** (38.6 g, 0.22 mol) was added to a solution of NaI (55.7 g, 0.29 mol) in acetone (1 L), and the solution was allowed to stand overnight. Hexane (500 mL) was added, and the precipitated solid was removed by filtration. The filtrate was concentrated and the residue partitioned between water (500 mL) and hexane (500 mL). The organic phase was separated, washed with saturated $Na_2S_2O_3$ (500 mL) and saturated brine (500 mL), and then dried ($MgSO_4$). The solvent was removed by evaporation and the residue vacuum distilled to give **10b** (44.9 g, 77%) as a dark liquid: bp 80–85 °C (0.15 mmHg); ^{1}H NMR ($CDCl_3$) 0.8–1.8 (complex m, 11 H), 1.9 (t, 2 H), 3.9 (m, 2 H), 5.7 (m, 2 H).

(4*S*)-4-Benzyl-3-[(2*S*,4*E*)-6-cyclohexyl-2-isopropylhex-4-enoyl]oxazolidin-2-one (12). A 1.6 M solution of butyllithium in hexane (93.9 ml, 0.15 mol) was added dropwise over 0.5 h to a stirred solution of diisopropylamine (15.3 g, 0.15 mol) in dry THF (200 mL) at 0 °C under an atmosphere of argon. The temperature was maintained at 0 °C for 0.5 h, and then the solution was cooled to −40 °C. A solution of **11**[14] (31.3 g, 0.12 mol) in THF (70 mL) was added dropwise over 0.5 h. The solution was kept at −40 °C for 0.5 h, and then the temperature was allowed to rise to 0 °C. A solution of **10b** (39.0 g, 0.15 mol) in THF (75 mL) was added dropwise over 0.5 h, and then the solution was stirred at 0 °C for 1 h. Saturated brine (250 mL) was added, and the mixture was extracted with ether (2 × 250 mL). The extracts were washed with saturated brine (2 × 250 mL) and dried ($MgSO_4$). The solvent was removed by evaporation and the residue purified by flash chromatography, eluting with EtOAc/hexane (8:92 v/v), to give **12** (34.5 g, 73%) as a clear oil: ^{1}H NMR ($CDCl_3$) 0.8–1.2 (complex m, 12 H), 1.6 (m, 6 H), 1.85 (t, 1 H), 2.0 (m, 2 H), 2.35 (m, 2 H), 2.6 (m, 1 H), 3.3 (dt, 1 H), 3.8 (m, 1 H), 4.1 (d, 1 H), 4.7 (m, 1 H), 5.4 (m, 2 H), 7.3 (m, 5 H); FABMS *m*/*e* 398 $(M + H^+)$; $[\alpha]^{22}_{436}$ + 45.8° (c, 1.04, $CHCl_3$). Anal. ($C_{25}H_{35}NO_3$) C, H, N.

(3*S*,5*S*)-5-[(1*R*)-1-Bromo-2-cyclohexylethyl]-3-isopropyltetrahydrofuran-2-one (13). *N*-Bromosuccinimide (13.7 g, 77.0 mmol) was added to **12** (27.9 g, 70.0 mmol) in water/di-

methoxyethane (1:1, 150 mL) stirred at 0 °C. The mixture was stirred at 0 °C for 10 min and then at ambient temperature overnight. Water (500 mL) was added, and the mixture was extracted with ether (3 × 250 mL). The extracts were washed with saturated $NaHCO_3$ (250 mL) and saturated brine (250 mL) and then dried ($MgSO_4$). The solvent was removed by evaporation and the residue purified by flash chromatography, eluting with EtOAc/hexane on a gradient from 1:9 v/v to 3:17 v/v, to give an 82:18 mixture of **13** and **15** (14.7 g, 67%). Recrystallization from hexane (70 mL) provided 10.1 g of material enriched to ca. 95% in **13**. The presence of the unwanted regioisomer **15**[11a] was quantified approximately by comparison of the integrals of the signals in the ^1H NMR spectrum at δ 4.55 and δ 4.4 due to **15** and **13**, respectively. A further recrystallization from hexane gave material containing no detectable amount of **15**: mp 94.5–95 °C (lit.[11a] mp 95–95.5 °C) $[\alpha]^{22}_D$ +31.9° (*c*, 1.0, $CHCl_3$) (lit.[11a] $[\alpha]^{22}_D$ +35.6° (*c*, 1.2, $CHCl_3$)).

