Dual Metalloprotease Inhibitors: Mercaptoacetyl-Based Fused Heterocyclic Dipeptide Mimetics as Inhibitors of Angiotensin-Converting Enzyme and Neutral Endopeptidase

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Received January 12, 1997[⊗]

A series of 7,6- and 7,5-fused bicyclic thiazepinones and oxazepinones were generated and incorporated as conformationally restricted dipeptide surrogates in mercaptoacyl dipeptides. These compounds are potent inhibitors of angiotensin-converting enzyme (ACE) and neutral endopeptidase (NEP) both *in vitro* and *in vivo*. Compound **1a**, a 7,6-fused bicyclic thiazepinone, demonstrated excellent blood pressure lowering in a variety of animal models characterized by various levels of plasma renin activity and significantly potentiated urinary sodium, ANP, and cGMP excretion in a cynomolgus monkey assay. On the basis of its potency and duration of action, compound **1a** (BMS-186716) was advanced into clinical development for the treatment of hypertension and congestive heart failure.

Introduction

Angiotensin-converting enzyme (ACE), a zinc-containing carboxydipeptidase, catalyzes the conversion of the decapeptide angiotensin I (AI) to the octapeptide angiotensin II (AII). AII is a potent vasoconstrictor which also triggers the release of aldosterone, a sodium-retaining steroid. Thus ACE raises blood pressure by increasing both vascular resistance and fluid volume. Effective inhibitors of ACE have found widespread use not only in the treatment of hypertension but in the clinical management of congestive heart failure.[1] Neutral endopeptidase (NEP), like ACE, is a zinc metalloprotease and is found in high concentration in the brush border of the renal proximal tubule. NEP is highly efficient in degrading atrial natriuretic peptide (ANP), a 28-amino acid peptide secreted by the heart in response to atrial distention. ANP has opposing hormonal actions to those of AII. By interaction with its receptor, ANP promotes the generation of cGMP via guanylate cyclase activation, thus resulting in vasodilatation, natriuresis, diuresis, and inhibition of aldosterone.[2]

The renin angiotensin system and the natriuretic peptide system have opposing actions on vascular resistance, sodium and water excretion, and aldosterone production. Therefore simultaneous potentiation of ANP via NEP inhibition and attenuation of AII via ACE inhibition should lead to complimentary effects in the management of hypertension and congestive heart failure.[3] Moreover, combining ACE inhibition with NEP inhibition would likely negate the complication of AII potentiation encountered with selective NEP inhibition.[4] On the basis of this rationale, our efforts have concentrated on the utilization of mercaptoacyl dipeptides related to the early lead SQ-26332 (Figure 1) as dual inhibitors of ACE and NEP.[5] We and others have demonstrated that potent dual inhibitors can be generated either by incorporation of nonendogenous amino acids in the dipeptide fragment or by incorporating conformationally restricted dipeptide mimetics for the dipeptide.[6] BMS-182657 was an early prototype of this latter class and has been shown to potently inhibit both ACE and NEP *in vitro* and *in vivo*.[7] Despite its encouraging activity, this compound was not developed primarily based on its relatively moderate duration of activity in animal models of hypertension. Further extension of this work has led to a series of bicyclic lactams characterized generically by structure **1**. In this report we describe the synthesis of a novel 7,6-fused bicyclic thiazepinone **1a** (BMS-186716) and its pharmacological evaluation both *in vitro* and *in vivo*.

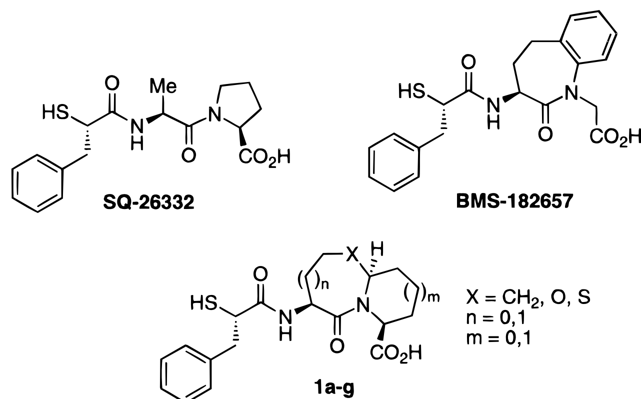

Figure 1.

Chemistry

The retrosynthesis of **1a** is depicted in Scheme 1. Bicyclic thiazepinones of the type **2** were previously unknown, and we envisioned that construction of the bicyclic ring system was possible via intramolecular *N*-acyliminium ion cyclization of intermediate **3**. Indeed we and others have demonstrated that formation of related 7,6(5)-fused ring systems can occur when the attacking nucleophile is an alcohol,[8] olefin,[9] phenyl,[6d] or heterocyclic[10] moiety. Iminium ion **3**, generated *in situ* via acid-catalyzed cyclization of dipeptide **4**, would

[⊗] Abstract published in *Advance ACS Abstracts,* May 1, 1997.

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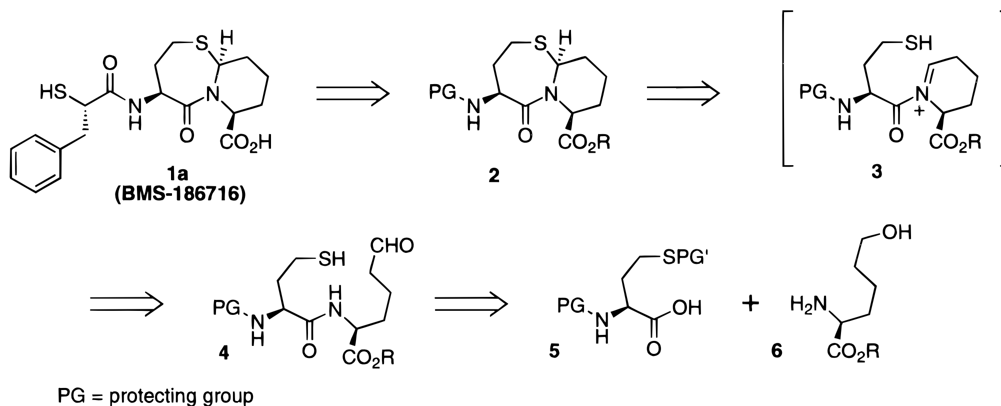
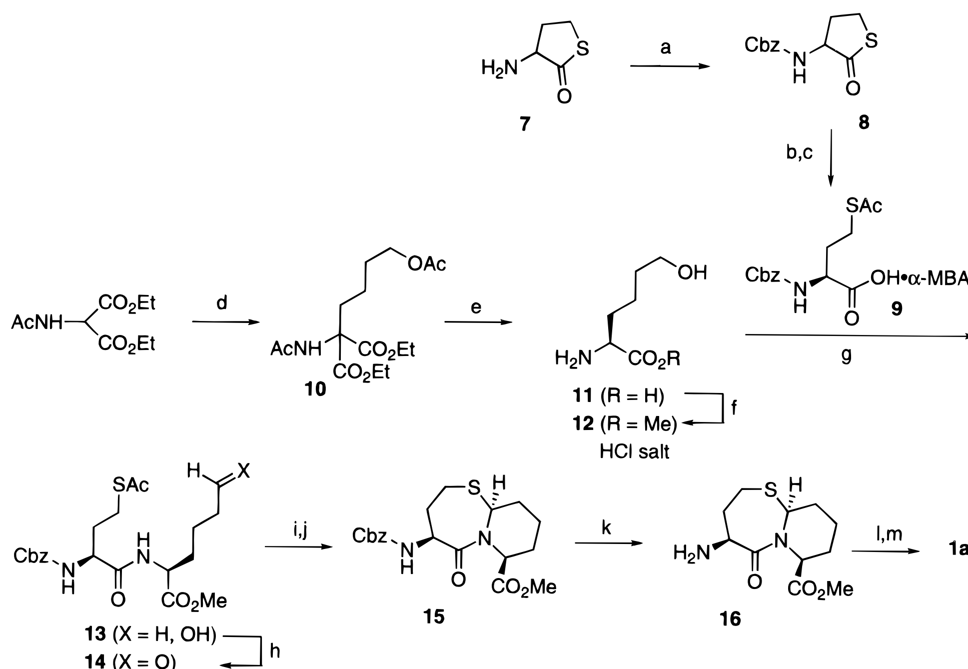
Scheme 1



