# Structural Modification of the P2′ Position of 2,7-Dialkyl-Substituted 5(*S*)-Amino-4(*S*)-hydroxy-8-phenyl-octanecarboxamides: The Discovery of Aliskiren, a Potent Nonpeptide Human Renin Inhibitor Active after Once Daily Dosing in Marmosets

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Due to its function in the rate limiting initial step of the renin-angiotensin system, renin is a particularly promising target for drugs designed to control hypertension, a growing risk to health worldwide. Despite vast efforts over more than two decades, no orally efficacious renin inhibitor had reached the market. As a result of a structure-based topological design approach, we have identified a novel class of small-molecule inhibitors with good oral blood-pressure lowering effects in primates. Further lead optimization aimed for improvement of in vivo potency and duration of action, mainly by P2′ modifications at the hydroxyethylene transition-state isostere. These efforts resulted in the discovery of aliskiren (**46**, CGP060536B, SPP100), a highly potent, selective inhibitor of renin, demonstrating excellent efficacy in sodium-depleted marmosets after oral administration, with sustained duration of action in reducing dose-dependently mean arterial blood pressure. Aliskiren has recently received regulatory approval by the U.S. Food and Drug Administration for the treatment of hypertension.

## Introduction

Despite the wide availability of many antihypertensive treatments, hypertension currently affects approximately 1 billion people worldwide,[1] and in 2004, an estimated 45% of patients being treated in the United States remained uncontrolled.[2,3] This lack of control is particularly concerning because hypertension is widely accepted today as a key prevalent risk factor for cardiovascular diseases including stroke and heart and kidney failure.

As a key regulator of blood pressure and fluid homeostasis, the renin-angiotensin system (RAS[a]) has long been recognized as highly attractive for therapeutic intervention and associated control of blood pressure.[4,5] Upon stimulation of the RAS by various mechanisms, such as decrease in blood pressure, circulating volume, or plasma sodium, renin is released from the kidney and acts by cleaving a decapeptide from the N-terminus of the substrate angiotensinogen to form angiotensin I (Ang I). Angiotensin converting enzyme (ACE) then acts upon Ang I, cleaving an additional two amino acids to produce the octapeptide angiotensin II (Ang II), which is a highly potent vasoconstrictor agent and the principal mediator of the RAS mainly through its interaction with the $AT_1$ receptor. Ang II also exerts direct action on the proximal tubule to promote sodium reabsorption and, furthermore, stimulates the adrenal cortex to secrete aldosterone, which in turn acts upon the distal nephron to retain sodium, leading to fluid retention.[6]

Realized as early as 1956 by Skeggs et al.,[7] blockade of the interaction of renin with its substrate, angiotensinogen, appears to be the most attractive of the three major targets within the RAS offering intervention strategies aimed at producing effective treatments to control hypertension. Angiotensinogen cleavage is the first and rate limiting step of the RAS cascade, which could be fully blocked even in situations of elevated circulating or tissue active renin, and moreover, renin displays remarkable specificity for its substrate.[5] First introduced to the market for the effective treatment of hypertension,[8] angiotensin converting enzyme inhibitors (ACEi) may give rise to incidences of persistent cough and angioedema associated with the broader substrate specificity of ACE,[9] and in addition, ACEi do not block Ang II formation via ACE-independent pathways.[10] Angiotensin $AT_1$ receptor blockers (ARBs), which interfere at the final stage of the RAS cascade and inhibit the effects of Ang II independent of its source (non-renin and non-ACE Ang II generation), have also become established for the effective management of cardiovascular diseases.[11] However, long-term ARB treatment results in chronically elevated levels of Ang II and its biodegradation products, which may exert their own pharmacological activities in vivo.[12]

Several peptide-like, second-generation renin inhibitors such as enalkiren,[13] remikiren,[14] and zankiren[15] were shown in early investigational clinical trials to be potent in reducing blood pressure similar to ACEi and ARBs, indicating furthermore that renin inhibition would be a safe and efficacious way to treat hypertension. Hence, enalkiren reduced blood pressure to a slightly greater extent than enalapril, whereas remikiren displayed similar efficacy to captopril.[16] While these results do

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[a] Abbreviations: ACE, angiotensin converting enzyme; ACEi, ACE inhibitors; Ang I, angiotensin I; Ang II, angiotensin II; ARBs, angiotensin $AT_1$ receptor blockers; $AT_1$, angiotensin II type I receptor; dTGR, double-transgenic rat; MAP, mean arterial blood pressure; ΔMAP, change in mean arterial blood pressure; RAS, renin-angiotensin system; rh-renin, recombinant human renin.

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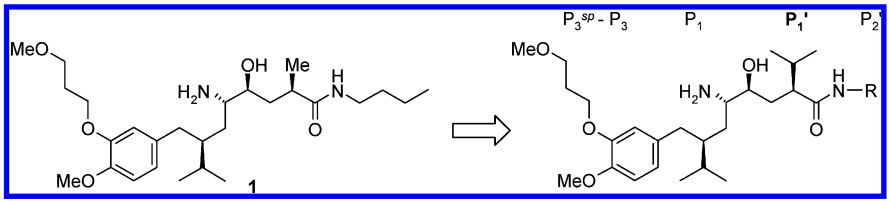



**Figure 1.** Inhibitor **1** and modification sites.

not make a conclusive case for the benefits of renin inhibitors, some further preclinical[17,18] and clinical[19] studies suggest that renin inhibitors may have also better efficacy against blood-pressure-independent renal organ damage as compared to treatment with either an ACEi or an Ang II receptor blocker. Furthermore, co-treatment with an ACEi and an ARB has demonstrated enhanced clinical benefits in chronic renal failure conditions[20] and chronic heart failure patients,[21] despite no enhanced effects on lowering blood pressure. It, therefore, could be hypothesized that a more complete and sustained blockage of the RAS by a single mechanism, for example through renin inhibition, may offer a superior therapeutic approach than the currently available ACEis or ARBs.[1,18]

Further clinical development of these and other second generation renin inhibitors has been hampered in most cases by their limited oral bioavailability in humans as well as technical hurdles due to complexity in synthesis of drug substance.[22,23] In a quest for the identification of more promising renin inhibitor lead scaffolds, an early medium throughput screening of a smaller corporate compound collection did not provide attractive hits as new entries into lead optimization, in contrast to reports by other groups.[24,25] Instead, we successfully developed, as the result of a target enzyme structure-oriented computational design approach and extensive structure−activity relationship (SAR) work, several novel, structurally distinct series of in vitro potent and selective topologically P3−P1-tethered transition-state mimetic renin inhibitors.[26] Common to all these inhibitors is their important binding interaction to a narrow nonsubstrate pocket extending from the P3 site of renin, as discovered by X-ray crystallography of several ligand−enzyme complexes.[27] In the preceding paper, the optimization of a highly attractive small-molecule lead series has been described, yielding the first preclinical candidate renin inhibitor **1** of this structure class (Figure 1), which demonstrated potent blood-pressure lowering activities in sodium-depleted marmosets after oral dosing.[28] We report here a fully detailed account[29,30] of our extended SAR exploration in the quest for analogues of **1** with increased in vivo potency and improved oral bioavailability. As a first step toward this goal, a P1′ isopropyl residue was incorporated into the transition-state isostere moiety of **1**, as this would mimic more closely the scissile dipeptide Leu-Val portion of the renin substrate[7] and, hence, would potentially lead to improved intrinsic potency in vitro. The stereoselective access to the advanced *N*,*O*-protected acid intermediate **7** then enabled us to conveniently investigate broadly on modifications at the P2′ position. Among several potent and selective analogues, the novel peptidomimetic inhibitor **46** was identified to be the most efficacious, long-acting, and orally active renin inhibitor in primates, as reported to date.

## Chemistry

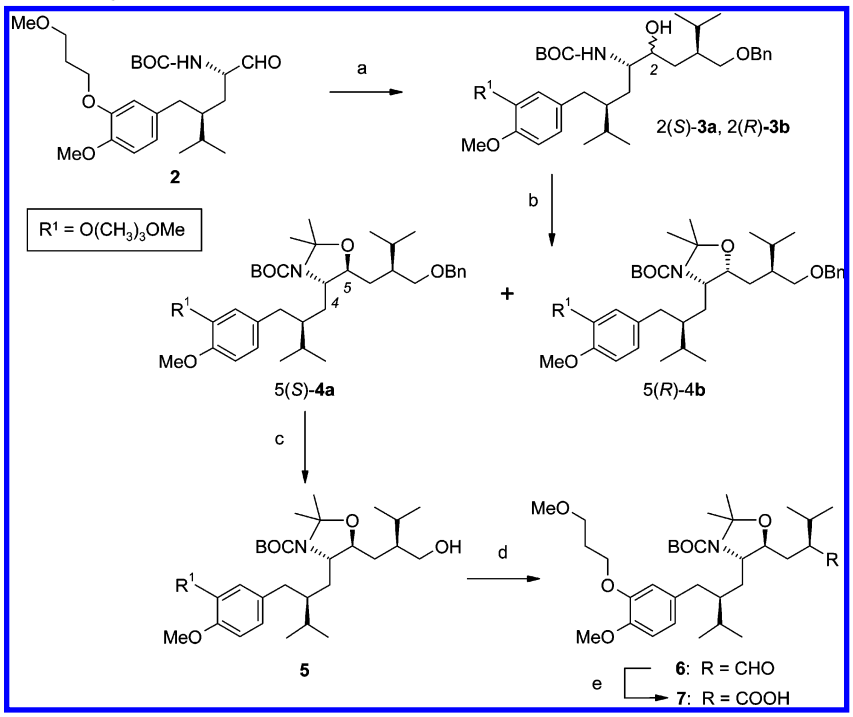
The synthesis of the *N*,*O*-protected key intermediate acid **7** is detailed in Scheme 1. The tetrahedral hydroxyethylene dipeptide isostere portion was assembled[29,31] by employing the method reported previously.[32] Conversion of the enantiopure 2(*S*)-bromomethyl-3-methylbutyl-benzyl ether[33] to the Grignard reagent and its addition to the *N*-Boc amino aldehyde **2** of defined absolute stereochemistry[28] resulted in a 4:6 ratio of the two diastereoisomers 2(*S*)-**3a** and 2(*R*)-**3b** (53% total yield), which were inseparable by chromatography on silica gel. The mixture **3a**/**3b** was completely converted to the mixture of *N*,*O*-acetals **4a**/**4b** by reaction with 2,2-dimethoxy-propane/cat. *p*-TsOH over 24 h at rt, which could be separated only with major effort by chromatography. However, we found that *N*,*O*-acetal formation can be achieved under mild reaction conditions (15 °C for 1 h) in a highly selective fashion on large scale, yielding almost exclusively the desired 5(*S*)-**4a** (97:3 crude mixture with 5(*R*)-**4b**). This allowed expeditious removal of the major unreacted 2(*R*)-**3b** and traces of 5(*R*)-**4b** by flash chromatography to afford the pure stereoisomer 5(*S*)-**4a**. The absolute 5(*S*)- and 5(*R*)-stereochemistry for **4a** and **4b**, respectively, was tentatively supported by proton NMR, based on decoupling/NOE experiments and the vicinal $^3J_{H,H}$ coupling constants for the 1,3-oxazolidine moiety,[34] as well as by the observed differences in the chemical reactivity as described herein; we reasoned that the 5(*S*)-configured *trans*-oxazolidine **4a** is formed more readily from 2(*S*)-**3a**, as compared to the sterically more demanding *cis*-isomer **4b** from 3(*R*)-**3b**.

The benzyl ether **4a** was cleaved by hydrogenolysis to afford alcohol **5**, which was then oxidized in a stepwise fashion first with *N*-methylmorpholine-*N*-oxide/TPAP to provide aldehyde **6** and, subsequently, to the carboxylic acid **7** by reaction with $KMnO_4/Bu_4N^+Br^-$ in 58% yield (two steps). Based on various optimization efforts, this protocol in our hands proved to be advantageous over the direct transformation of **5** to **7**, in particular, when performed on a larger reaction scale.

The final renin inhibitors (Tables 1−3) were conveniently prepared by the sequence representatively shown in Scheme 2. Thus, standard mixed anhydride coupling of a variety of P2′ amines to carboxylic acid **7**, using either diethyl cyanophosphonate (DEPC) as the activating reagent, or HBTU[35] for the reaction with sterically hindered secondary amines, afforded carboxamides **8a**−**f**. Final *N*,*O*-deprotection was achieved smoothly in a sequential manner under mild conditions, first by reaction with catalytic *p*-TsOH in MeOH to give intermediates **9**, followed by removal of the *N*-Boc group in 4 N HCl/dioxane to afford the hydroxyethylene dipeptide isosteres as amorphous HCl salts. Alternatively, aminolysis of a carboxylic ester precursor was employed for efficient elaboration of the SAR at the N-terminal P2′ position, as exemplified by the transformation of **8c** to the carboxamide **8d** (Scheme 2).