(3*S*,5*S*)-5-[(1*S*)-1-Azido-2-cyclohexylethyl]-3-isopropyltetrahydrofuran-2-one (16a) was prepared from **13** described previously.[11a]

Cha^{Lac}Val (16b).[15] **16a** (2.69 g, 9.6 mmol) in EtOH (50 mL) was catalytically hydrogenated over 10% Pd/C catalyst (280 mg) at 1 atm and 20 °C. After uptake of hydrogen ceased, the catalyst was removed by filtration through Celite. Evaporation of the filtrate gave **16b** (2.38 g, 100%), as an oil which solidified on standing: mp 47–48 °C (from hexane) (lit.[11d] mp 48–49 °C); $[\alpha]^{22}_D$ +20.8° (*c*, 1.0, EtOH); IR (Nujol) 3280, 1760; ^1H NMR ($CDCl_3$) 0.8–1.9 (complex m, 19 H), 2.1 (m, 3 H), 2.1 (m, 3 H), 2.6 (ddd, 1 H), 2.85 (m, 1 H), 4.2 (m, 1 H); CIMS *m/e* 254 $(M + H)^+$. Anal. ($C_{15}H_{27}NO_2$) C, H, N.

***N*-[(2*RS*)-2-(8-Propyl-6-pyridin-3-yl-1,2,4-triazolo[4,3-*a*]pyrazin-3-yl)-3-pyridin-3-ylpropanoyl]Cha^{Lac}Val (17).** A solution of **6c**[1] (3.95 g, 9.6 mmol) in water (10 mL) was added to a solution of **16b** (2.38 g, 9.6 mmol) in DMF (90 mL). HOBT (1.30 g, 9.6 mmol) and EDC (1.85 g, 9.6 mmol) were added, and the solution was left to stand overnight. Volatile material was removed by evaporation and the residue partitioned between EtOAc (200 mL) and saturated $NaHCO_3$ (200 mL). The organic phase was separated, washed with water (200 mL) and saturated brine (200 mL), and then dried ($MgSO_4$). The solvent was removed by evaporation and the residue purified by flash chromatography, eluting with $MeOH/CH_2Cl_2$ (1:9 v/v), to give **17** (4.14 g, 69%) as a white powder: mp 200–202 °C (after trituration with ether); ^1H NMR (DMSO-d_6, CD_3CO_2D) 0.6 (d, 6 H), 0.7–1.9 (complex m, 20 H), 2.0 (m, 2 H), 3.4 (m, 3 H), 3.9 (m, 2 H), 4.3 (m, 1 H), 4.85 (m, 1 H), 7.4 (m, 1 H), 7.6 (m, 1 H), 7.9 (m, 1 H), 8.5 (m, 2 H), 8.7 (m, 2 H), 9.1, 9.3 (both s, ratio 1:1, total 1 H), 9.3, 9.35 (both d, ratio 1:1, total 1 H); CIMS *m/e* 624 $(M + H)^+$. Anal. ($C_{36}H_{45}N_7O_3$) C, H, N.

***N*-[(2*RS*)-2-(8-Propyl-6-pyridin-3-yl-1,2,4-triazolo[4,3-*a*]pyrazin-3-yl)-3-pyridin-3-ylpropanoyl]Cha^{OH}ValNH$(CH_2)_3CH_3$ (7d).** With the use of the procedure described for preparation of **17**, reaction of **5a** (98 mg, 0.30 mmol) with **6c**[1] (120 mg, 0.30 mmol) in the presence of HOBT (40 mg, 0.30 mmol) and EDC (58 mg, 0.30 mmol) gave, after flash chromatography eluting with $MeOH/CH_2Cl_2$ (7:93 v/v), **7d** (77 mg, 37%) as a white powder: mp 134–137 °C (after trituration with ether); ^1H NMR (DMSO-d_6, CD_3CO_2D) 0.5–2.1 (complex m, 36 H), 2.5 (m, 1 H), 2.9–3.3 (complex m, 4 H), 3.4 (m, 3 H), 3.7 (m, 1 H), 3.9 (m, 2 H), 5.0 (m, 1 H), 7.5 (m, 3 H), 7.7 (m, total 1 H), 8.1 (m, 2 H), 8.3 (d, 1 H), 8.6 (m, 1 H), 8.8 (s, 1 H), 8.9 (2 × s, ratio 1:1, total 1 H); FABMS *m/e* 697 $(M + H)^+$, 624. Anal. ($C_{41}H_{56}N_8O_3 \cdot H_2O$) C, H, N.

Similarly prepared (Table I) were compounds **7a** (from **5a** and **6a**[1]), **7b** (from **5a** and **6b**), and **7c** (from **5b** and **6b**).