Scheme 2[a]



[a] Reagents: (a) Cbz-OSu, $NaHCO_3$, H_2O, acetone, 97%; (b) KOH, H_2O, then excess Ac_2O, 85%; (c) resolve via (*S*)-α-methylbenzylamine, 41% of theory; (d) $Br(CH_2)_4OAc$, NaH, DMF, 98%; (e) (i) NaOH, H_2O, (ii) H_3O^+, Δ, (iii) porcine kidney acylase, 66% of theory overall; (f) HCl, MeOH; (g) WSC, HOBT, 4-methylmorpholine, DMF, CH_2Cl_2, 88% (two steps); (h) Swern oxidation, 86%; (i) NaOMe, MeOH, rt, 10 min; (j) cat. TFA, CH_2Cl_2, Δ, 72% (two steps); (k) TMSI, CH_2Cl_2, 92%; (l) (*S*)-$AcSCH(CH_2Ph)CO_2H$, BOP reagent, TEA, CH_2Cl_2, 69%; (m) NaOH, H_2O, MeOH, then H_3O^+, 83%.

be derived from the protected L-homocysteine and L-hydroxynorleucine fragments **5** and **6**, respectively.

Commercially available racemic homocysteine thiolactone (**7**) was protected to give **8** (Scheme 2) and subsequently saponified and acetylated *in situ* to give racemic **9** (as the free acid) in high yield.[11] Resolution of the acid via the (*S*)-α-methylbenzylamine salt crystallized from MeOH/EtOAc afforded **9** in >98% ee and 41% of theoretical yield. Literature methods[12] for the generation of the requisite L-hydroxynorleucine (**11**) were inefficient, lengthy, and problematic to run on a large scale and thus necessitated development of an alternate route to this nonendogenous amino acid. Sodium hydride-induced alkylation of diethyl acetamidomalonate with 4-bromobutyl acetate afforded **10**. In a subsequent one-pot transformation, the adduct was saponified, decarboxylated, and enzymatically resolved to give L-hydroxynorleucine **11**. Coupling of the methyl ester **12** with the free acid of **9** provided dipeptide **13**. Swern oxidation to give **14**, followed by deprotection of the thiol and subsequent TFA-induced cyclization afforded bicyclic lactam **15** in good yield and high (>99%) diastereomeric purity.[13] The stereoconfiguration was confirmed by NOE and ultimately single-crystal X-ray analysis of **1a**. Removal of the Cbz protecting group afforded the amine **16** which was coupled to (*S*)-2-(acetylthio)-3-benzenepropanoic acid[14] and deprotected to give **1a**. A variant of this methodology was also used to prepare the corresponding 7,5- and 6,6-fused bicyclics in both the oxygen and sulfur series.[15]

Discussion

Table 1 lists *in vitro* data and AI pressor responses (intravenous administration in rats) for compounds **1a**–**g**. In vitro, all of the fused bicyclic mimetic-based inhibitors demonstrated superior activity against both ACE and NEP as compared to their parent Ala-Pro-based counterpart SQ-26332[5] (ACE I_{50} = 30 nM, NEP I_{50} = 400 nM), validating the concept of conformational restriction in this series. Essentially all of the com-

Table 1. Inhibition of ACE and NEP *in Vitro* and AI Pressor Responses for Compounds **1a–g**

no.	X	*n*	*m*	fusion	formula[a]	I_{50} (nM) NEP[b]	I_{50} (nM) ACE[c]	AI pressor ED_{50}[d] (μmol/kg, iv)	% inhibition of AI pressor response at various time points (min) after 0.5 μmol/kg iv administration: max.[e]	30	60	120
1a	S	1	1	7,6	$C_{19}H_{24}N_2O_4S_2$	8	5	0.07	76 ± 4	66 ± 9	60 ± 16	58 ± 12
1b	S	1	0	7,5	$C_{18}H_{22}N_2O_4S_2$[f]	4	4	0.03	82 ± 6	76 ± 7	60 ± 5	25 ± 6
1c	S	0	1	6,6	$C_{18}H_{22}N_2O_4S_2$·0.12EtOAc	42	5	0.23	78 ± 3	3 ± 5	nd[g]	nd
1d	O	1	1	7,6	$C_{19}H_{24}N_2O_5S$·0.12EtOAc	9	8	0.05	83 ± 4	3 ± 3	nd	nd
1e	O	1	0	7,5	$C_{18}H_{22}N_2O_5S$·0.85H_2O	26	7	0.06	79 ± 6	63 ± 5	3 ± 2	nd
1f	CH_2	1	1	7,6	$C_{20}H_{26}N_2O_4S$·0.45H_2O[h]	25	11	0.12	71 ± 4	65 ± 5	35 ± 6	11 ± 5
1g	CH_2	1	0	7,5	$C_{19}H_{24}N_2O_4S$	18	5	0.06	75 ± 4	34 ± 4	3 ± 10	nd

[a] All spectral data were consistent with the assigned structures. All compounds were analyzed for C, H, N, and S for the formula shown. [b] Compounds were assayed against purified rat kidney neutral endopeptidase using a fluorometric assay with dansyl-Gly-Phe-Arg as the substrate. [c] Compounds were assayed against angiotensin-converting enzyme isolated from rabbit lung extract using hippuryl-L-histidyl-L-leucine (HHL) as the substrate. [d] Represents dose required for 50% inhibition of the AI pressor response in normotensive rats. [e] Maximal response in assay usually 2 min after administration of compound. [f] Anal. ($C_{18}H_{22}N_2O_4S_2$) C, H, N; S: calcd, 16.25; found, 15.80. [g] Not determined at this time point. [h] Anal. ($C_{20}H_{26}N_2O_4S$·0.45H_2O) C, H, S; N: calcd, 7.03; found, 6.57.