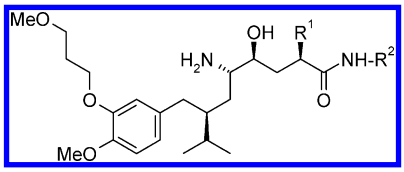
The carba-analogue inhibitors **59** and **60** (Table 4), both bearing a 4-methoxybutyl side chain at the phenyl moiety, were prepared by a different synthetic route (Scheme 3).[36] Wittig reaction of aldehyde **11** with phosphonium bromide **10** afforded olefin **12** as a mixture of *E*/*Z* isomers, which was hydrogenated in the presence of 5% platinum on carbon to give bromide **13**. Lithiation with *n*-butyllithium (THF, −78 °C) and subsequent metal exchange by addition of Mg/1,2-dibromoethane at low temperature generated the Grignard species of **13**. Condensation with the azido-lactone aldehyde **14**[36] proceeded to about a 3:1 diastereomeric mixture of inseparable secondary alcohols **15**.

**Scheme 1.** Synthesis of the Carboxylic Acid Intermediate **7**[a]




[a] Reagents and conditions: (a) 2(*S*)-bromomethyl-3-methylbutyl-benzyl ether, Mg, 1,2-dibromoethane, THF (53%); (b) 2,2-dimethoxypropane, *p*-TsOH, $CH_2Cl_2$, 24 h, rt (36% for 5(*R*)-**4b** and 50% for 5(*S*)-**4a**; isolated yields); (c) $H_2$, Pd/C, THF, 15 min (91%); (d) *N*-methylmorpholine-*N*-oxide, TPAP, $CH_2Cl_2$, 30 min, quant.; (e) $KMnO_4$, $Bu_4N^+Br^-$, toluene−$H_2O$, 48 h, 0 °C (58%).

**Table 1.** In Vitro Activity of Renin Inhibitors with P1′ Methyl versus *i*-Propyl Moiety[a]




| | | | binding affinity $IC_{50}$ (nM), renin (pH 7.2) | | |
|---|---|---|---|---|---|
| | | | human | | marmoset |
| cmpd | $R^1$ = | $R^2$ = | purified[b] | plasma[c] | plasma[c] |
| **1**[d] | Me | $(CH_2)_3CH_3$ | 1 | 1 | 3 |
| **28** | *i*-propyl | $(CH_2)_3CH_3$ | 1 | 4 | 8 |
| **29**[d] | Me | $(CH_2)_2CONH_2$ | 7 | 17 | 13 |
| **30** | *i*-propyl | $(CH_2)_2CONH_2$ | 1 | 3 | 6 |
| **31**[d] | Me | $(CH_2)_3CONH_2$ | 1 | 3 | 11 |
| **32** | *i*-propyl | $(CH_2)_3CONH_2$ | 0.7 | 0.9 | 3 |

[a] Compounds obtained as HCl salts; for details, see Experimental Section. [b] Purified human renin, measured at pH 7.2. [c] Human or marmoset plasma renin assay; for details, see ref 28. [d] See ref 28.

Removal of the benzylic OH required *O*-acetylation prior to hydrogenation, which proceeded to completion in the presence of Pd/C 5% in EtOH after four days with concomitant reduction of the azide group. *N*-Boc protection of the primary amine afforded intermediate **16** in 48% yield over three steps. Aminolysis of the lactone **16** with 2-aminoethyl-morpholine in the presence of catalytic acetic acid[37] provided **17** in 86% yield, which after *N*-deprotection eventually gave inhibitor **59** as its dihydrochloride salt.

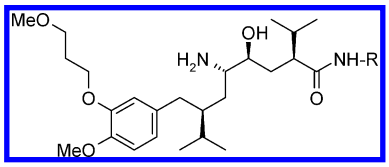
In contrast, direct lactone-opening with amines **23** (Scheme 4) as their free base under identical conditions surprisingly proved unsuccessful. Therefore, we adopted the approach reported by Evans et al.,[38] to form the *O*-TBDMSi-protected γ-hydroxy carboxylic acid **18** after alkaline hydrolysis of **16** (66% yield, two steps). HBTU coupling of **18** with **23a**, followed by desilylation with $Bu_4N^+F^-$, afforded **19** and, after subsequent removal (4 N HCl/dioxane, 0 °C) of the *N*-Boc group, the final inhibitor **60** as an amorphous HCl salt.

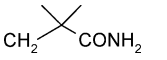
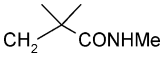
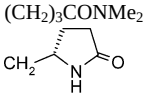
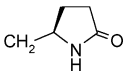
The preparation of the P2′ primary amines **23a**,**b** and **27** is shown in Scheme 4 (see Supporting Information). Aminolysis of the Cbz-protected ethyl ester **21**, readily obtained from cyanoester **20** via Ra−Ni hydrogenation and subsequent Cbz protection, with ammonia gas gave the carboxamide **22a** in moderate but acceptable yields, although the reaction proceeded very sluggishly at 70 °C under pressure in an autoclave reactor with methanol as the solvent (no reaction observed in EtOH). Similarly, **21** was reacted under pressure with $NH_2Me$ (60 °C) in EtOH to afford **22b**. Hydrogenation of compounds **22a**,**b** was performed under pH control (pH 6) to give the crystalline HCl salts **23a**,**b**. Transformation of the chiral hydroxy ester **24** was achieved in three steps by *O*-tosylation, azide displacement, and $H_2$/Pd reduction to afford amine **27** as HCl salt.

## Results and Discussion

**Replacement of Methyl versus *i*-Propyl at P1′.** The $IC_{50}$ data shown in Table 1 for a set of representative analogues bearing either an aliphatic *n*-butyl or an N-terminal carboxamide P2′ residue indicated that replacing the methyl group at P1′ of the dipeptide transition-state isostere by the more bulky isopropyl residue (**1** and **28**, **29** and **30**, **31** and **32**) resulted in compounds with at least similar or improved in vitro potencies. For example, a 3−5-fold increase in binding affinity was observed for **30** as compared to **29** against human renin. Most importantly, the more lipophilic P1′ isopropyl analogues revealed very similar enzyme affinities in the human and marmoset plasma assays, with an only up to 4-fold increase in $IC_{50}$ as compared to the purified human renin buffer assay. We considered the retention of high binding affinity under these physiologically more relevant assay conditions as a key require-

**Table 2.** In Vitro Activity of Renin Inhibitors Bearing Carboxamides at P2′ Position[a]



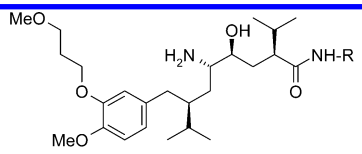

| | | | binding affinity $IC_{50}$ (nM), renin (pH 7.2) | | |
|---|---|---|---|---|---|
| | | | human | | marmoset |
| cmpd | R = | config[b] | purified | plasma | plasma |
| **33** | $CH_2CONH_2$ | | 3 | 10 | 16 |
| **34** | $CHMeCONH_2$ | *S* | 4 | 12 | 13 |
| **35** | $CHEtCONH_2$ | *S* | 0.8 | 0.6 | 1 |
| **36** | $CH(CH_2OH)CONH_2$ | *S* | 2 | 3 | 6 |
| **37** | $CMe_2CONH_2$ | | 13 | 100 | 200 |
| **38** | $(CH_2)_2CONHMe$ | | 3 | 2 | 4 |
| **39** | $(CH_2)_2CONMe_2$ | | 3 | 7 | 6 |
| **40** | $CHMeCH_2CONH_2$ | *S* | 1 | 2 | 3 |
| **41** | $CHMeCH_2CONH_2$ | *R* | 6 | 57 | 28 |
| **42** | $CH_2CHMeCONH_2$ | *S* | 1 | 1 | 3 |
| **43** | $CH_2CHMeCONH_2$ | *R* | 0.9 | 2 | 2 |
| **44** | $CH_2CHMeCONHMe$ | *S* | 1 | 2 | 10 |
| **45** | $CH_2CHMeCONHMe$ | *R* | 0.9 | 3 | 1 |
| **46** |  | | 0.6 | 0.6 | 2 |
| **47** |  | | 0.4 | 0.7 | 2 |
| **48** | $(CH_2)_3CONHMe$ | | 0.8 | 3 | 3 |
| **49** | $(CH_2)_3CONMe_2$ | | 1 | 4 | 3 |
| **50** |  | *S* | 0.6 | 0.9 | 3 |
| **51** |  | *R* | 2 | 1 | 2 |

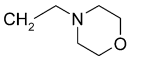
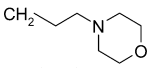
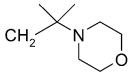
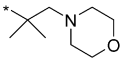
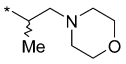
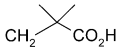
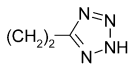
[a] Compounds obtained as HCl salts; for details, see Experimental Section. [b] Absolute configuration at the P2′ moiety.

ment for the identification of inhibitors with high in vivo potency in our animal model. The new compounds **28**, **30**, and **32** were furthermore highly active against marmoset plasma renin (Table 1). As will be discussed later, the P1′ isopropyl inhibitors were generally found to have a more pronounced potency and longer duration of action, as compared to their P1′ methyl congeners, in sodium-depleted renin-activated marmosets after oral administration.

**In Vitro Structure−Activity Relationship at P2′ Position.** We noted in the course of our work[28] that N-terminal carboxamides at the P2′ position were particularly favorable in providing highly potent inhibitors in vitro in the presence of plasma, as well as oral potency in our pharmacodynamic primate model. Therefore, and based on the in vitro results for the initial P1′ isopropyl analogues **30** and **32**, we focused our attention on further tuning such P2′ carboxamide moieties, mainly with the intention of optimizing their in vivo potency and duration of action. The $IC_{50}$ values for human and marmoset renin for a range of closely related unsubstituted versus N-methylated analogues with modified length and branching of the aliphatic chain, are summarized in Table 2. The vast majority of these inhibitors demonstrated very high in vitro binding affinities, which were even in the subnanomolar range in several cases. The glycine- and (*S*)-alanine-derived carboxamides **33** and **34** showed similar activities, whereas for the (*S*)-ethyl branched analogue **35** a significant improvement in potency was observed in particular in the plasma assay. The physiological substrate of renin, angiotensinogen, contains an Ile residue at P2′,

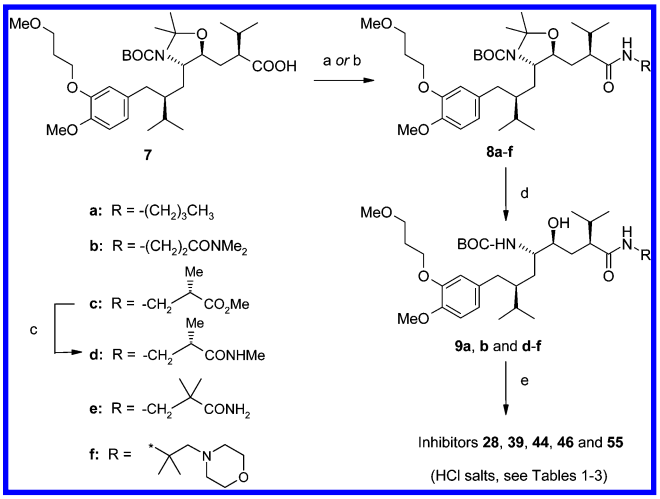
**Table 3.** In Vitro Activity of Renin Inhibitors[a]




| | | binding affinity $IC_{50}$ (nM), renin (pH 7.2) | | |
|---|---|---|---|---|
| | | human | | marmoset |
| cmpd | R = | purified[b] | plasma[c] | plasma[c] |
| **52** |  | 0.4 | 1 | 3 |
| **53** |  | 3 | 3 | 3 |
| **54** |  | 0.6 | 2 | 18 |
| **55** |  | 16 | 27 | nd[d] |
| **56** |  | 3 | 7 | 9 |
| **57** |  | 3 | 13 | 13 |
| **58** |  | 2 | 2 | 2 |

[a] Compounds obtained as HCl salts; for details, see Experimental Section. [b] Purified human renin, measured at pH 7.2. [c] Human or marmoset plasma renin assay; for details, see ref 28. [d] nd = not determined.