***N*-[(2*S*)-2-(8-Propyl-6-pyridin-3-yl-1,2,4-triazolo[4,3-*a*]pyrazin-3-yl)-3-pyridin-3-ylpropanoyl]Cha^{OH}ValNH$(CH_2)_2N(CH_3)_2$ (7e)[26] and *N*-[(2*R*)-2-(8-Propyl-6-pyridin-3-yl-1,2,4-triazolo[4,3-*a*]pyrazin-3-yl)-3-pyridin-3-ylpropanoyl]Cha^{OH}ValNH$(CH_2)_2N(CH_3)_2$ (7f).**[26] A solution of **17** (256 mg, 0.41 mmol) in *N,N*-dimethylethylenediamine (5 mL) and dioxan (5 mL) was heated at 100 °C for 6 h. Volatile material was removed by evaporation and the residue dissolved in $CHCl_3$ (20 mL). The solution was washed with water (4 × 10 mL) and saturated brine (10 mL) and then dried ($MgSO_4$). The solvent was removed by evaporation and the residue purified by flash chromatography, eluting with $MeOH/CH_2Cl_2$ as a gradient from 1:9 v/v to 4:6 v/v, to give initially **7f** (91 mg, 31%) as a white powder: mp 130–134 °C (after trituration with ether); ^1H NMR (DMSO-d_6, CD_3CO_2D) 0.3 (d, 3 H), 0.5 (d, 3 H), 0.6–1.8 (complex m, 20 H), 2.0 (m, 2 H), 2.8 (s + m, 7 H), 2.9 (m, 1 H), 3.0–3.4 (complex m, 6 H), 3.6 (m, 1 H), 3.9 (m, 1 H), 4.8 (dd, 1 H), 7.4 (dd, 1 H), 7.6 (dd, 1 H), 7.8 (d, 1 H), 8.5 (m, 2 H), 8.65 (m, 2 H), 9.3 (s, 1 H), 9.35 (d, 1 H); FABMS *m/e* 712 $(M + H)^+$, 624, 496, 388, 371, 343, 280. Anal. ($C_{40}H_{57}N_9O_3 \cdot H_2O$) C, H, N. Further elution of the chromatography column provided **7e** (107 mg, 37%) as a white powder: mp 118–120 °C (after trituration with ether); ^1H NMR (DMSO-d_6, CD_3CO_2D) 0.7–1.8 (complex m, 26 H), 2.0 (m, 2 H), 2.8 (s, 6 H), 3.1–3.5 (complex m, 8 H), 3.7 (m, 1 H), 3.9 (m, 1 H), 4.85 (dd, 1 H), 7.4 (dd, 1 H), 7.6 (dd, 1 H), 7.8 (m, 1 H), 8.5 (m, 2 H), 8.65 (m, 2 H), 9.3 (s + d, 2 H); FABMS *m/e* 712 $(M + H)^+$, 624. Anal. ($C_{40}H_{57}N_9O_3 \cdot 1.5H_2O$) C, H, N.

Compounds **7g–k** (Table I) were prepared similarly by reaction of **17** with the appropriate primary amines. All were isolated as ca. 1:1 mixtures of diastereoisomers at the asymmetric center α to the 1,2,4-triazolo[4,3-*a*]pyrazine heterocycle.

***N*-[(2*RS*)-2-(8-Propyl-6-pyridin-3-yl-1,2,4-triazolo[4,3-*a*]pyrazin-3-yl)-3-pyridin-3-ylpropanoyl]Cha^{OH}ValN$(CH_3)(CH_2)_3CH_3$ (7l).** A 1 M solution of dimethylaluminum chloride in hexane (1.4 mL, 1.40 mmol) was added to a solution of *N*-methylbutylamine (126 mg, 1.44 mmol) in dry CH_2Cl_2 (10 mL) stirred at 0 °C under an atmosphere of argon. The temperature was maintained at 0 °C for 0.25 h, and then a solution of **17** (220 mg, 0.35 mmol) in CH_2Cl_2 (10 mL) was added. The solution was heated under reflux for 6 h, and then pH 7 phosphate buffer solution (20 mL) was added. The organic layer was separated, washed with saturated brine (20 mL), and dried ($MgSO_4$). The solvent was removed by evaporation and the residue purified by flash chromatography, eluting with $MeOH/CH_2Cl_2$ on a gradient from 3:97 v/v to 1:9 v/v, to give **7l** (50 mg, 20%) as a white powder: mp 100–103 °C (after trituration with ether); ^1H NMR (DMSO-d_6, CD_3CO_2D) 0.2–1.7 (complex m, 32 H), 2.0 (m, 3 H), 2.5–3.5 (complex m, 9 H), 3.65 (m, 1 H), 3.8 (m, 1 H), 4.8 (m, 1 H), 7.4 (m, 1 H), 7.6 (m, 1 H), 7.9 (m, 1 H), 8.5 (m, 2 H), 8.7 (m, 2 H), 9.3 (m, 2 H); FABMS *m/e* 711 $(M + H)^+$, 624, 343, 280. Anal. ($C_{41}H_{58}N_8O_4 \cdot H_2O$) C, H, N.

Compounds 7m and **7n** (Table I) were prepared similarly by reaction of **17** with the appropriate secondary amines.

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(26) Provisional assignment of stereochemistry at the asymmetric centre α to the 1,2,4-triazolo[4,3-*a*]pyrazine heterocycle. See results and discussion.