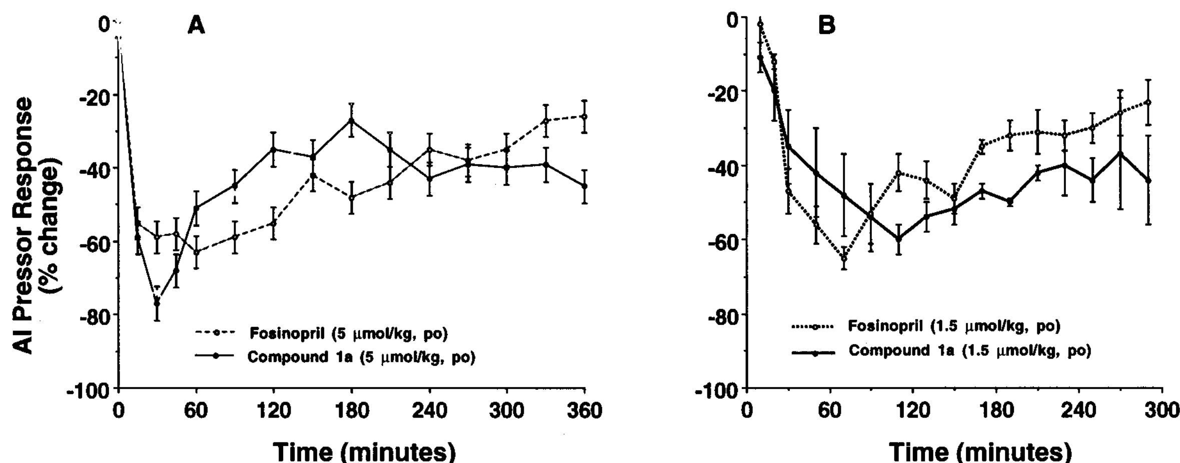


Figure 2. Inhibition of the MAP response to angiotensin I (AI, iv administration) in conscious rats (A) and cynomolgus monkeys (B) after oral (po) administration of fosinopril or compound **1a** was determined according to procedures previously described.[7] Conscious animals ($n = 4$ per group), instrumented with implanted arterial and venous catheters at least 2 weeks prior to study, were prepared for direct recording of aterial blood pressure using a pressure transducer. Changes in MAP in response to iv injections of AI (310 ng/kg) were obtained before (control) and at intervals after the administration of vehicle, fosinopril, or compound **1a**. The percent change (mean ± SE) from the control response (% inhibition) was determined for each response after drug or vehicle administration.

pounds potently inhibited ACE *in vitro* and robustly inhibited the acute pressor responses to AI in the rat, although there were some variations with respect to potency against NEP *in vitro*. The 6,6-fused thiazine **1c** was substantially less potent versus NEP *in vitro* and ACE *in vivo* than its 7,6- or 7,5-fused analogs, suggesting that incorporation of a seven-membered azepinone ring in the mimetic is critical for optimal activity. A similar observation was made when comparing azepinone-based inhibitors with their corresponding eight-membered azocinone derivatives.[16] Differentiation among compounds was also made by comparing duration of activity in the AI pressor assay at a comparative dose of 0.5 μmol/kg, iv. Despite their high maximal responses in this assay, compounds **1c**, **1d**, **1e**, and **1g** were short-acting, failing to display significant activity 60 min postdosing. The duration of activity was especially short with **1c** and **1d**, in which response levels returned to baseline within 30 min. In contrast, the 7,6(5)-fused thiazepinones **1a** and **1b** effected significant changes in the pressor response 120 min posttreatment at this dose. Upon oral administration to rats and monkeys in the AI pressor assay (Figure 2), **1a** effected a response comparable both in magnitude and duration of activity to that of the clinically efficacious, once-a-day, selective ACE inhibitor fosinopril.[17] On the basis of its excellent potency against NEP and ACE *in vitro* and its superior duration of activity in the AI pressor assay, compound **1a** was selected for extensive evaluation *in vivo*.

ACE inhibitors have historically demonstrated depressor effects in the sodium-deplete spontaneously hypertensive rat (sd-SHR) whereas selective NEP inhibitors have generally been shown to be ineffective in this assay.[18] At a single oral dose (30 μmol/kg, ~12 mg/kg), **1a** decreased mean arterial pressure (MAP) approximately 40 mmHg below baseline from 10 to 24 h, whereas fosinopril averaged a 20 mmHg decrease dur-

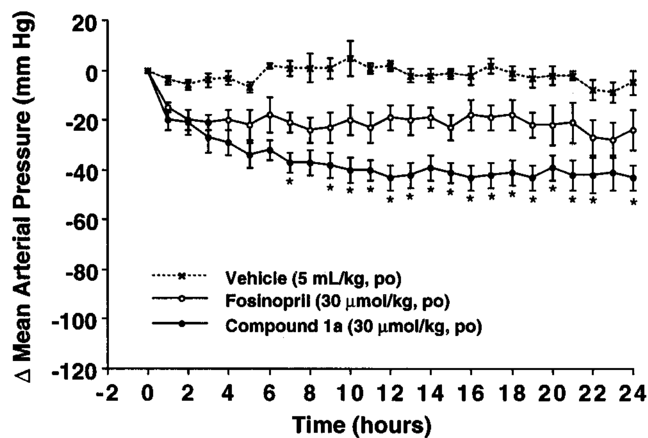


Figure 3. Changes in MAP in conscious sodium-depleted spontaneously hypertensive rats (sd-SHR) after oral administration of vehicle (5% $NaHCO_3$, $n = 10$), fosinopril ($n = 7$), or compound **1a** (n = 7). Mean arterial pressures before administration of agents were (mean ± SE): 146 ± 3, 150 ± 7, and 148 ±5 mmHg in the vehicle, fosinopril, and compound **1a** groups, respectively. For fosinopril, $p < 0.05$ vs vehicle for hours 2–24. For compound **1a**, $p < 0.05$ vs vehicle for hours 1–24. For compound **1a**, $p < 0.05$ vs fosinopril (*).

ing this same period (Figure 3). Thus, as an ACE inhibitor, **1a** was at least as effective as fosinopril at 30 μmol/kg, po, with regard to magnitude and duration of lowering MAP in this model of high renin hypertension. *In vivo* inhibition of NEP for **1a** was demonstrated in the 1-kidney DOCA salt rat hypertension assay. Selective NEP inhibitors have been shown to lower MAP in this low renin model of hypertension, whereas selective ACE inhibitors (i.e. fosinopril) are usually ineffective. Oral administration of **1a** at 100 μmol/kg once daily resulted in a 38 mmHg decrease in systolic blood pressure at day three as compared to vehicle (Figure 4). No significant difference versus vehicle was observed with fosinopril. Again, the duration of the observed antihypertensive effect with **1a** was robust; at day four MAP, as measured directly from indwelling catheters, was 36 mmHg lower than that in the vehicle group 24 h postdosing.

The potent response in both the AI pressor assay and the sd-SHR indicated that compound **1a** is an effective ACE inhibitor with good duration of action. In order to better assess the NEP inhibitory component on renal parameters, **1a** was administered to cynomolgus monkeys infused with saline and 30 ng/min of AII and subsequently challenged with a bolus dose of human-ANP. The constant infusion of AII masked the effect of ACE inhibition by suppressing renin release and replacing angiotensin II so that a clearer measure of *in vivo* inhibition of NEP would be obtained.[19] As anticipated, 0.1–3 μmol/kg doses of **1a** potentiated urinary sodium, urinary ANP, and urinary cGMP excretion (Figure 5). The responses were dose related except for ANP since the time course of the experiment did not allow ANP levels to return to baseline and thus the full ANP response to the drug was not obtained. In a subsequent study in cynomolgus monkeys (manuscript in preparation), daily oral administration of **1a** at 50 μmol/kg for four days potentiated the urinary ANP response to exogenous human-ANP. The results are consistent with the oral activity of **1a** in the DOCA-salt hypertensive rats.