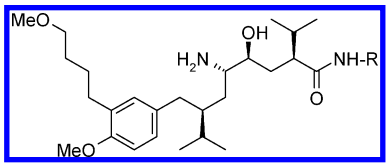
**Scheme 2.** Final Reaction Sequence to Renin Inhibitors[a]

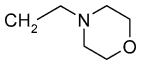
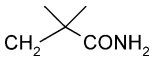



[a] Reagents and conditions: (a) $H_2N$-R (HCl salt or free base), $(EtO)_2P(O)CN$, $Et_3N$, DMF, rt; (b) $H_2N$-R, HBTU, $Et_3N$, MeCN−DMF (90% for **8f**); (c) **8c**, $NH_2Me$ 33% in EtOH, 40 °C, 40 h (83%); (d) *p*-TsOH (hydrate), MeOH, 0 °C or rt; (e) 4 N HCl−dioxane, 0 °C.

suggesting that analogues of **35** with larger and more lipophilic substituents at this position could provide more potent in vitro inhibitors. However, we did not investigate this possibility further in the specific context of this compound series to keep the molecular weight and overall lipophilicity low. Introducing a side-chain OH group (**36** vs **34**) had no impact on the binding affinity. On the other hand, a marked drop of in vitro potency

**Table 4.** In Vitro Activity of Side-Chain Carba Analogue Inhibitors **59** and **60**[a]




| | | binding affinity $IC_{50}$ (nM), renin (pH 7.2) | | |
|---|---|---|---|---|
| | | human | | marmoset |
| cmpd | R = | purified[b] | plasma[c] | plasma[c] |
| **59** |  | 2 | 0.9 | 1 |
| **60** |  | 1 | 3 | 4 |

[a] Compounds obtained as HCl salts; for details, see Experimental Section. [b] Purified human renin, measured at pH 7.2. [c] Human or marmoset plasma renin assay; for details, see ref 28.

was observed for the isobutyramide **37**, which was most striking when determined in both plasma renin assays.

Extension of the P2′ moiety by one carbon and *N*-substitution by one (**38**) or two methyl groups (**39**) was of little influence on the affinities to the enzyme as compared to **33**. Similarly, the impact of branching the $\beta$-position by methyl was only minor, with the expected preference for the (*S*)- over the (*R*)-configuration (**40** vs **41**). Introduction of a methyl group at the α-carboxamide position, either with absolute (*S*)- (**42** and **44**) or (*R*)-configuration (**43** and **45**) resulted in virtually equipotent inhibitors independent of N-substitution. However, and most intriguingly, the corresponding geminal-dimethyl-substituted carboxamides **46** and **47** demonstrated subnanomolar binding affinities for both purified and plasma human renin.

Further extension of the linear P2′ chain by one more carbon (**48** and **49**) retained the high enzyme affinity (compared to **33** and **39**). The conformationally more rigid (*S*)- and (*R*)-configured lactams **50** and **51**, respectively, showed very similar potencies in vitro. It is important to note that most of the inhibitors listed in Table 2 demonstrated very comparable in vitro binding affinities toward marmoset renin with low-nanomolar $IC_{50}$ values in the presence of plasma.

In vitro data for a series of analogues bearing a basic P2′ morpholine moiety tethered by different alkyl spacers is listed in Table 3. The selection of these compounds was based on the results of a more extended SAR undertaken in the context of the related P1′ methyl dipeptide isostere series.[28,39] The ethyl side-chain derivative **52** appeared to be slightly more potent in vitro than the propyl analogue **53** toward human renin, but was equipotent against marmoset renin. The α,α-dimethylated derivative **54** turned out to have similar affinity to human renin as **52**, albeit being somewhat less active against marmoset renin. Similar to the findings in the P2′ carboxamide sub-series, a tertiary carbon adjacent to the transition-state mimetic amide nitrogen, as in inhibitor **55**, dramatically reduced the binding affinity when compared to the monomethylated derivative **56**, most likely as result of an unfavorable conformational constraint.

As was demonstrated previously,[28] acidic functionalities are well accommodated at the P2′ position. Accordingly, the carboxylic acid derivative **57** and the tetrazole **58** of the P1′ isopropyl series (Table 3) yielded potent in vitro inhibitors.

**P3$^{sp}$ Side-Chain Carba Analogues.** The X-ray structure of **46** bound to recombinant human renin (rh-renin; at 2.2 Å resolution, vide infra) revealed an indirect, water molecule mediated H-bonding of the phenoxy ether oxygen to the side chain hydroxyl of Ser219. In order to probe its contribution to the binding affinity, the carba-analogues **59** and **60**, both bearing a methoxybutyl chain at P3$^{sp}$, were synthesized and tested against human and marmoset renin (Table 4). Both compounds displayed very similar in vitro potencies in both the buffer and the plasma enzyme assays as compared to the ether analogues **52** and **46**, respectively, thus indicating that the "proximal" ether oxygen is not critical for binding to the enzyme. Furthermore, both **59** and **60** were found to be highly potent in vivo with a long duration of action in reducing blood pressure in marmosets (vide infra).[29]

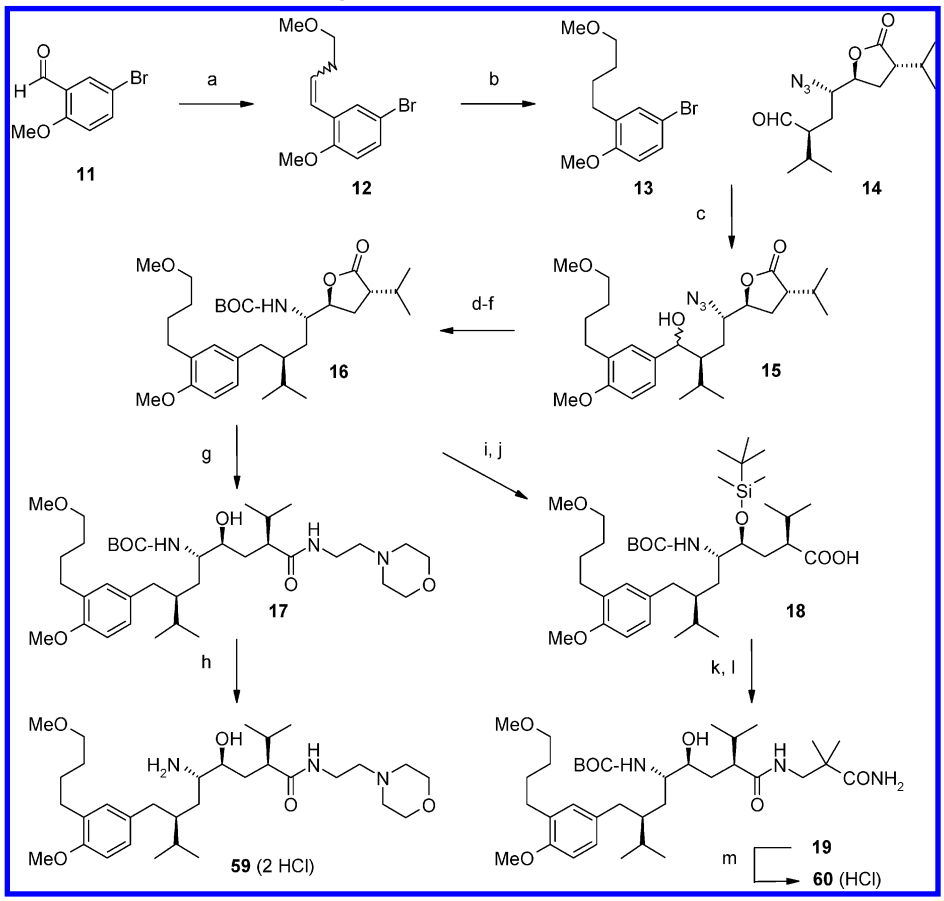
**Enzyme Selectivity.** All new inhibitors were routinely tested for their in vitro selectivity against porcine pepsin and bovine cathepsin D[40] and found to be completely inactive at 10 $\mu$M concentrations (data not shown). Inhibitor **46** displayed very high enzyme selectivity for rh-renin, with $IC_{50}$ values > 10 $\mu$M for human pepsin A, cathepsin D, cathepsin E, BACE-1, and other human enzyme family members,[41] as well as for the viral HIV-1 aspartyl protease. Furthermore, **46** revealed high species specificity for primate renin (human, monkey) and was less active against plasma renin from various other mammalians, such as dog, rabbit, pig, guinea pig, and rat plasma renin ($IC_{50}$ = 7, 11, 150, 63, and 80 nM, respectively).[30]

**Physicochemical Parameters.** Experimental data for the aqueous solubility, the logP octanol−water partition coefficient at physiological and weakly acidic pH, as well as p$K_a$ values of several potent inhibitors, are listed in Table 5. All compounds are characterized by a low to moderate lipophilicity, with $logP_{pH7.4}$ ranging from 2.1 to 3.1, and furthermore by very high solubility of their salts in water or in aqueous buffer at neutral pH 7.4 (>100 g/L for **46** as its hemi-fumarate salt) as well as high intrinsic solubility for **46** of 0.44 g/L. In this respect, our newly designed structural class of renin inhibitors revealed an improved physicochemical profile clearly distinct from that of many of the previous generation, peptide-like transition-state mimetics.[37] We reasoned that such compound characteristics of low lipophilicity (particularly low logD) and high intrinsic aqueous solubility could be of favorable impact on the drug metabolism and pharmacokinetics (DMPK) profile of these renin inhibitors. The large volume of distribution observed for **46** in some species, including human, could be beneficial for a more target organ specific tissue distribution, such as the kidney, and hence may, at least partially, contribute to the excellent in vivo efficacy of **46** (vide infra).[42]

A broader salt screening program was performed for the preclinical candidate inhibitor **46** from which the crystalline hemi-fumarate salt (CGP60536B, SPP100, aliskiren) was identified to be suitable for formulation.

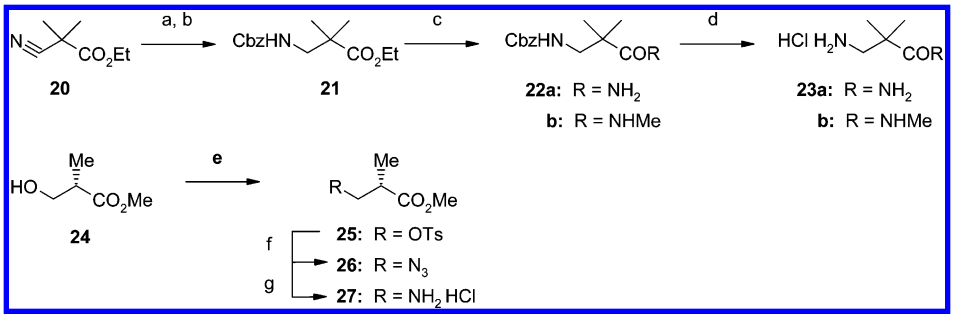
**X-ray Crystallography.** The crystal structure of compound **46** has been resolved by cocrystallization of the inhibitor with recombinant glycosylated human renin at 2.2 Å resolution (Figure 2a−c).[27] We wish to illustrate here some of the key features of the binding interactions of **46** in more detail, also in view of the in vitro SAR data that has emerged within this compound series. In contrast to known second generation peptide-based renin inhibitors, compound **46** does not interact with the S2 or S4 binding sites. Instead, its hydrophobic P3−P1 pharmacophore, comprising of the P1 isopropyl group and the phenyl spacer substituted by the small polar P3 methoxy group forming contacts to the side chains of Phe117 and Pro111 at approximately 4 Å, is accommodated nicely by the large contiguous S3−S1 "superpocket". The methoxypropoxy side chain of **46** is binding to the nonsubstrate cavity S3$^{sp}$, extending

**Scheme 3.** Synthesis of P3$^{sp}$ Side-Chain Carba-Analogues **59** and **60**[a]




[a] Reagents and conditions: (a) MeO(CH$_2$)$_3$P$^+$Ph$_3$Br$^-$ (**10**), NaHMDS, 0 °C (80%); (b) Pt/C 5%, THF (76%); (c), **13**, *n*-BuLi, THF, −75 °C, then Mg/1,2-C$_2$H$_4$Br$_2$, −75 °C; then add **14**; (d) Ac$_2$O, Et$_3$N, 4-DMAP, CH$_2$Cl$_2$; (e) Pd/C 5%, EtOH, 4 days; (f) BOC$_2$O, Et$_3$N, CH$_2$Cl$_2$ (48%, 3 steps); (g) NH$_2$-R, AcOH cat., 80 °C, 24 h (86%); (h) 4 N HCl−dioxane, 0 °C; (i) LiOH, DME−H$_2$O (97%); (j) TBDMSiCl, imidazole, DMF, 6 days (68%); (k) **23a**, HBTU, Et$_3$N, MeCN; (l) *n*-Bu$_4$NF, THF (87%, 2 steps); (m) 4 N HCl−dioxane, 0 °C.

