Pergamon

Bioorganic & Medicinal Chemistry, Vol. 2, No. 9, pp. 971-985, 1994

0968-0896/94 $7.00 + .00

0968-0896(94)00116-2

Conformationally Constrained *o*-Tolylpiperazine Camphorsulfonamide Oxytocin Antagonists. Structural Modifications that Provide High Receptor Affinity and Suggest a Bioactive Conformation.

Peter D. Williams,* Richard G. Ball,[c] Bradley V. Clineschmidt,[a]
J. Chris Culberson,[b] Jill M. Erb, Roger M. Freidinger, Joseph M. Pawluczyk, Debra S. Perlow,
Douglas J. Pettibone[a] and Daniel F. Veber

Departments of Medicinal Chemistry, [a]New Lead Pharmacology,
[b]Molecular Systems, Merck Research Laboratories, West Point, PA19486, U.S.A.
[c]Department of Biophysical Chemistry, Merck Research Laboratories, Rahway, NJ 07065, U.S.A.

Abstract—A series of new *o*-tolylpiperazine camphorsulfonamide OT antagonists is described. Analogs containing conformationally constrained 1-acylamino-2-propyl substituents at the camphor C2 *endo* position exhibit high affinity for OT and AVP-V_{1a} receptors or high affinity and selectivity for OT receptors, depending on functionalities present in the acyl group. Determination of the preferred conformation of potency-enhancing 1-acylamino-2-propyl substituents using molecular mechanics energy calculations and X-ray crystallography, along with topological similarities to a conformationally constrained cyclic hexapeptide OT antagonist, suggests a receptor-bound conformation for this series of non-peptide OT antagonists.

Introduction

Peptides play important roles in the regulation of diverse physiological processes. Modulation of the function of biologically important peptides, either by interrupting their biosynthesis or by blocking or activating their receptors, offers many opportunities for the development of new therapeutic agents. Advances in chemical methodology have enabled the synthesis of increasingly complex peptide structures, which in turn has greatly facilitated the ability to determine the roles of biologically important peptides in normal and disease states. The development of peptides as effective therapeutic agents, however, has been difficult in many cases due to limiting pharmacokinetic properties and poor oral bioavailability.[1] Over the last decade, the design and discovery of non-peptide structures (peptidomimetics) for peptide receptors has become an area of increasingly active investigation. Already there are many examples of potent and selective peptidomimetic compounds which exhibit encouraging pharmacokinetic and absorption properties, thus offering the potential for the development of new, breakthrough therapeutic agents.[2]

The challenge of obtaining peptidomimetic structures has been met in three general ways: design based on the structure of the natural peptide and its analogs,[3] design based on structure of the enzyme or receptor molecule,[4] and empirical screening of natural and/or synthetic chemicals.[5] We have utilized a receptor-based screening approach[6,7] for obtaining structurally novel antagonists of the nonapeptide hormone, oxytocin (OT, Figure 1). OT is an important hormone early in parturition because of its direct and indirect contractile effects on the uterus.[8-10] The potential therapeutic utility of an OT antagonist for treating preterm labor to prevent premature birth has been demonstrated with ORF 22164 (atosiban),[11-14] an antagonist analog of OT. Evans and Pettibone recently described the first non-peptide OT antagonist.[7,15] Modification of a weak OT receptor ligand discovered through receptor-based screening of the Merck chemical collection provided L-366,509 (Figure 1), an orally active, prototypic member of a new class of OT antagonists based on the spiroindenylpiperidine camphor-10-sulfonamide framework. Certain structural modifications in the C2 *endo* substituent on the camphor ring of L-366,509 have provided analogs with greatly enhanced receptor affinity[16,17] which, when combined with replacement of the spiroindenylpiperidine with a *o*-tolylpiperazine moiety, has led to the identification of an analog with suitable properties for clinical testing as an oral and intravenous tocolytic agent.[18,19] Herein we report a series of *o*-tolylpiperazine camphorsulfonamides containing conformationally restricted 1-acylamino-2-propyl substituents at the C2 *endo* position that offers insight into the bioactive conformation of this series of antagonists. In addition, we describe functionalities in the *endo* substituent that result in high affinity and specificity for binding to OT receptors, as well as functionalities which enhance binding to AVP-V_{1a} receptors, one of the important receptor subtypes for the structurally related peptide hormone, arginine vasopressin (AVP, Figure 1).