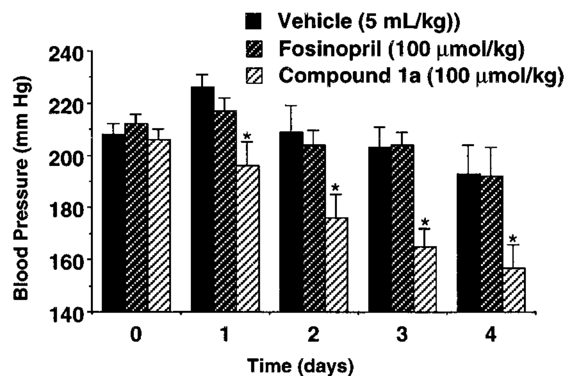


Figure 4. Effect on blood pressure upon oral administration (qd) of vehicle (5% $NaHCO_3$, $n = 5$), fosinopril ($n = 9$), or compound **1a** ($n = 9$) in the 1K-DOCA salt rat assay. At day 0, systolic blood pressure was measured indirectly by the tail-cuff method after conditioning rats to the procedure for three consecutive days prior. Dosing was initiated on day 1. At days 1, 2, and 3, systolic blood pressure was measured indirectly 4 h after each dose. At day 4, mean arterial pressure was measured directly from indwelling catheters 24 h after the last dose. $^*p < 0.05$ vs vehicle.

Conformationally restricted mercaptoacyl dipeptide **1a** has been demonstrated to be a potent inhibitor of both ACE and NEP *in vitro* and was shown to be an effective antihypertensive agent in models characterized by various levels of plasma renin activity. Additional antihypertensive, hemodynamic, and renal pharmacology studies on **1a** will be presented in future reports as well as animal studies demonstrating the potential of this compound in the treatment of congestive heart failure. Based in part on these studies, compound **1a** (BMS-186716) was selected for further clinical development. The compound is currently in phase II clinical trials for the treatment of hypertension and congestive heart failure.

Experimental Section

All reactions were carried out under a static atmosphere of argon and stirred magnetically unless otherwise noted. All reagents used were of commercial quality and were obtained from Aldrich Chemical Co. or Sigma Chemical Co. Melting points were obtained on a Hoover Uni-melt melting point apparatus and are uncorrected. Infrared spectra were recorded on a Mattson Sirius 100-FTIR spectrophotometer. ^1H (400 Mz) and ^{13}C (100 Mz) NMR spectra were recorded on a JEOL GSX400 spectrometer using Me_4Si as an internal standard. Optical rotations were measured in a 1-dm cell on a Perkin-Elmer 241 polarimeter, and *c* is expressed in g/100 mL. All flash chromatographic separations were performed using E. Merck silica gel (60, particle size, 0.040–0.063 mm). Reactions were monitored by TLC using 0.25 mm E. Merck silica gel plates (60 F_{254}) and were visualized with UV light or 5% phosphomolybdic acid in 95% EtOH.

***N*-(Carbobenzyloxy)-D,L-homocysteine thiolactone (8).** To a 4 L flask equipped with a large magnetic stirring bar was added 750 mL of H_2O followed by DL-homocysteine thiolactone hydrochloride (**7**) (362.07 g, 2.36 mol). After most of the salt had dissolved, complete dissolution was achieved by the addition of 750 mL of acetone. To the resulting solution was added *N*-((benzyloxycarbonyl)oxy)succinimide (Cbz-OSu, 587.38 g, 2.36 mol) as a solid. The resulting heterogenous mixture was carefully treated with solid $NaHCO_3$ (201.93 g, 2.40 mol) added portionwise over a period of 5 min, loosely covered, and stirred at ambient temperature for 20 h. The reaction mixture was then diluted with H_2O to a volume of 4 L, and the precipitate was collected by filtration and rinsed three times with 1 L of H_2O. Drying *in vacuo* at 35 °C for 16 h afforded compound **8** (578.6 g, 97%) as a white solid: TLC

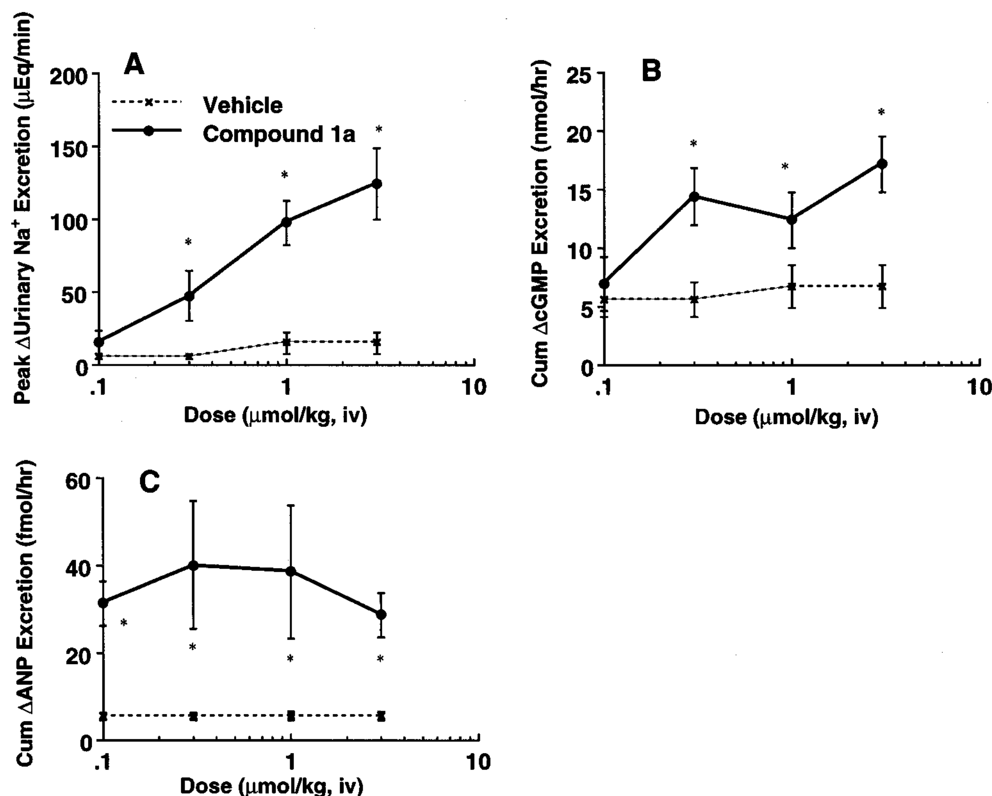


Figure 5. Natriuretic, cGMP, and ANP excretory responses to 0.3 nmol/kg ANP in conscious, sodium-loaded monkeys infused with 30 ng/min angiotensin II and treated with compound **1a** or vehicle (0.84% NaHCO3) by iv administration. The dose–response relationships on the peak change in urinary sodium excretion (A), cumulative changes in urinary cGMP excretions (B), and cumulative changes in urinary ANP excretions (C) following ANP challenge.