**Scheme 4**[a]




[a] Reagents and conditions: (a) H$_2$, RaNi, NH$_3$/MeOH 4% (67%); (b) BnOCOCl, 1 M NaHCO$_3$, EtOAc (85%); (c) NH$_3$/MeOH, 70 °C, 300 h (53%), or NH$_2$Me 33% in EtOH, 70 °C, 8 days (49%); (d) H$_2$, Pd/C 10%, MeOH, dilut. aq HCl (pH stat control; pH 6); (e) TsCl, Et$_3$N, CH$_2$Cl$_2$ (80%); (f) NaN$_3$, DMF, 40−45 °C; (g) H$_2$, Pd/C 10%, THF (35%, 2 steps).

by approximately 9 Å from the S3 site toward the protein core, in a similar fashion as reported for inhibitor **1**.[28] An enzyme-bound water (W092) at the entrance to S3$^{sp}$ forms a bridging H-bond to the phenolic oxygen of **46** (3 Å) and the OH of Ser219 (2.8 Å). However, the similar in vitro potency of the related inhibitor **60** in which the ether oxygen has been replaced by a carbon atom (Table 4; compare also **52** vs **59**) suggests only a minor contribution to the overall binding affinity.

Figure 2b illustrates the binding interactions of the hydroxyethylene dipeptide isostere portion of **46** in comparison to the super-positioned close analogue **1**. Strikingly, the complex structure of **46** revealed a marked shift in the position of the carbon backbone of the mimetic portion along the axis of the catalytic cleft as well as significant differences in the conforma-

**Table 5.** Physicochemical Characteristics[a]

| compound | **39**[b] | **46**[c] | **51** | **59** | **60** |
|---|---|---|---|---|---|
| log P (pH 7.4)[d] | 2.48 | 2.5 | 2.06 | 2.85 | 3.31 |
| log D (pH 6.8) | | 0.1 | | | |
| intrinsic solubility [g/L] | | 0.44 | | | |
| solubility[e] [g/L] | >100 | >100 | >100 | | |
| p$K_a$ | 9.4[g] | 9.2[f] | 9.09 | 6.01/9.55 | 9.44 |

[a] Determined for amorphous HCl salts, if not otherwise indicated. [b] As amorphous monosodium citrate salt. [c] As crystalline hemi-fumarate salt. [d] Apparent octanol-aqueous phosphate buffer partition coefficient at 22 °C. Determined for **46** by dual-phase potentiometric titration in 0.15 M KCl at 25 °C.[53] [e] Determined in water at 22 °C; for **46**, determined in aqueous phosphate buffer (pH 7.4) at 22 °C. [f] Potentiometric titration in 0.15 M KCl at 25 °C.[54] [g] p$K_a$ = 5.7 for citrate.

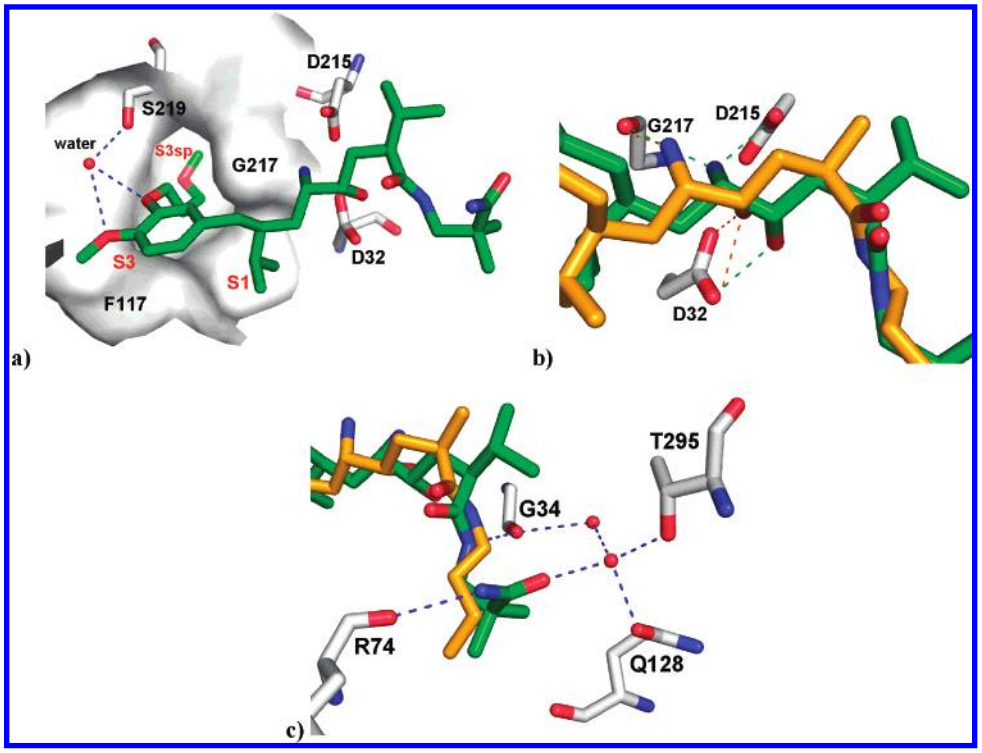



**Figure 2.** (a) X-ray structure of inhibitor **46** (green color) with rh-renin.[27] (b) Superposition of the hydroxyethylene transition-state dipeptide isostere portions (spanning P1 to P1′) of inhibitors **46** (green color) and **1** (orange color) in the catalytic site of renin. Inhibitors are shown in a stick model with oxygen and nitrogen atoms in red and blue, respectively. The catalytic aspartic acids (D32 and D215) as well as Gly217 are shown in gray color, selected H-bond interactions are shown as dotted lines using the color code corresponding to that of the inhibitor. (c) Binding interactions of **46** (green color) to the S2′ subpocket. The *n*-butyl P2′ group of **1** is superimposed in orange color. All graphs were generated using the PyMOL software package.[43]

tion of the center scaffold. As a consequence, the mimetic OH group gets positioned in a more symmetrical fashion to both aspartates D32 (H-bond distances to oxygen atoms 3.1 and 2.9 Å, respectively) and D215 (3.5 Å), in contrast to **1** with a H-bond only to D32.[28] Also, the primary $NH_2$ in **46** is within H-bond distance both to Gly217 (3.0 Å) and Asp215 (2.8 Å). The P1′ isopropyl group is now ideally positioned to closely fit to and deeply penetrate into the hydrophobic S1′ site; this is again in contrast to what is observed for inhibitor **1**, which binds only partially to S1′ (Figure 2b).

The binding mode of the α,α-diMe β-alanine carboxamide of **46** within the S2′ pocket is shown in Figure 2c. The terminal $CONH_2$ forms a tight network of direct and solvent-mediated H-bonds to several amino acids of this pocket, for example, Arg74 of the flap, Gly34, Gln128, and Thr295, involving two enzyme-bound water molecules (W010 and W096). The geminal methyl groups provide additional hydrophobic van der Waals contacts to the S2′ site, as compared to the aliphatic *n*-butyl chain of **1**. Overall, the specific hydrophobic and polar binding interactions of the unique P2′ pharmacophore are thought to contribute substantially to the remarkably strong enzyme affinity of **46**, being in our hands one of the most potent inhibitors of rh-renin discovered to date.

**In Vivo Activity in $Na^+$-Depleted Marmosets.** The in vitro potent renin inhibitors from this series were routinely administered orally, at a 3 mg/kg dose, to conscious, unrestrained $Na^+$-depleted marmosets to evaluate their efficacy as blood pressure (BP) reducing agents. In this pharmacological model, systolic, diastolic, and mean arterial pressures (MAPs) and heart rate were noninvasively and continuously recorded over a 24 h time period using an implanted telemetry device.[44] Time courses of changes (Δ) in MAP values from baseline for the test compounds and vehicle were compared. Several compounds, as

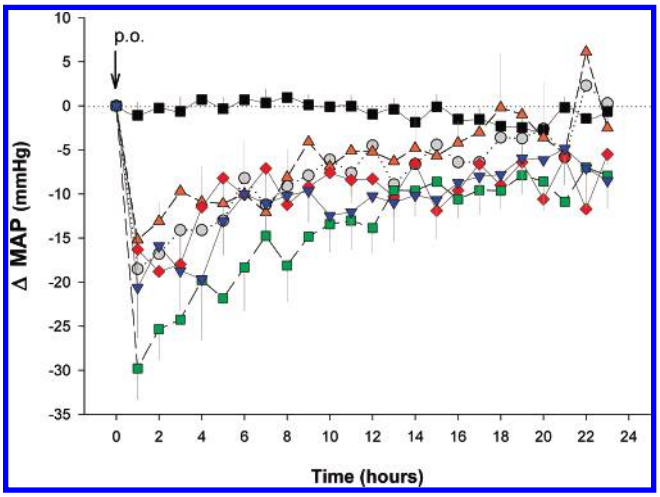



**Figure 3.** Changes in ΔMAP after single oral administration of renin inhibitors at a dose of 3 mg/kg, or vehicle control, to $Na^+$-depleted freely moving telemetered marmosets. Values are means ± SEM of the change (△) in MAP from baseline over 24 h after p.o. dosing (time 0) with **1** (HCl salt; $n = 4$, orange triangle), **28** (HCl salt; $n = 4$, gray circle), **39** (citrate salt; $n = 6$, upside-down blue triangle), **46** (HCl salt; $n = 6$, green square), compound **50** (HCl salt; $n = 3$, red diamond), and vehicle control ($n = 20$, black square). Mean baseline arterial blood pressure and heart rate values in all groups were similar to 81 ± 2 mmHg and 205 ± 8 beats/min observed in the 10 mg/kg po dose group.

exemplified in Figure 3 for inhibitors **28**, **39**, **46**, and **50**, were found to be highly potent in this renin-dependent normotensive primate model, leading to a pronounced and sustained reduction in ΔMAP. Inhibitor **28** bearing a P1′ isopropyl residue showed a peak ΔMAP of −20 mmHg 1−3 h post-dose, which appeared to be significantly more potent, albeit not longer acting, as compared to the corresponding P1′ methyl analogue **1**. Interest-

ingly, we observed a consistent trend for a markedly stronger effect on BP and a longer duration of action for the respective P1′ isopropyl versus methyl analogues (data not shown), which were considered to be likely due to differences in the pharmacokinetics profiles of the corresponding analogues.

Some of the most potent in vivo and long-acting inhibitors were identified from the P2′ carboxamide sub-series. Inhibitors **39** and **50** (and similarly **43**, **45**, **47**, as well as the $P3^{sp}$ side chain carba analogues **59** and **60**; data not shown) induced similar peak BP reduction as **1** and **28**, but revealed a clearly more sustained pharmacological activity, because blood pressure levels did not fully recover to baseline even 24 h post-dose (Figure 3). Compound **46** (as its HCl salt) was found to be most potent, with a rapid onset of action leading to a remarkable peak ΔMAP of −30 mmHg 1 h after dosing. This inhibitor also revealed a very prolonged activity, with MAP being still lowered by −10 mmHg at the 24 h time point and, hence, appeared to be one of the longest acting inhibitors in this primate model. The absolute oral bioavailability of **46** in non-$Na^+$-depleted marmosets, dog, and rat was determined to be 16, 32, and 2.4%, respectively, as determined from plasma concentrations using an enzymatic assay.[45−47] All analogues bearing an acidic functionality at P2′ (cf. Table 3) were completely inactive in vivo, suggesting a poor bioavailability profile in marmosets.

The extent of ΔMAP reduction measured in $Na^+$-depleted marmosets paralleled the % inhibition of plasma renin activity (PRA),[48] thus strongly indicating that the pharmacological effect was mechanistically related to renin inhibition. For example, PRA was completely blocked over the initial 6 h after giving a 3 mg/kg oral dose of inhibitor **28**, with significant reduction in ΔMAP observed (Figure 3), and was only partially inhibited by 79% at 24 h when BP had basically recovered to normal levels. On the other hand, PRA was completely inhibited over the 24 h period for all inhibitors having a longer duration of action including **46**, which can be most likely attributed to more extended in vivo plasma and/or tissue exposures of these compounds.