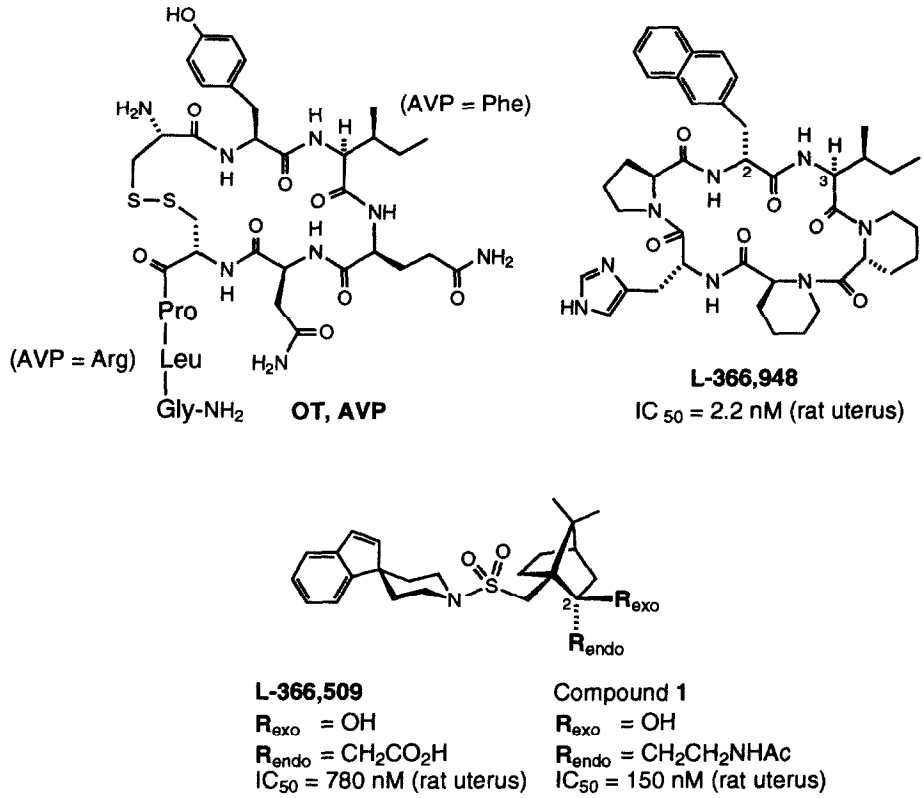


Figure 1.

Chemical Methods[20]