R_f 0.55 (6:4 EtOAc:hexane); mp 109–111 °C; ^{1}H NMR ($CDCl_3$) δ 1.97 (m, 1H), 2.71 (m, 1H), 3.14 (m, 1H), 3.21 (m, 1H), 4.30 (m, 1H), 5.08 (s, 2H), 5.58 (s, 1H), 7.31 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 204.86, 156.03, 135.88, 128.32, 128.00, 127.86, 66.93, 60.45, 31.27, 26.88. Anal. Calcd for $C_{12}H_{13}NO_3S$: C, 57.35; H, 5.21; N, 5.57; S, 12.76. Found: C, 57.41; H, 5.10; N, 5.51; S, 12.67.

***S*-Acetyl-*N*-[(phenylmethoxy)carbonyl]-L-homocysteine, *S*-(−)-α-Methylbenzylamine Salt (9).** A solution of compound **8** (750 g, 3.02 mol) in THF (3.75 L) and H_2O (1.2 L) was degassed three times (vacuum/nitrogen purges). A solution of KOH (88%, 577 g, 9.05 moles) in H_2O (1.5 L), degassed as above, was added to the thiolactone solution. After the solution was stirred at room temperature for 1.5 h, acetic anhydride (1623 g, 15.9 mol) was then added over 1.75 h with continued cooling (ice bath), maintaining a temperature of <27 °C. After an additional 30 min at room temperature, the reaction was acidified with 1 L of 6 N aqueous HCl (final pH = 4.32) and then concentrated *in vacuo*, collecting ~3.5 L of distillate. The concentrate was acidified further with additional 6 N aqueous HCl (400 mL) to a pH of 2.6. The product was extracted with EtOAc (2 × 2.5 L). The combined organic extracts were washed three times with saturated brine, dried (Na_2SO_4), filtered, and concentrated under vacuum to afford a tacky white solid. The residue was azeotroped three times with toluene (2 L portions) to remove residual acetic acid. The solid slurry was collected by filtration using ~500 mL hexane/EtOAc to transfer and dried under vacuum at 40 °C for 16 h to afford racemic **9** (free acid form, 799 g, 85%) as a white solid: TLC R_f 0.48 (2:98 HOAc:EtOAc).

To a solution of racemic free acid of **9** (1243 g, 3.99 mol) in EtOAc (5.0 L) was added (*S*)-(−)-α-methylbenzylamine (459.7 g, 3.8 mol, Lancaster Synthesis) dropwise maintaining a temperature ≤26 °C with cooling (ice bath) over 1.5 h. The mixture was seeded with 98% enantiomerically pure **9** and stirred at room temperature for 4 h. The precipitate was collected by filtration and dried for 24 h at room temperature to give enantiomerically enriched **9** (595 g, 72.5% of theory, 36.3% from racemic free acid) as a white solid with an enantiomeric purity of 82.3% by chiral HPLC analysis. A portion of this salt (23 g) was suspended in a mixture of EtOAc:MeOH (3:1, 120 mL) and stirred for 3 h. The solid was collected by filtration, washed with cold EtOAc (25 mL), and dried *in vacuo* to give **9** (13 g) with an enantiomeric purity of 98.7% by chiral HPLC analysis. Repeating the above procedure with EtOAc:MeOH (3:1, 60 mL) afforded the salt (11 g) with an enantiomeric purity of 99.5%: $[\alpha]_D$ +2.6° (*c* 1.0, EtOH); mp 117–119 °C dec; ^{1}H NMR (CD_3OD) δ 1.59 (d, J = 7 Hz, 3H), 1.88 (m, 1H), 2.05 (m, 1H), 2.26 (s, 3H), 2.89 (m, 2H), 4.06 (m, 1H), 4.42 (q, J = 7 Hz, 1H), 5.06 (dd, 2H), 7.26–7.46 (m, 10H); ^{13}C NMR (CD_3OD) δ 197.22, 178.25, 158.08, 140.03, 138.25, 130.13, 129.89, 129.40, 128.88, 128.72, 127.62, 67.39, 56.95, 52.15, 34.24, 30.48, 26.63, 20.93. Anal. Calcd for $C_{22}H_{28}N_2O_5S$: C, 60.84; H, 6.54; N, 6.45; S, 7.38. Found: C, 60.82; H, 6.39; N, 6.25; S, 7.37. Partitioning the salt between EtOAc and 10% aqueous $KHSO_4$ afforded the free acid of **9** in quantitative yield as an oil which solidified on standing: $[\alpha]_D$ = −1.3° (*c* 1.0, 95% EtOH); mp 73–74 °C; ^{1}H NMR (CD_3OD) δ 1.94 (m, 1H), 2.08 (m, 1H), 2.28 (s, 3H), 2.86–3.00 (m, 2H), 4.25 (m, 1H), 5.08 (s, 2H), 7.27–7.36 (m, 5H); ^{13}C NMR (CD_3OD) δ 197.07, 175.11, 158.57, 138.07, 129.41, 128.94, 128.73, 67.64, 54.23, 32.69, 30.47, 26.43. Anal. Calcd for $C_{14}H_{17}NO_5S$: C, 54.01; H, 5.50; N, 4.50; S, 10.30. Found: C, 53.97; H, 5.45; N, 4.36; S, 10.40.

2-(Acetylamino)-2-[4-(acetyloxy)butyl]propanedioic Acid, Diethyl Ester (10). A stirred suspension of 95% sodium hydride (60.8 g, 2.532 mol) in anhydrous DMF (500 mL) under an atmosphere of argon was cooled to 0 °C (ice bath). A solution of diethyl acetamidomalonate (500 g, 2.302 mol) in anhydrous DMF (1.2 L) was added over a period of 45 min while keeping the reaction temperature below 18 °C. After the addition was complete, the turbid solution was gradually warmed to room temperature. After 1 h of stirring at room temperature, 4-bromobutyl acetate (471.5 g, 2.417 mol) was added. The mixture was then stirred at 59–60 °C for 18 h. The resulting slurry was cooled to room temperature, quenched with absolute EtOH (40 mL) and glacial acetic acid (4 mL), stirred for about 15 min, poured into a 10% LiCl solution, and

extracted with EtOAc (2 × 3 L). The combined EtOAc extracts were washed with 10% LiCl (3 × 3 L), dried (Na_2SO_4), and evaporated *in vacuo* to give compound **10** (750 g, 98%) as an oil: TLC R_f 0.19 (1:1 EtOAc:hexane); ^{1}H NMR ($CDCl_3$) δ 1.95 (m, 2H), 2.05 (t, J = 7.1 Hz, 6H), 1.63 (tt, J = 7.0 and 7.0 Hz, 2H), 2.03 (s, 3H), 2.04 (s, 3H), 2.38 (m, 2H), 4.03 (t, J = 6.5 Hz, 2H), 4.25 (q, J = 7.0 Hz, 4H), 6.83 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 170.86, 168.89, 167.87, 66.25, 63.89, 62.36, 31.63, 28.09, 22.86, 20.76, 20.08, 13.81; IR (film) 2926, 1740, 1680, 1507, 1370, 1248, 1198 cm^{-1}.