The blood pressure lowering effects of inhibitor **46** as its hemi-fumarate salt has been more extensively investigated in sodium-depleted marmosets after single and multiple doses over 8 days,[30,46] in spontaneously hypertensive rats (SHR) following subchronic administration for 14 days,[46] and in double-transgenic rats (dTGR) expressing human renin and human angiotensinogen.[49] In these studies in sodium-depleted marmosets, orally administered **46** reduced blood pressure dose-dependently and proved to be more effective and long-lasting than two known orally active peptide-like renin inhibitors that have been in clinical trials previously. Furthermore, **46** was shown to be at least as effective in reducing MAP as an ACE inhibitor and an AT1 receptor blocker.[46] Based on its overall characteristics and preclinical in vitro/in vivo profile, inhibitor **46** (hemi-fumarate salt) was selected as a candidate for further development in clinical trials.

## Conclusions

The structural modification of the P1′ and P2′ group of the parent nonpeptide transition state renin inhibitor **1** had little influence on the observed in vitro $IC_{50}$ values (plasma assays), however, this did provide compounds with significantly improved oral potency when measured in vivo. Several inhibitors that exhibited moderate lipophilicity and high water solubility were found to be very efficacious in reducing mean arterial blood pressure in salt-depleted, telemetered marmosets with a long duration of action. In particular, in our renin-dependent primate model, which used conscious, unrestrained, sodium-depleted marmosets, the oral administration of inhibitor **46** resulted in a very pronounced (−30 mmHg), sustained (at 24 h) drop in MAP. This reduction in blood pressure was paralleled by a complete blockage of PRA that lasted for at least 24 h. Inhibitor **46** was found to display very high enzyme selectivity for rh-renin, as well as for primate renin and was less active against plasma renin from other mammalian sources. Many of the newly designed class of inhibitors, described herein, exhibited low to medium lipophilicity with high aqueous solubility and were, therefore, clearly distinct from many of the previous generations of described peptide-like mimetics. We conclude that these physical characteristics may, at least in part, be responsible for the excellent observed in vivo efficacies.

In summary, inhibitor **46** proved to be among the most potent renin inhibitors after oral administration that has as yet been described. Based upon its excellent overall pharmacological profile, **46** has been selected for clinical trials, has recently received regulatory approval by the U.S. Food and Drug Administration (FDA) for the treatment of hypertension, and has become the first marketed orally active renin inhibitor.

## Experimental Section

General methods for experimental and analytical procedures and for purifications can be found in the Supporting Information. Noncommercial amines used for the coupling to acid intermediate **7** or for aminolysis of **8c** and related analogues, and which are not mentioned explicitly in the section below, were prepared according to our preceding paper[28] or to standard literature procedures.

**(1*S*,2*S*,4*S*,2′*S* and 1*S*,2*R*,4*S*,2′*S*)-(4-Benzyloxymethyl-2-hydroxy-1-{2′-[4-methoxy-3-(3-methoxy-propoxy)-benzyl]-3-methyl-butyl}-5-methyl-hexyl)-carbamic Acid *tert*-Butyl Ester (3a and 3b).** To Mg turnings (51.1 g, 2.10 mol) in THF (1.4 L) at 55 °C was added over 30 min a solution of 2(*S*)-bromomethyl-3-methyl-butyl-benzylether[33] (380 g, 1.40 mol) and 1,2-dibromoethane (30.2 mL, 0.35 mol) in THF (0.8 L). Stirring was continued at 55 °C for 20 min, followed by the dropwise addition of crude aldehyde **2**[28] (190 g, theoretical 0.42 mol) in THF (0.7 L) at 5 °C. The mixture was stirred for 3 h at ambient temperature and, after cooling to 5 °C, was quenched by the addition of a saturated $NH_4Cl$ solution (2 L). Extractive workup with $Et_2O$ and FC purification (EtOAc/hexane l:3) afforded a 4:6 ratio of inseparable diastereomers (1*S*,2*S*,4*S*,2′*S*)-**3a** and (1*S*,2*R*,4*S*,2′*S*)-**3b** (139 g, 53%) as a colorless oil: TLC $R_f$ 0.26 (AcOEt/hexane l:2); HPLC $t_R$ 22.7/22.8 min (peak ratio 6:4); $^1H$ NMR ($CDCl_3$, 360 MHz, most of the signals were doubled) $\delta$ 0.78 (m, 12H), 1.1−1.7 (m, 9H), 2.00 (m, 2H), 2.28 (m, 1H), 2.60 (m, 1H), 3.27 (s, 3H), 3.3−3.6 (m, 4H), 3.75 (s, 3H), 3.95−4.2 (m, 4H), 4.4−4.5 (m, 2H), 4.65 (d, $J$ = 12, 0.6H), 4.73 (d, $J$ = 12, 0.4H), 6.6−6.8 (m, 3H), 7.1−7.3 (m, 5H).

**(4*S*,5*S*,2′*S*,2″*S*)- and (4*S*,5*R*,2′*S*,2″*S*)-5-(2′-Benzyloxymethyl-3-methyl-butyl)-4-{2″-[4-methoxy-3-(3-methoxy-propoxy)-benzyl]-3-methyl-butyl}-2,2-dimethyl-oxazolidine-3-carboxylic Acid *tert*-Butyl Ester (4a and 4b).** A solution of **3a**, **3b** (7.0 g, 11.1 mmol), 2,2-dimethoxypropane (10.9 mL, 88.9 mmol), and catalytic *p*-TsOH·$H_2O$ (5 mg) in $CH_2Cl_2$ (70 mL) was stirred for 24 h at ambient temperature. Evaporation and FC purification of the residue ($CH_2Cl_2$/$EtO_2$ 96:4) afforded both pure diastereoisomers **4a** (3.72 g, 50%) and **4b** (2.68 g, 36%) as colorless oils. Data for (4*S*,5*S*,2′*S*,2″*S*)-**4a**: TLC $R_f$ 0.35 ($CH_2Cl_2$/$EtO_2$ 95:5); HPLC $t_R$ 27.7 min; $^1H$ NMR ($CDCl_3$, 360 MHz) $\delta$ 0.8−0.9 (m, 12H), 1.15−1.9 (m, 10H), 1.42 (s, 3H, $CH_3$), 1.49 (s, 9H, $C(CH_3)_3$), 1.55 (s, 3H, $CH_3$), 2.07 (m, 2H, $CH_2CH_2O$), 2.3−2.6 (m, 2H, $ArH_aH_b$), 3.35 (s, 3H, $OCH_3$), 3.41 (m, 1H, *CH*OH), 3.38 (t, $J$ = 7, 2H, $OCH_2$), 3.6−3.8 (br m, 1H, *CH*N), 3.81 (s, 3H, $OCH_3$), 4.06 (t, $J$ = 7, 2H, $OCH_2$), 4.44 (s, 2H, $OCH_2C_6H_5$), 6.6−6.8 (m, 3H, arom), 7.2−7.35 (m, 5H, arom). Data for (4*S*,5*R*,2′*S*,2″*S*)-**4b**: TLC $R_f$ 0.46 ($CH_2Cl_2$/$EtO_2$ 95:5); HPLC $t_R$ 28.1 min; $^1H$ NMR ($CDCl_3$, 360 MHz) $\delta$ 0.78 (d, $J$ = 7, 3H, $CH_3$), 0.79 (t, $J$ = 7, 3H, $CH_3$), 0.86

(d, $J = 7$, 3H, $CH_3$), 0.91 (d, $J = 7$, 3H, $CH_3$), 1.17 (m, 1H, CH), 1.25−1.8 (m, 20H), 1.91 (m, 1H, $ArCH_2CH$), 2.09 (m, 2H, $CH_2CH_2O$), 2.20 (m, 1H), 2.47 (br m, 1H, $ArH_aH_b$), 2.95 (m, 1H, $ArH_aH_b$), 3.35 (s, 3H, $OCH_3$), 3.41 (m, 1H, *CH*OH), 3.45 (m, 1H, *CH*N), 3.57 (t, $J = 7$, 2H, $OCH_2$), 3.82 (s, 3H, $OCH_3$), 4.05−4.2 (m, 2H, $CHCH_2O$), 4.12 (t, $J = 7$, 2H, $OCH_2$), 4.49 (br s, 2H, $OCH_2C_6H_5$), 6.65−6.8 (m, 2H, arom), 6.97 (br s, 1H, arom), 7.2−7.4 (m, 5H, arom).

Selective *N*,*O*-protection of the desired diastereoisomer **3a** on a larger reaction scale was achieved by the following procedure: To a cooled solution of stereoisomers **3a**,**b** (4:6 ratio; 186 g, 0.295 mol) in $CH_2Cl_2$ (1.86 L) were added 2,2-dimethoxypropane (290 mL, 2.362 mol) and *p*-TsOH·$H_2O$ (250 mg) by keeping the reaction temperature at 15 °C (slightly exothermic; the proceeding of the reaction was checked by analytical RP-HPLC to keep formation of **4b** to a minimum). After stirring for 60 min, $Et_3N$ (0.5 mL) was added to the mixture, which was then concentrated at 30 °C under reduced pressure. The residue was flash chromatographed on silica gel (2.4 kg, $CH_2Cl_2$/*t*-BuOMe gradient 96:4 to 1:2) to afford a 93:7 mixture of products **4a** and **4b** (total 128 g), respectively, besides unreacted **3b** (55 g, pale yellow oil). From this product mixture, diastereometrically pure **4a** (97.2 g, 49%) and **4b** (7.5 g) were isolated by repeated FC on silica gel ($CH_2Cl_2$/*t*-BuOMe 96:4).

**(4*S*,5*S*,2′*S*,2″*S*)-5-(2′-Hydroxymethyl-3-methyl-butyl)-4-{2″-[4-methoxy-3-(3-methoxy-propoxy)-benzyl]-3-methyl-butyl}-2,2-dimethyl-oxazolidine-3-carboxylic Acid *tert*-Butyl Ester (5).** A solution of **4a** (3.70 g, 5.52 mmol) in THF (50 mL) was hydrogenated in the presence of 5% palladium on charcoal (1.0 g) for 15 min at ambient temperature. The catalyst was removed by filtration over Hyflo, the combined filtrates were concentrated, and the residue was purified by FC (EtOAc/hexane 1:2) to afford **5** (2.92 g, 91%) as a colorless oil: TLC $R_f$ 0.28 TLC (AcOEt/hexane 1:2); HPLC $t_R$ 23.1 min; $[\alpha]^{20}_D$ +44.3 (*c* 1.3, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 0.8−1.05 (m, 12H), 1.48 (s, 9H), 1.2−1.9 (m, 15H), 2.09 (m, 2H), 2.2−2.8 (m, 2H), 3.35 (s, 3H), 3.45−3.65 (m, 6H), 3.84 (s, 3H), 4.10 (m, 2H), 6.67 (d, $J = 8$, 1H), 6.73 (s, 1H), 6.78 (d, $J = 8$, 1H). Anal. ($C_{33}H_{57}NO_7$) H, N; C: calcd, 68.36; found, 67.78.

**(4*S*,5*S*,2′*S*,2″*S*)-5-(2′-Formyl-3-methyl-butyl)-4-{2″-[4-methoxy-3-(3-methoxy-propoxy)-benzyl]-3-methyl-butyl}-2,2-dimethyl-oxazolidine-3-carboxylic Acid *tert*-Butyl Ester (6).** To a solution of **5** (53.0 g, 91.4 mmol) in $CH_2Cl_2$ (1.8 L) was added *N*-methyl-morpholine-*N*-oxide (16.6 g, 137 mmol) and molecular sieves 0.3 nm (100 g). After 10 min, tetrapropylammonium-perruthenate (1.60 g, 4.55 mmol) was added, and the mixture was stirred for another 30 min at ambient temperature. After removal of the molecular sieves by filtration, the mixture was diluted with $CH_2Cl_2$ and then subsequently washed with a 2 M solution of $Na_2SO_3$, brine, a 1 M solution of $CuSO_4$, and again with brine. The dried ($MgSO_4$) organics were evaporated and dried in vacuo to yield crude aldehyde **6** (53 g, used in the next step without further purification): TLC $R_f$ 0.43 (EtOAc/hexane 1:2); HPLC $t_R$ 23.8 min; $[\alpha]^{20}_D$ +76.1 (*c* 1.2, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 0.9−1.0 (m, 12H), 1.48 (s, 9H), 1.2−1.9 (m, 14H), 2.09 (m, 2H), 2.15−2.65 (m, 3H), 3.35 (s, 3H), 3.45−3.7 (m, 1H), 3.57 (t, $J = 7$, 2H), 3.83 (s, 3H), 4.10 (m, 2H), 6.65−6.8 (m, 3H), 9.67 (s, 1H).