The methodology used to obtain the compounds listed in Tables 1 and 2 is exemplified by the synthesis given in Scheme I. *o*-Tolylpiperazine was sulfonylated with (+)-10-camphorsulfonyl chloride to give **2**. Addition of the lithium salt of acetonitrile to the carbonyl group of **2** occurred stereoselectively from the *endo* face of the camphor ring, giving **3a** in > 95 % isolated yield. The corresponding *exo* adduct was not detected. The stereochemical assignment of **3a** was based on ^{1}H NMR spectroscopic characteristics of the camphor methyl groups as previously noted by Evans[15] for similar addition products in the spiroindenylpiperidine camphorsulfonamide series. One of the two camphor methyl groups is deshielded due to the proximity of the *exo* hydroxyl group (1.23 ppm and 0.95 ppm). Addition of the lithium salt of propionitrile to **2** gave a mixture of four β-hydroxy nitrile diastereomers that was greatly enriched in one isomer. The mixture also contained a small amount (*ca* 5 %) of starting ketone that was likely the result of competitive enolization. The major, slowest eluting adduct **3b** was obtained in 80–85 % yield after chromatographic separation on silica gel. Alternatively, **3b** could be obtained directly from the crude product mixture in 50–55 % yield by crystallization from ether. The minor propionitrile adducts were difficult to obtain in pure form by silica gel chromatography and their stereochemical structures were not assigned. Reduction of **3b** with LAH provided amino alcohol **4b**, the stereochemistry of which was determined by single crystal X-ray diffraction analysis (Figure 3). Addition of the lithium salt of butyronitrile to **2** proceeded in a manner analogous to the reaction with propionitrile: a mixture of isomers was obtained in which the least mobile isomer by silica gel TLC greatly predominated. Chromatographic separation and reduction provided **3c** and **4c**, the stereochemistries of which were assigned by analogy to the propionitrile case. Carboxamide derivatives of amino alcohols **4a–c** were prepared by acylation using anhydrides (**5a–c**, Table 1; **7**, Table 2) or various carboxylic acid derivatives activated with the BOP reagent.[21] Amino amides **8–12** (Table 2) were obtained by coupling an N-Fmoc amino acid derivative to **4b** using the BOP reagent, followed by removal of the Fmoc protecting group using diethylamine, as exemplified by the preparation of **11** in Scheme I. Deprotection of coupling products derived from N-Boc amino acids using TFA was complicated by competitive dehydration to the 2,3-dehydro camphor derivative. Acylation of **4b** with racemic quinuclidine-3-carboxylic acid[22] gave **13** (Table 2) as a mixture of diastereomers that were inseparable on silica gel or reverse phase HPLC. Alkylation of **13** with an alkyl bromide or iodide in DMF containing DIEA gave the quaternary ammonium derivatives **14** and **17–19** (Table 2) as mixtures of diastereomers which were also chromatographically inseparable. However, separation of **14** into its diastereomeric components was accomplished by crystallization of the chloride salt from chloroform. In this manner, essentially pure **15** was obtained as a crystalline solid, leaving enriched **16** in the filtrate. Diastereomers **15** and **16** were readily differentiated by ^{1}H NMR on the basis of the chemical shift of the aminopropyl methyl doublet (**15**, 0.98 ppm; **16**, 0.97 ppm), which allowed quantitation of the isomer ratio after crystallization. The stereochemical configuration at the 3-position of the

quinuclidine ring in **15** and **16** was not determined. Amino acids **20–25** (Table 2) were obtained by alkylation of the corresponding amine precursors **8–12** with ethyl bromoacetate in DMF containing DIEA, or by Michael addition of methyl acrylate in methanol, followed by saponification as exemplified by the preparation of **23** and **24** in Scheme 1.

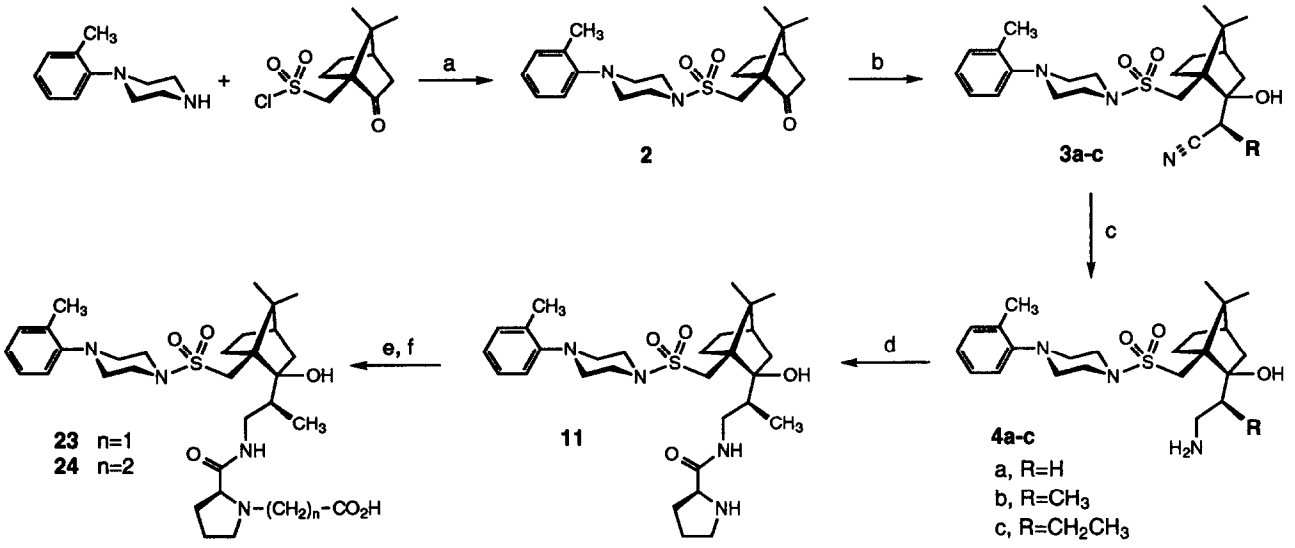
Biological Methods[20]