(*S*)-2-Amino-6-hydroxyhexanoic Acid (11). To a solution of compound **10** (730 g, 2.2 mol) in a 5 L 3-neck flask (equipped with a thermometer, magnetic stirrer, and air-cooled condenser) in absolute EtOH (300 mL) was added aqueous 6 N sodium hydroxide (1.6 L, 9.6 mol). The reaction mixture was heated at 68−70 °C for 5 h to afford a homogeneous solution. The reaction was cooled to room temperature, and 6 N HCl (1.32 L) was added slowly to adjust the pH to 1.3. The flask was equipped with a short path distillation head, and the EtOH was distilled off as the temperature was slowly increased to 87−90 °C and maintained at this temperature for 8.5 h. Slow carbon dioxide evolution was observed. The total volume of distillate was 600 mL. The reaction mixture was stripped and then concentrated from toluene (2 × 500 mL) to give a semisolid mass. The solid was triturated with absolute EtOH (1 L), filtered, and rinsed with additional absolute EtOH (500 mL). The filtrate was concentrated *in vacuo* to yield 509 g of the crude intermediate (82% purity) which contained EtOH and toluene.

The residue product (443 g of crude product) was dissolved in H_2O (3.3 L), and 1 N lithium hydroxide was added until the pH was 7.5 (1.53 L required). The mixture was heated to 35 °C, porcine kidney acylase I (0.4 g, Sigma) was added, and the reaction mixture was stirred for 24 h. The pH was readjusted to 7.5 with 1 N lithium hydroxide (about 2 mL), additional acylase (0.4 g) was added, and the reaction mixture was stirred for an additional 17 h. The pH of the solution was adjusted to 5.9 with acetic acid. Celite (20 g) and charcoal (20 g) were added, and the reaction mixture was heated to 92 °C and maintained at this temperature for 5 min. The reaction was filtered through a pad of Celite and concentrated *in vacuo* to a paste (441 g) which was triturated with 900 mL of 1:5:10 H_2O:EtOH:DMF. The slurry was refrigerated overnight, filtered, and washed with 200 mL of the above solvent mixture to yield 214 g of crude product. This material was suspended in MeOH (500 mL), warmed on a steam bath, allowed to stand for 2 h, and filtered. Repeat treatment of the solid afforded after drying compound **11** (108 g, 33% chemical yield, 66% theoretical) as a white powder: TLC R_f 0.62 (MeOH:H_2O:HOAc, 10:1:1); $[\alpha]_D$ = +22° (c 1.44, 6 N HCl); ^{1}H NMR (D_2O) δ 1.27 (m, 2H), 1.43 (tt, J = 7.0 and 7.0 Hz, 2H), 1.72 (m, 2H), 3.45 (t, J = 6.3 Hz, 2H), 3.58 (t, J = 6.0 Hz, 1H); ^{13}C NMR (D_2O) δ 175.85, 62.24, 55.74, 31.92, 31.20, 21.85; IR (KBr) 3418, 2945, 1605, 1582, 1518, 1408, 1354 cm^{-1}; MS (CI) 148 (M + H)$^+$. Anal. Calcd for $C_6H_{13}NO_3$: C, 48.25; H, 8.94; N, 9.38. Found: C, 48.66; H, 8.77; N, 9.43.

[*S*-(*R,*R**)]-2-[[4-(Acetylthio)-1-oxo-2-[[(phenylmethoxy)carbonyl]amino]butyl]amino]-6-hydroxyhexanoic Acid, Methyl Ester (13).** A stream of hydrogen chloride gas was bubbled into a suspension of **11** (7.35 g, 0.05 mol) in MeOH (150 mL) cooled at 0 °C (ice bath) for 15 min. The resulting solution was refluxed for 3.5 h. After being cooled to room temperature, the reaction mixture was concentrated *in vacuo*, and the obtained oily residue was azeotroped with toluene (3 × 50 mL) and dried *in vacuo* to give ester **12** (HCl salt) (10 g, 100%) as a colorless syrup, which was used in the following reaction without further purification: TLC R_f 0.33 (2:1:1:1 EtOAc:*n*-BuOH:AcOH:H_2O); ^{1}H NMR (DMSO-d^6) δ 1.25−1.50 (m, 4H), 1.80 (m, 2H), 3.38 (t, J = 6.0 Hz, 2H), 3.75 (s, 3H), 3.98 (m, 1H), 4.63 (br s, 1H), 8.65 (br s, 3H); MS (ESI) (M + H)$^+$ 162.

To a solution of the free acid of **9** (14.40 g, 0.046 mol) in CH_2Cl_2 (100 mL) at 0 °C (ice bath) was added 1-hydroxybenzotrizole hydrate (HOBt, 6.756 g, 0.05 mol), followed by 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (WSC, 8.90 g, 0.046 mol). The resulting suspension was stirred at 0 °C for 30 min and then a solution of **12** in DMF (50 mL) was added, followed by dropwise addition of 4-methylmorpholine (6.05 mL, 0.055 mol). The resulting suspension was stirred at room temperature for 20 h. The reaction mixture was partitioned between EtOAc (500 mL) and 5% aqueous $KHSO_4$ (100 mL). The separated organic layer was washed with 5% aqueous $KHSO_4$ (100 mL), saturated aqueous $NaHCO_3$ (2 × 100 mL), H_2O (2 × 200 mL), and brine (200 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated *in vacuo*, and the residue was flash chromatographed on a short silica gel column (4:1 EtOAc:hexanes as eluent) to afford dipeptide **13** (18.56 g, 88%) as a colorless oil which solidified on standing: $[\alpha]_D$ −6.5° (c 1.0, CH_2Cl_2); mp 87−88 °C; TLC R_f 0.25 (4:1 EtOAC:hexane); ^{1}H NMR ($CDCl_3$) δ 1.42 (m, 2H), 1.55 (m, 2H), 1.73 (m, 1H), 1.91 (m, 2H), 2.10 (m, 2H), 2.34 (s, 3H), 2.87 (m, 1H), 3.06 (m, 1H), 3.60 (m, 2H), 3.73 (s, 3H), 4.22 (m, 1H), 4.56 (m, 1H), 5.10 (s, 2H), 5.71 (d, J = 7.7 Hz, 1H), 7.16 (d, J = 7.2 Hz, 1H), 7.34 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 196.61, 172.57, 171.16, 156.24, 136.16, 128.51, 128.16, 128.00, 67.08, 62.00, 53.77, 52.40, 52.35, 33.04, 31.86, 31.51, 30.58, 25.25, 21.60; IR (KBr) 3518, 3296, 2934, 1740, 1695, 1647, 1531, 1263, 1064, 633 cm^{-1}; MS (ESI) (M + H)$^+$ 455. Anal. Calcd for $C_{21}H_{30}N_2O_7S$: C, 55.49; H, 6.65; N, 6.16; S, 7.05. Found: C, 55.22; H, 6.54; N, 5.97; S, 6.98.