**(4*S*,5*S*,2′*S*,2″*S*)-5-(2′-Carboxy-3-methyl-butyl)-4-{2″-[4-methoxy-3-(3-methoxy-propoxy)-benzyl]-3-methyl-butyl}-2,2-dimethyl-oxazolidine-3-carboxylic Acid *tert*-Butyl Ester (7).** To a solution of **6** (53 g, theor. 91.4 mmol) in toluene (470 mL) was added water (470 mL), $KMnO_4$ (79.1 g, 500 mmol), and *n*-$Bu_4NBr$ (9.7 g, 30 mmol) at 0 °C. After 48 h, a 10% aqueous solution of $Na_2SO_3$ (1.2 L) was added dropwise by keeping the temperature at 0−10 °C. The colored mixture was stirred for 30 min (aqueous phase pH ∼ 12), followed by addition of a 10% solution of citric acid monohydrate (1.95 L) and water (1.2 L). The aqueous layer was extracted with EtOAc (3 × 2.5 L), and the combined organics were washed with water and brine, dried ($MgSO_4$), and concentrated. Purification by FC (EtOAc/hexane 3:7) afforded **7** (31.7 g, 58%) as an oil: TLC $R_f$ 0.27 (EtOAc/hexane 1:2); HPLC $t_R$ 22.3 min; $^1$H NMR (DMSO-$d_6$, 80 °C) $\delta$ 0.9−0.95 (m, 12H), 1.33 (s, 3H), 1.45 (s, 9H), 1.49 (s, 3H), 1.93 (t, $J = 7$, 2H), 1.25−2.00 (m, 7H), 2.25−2.3 (m, 1H), 2.3−2.6 (m, 2H), 3.00 (br s, 1H), 3.26 (s, 3H), 3.49 (t, $J = 7$, 2H), 3.6−3.7 (m, 2H), 3.75 (s, 3H), 4.01 (t, $J = 7$, 2H), 6.69 (dd, $J = 2$, 8, 1H), 6.77 (d, $J = 2$, 1H), 6.86 (d, $J = 8$, 1H). Anal. ($C_{33}H_{55}NO_8$) C, H, N.

**Compound 9e.** The mixture of **7** (8.9 g, 15.0 mmol), $Et_3N$ (5.02 mL, 36.0 mmol), 3-amino-2,2-dimethyl-propionamide, HCl salt (**23a**; 2.75 g, 18.0 mmol), and DEPC (95%, 2.87 mL, 18.0 mmol) in DMF (360 mL) was reacted overnight at rt as described for **8a** (see Supporting Information). The mixture was concentrated, and the residue was partitioned between a saturated aqueous $NaHCO_3$ solution (40 mL) and EtOAc (3 × 40 mL). The combined organics were washed with brine, dried ($MgSO_4$), and concentrated in vacuo. FC purification (EtOAc/hexane 1:2) afforded **8e** (10.4 g) as a white foam: TLC $R_f$ 0.27 ($CH_2Cl_2$/MeOH 95:5); $^1$H NMR (DMSO-$d_6$, 80 °C; 360 MHz) $\delta$ 0.85−0.91 (m, 12H, $4CH_3$), 1.07 (s, 3H, $(CH_3)_2$-$CONH_2$), 1.08 (s, 3H, $(CH_3)_2CONH_2$), 1.2−1.85 (m, 7H, aliphat), 1.33 (s, 3H, $CH_3$), 1.45 (s, 9H, $C(CH_3)_3$), 1,51 (s, 3H, $CH_3$), 1.93 (m, 2H, $CH_2CH_2O$), 2.24 (m, 1H, *CH*CONH), 2.33 (m, 1H, $ArCH_aH_b$,), 2.45−2.5 (m, 1H, $ArCH_aH_b$; signal overlapped), 3.15 (dd, $J = 6$, 13, 1H, $CONHCH_a$), 3.26 (s, 3H, $OCH_3$), 3.33 (dd, $J = 6$, 13, 1H, $CONHCH_b$), 3.49 (t, $J = 6.4$, 2H, $OCH_2$), 3.57 (m, 1H, *CH*OH), 3.55 m, 1H, *CH*NH), 3.74 (s, 3H, $OCH_3$), 4.03 (t, $J = 6.5$, 2H, $ArOCH_2$), 6.66 (br s, 2H, $CONH_2$), 6.71 (dd, $J = 2$, 8, 1H, arom), 6.79 (d, $J = 2$, 1H, arom), 6.84 (d, $J = 8$, 1H, arom), 7.20 (br t, 1H, CO*NH*).

A solution of **8e** (10.3 g, 14.9 mmol) and a catalytic amount of *p*-TsOH·$H_2O$ (0.10 g) in absolute MeOH (210 mL) was stirred at 0 °C overnight. Extractive workup, FC purification on silica gel (400 g; eluant: $CH_2Cl_2$−MeOH 9:1), and recrystallization from $Et_2O$−hexane (1:1, 40 mL) afforded the title compound **9e** (7.70 g, 79% over 2 steps) as a white solid: mp 141−142 °C; TLC $R_f$ 0.45 ($CH_2Cl_2$/MeOH 9:1); $^1$H NMR (DMSO-$d_6$, 360 MHz) $\delta$ 0.74 (d, $J = 6.6$, 3H, $CH_3$), 0.75 (d, $J = 6.6$, 3H, $CH_3$), 0.84 (d, $J = 6.6$, 6H, $2CH_3$), 1.04 (s, 6H, $(CH_3)_2CONH_2$), 1.15−1.7 (m, 7H, aliphat), 1.40 (s, 9H, $C(CH_3)_3$), 1.91 (m, 2H, $CH_2CH_2O$), 2.18−2.25 (m, 2H, $ArCH_aH_b$, *CH*CONH), 2.61 (m, 1H, $ArCH_aH_b$), 3.12 (dd, $J = 6$, 13.3, 1H, $CONHCH_a$), 3.24 (s, 3H, $OCH_3$), 3.25−3.35 (m, 2H, CH, $CONHCH_b$; signals overlapped), 3.46 (t, $J = 6.3$, 2H, $OCH_2$), 3.45−3.55 (m, 1H, *CH*OH), 3.71 (s, 3H, $OCH_3$), 3.95 (t, $J = 6.5$, 2H, $ArOCH_2$), 4.30 (d, $J = 6.2$, 1H, OH), 6.15 (d, $J = 9.8$, 1H, BOC-*NH*), 6.64 (m, 1H, arom), 6.75−6.85 (m, 3H, arom, $CONH_aH_b$), 7.10 (br s, 1H, $CONH_aH_b$), 7.38 (t, $J = 6$, 1H, CO*NH*). Anal. ($C_{35}H_{61}N_3O_8$) C, H, N.

**(2*S*,4*S*,5*S*,7*S*)- 5-Amino-4-hydroxy-2-isopropyl-7-[4-methoxy-3-(3-methoxy-propoxy)-benzyl]-8-methyl-nonanoic Acid Butyl-amide, Hydrochloride Salt (28).** A solution of **9a** (2.45 g, 4.02 mmol) in dioxane (10 mL) was concentrated to a volume of 5 mL, cooled to 0 °C in an ice bath, and 4 N HCl−dioxane (30 mL) was added dropwise. Stirring was continued for 3 h at 0 °C, followed by lyophilisation of the white suspension in high vacuo overnight. The residue was dissolved in $CH_2Cl_2$, filtered through a glass fiber filter, the solvent was evaporated, and the residue was dried in high vacuo at rt to give title compound **28** (2.15 g, 96%) as an amorphous solid: TLC $R_f$ 0.31 ($CH_2Cl_2$/MeOH 9:1); HPLC $t_R$ 14.7 min (100%); $^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$ 0.7−0.9 (m, 15H, $5CH_3$), 1.2−1.45 (m, 7H, aliphat), 1.5−1.7 (m, 3H, aliphat), 1.78 (m, 1H, $ArCH_2CH$), 1.92 (m, 2H, $CH_2CH_2O$), 2.16 (m, 1H, *CH*CONH), 2.38 (dd, $J = 8$, 13.5, 1H, $ArCH_a$), 2.44 (dd, $J = 7$, 13.5, 1H, $ArCH_b$), 2.68 (m, 1H, $CHNH_3^+$), 2.96 (m, 1H, $CONHCH_a$), 3.11 (m, 1H, $CONHCH_b$), 3.20−3.25 (m, 1H, *CH*OH), 3.27 (s, 3H, $OCH_3$), 3.46 (t, $J = 6.3$, 2H, $OCH_2$), 3.71 (s, 3H, $OCH_3$), 3.97 (t, $J = 6.5$, 2H, $ArOCH_2$), 5.31 (br s, 1H, OH; exchangeable), 6.69 (dd, $J = 1.7$, 8, 1H, arom), 6.80 (d, $J = 1.7$, 1H, arom), 6.82 (d, $J = 8$, 1H, arom), 7.3−7.9 (br s, 3H, $NH_3^+$), 7.83 (t, $J = 5.5$, 1H, NH amide); HRMS *m*/*z* 509.3973 $[(M + H)^+$ calcd for $C_{29}H_{53}N_2O_5^+$, 509.3954]. Anal. ($C_{29}H_{52}N_2O_5$·HCl·0.64$H_2O$) C, H, N; Cl: calcd, 6.37; found, 7.09.

**Compound 39, Mono-Na-Citrate Salt.** Compound **9b** (0.86 g, 1.32 mmol), dissolved in 4 N HCl−dioxane (17 mL), was stirred at 0 °C for 2 h, followed by freeze-drying, to afford the

hydrochloride salt of compound **39** as a white solid. This material was dissolved in ice-cold 1 N NaOH (50 mL), and the aqueous layer was extracted with $Et_2O$ (3 × 70 mL). The combined organics were washed with water and brine, dried ($MgSO_4$), and concentrated to afford the free base of **39** as a pale yellow oil. To a solution of this material (0.72 g, 1.31 mmol) in EtOH (50 mL) was added citric acid monohydrate (0.274 g, 1.31 mmol) at rt. After stirring for 10 min, water (50 mL) and 1 N NaOH (1.31 mL, 1.31 mmol) were added. Then volatiles were removed in vacuo at 30 °C, the water phase (ca. 60 mL) was filtered through a glass fiber filter to remove remaining particles, and the filtrates were lyophilised overnight. The residue was then taken up in $Et_2O$ (60 mL) and stirred for 3 h at rt and the precipitate was filtered. After drying in high vacuo at 30 °C overnight, the title compound **39**, monosodium citrate salt, (0.70 g, 69%) was obtained as white powder: TLC $R_f$ 0.38 ($CH_2Cl_2$/MeOH 8:2); HPLC $t_R$ 11.4 min (99.2%); $^1$H NMR (DMSO-$d_6$, 80 °C; 500 MHz) $\delta$ 0.81−0.87 (m, 12H), 1.27−1.37 (m, 3H), 1.6−1.68 (m, 2H), 1.70 (m, 1H), 1.76 (m, 1H), 1.92 (m, 2H), 2.08 (m, 1H), 2.4−2.58 (m, 14H), 3.12−3.18 (m, 1H), 3.25 (s, 3H), 3.2−3.3 (m, 1H; overlapped), 3.3−3.4 (m, 2H), 3.48 (t, $J$ = 6.3, 2H), 3.73 (s, 3H), 4.00 (t, $J$ = 6.5, 2H), 6.71 (dd, $J$ = 2, 8, 1H), 6.77 (d, $J$ = 2, 1H), 6.83 (d, $J$ = 8, 1H), 7.36 (br t, 1H); HRMS $m/z$ 552.4014 [$(M + H)^+$ calcd for $C_{30}H_{53}N_3O_6^+$, 552.4012]. Anal. ($C_{36}H_{60}NaN_3O_{13}\cdot 0.59H_2O$) C, H, N.