The high affinity binding of [^{3}H]OT to uterine tissue and [^{3}H]AVP to liver (AVP-V_{1a} site) and kidney medulla (AVP-V_2 site) formed the basis for competition experiments to determine receptor affinities of the test compounds listed in Tables 1–3. Detailed experimental procedures for the radioligand binding assays have been described previously.[6,7] Uterine tissue was taken from DES-pretreated nonpregnant rats and human uterine myometrial tissue was obtained from non-labor pregnant women with informed consent undergoing cesarean section at 38–39 weeks gestation. Livers and kidney medullae were taken from male rats and from human surgical and early postmortem donors. Competition studies were conducted at equilibrium using 1 nM [^{3}H]OT or 0.5 nM [^{3}H]AVP. IC_{50} values, the molar concentrations of test compound required to reduce binding of radioligand by 50 %, were determined by linear regression analysis of per cent inhibition of specific binding versus log concentration of antagonists (seven antagonist concentrations per IC_{50} determination). K_i values (Table 3) were calculated from the equation $K_i = IC_{50}/[1 + c/K_d]$.[23] K_d values were calculated from equilibrium saturation assays: [^{3}H]OT: 0.69 nM (rat uterus), 1.1 nM (human myometrium); [^{3}H]AVP: 0.21 nM (rat liver), 0.27 nM (human liver), 0.27 nM (rat kidney), 1.4 nM (human kidney).

Results for antagonism of OT-stimulated contractions of the isolated rat uterus by selected compounds are given in Table 3. Uteri were isolated from DES-pretreated rats and prepared for recording of contractile responses as previously described.[6,7] A cumulative concentration–response curve was generated using a 4-min exposure at each concentration of OT. Once the E_{max} was obtained, the tissues were washed repeatedly for 75 min, at which time antagonist or vehicle was added to the tissue bath and, 45 min later, a second concentration–response curve to OT was constructed. The concentration of OT producing 50 % of E_{max} before and after treatment with antagonist was determined by regression analysis. Dose ratios (EC_{50} after treatment/EC_{50} before) were corrected, if indicated, by a factor derived from concurrent vehicle-treated tissues. The results were analyzed for competitiveness (Schild slope), and antagonist potencies were expressed as pA_2 values, the negative logarithm of the molar concentration of antagonist that causes a two-fold rightward shift in the dose–response curve of the antagonist (i.e., a dose ratio of 2).[24] Each antagonist was tested at three to four concentrations, with four to seven tissues per concentration.

Results for antagonism of AVP-stimulated contractions of isolated rat tail (caudal) artery rings by compound **9** are given in Table 3. The contractile response is mediated via AVP-V_{1a} receptors. Experimental details for this assay have been reported previously.[25] Concentration–response curves to AVP were constructed before and after treatment with vehicle or **9**, according to the procedure described above for the isolated uterus. The results were analyzed (pA_2, Schild slope) also as described above.

Results for antagonism of OT-stimulated contractions of the *in situ* rat uterus by selected compounds are given in Table 3. The experiments were performed as described previously.[7] DES-Pretreated rats (n = 4–6) were prepared for recording of isometric contraction of the uterus *in situ*. OT was injected intravenously every 35 min (1 mg/kg; approximately an ED_{50} dose) for a total of eight times. The contractile response obtained after the third injection was set as 100 %, i.e., contractile responses determined after each injection were expressed as a percentage of the response obtained for the third injection of OT. Fifteen minutes before the fourth injection of OT, vehicle with or