[*S*-(*R,*R**)]-2-[[4-(Acetylthio)-1-oxo-2-[[(phenylmethoxy)carbonyl]amino]butyl]amino]-6-oxohexanoic Acid, Methyl Ester (14).** To a solution of oxalyl chloride (4.17 mL, 0.048 mol) in CH_2Cl_2 (75 mL) cooled at −78 °C was added dropwise anhydrous methyl sulfoxide (DMSO, 6.78 mL, 0.096 mol). The mixture was stirred for 20 min and then treated with a solution of **13** (15.50 g, 0.034 mol) in CH_2Cl_2 (50 mL) via cannula. The resulting white suspension was stirred at −78 °C for 1 h, and then triethylamine (21 mL, 0.15 mol) was added. After being stirred at −78 °C for 15 min, the reaction mixture was slowly warmed to room temperature and then partitioned between EtOAc (500 mL) and 5% aqueous $KHSO_4$ (100 mL). The organic layer was washed with 5% aqueous $KHSO_4$ (100 mL), saturated aqueous $NaHCO_3$ (100 mL), H_2O (2 × 200 mL), and brine (200 mL), then dried over $MgSO_4$, and filtered. The filtrate was concentrated *in vacuo*, and the residue was flash chromatographed (40−60% EtOAc:hexanes as eluent) to afford aldehyde **14** (13.18 g, 86%) as a white solid: $[\alpha]_D$ −6.8° (c 1.2, CH_2Cl_2); mp 74−75 °C; TLC R_f 0.49 (4:1 EtOAC:hexane); ^{1}H NMR ($CDCl_3$) δ 1.67 (m, 3H), 1.90 (m, 2H), 2.11 (m, 1H), 2.35 (s, 3H), 2.47 (t, J = 6.4 Hz, 2H), 2.85 (m, 1H), 3.10 (m, 1H), 3.75 (s, 3H), 4.20 (m, 1H), 4.57 (m, 1H), 5.12 (s, 2H), 5.58 (d, J = 7.7 Hz, 1H), 7.08 (d, J = 7.7 Hz, 1H), 7.34 (m, 5H), 9.73 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 201.57, 196.35, 172.12, 170.91, 155.98, 136.10, 128.39, 128.03, 127.88, 66.92, 53.60, 52.36, 51.88, 42.85, 32.94, 31.17, 30.44, 25.10, 17.69; IR (KBr) 3302, 2928, 1751, 1696, 1649, 1539, 1262, 1215, 1128 cm^{-1}; MS (ESI) (M + H)$^+$ 453. Anal. Calcd for $C_{21}H_{28}N_2O_7S$: C, 55.74; H, 6.24; N, 6.19; S, 7.08. Found: C, 55.66; H, 6.11; N, 6.08; S, 7.12.

[4*S*-(4α,7α,10a*β*)]-Octahydro-4-[[(phenylmethoxy)carbonyl]amino]-5-oxo-7*H*-pyrido[2,1-*b*][1,3]thiazepine-7-carboxylic Acid, Methyl Ester (15). A solution of **14** (11.845 g, 0.026 mol) in MeOH (110 mL) cooled to 0 °C (ice bath) was purged with argon for 20 min. Sodium methoxide (25% wt/wt in MeOH, 6.00 mL, 0.026 mol) was added rapidly with continual argon purging, and the reaction mixture was stirred for an additional 10 min and then quenched with saturated aqueous ammonium chloride (160 mL). The resulting suspension was partitioned between EtOAc (200 mL) and H_2O (100 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (3 × 100 mL). The combined EtOAc extracts were washed with saturated aqueous ammonium chloride (100 mL), H_2O, and brine, then dried over $MgSO_4$, and filtered. The filtrate was concentrated *in vacuo*, and the oily residue was azeotroped twice with toluene/CH_2Cl_2, dried *in vacuo*, and used immediately in the next reaction without further purification: ^{1}H NMR ($CDCl_3$) δ 1.60 (m, 3H), 1.80−2.15 (m, 4H), 2.45 (m, 2H), 2.60 (m, 2H), 3.74 (s, 3H), 4.47 (m, 1H), 4.56 (m, 1H), 5.10 (s, 2H), 5.67 (d, J = 7.9 Hz, 1H), 6.88 (d, J = 7.2 Hz, 1H), 7.32 (m, 5H), 9.71 (s, 1H).

To a solution of the above thiol in CH_2Cl_2 (200 mL) was added dropwise trifluoroacetic acid (2.0 mL, 0.026 mol), and the mixture was refluxed under argon for 16 h. The resulting slightly turbid solution was concentrated *in vacuo*, diluted with EtOAc (300 mL), and washed successively with saturated aqueous $NaHCO_3$, H_2O, and brine, then dried over $MgSO_4$, and filtered. The filtrate was concentrated *in vacuo*, and the resulting light brown oil was flash chromatographed (25−30% EtOAc:hexanes as eluent) to give diastereomerically pure bicyclic thiazepinone **15** (7.38 g, 72%) as a white foam: $[\alpha]_D$ −93.1° (*c* 1.0, CH_2Cl_2); TLC R_f 0.40 (1:1 EtOAC:hexanes); ^1H NMR ($CDCl_3$) δ 1.67 (m, 3H), 2.03 (m, 3H), 2.33 (m, 1H), 2.44 (m, 1H), 2.95 (m, 1H), 3.26 (m, 1H), 3.72 (s, 3H), 4.86 (m, 1H), 5.11 (m, 2H), 5.21 (d, J = 4.8 Hz, 1H), 5.30 (m, 1H), 6.22 (d, J = 6.5 Hz, 1H), 7.32 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 172.80, 171.29, 155.38, 136.34, 128.42, 128.01, 127.85, 66.68, 58.86, 52.67, 52.13, 50.89, 33.08, 31.07, 24.69, 16.96; IR (CH_2Cl_2) 3403, 3327, 2949, 1724, 1657, 1497, 1433, 1414, 1209, 1053, 1001 cm^{-1}; MS (ESI) $(M + H)^+$ 393 Anal. Calcd for $C_{19}H_{24}N_2O_5S.0.15H_2O$: C, 57.75; H, 6.20; N, 7.09; S, 8.11. Found: C, 57.76; H, 6.39; N, 7.04; S, 8.26.

[4S-(4α,7α,10a*β*)]-Octahydro-4-amino-5-oxo-7*H*-pyrido-[2,1-*b*][1,3]thiazepine-7-carboxylic Acid, Methyl Ester (16). Iodotrimethylsilane (76.6 mL, 0.538 mol) was added to a solution of compound **15** (162.43 g, 0.414 mol) in CH_2Cl_2 (1.5 L) at room temperature. After 1.5 h of stirring, the reaction mixture was concentrated *in vacuo* and the residue was partitioned between 1 L of EtOAc and 700 mL of 1 N HCl. The EtOAc layer was separated and extracted with 300 mL of 1 N HCl. The combined acidic aqueous extracts were washed with additional EtOAc (1 L), then cooled to 0 °C, and basified with 4 N sodium hydroxide (about 275 mL) to pH 10. The aqueous layer was saturated with solid NaCl and then extracted with five 1 L portions of CH_2Cl_2. The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was redissolved in 1 L of CH_2Cl_2, washed with 0.5 L of brine, dried (Na_2SO_4), filtered, and concentrated to give pure amine **16** (98.8 g, 92%) as a yellow oil: TLC R_f 0.31 (10:90 MeOH: CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 1.59−1.72 (m, 3H), 1.83 (br s, 2H), 1.89−2.01 (m, 3H), 2.20−2.25 (m, 1H), 2.35-2.46 (m, 1H), 3.00−3.06 (m, 2H), 3.72 (s, 3H), 4.05 (dd, 1H), 5.20 (m, 1H), 5.34 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 176.92, 172.74, 57.68, 52.84, 52.09, 51.05, 35.07, 30.78, 30.55, 24.99, 16.93.