**(2*S*,4*S*,5*S*,7*S*)-5-Amino-4-hydroxy-2-isopropyl-7-[4-methoxy-3-(3-methoxy-propoxy)-benzyl]-8-methyl-nonanoic Acid (2-Carbamoyl-2-methyl-propyl)-amide, Hydrochloride Salt (46).** A solution of **9e** (7.60 g, 11.7 mmol) in 4 N HCl−dioxane (100 mL) was stirred at 0 °C for 2 h. The mixture was concentrated under reduced pressure at rt to a small volume, which was lyophilised overnight. The residue was dissolved in water (200 mL), filtered through a glass fiber filter, and the combined filtrates were freeze-dried in high vacuo overnight at ambient temperature to yield the title compound **46**, HCl salt, (7.00 g) as a hygroscopic white powder: TLC $R_f$ 0.33 ($CH_2Cl_2$/MeOH 8:2); HPLC $t_R$ 12.7 min (99.4%); $^1$H NMR (DMSO-$d_6$, 360 MHz): $\delta$ 0.7−0.9 (m, 12H, 4$CH_3$), 1.05 (s, 6H, ($CH_3$)$_2$CONH$_2$), 1.2−1.45 (m, 3H, aliphat), 1.5−1.7 (m, 3H, aliphat), 1.78 (m, 1H, ArCH$_2$*CH*), 1.92 (m, 2H, *CH*$_2$CH$_2$O), 2.26 (m, 1H, *CH*CONH), 2.36−2.47 (m, 2H, Ar*CH*$_2$), 2.68 (m, 1H, *CH*$NH_3^+$), 3.07 (dd, $J$ = 6, 13.3, 1H, CONH*CH*$_a$), 3.20−3.25 (m, 1H, *CH*OH), 3.27 (s, 3H, $OCH_3$), 3.31 (dd, $J$ = 6, 13.3, 1H, CONH*CH*$_b$), 3.46 (t, $J$ = 6.3, 2H, $OCH_2$), 3.71 (s, 3H, $OCH_3$), 3.98 (t, $J$ = 6.5, 2H, ArO*CH*$_2$), 5.35 (d, $J$ = 5.5, 1H, OH), 6.69 (dd, $J$ = 1.5, 8, 1H, arom), 6.80−6.82 (m, 3H, arom, CON*H*$_a$H$_b$), 7.13 (br s, 1H, CONH$_a$*H*$_b$), 7.54 (t, $J$ = 6, 1H, CO*NH*), 7.65 (br s, 3H, $NH_3^+$); HRMS $m/z$ 552.4015 [$(M + H)^+$ calcd for $C_{30}H_{54}N_3O_6^+$, 552.4013]. Anal. ($C_{30}H_{54}N_3O_6\cdot HCl\cdot 0.32H_2O$) C, H, Cl, N.

**Compound 46, Hemi-Fumarate Salt (Aliskiren).** To a solution of **46**, HCl salt, (0.24 g, 0.40 mmol) in water (2.5 mL) were added crushed ice (2 g) and 2 N NaOH (1.2 mL, 2.4 mmol). The white suspension was extracted with $Et_2O$ (3 × 15 mL), and the combined organics were dried ($MgSO_4$) and evaporated. The free base (220 mg) was dissolved in *i*-PrOH, and a solution of fumaric acid (23 mg, 0.20 mmol) in *i*-PrOH (2 mL) was added. Insoluble materials were removed by filtration through a glass fiber filter, and the filtrate was concentrated to a volume of 1.5 mL. $Et_2O$ (2 mL) was slowly added, followed by the addition of a seed crystal, and stirring of the gradually forming fine suspension was continued overnight at rt. The white precipitate was filtered, washed with a small volume of *i*-PrOH−$Et_2O$, and dried in high vacuo at 50 °C to afford the title compound (150 mg) as a white solid; mp 108−115 °C; HPLC $t_R$ 12.7 min (99.1%). Anal. ($C_{30}H_{53}N_3O_6\cdot C_2H_2O_2\cdot 0.88H_2O$) C, H, N.

**General Method−Coupling of Sterically Hindered Amines. Compound 9f.** To a solution of acid **7** (119 mg, 0.20 mmol) in MeCN (5 mL) were subsequently added $NEt_3$ (34 $\mu$L, 0.24 mmol), 1,1-dimethyl-2-morpholin-4-yl-ethylamine (114 mg, 0.72 mmol),[50] and *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium-hexafluorophosphate (HBTU) (91 mg, 0.24 mmol). After a 30 min reaction time, DMF (2 mL) was added to the suspension and stirring was continued overnight at rt. The mixture was concentrated and the residue was partitioned between EtOAc and satd aq $NaHCO_3$ solution. The combined organics were washed with brine, dried ($MgSO_4$), and concentrated, followed by FC purification ($CH_2Cl_2$/MeOH 95:5), to afford compound **8f** as a colorless oil (132 mg, 90%): TLC $R_f$ 0.47 ($CH_2Cl_2$/MeOH 95:5); HPLC $t_R$ 19.0 min (97.8% purity); $^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$ 0.8−0.9 (m, 12H), 1.19 (s, 3H), 1.20 (s, 3H), 1.15−1.55 (m, 3H), 1.32 (s, 3H), 1.42 (s, 9H), 1.48 (s, 3H), 1.6−1.75 (m, 4H), 1.90 (m, 2H), 2.14 (m, 1H), 2.2−2.55 (m, 8H), 3.22 (s, 3H), 3.45 (t, $J$ = 6.5, 2H), 3.45−3.65 (m, 6H), 3.70 (s, 3H), 3.98 (m, 2H), 6.66 (dd, $J$ = 1.5, 8, 1H), 6.77 (d, $J$ = 1.5, 1H), 6.80 (d, $J$ = 8, 1H), 7.06 (s, 1H, NH). FAB-MS $m/z$ 734 $(M + H)^+$. The thus obtained **8f** (128 mg, 0.174 mmol), dissolved in MeOH (5 mL), was stirred in the presence of *p*-TsOH·$H_2O$ (32 mg, 0.20 mmol) overnight at rt. After evaporation of the solvent, the residue was taken up in $CH_2Cl_2$, the organic layer was washed with ice-cold 0.1 N NaOH, filtered through cotton wool, and concentrated. FC purification ($CH_2Cl_2$/MeOH 95:5) gave the title compound **9f** (99 mg, 82%) as colorless foam: TLC $R_f$ 0.54 ($CH_2Cl_2$/MeOH 9:1); HPLC $t_R$ 15.4 min (>98% purity); $^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$ 0.75 (t, $J$ = 6.5, 6H), 0.84 (t, $J$ = 6.5, 6H), 1.10 (m, 1H), 1.20 (s, 3H), 1.21 (s, 3H), 1.15−1.6 (m, 5H), 1.39 (s, 9H), 1.64 (m, 1H), 1.90 (m, 2H), 2.11 (m, 1H), 2.25 (m, 1H), 2.4−2.6 (m, 7H), 3.2−3.3 (m, 1H), 3.23 (s, 3H), 3.43−3.5 (m, 1H), 3.45 (t, $J$ = 6.5, 2H), 3.53 (m, 4H), 3.70 (s, 3H), 3.95 (t, $J$ = 6.5, 2H), 4.18 (d, $J$ = 6, 1H, OH), 6.04 (d, $J$ = 9.8, 1H, NH), 6.64 (dd, $J$ = 1.5, 8, 1H), 6.75 (d, $J$ = 1.5, 1H), 6.78 (d, $J$ = 8, 1H), 6.92 (s, 1H, NH). FAB-MS $m/z$ 695 $(M + H)^+$.

**(3*S*,5*S*,1′*S*,3′*S*,1″*R* and 3*S*,5*S*,1′*S*,3′*S*,1″*S*)-5-(1′-Azido-3′-{1″-hydroxy-[4-methoxy-3-(4-meth-oxy-butyl)-phenyl]-methyl}-4-methyl-pentyl)-3-isopropyl-dihydro-furan-2-one (15).** To the solution of bromide **13** (8.94 g, 32.7 mmol) in THF (70 mL) was added over 15 min at −75 °C a 1.5 M solution of *n*-BuLi in hexane (20.8 mL, 31.2 mmol), followed by stirring for 1 h at −75 °C. To a stirred suspension of Mg powder (1.14 g, 46.7 mmol) in THF (18 mL) was added over 10 min a solution of 1,2-dibromoethane (4.03 mL 46.7 mmol) in THF (40 mL) at rt, and stirring was continued for 1 h. To this suspension was added, after cooling to −75 °C, via syringe, the clear solution obtained above. After 1 h, a solution of aldehyde **14**[36] (4.38 g, 15.58 mmol) was added dropwise by keeping the temperature below −65 °C. After 40 min at −65 °C, the reaction mixture was poured into an ice-cooled saturated aqueous $NH_4Cl$ solution (400 mL), followed by extraction of the water layer with EtOAc. Standard workup and FC purification (EtOAc/hexane 1:4) gave the title compound in the form of a 3:1 ratio of inseparable diastereoisomers (7.41 g) as a colorless oil: TLC $R_f$ 0.24 (EtOAc/hexane 1:4); HPLC $t_R$ 19.1 min (26%) and 20.1 min (72%); IR ($CH_2Cl_2$) 3600 (OH), 2110 ($N_3$), 1770 (CO); $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 0.78−2.43 (m, 24H), 2.61−2.68 (m, 3H), 3.33 (s, 3H), 3.40 (t, $J$ = 6, 2H), 3.64−3.70 (m, 0.75H), 3.82 (s, 3H), 3.98−4.02 (m, 0.25H), 4.35−4.42 (m, 1H), 4.44 (dd, $J$ = 9, 3.5, 0.25H), 4.59 (dd, $J$ = 8, 2, 0.75H), 6.80−6.84 (m, 1H), 7.09−7.18 (m, 2H); FAB-MS, $m/z$ 430 $[M - N_2 - OH]^+$.

**(1*S*,3*S*,2′*S*,4′*S*)-{1-(4-Isopropyl-5-oxo-tetrahydro-furan-2-yl)-3-[4-methoxy-3-(4-methoxy-butyl)-benzyl]-4-methyl-pentyl}-carbamic Acid *tert*-Butyl Ester (16).** The mixture of **15** (7.41 g, 15.6 mmol), $Et_3N$ (3.26 mL, 23.4 mmol), acetic anhydride (2.21 mL, 23.4 mmol), and a catalytic amount of 4-DMAP (95 mg) in $CH_2Cl_2$ was stirred for 30 min at 0 °C, and for 15 h at rt. Then, $CH_2Cl_2$ (150 mL) was added and the organic phase was washed with 1 N HCl, a saturated $NaHCO_3$ solution, and brine, dried ($Na_2SO_4$), and evaporated. FC (EtOAc/hexane 1:3) gave the crude *O*-acetyl derivative of **15** (8.21 g, >100%) as a colorless oil: TLC $R_f$ 0.45 (EtOAc/hexane 1:2); HPLC $t_R$ 21.8 min; $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 0.83−2.45 (m, 26H), 2.58−2.65 (m, 3H), 3.17−3.20 (m, 1H), 3.33 (s, 3H), 3.38−3.42 (m, 2H), 3.80 (s, 3H), 4.00−4.05 (m, 0.25H), 4.32−4.35 (m, 0.75H), 5.61 (d, $J$ = 9, 0.25H), 5.73 (d, $J$ = 8, 0.75H), 6.78−6.83 (m, 1H), 7.04−7.18 (m, 2H).

This material, dissolved in EtOH (400 mL), was hydrogenated in the presence of 5% palladium on charcoal (Engelhardt) for 4

days under atmospheric pressure at rt. The catalyst was removed by filtration over Celite, followed by rigorous washing with EtOH (1 L). Concentration of the combined filtrates and drying in vacuo afforded a yellow oil (TLC $R_f$ 0.56 ($CH_2Cl_2$/MeOH 10:1)). Without further purification, this material was dissolved in $CH_2Cl_2$ (80 mL), and $BOC_2O$ (5.10 g, 23.4 mmol) and $Et_3N$ (5.6 mL, 32.7 mmol) were added at 0 °C. After warming to rt, stirring was continued for 16 h before removing volatiles under reduced pressure. The residue was purified by FC (EtOAc/hexane 1:4) to afford title compound **16** (4.00 g, 48%; 3 steps) as a white crystalline solid: mp 81−82 °C; TLC $R_f$ 0.36 (EtOAc/hexane 1:4); HPLC $t_R$ 23.1 min; IR ($CH_2Cl_2$) 1765 (CO), 1710 (BOC); $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 0.82 (d, $J$ = 7, 6H), 0.93 (d, $J$ = 7, 3H), 1.01 (d, $J$ = 7, 3H), 1.23−1.32 (m, 1H), 1.47 (s, 9H), 1.52−1.69 (m, 7H), 2.04−2.20 (m, 3H), 2.31−2.39 (m, 1H), 2.52−2.66 (m, 4H), 3.33 (s, 3H), 3.37−3.41 (m, 2H), 3.79 (s, 3H), 3.80−3.88 (m, 1H), 4.37−4.40 (m, 2H), 6.72 (d, $J$ = 8, 1H), 6.91−6.95 (m, 2H); FAB-MS $m/z$ 534 $[M + H]^+$, 434 $[M - BOC]^+$.