Scheme I. (a) DIEA, $CHCl_3$, 0 °C; (b) RCH(Li)CN, THF, -78 °C; (c) LAH, THF, -78 °C to 25 °C; (d) i. Fmoc-L-Pro-OH, BOP, DIEA, DMF; ii. Et_2NH; (e) $BrCH_2CO_2Et$, DIEA, DMF (n=1) or CH_2=$CHCO_2CH_3$, MeOH (n=2); (f) NaOH, EtOH, H_2O.

without antagonist was infused intravenously in saline (**13**, **14**, **24**) or in 70:15:15 saline:DMSO:emulphor (**6b**) over a 10 min period. The dose of antagonist required to reduce the response to OT by 50 % (AD_{50}) was calculated from the contractile response obtained after the fourth injection of OT (i.e., 5 min post-infusion of the antagonist). Percentage antagonism relative to the concurrent vehicle treated group was determined for each dose of antagonist and the AD_{50} was estimated by regression analysis.

Results and Discussion

Evans and co-workers recently reported a number of modifications to the C2 *endo* camphor substituent in the prototype OT antagonist L-366,509 that resulted in substantially improved receptor affinity,[16] an example of which is the *endo*-acetylaminoethyl analog **1** (Figure 1). It seemed possible that further enhancements in potency might be realized by introducing rigidity into the conformationally flexible aminoethyl group in **1** and/or by employing other acyl groups containing different types of functionalities. These types of modifications were indeed ultimately successful in improving receptor affinity as described below for an alternative series of camphorsulfonamides in which the spiroindenylpiperidine is replaced with an *o*-tolylpiperazine group.[19]

The *o*-tolylpiperazine **5a** (Table 1) exhibited modestly improved receptor affinity compared to the spiroindenylpiperidine analog **1**. As can be seen from the results in Table 1, introduction of an alkyl group (R^2) on the *endo* substituent of the camphor ring had a modest beneficial effect on OT receptor affinity at the amine (**4a–c**) and acetamide (**5a–c**) stages. With the potency-enhancing 4(5)-imidazoleacetyl end group, a more pronounced beneficial effect was observed for the branched analogs **6b** and **6c** relative to the unbranched compound **6a**. That **6b** and **6c** are equipotent suggested that the beneficial effect of branching may be due to a conformational effect. To assess the conformational consequences of branching, the relative energies of the three staggered *endo* substituent rotamers of **6a** and **6b** (see Figure 2) were determined using the the MacroModel[26] program. High energy conformers resulted when a non-hydrogen substituent was oriented beneath the camphor ring, leaving two low energy rotamers (rotamers A and B) for the unbranched compound **6a** and one low energy rotamer (rotamer A) for the branched analog **6b**. Thus, the energy calculations suggest that branching of the type found in compounds **5b–c**, **6b–c** (Table 1), and **7–25** (Table 2) should limit the rotational freedom of the *endo* substituent on camphor such that the conformation represented by rotamer A would be expected to be the most highly populated. Indeed, the X-ray crystal structure of the branched amino alcohol **4b** (Figure 3) shows that the *endo*-aminopropyl substituent adopts the orientation of rotamer A.

The preferred *endo* substituent orientation in compound **6b** fits well with our modeling hypothesis[19] that relates important pharmacophoric elements in camphor-based non-peptide and conformationally constrained cyclic hexapeptide OT antagonists. In this model, the *o*-tolylpiperazine camphorsulfonamide portion of the molecule serves as a mimetic of the critical D-Nal-L-Ile 2,3-dipeptide found in potent cyclic hexapeptide OT antagonists[27] such as L-366,948 (structure given in Figure 1). Figure 4 shows an alignment of a low energy conformer of **6b** with an NMR-consistent, low energy conformer of L-366,948. The *o*-tolylpiperazine camphorsulfonamide portion of **6b** aligns well with the 2,3-dipeptide as found previously, and the energetically preferred *endo* substituent rotamer orients the imidazoleacetyl group into the region occupied by the D-His^6 side chain of the cyclic peptide. It is interesting to note that in both structural classes the imidazole group is found to be a potency-enhancing structural feature. Thus,