[4*S*-[4α(*R),7α,10a*β*]]-Octahydro-4-[(2-mercapto-1-oxo-3-phenylpropyl)amino]-5-oxo-7*H*-pyrido[2,1-*b*][1,3]-thiazepine-7-carboxylic Acid (1a).** (*S*)-2-(Acetylthio)benzenepropanoic acid, dicyclohexylamine salt[14,15] (173.1 g, 0.427 mol) was partitioned between EtOAc (1 L) and 10% potassium bisulfate (800 mL). The organic layer was separated, washed with 5% potassium bisulfate (1 L), 50% brine (1 L), and brine (1 L), dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was azeotroped several times with CH_2Cl_2 and then dried overnight *in vacuo* to yield 97.3 g of crude (*S*)-2-(acetylthio)benzenepropanoic acid.

The acid (0.427 mol) in CH_2Cl_2 (900 mL) was chilled (ice bath) and treated sequentially with a solution of amine **16** (100.28 g, 0.388 mol) in CH_2Cl_2 (600 mL), neat triethylamine (154.1 mL, 0.388 mol), and solid (benzotriazol-1-yloxy)tris-(dimethylamino)phosphonium hexafluorophosphate (BOP reagent, 188.9 g, 0.427 mol). After 1 h at 0 °C and 2 h at room temperature, the reaction mixture was concentrated *in vacuo* and dissolved in 2 L of EtOAc. The solution was again concentrated *in vacuo* and dissolved in 2 L of EtOAc. The EtOAc solution was washed successively with brine (0.5 L), 0.5 N aqueous HCl (1 L), H_2O (1 L), saturated $NaHCO_3$ (2 L), H_2O (1 L), and brine (1 L), dried (Na_2SO_4), filtered, and concentrated. Those aqueous rinses which contained product (TLC indication) were reextracted with EtOAc. These extracts were worked up in the usual manner, and all EtOAc extracts were combined and stripped to give a yellow oily residue. The oil was applied to a 15 × 15 cm silica gel pad prepared in 1:1 EtOAc:hexanes and eluted with 7 L of 1:1 EtOAc:hexanes followed by 4 L of 6:4 EtOAc:hexanes and finally 2 L of 7:3 EtOAc:hexanes. The filtrates containing the desired product were concentrated to give the coupling product [4*S*-[4α(*R**),7α,10a*β*]]-octahydro-4-[[2-(acetylthio)-1-oxo-3-phenylpropyl]amino]-5-oxo-7*H*-pyrido[2,1-*b*][1,3]thiazepine-7-carboxylic acid, methyl ester (123.57 g, 69%) as an oil: TLC R_f 0.37 (1:1 EtOAc: hexanes); ^1H NMR ($CDCl_3$) δ 1.59−1.72 (m, 3H), 1.85−2.04 (m, 3H), 2.21 (m, 1H), 2.33 (s, 3H), 2.42 (m, 1H), 2.89 (m, 1H), 3.00 (dd, 1H), 3.24−3.33 (m, 2H), 3.71 (s, 3H), 4.3 (t, 1H, J = 7.7 Hz), 4.95 (m, 1H), 5.18 (m, 1H), 5.28 (m, 1H), 7.20−7.29 (m, 5H), 7.49 (d, 1H, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$) δ 195.00, 173.18, 169.73, 138.14, 129.82, 129.07, 127.51, 59.55, 52.87, 52.06, 51.63, 48.81, 37.44, 32.92, 31.82, 31.73, 31.04, 25.43, 17.71.

To a 12 L three-necked flask, fitted with an additional funnel and mechanical stirrer, was added a solution of the ester (96.0 g, 0.207 mol) in MeOH (1.1 L). The solution was purged with argon for 30 min and then cooled in an ice bath until the internal temperature was 7 °C. A 1 N NaOH solution (1.45 L, previously sparged with argon for 30 min) was added over 1 h. The reaction mixture was continuously purged with argon during the addition. The reaction temperature rose to 12 °C and was maintained during the addition. After stirring for an additional 30 min, the mixture was warmed to room temperature, and stirred for 2.5 h. Approximately 250 mL of 6 N HCl was added dropwise over a 15−20 min period to adjust the pH to 2. A gummy precipitate formed during acidification. After the mixture was continually stirred for an additional 2 h, the precipitate changed to a fine white solid with some larger chunks of solid product present. The product was collected by filtration and washed with 1 L of H_2O followed by 2 L of anhydrous Et_2O and dried *in vacuo* to afford **1a** (70.3 g, 83%) as a fine white solid: TLC R_f 0.48 (5:95 AcOH:(1:1 EtOAc: hexanes)); $[\alpha]_D$ −78.9° (*c* 0.46, DMF); mp 218−220 °C dec; ^1H NMR (DMSO-d^6) δ 1.45−1.63 (m, 3H), 1.80- 2.00 (m, 3H), 2.10 (m, 1H), 2.40 (m, 1H), 2.74 (d, J = 9.0 Hz, 1H), 2.80 (dd, 1H), 2.94 (m, 1H), 3.10 (m, 1H), 3.19 (dd, 1H), 3.82 (q, 1H), 4.90 (m, 1H), 5.05 (m, 1H), 5.64 (m, 1H), 7.17−7.29 (m, 5H), 8.30 (d, J = 6.8 Hz, 1H), 12.55 (brs, 1H); ^{13}C NMR (DMSO-d^6) δ 172.1, 170.8, 138.6, 129.0, 128.0, 126.2, 57.1, 50.6, 50.4, 42.1, 40.8, 31.5, 30.6, 29.3, 24.7, 16.6; IR (KBr) 3437, 2942, 2562, 1744, 1653, 1630, 1516, 1416, 1186, 1140, 750, 702 cm^{-1}. Anal. Calcd for $C_{19}H_{24}N_2O_4S_2$: C, 55.86; H, 5.92; N, 6.86; S, 15.70. Found: C, 55.87; H, 5.90; N, 6.94; S, 15.80.

Acknowledgment. We gratefully acknowledge Dr. Donald S. Karanewsky, Dr. Edward W. Petrillo, and Dr. James Powell for their contributions to this project. We are also grateful for the contributions of Jerry Baumann and Richard Knappenberger to this work.

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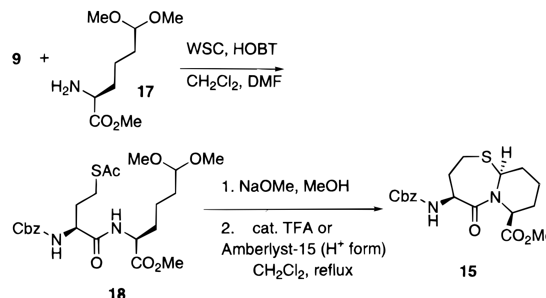

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JM970041E