**(1*S*,2*S*,4*S*,2′*S*)-[2-Hydroxy-1-{2′-[4-methoxy-3-(4-methoxy-butyl)-benzyl]-3-methyl-butyl}-5-methyl-4-(2-morpholin-4-yl-ethylcarbamoyl)-hexyl]-carbamic Acid *tert*-Butyl Ester (17).** To the mixture of lactone **16** (4.00 g, 7.49 mmol) and 4-(2-aminoethyl)-morpholine (15 mL) was added a catalytic amount of acetic acid (4 $\mu$L), followed by heating for 24 h at 80 °C. After cooling, excess amine was removed under reduced pressure, and the residue was purified by FC on silica gel ($CH_2Cl_2$/MeOH 10:1) and recrystallization from diisopropyl ether−hexane (1:1, 60 mL) to afford **17** (4.28 g, 86%) as a white solid: mp 108−110 °C; TLC $R_f$ 0.36 ($CH_2Cl_2$/MeOH 10:1); HPLC $t_R$ 17.4 min; $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$ 0.80 (d, $J$ = 7, 3H), 0.82 (d, 3H, $J$ = 7, 3H), 0.92 (d, 3H, $J$ = 7, 3H), 0.94 (d, $J$ = 7, 3H), 1.11−1.17 (m, 1H), 1.48 (s, 9H), 1.45−1.78 (m, 10H), 1.85−1.92 (m, 1H), 2.00−2.05 (m, 1H), 2.30−2.68 (m, 10H), 3.20−3.28 (m, 1H), 3.32 (s, 3H), 3.39 (m, 2H), 3.52−3.60 (m, 3H), 3.68−3.76 (m, 4H), 3.79 (s, 3H), 4.74 (br d, $J$ = 10, 1H), 6.00 (br t, $J$ = 5, 1H), 6.72 (d, $J$ = 8, 1H), 6.95−6.97 (m, 2H); FAB-MS $m/z$ 664 $[M + H]^+$. Anal. ($C_{37}H_{65}N_3O_7$) C, H, N.

**(2*S*,4*S*,5*S*,7*S*)-5-Amino-4-hydroxy-2-isopropyl-7-[4-methoxy-3-(4-methoxy-butyl)-benzyl]-8-methyl-nonanoic Acid (2-Morpholin-4-yl-ethyl)-amide, Dihydrochloride Salt (59).** A solution of **17** (4.25 g, 6.40 mmol) in 4 N HCl−dioxane (20 mL) was stirred for 2 h at 0 °C. The mixture was lyophilized in high vacuo to give the title compound **59** (4.07 g) as an amorphous solid: TLC $R_f$ 0.21 ($CH_2Cl_2$/MeOH 10:1); HPLC $t_R$ 12.7 min (96% purity); $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 0.87−0.94 (m, 12H), 1.23−2.05 (m, 18H), 2.22−2.30 (m, 1H), 2.51−2.61 (m, 3H), 2.80−2.95 (m, 2H), 3.20−3.32 (m, 1H), 3.32 (s, 3H), 3.37−3.41 (m, 2H), 3.44−3.75 (m, 4H), 3.77 (s, 3H), 3.94−4.20 (m, 7H), 6.74 (d, $J$ = 8, 1H), 7.01 (d, $J$ = 1.5, 1H), 7.09 (dd, $J$ = 8, 1.5, 1H), 7.92−8.20 (m, 2H), 8.42 (m, 1H); FAB-MS $m/z$ 564 $[M + H]^+$. Anal. ($C_{32}H_{57}N_3O_5 \cdot 2HCl \cdot 0.5H_2O$) C, H, N.

**(2*S*,4*S*,5*S*,7*S*)-5-*tert*-Butoxycarbonylamino-4-(*tert*-butyl-dimethyl-silanyloxy)-2-isopropyl-7-[4-methoxy-3-(4-methoxy-butyl)-benzyl]-8-methyl-nonanoic Acid (18).** To a solution of **16** (0.54 g, 1.01 mmol) in 1,2-dimethoxyethane−water (2:1, 30 mL) was added a 1 M aqueous solution of LiOH (4.05 mL, 4.04 mmol) at rt. After 1 h, the reaction was complete according to TLC control. The volatiles were removed under reduced pressure, and the water phase was cooled to 0−5 °C and adjusted to pH 4 by the addition of a 10% aqueous solution of citric acid (ca. 11 mL). Repeated extraction with EtOAc, drying of the combined organics ($Na_2SO_4$), and evaporation afforded the crude $\gamma$-hydroxy acid (0.54 g, 97%) as a colorless gum: TLC $R_f$ 0.43 ($CH_2Cl_2$/MeOH 10:1). This material was dissolved in DMF (8.0 mL), TBDMSiCl (0.335 g, 2.22 mmol) and imidazole (0.303 g, 4.45 mmol) were added, and the mixture was stirred for 6 days at rt. The solvent was removed in vacuo at ambient temperature, and the residue was taken up in ice−water (50 mL) followed by repeated extraction with EtOAc. The combined organics were washed with ice−water (50 mL), ice-cold 10% citric acid (50 mL), ice−water (50 mL), and brine, dried ($Na_2SO_4$), and evaporated. FC purification (EtOAc/hexane 1:4) afforded **18** (0.46 g, 68%) as an amorphous solid: TLC $R_f$ 0.45 (EtOAc/hexane 1:2); IR ($CH_2Cl_2$) 1710 (CO); $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 0.02 (s, 3H), 0.07 (s, 3H), 0.81−0.97 (m, 21H), 1.48 (s, 9H), 1.10−1.93 (m, 12H), 2.30−2.42 (m, 2H), 2.50−2.66 (m, 3H), 3.34 (s, 3H), 3.41−3.44 (m, 2H), 3.55−3.60 (m, 1H), 3.65−3.74 (m, 1H), 3.78 (s, 3H), 4.58 (d, $J$ = 10, 1H), 6.70−6.73 (m, 1H), 6.89−6.96 (m, 2H); FAB-MS $m/z$ 689 $[M + Na]^+$, 666 $[M + H]^+$, 586 $[M - BOC]^+$.

**(1*S*,2*S*,4*S*,2′*S*)-(4-(2-Carbamoyl-2-methyl-propylcarbamoyl)-2-hydroxy-1-{2′-[4-methoxy-3-(4-methoxy-butyl)-benzyl]-3-methyl-butyl}-5-methyl-hexyl)-carbamic Acid *tert*-Butyl Ester (19).** To a solution of acid **18** (0.45 g, 0.68 mmol) and 3-amino-2,2-dimethyl-propionamide, HCl salt (**23a**; 0.114 g, 0.746 mmol), in acetonitrile (20 mL) were added $Et_3N$ (0.45 mL, 3.19 mmol) and, after 5 min, coupling reagent HBTU (0.309 g, 0.814) at 0 °C. Stirring was continued for 30 min at 0 °C and then at rt for 15 h. Volatiles were removed under reduced pressure at <35 °C, brine (50 mL) was added, and the water layer was extracted with EtOAc. The combined organics were washed with 1 N HCl, a saturated $NaHCO_3$ solution, and brine, dried ($Na_2SO_4$), and evaporated. FC purification (EtOAc/hexane 2:1) gave the *O*-TBDMSi-protected coupling product (0.54 g, >100%) as an amorphous solid: TLC $R_f$ 0.48 ($CH_2Cl_2$/MeOH 10:1); $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 0.06 (s, 3H), 0.09 (s, 3H), 0.79 (d, $J$ = 6, 6H), 0.89 (s, 9H), 0.92−0.96 (m, 6H), 1.21 (s, 3H), 1.22 (s, 3H), 1.48 (s, 9H), 1.10−1.80 (m, 11H), 1.95−2.00 (m, 1H), 2.27−2.32 (m, 1H), 2.51−2.70 (m, 3H), 3.18−3.23 (m, 1H), 3.33 (s, 3H), 3.38−3.41 (m, 2H), 3.50−3.58 (m, 2H), 3.68−3.76 (m, 1H), 3.78 (s, 3H), 4.53 (d, $J$ = 9.5, 1H), 5.21 (m, 1H), 6.71 (d, $J$ = 8, 1H), 6.27−6.33 (m, 1H), 6.58−6.62 (m, 1H), 6.92−6.97 (m, 2H); FAB-MS $m/z$ 786 $[M + Na]^+$, 764 $[M + H]^+$, 664 $[(M - BOC]^+$.

To the solution of the above *O*-silyl ether (0.52 g, 0.67 mmol) in THF (10 mL) was added a 1 M solution of *n*-$Bu_4NF$ in THF (0.71 mL, 0.71 mmol) at 0 °C. After warming to rt, the mixture was stirred for 36 h, followed by the addition of 0.71 mL of 1 M *n*-$Bu_4NF$ in THF. After completion of the reaction (TLC), the mixture was poured into water (60 mL), followed by extractive workup with EtOAc. FC purification (eluent: EtOAc) gave the title compound **19** (0.38 g, 87%) as an amorphous glass: TLC $R_f$ 0.33 (EtOAc); HPLC $t_R$ 19.7 min; $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 0.83 (d, $J$ = 7, 6H), 0.92 (d, $J$ = 6, 6H), 1.45 (s, 9H), 1.00−1.70 (m, 16H), 1.87−1.91 (m, 1H), 2.00−2.10 (m, 1H), 2.42 (m, 1H), 2.53−2.60 (m, 3H), 3.33 (s, 3H), 3.35−3.53 (m, 6H), 3.59−3.65 (m, 1H), 3.78 (s, 3H), 4.66 (d, $J$ = 9, 1H), 5.43 (m, 1H), 6.15 (m, 1H), 6.38−6.42 (m, 1H), 6.72 (d, $J$ = 8, 1H), 6.91−6.96 (m, 2H); FAB-MS $m/z$ 672 $[M + Na]^+$, 650 $[M + H]^+$, 550 $[M - BOC]^+$.

**(2*S*,4*S*,5*S*,7*S*)-5-Amino-4-hydroxy-2-isopropyl-7-[4-methoxy-3-(4-methoxy-butyl)-benzyl]-8-methyl-nonanoic Acid (2-Carbamoyl-2-methyl-propyl)-amide, Hydrochloride Salt (60).** A solution of **19** (0.37 g, 5.77 mmol) in 4 N HCl−dioxane (2 mL) was stirred for 2 h at 0 °C. The mixture was lyophilised in high vacuo to give the title compound (0.33 g) as an amorphous solid: TLC $R_f$ 0.28 ($CH_2Cl_2$/MeOH/$NH_4OH$ 350:50:1); HPLC $t_R$ 14.3 min (98% purity); $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 0.83 (d, $J$ = 6, 3H), 0.89 (d, 9H, $J$ = 6, 9H), 1.21 (s, 3H), 1.23 (s, 3H), 0.95−1.84 (m, 11H), 1.90−1.95 (m, 1H), 2.36−2.58 (m, 6H), 2.77−2.84 (m, 1H), 2.93−2.98 (m, 1H), 3.31 (s, 3H), 3.35−3.38 (m, 2H), 3.44−3.51 (m, 1H), 3.75 (s, 3H), 3.65−3.86 (m, 3H), 6.71 (d, $J$ = 8, 1H), 6.98 (d, $J$ = 1.5, 1H), 7.07 (dd, $J$ = 1.5, 8, 1H), 7.17 (br s, 1H), 7.29 (br s, 3H), 7.92 (br s, 2H); MS FAB-MS $m/z$ 550 $[M + H]^+$. Anal. ($C_{31}H_{55}N_3O_5 \cdot HCl \cdot 1.5H_2O$) C, H, N.

**Enzyme Inhibition Measurements.** Human BACE-1 activity was assayed in 100 mM acetate buffer, pH 4.5, containing 0.1% CHAPS, using Mca-SEVNLDAEFK(DNP)-OH (3 $\mu$M), as described.[51] The HIV-1 protease assay was performed as described previously.[52] $IC_{50}$ determination against nonprimate plasma renin of various species is described elsewhere.[48]

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**Supporting Information Available:** Additional data on analytical characterization of the final inhibitor compounds and experimental procedures for the preparation of **8a**−**d**, **9a**, **9b**, **9d**, **10**, **12**, **13**, **23a**, **23b**, **27**, and the P2′ amine of inhibitor **49**. This material is available free of charge via the Internet at http://pubs.acs.org.

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