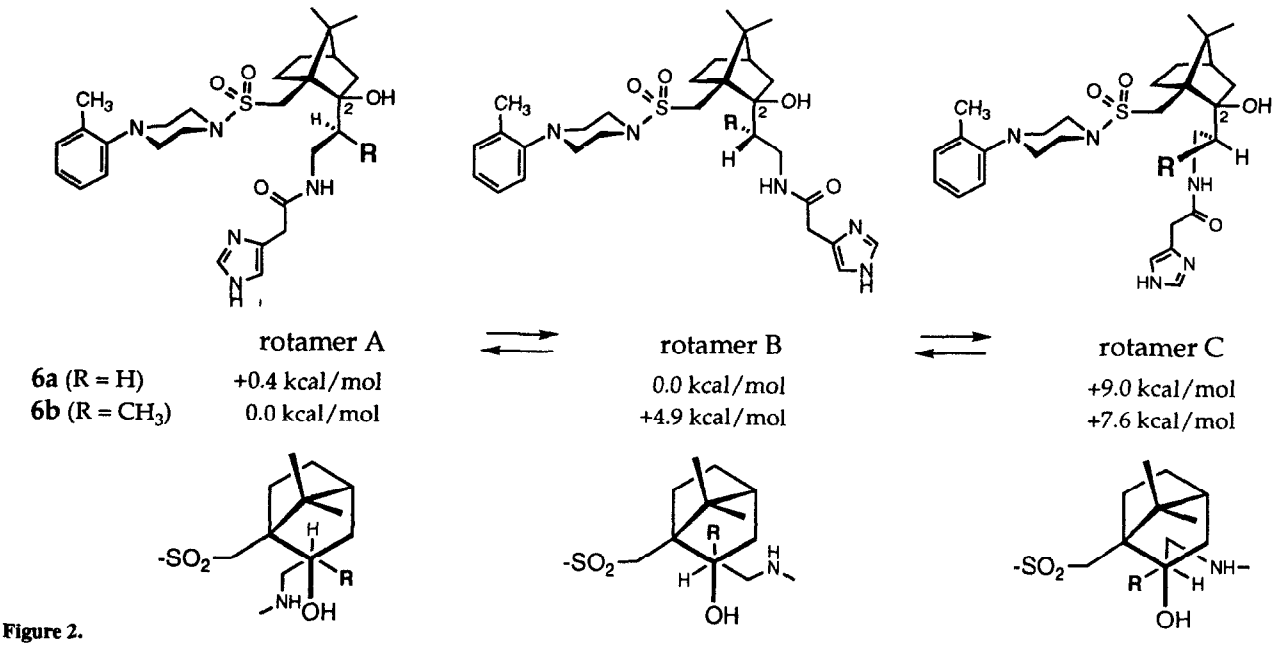
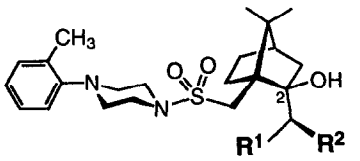


Figure 2.

Table 1.



no.	R^1	R^2	Receptor Affinities (IC_{50}, nM)[a] OT	V_{1a}	V_2
3a	CN	H	190	4,900	12,000
3b	CN	CH_3	160	1,800	1,700
3c	CN	CH_2CH_3	290	2,000	2,900
4a	CH_2NH_2	H	550	4,200	20,000
4b	CH_2NH_2	CH_3	180	350	1,600
4c	CH_2NH_2	CH_2CH_3	310	720	5,100
5a	$CH_2NHCOCH_3$	H	89	1,800	4,400
5b	$CH_2NHCOCH_3$	CH_3	39	260	540
5c	$CH_2NHCOCH_3$	CH_2CH_3	63	290	1,600
6a	$CH_2NHCOCH_2$-imidazole	H	37	860	2,400
6b	$CH_2NHCOCH_2$-imidazole	CH_3	8.1 ± 0.67	190 ± 25	330 ± 35
6c	$CH_2NHCOCH_2$-imidazole	CH_2CH_3	6.9	370	850

[a] IC_{50} values refer to the concentration of test compound in nanomoles per litre that displaces by 50 % the binding of [^{3}H]OT to rat uterus (OT column, [^{3}H]AVP to rat liver (V_{1a} column), and [^{3}H]AVP to rat kidney (V_2 column). Compounds for which there were ≥3 separate determinations, IC_{50} values are reported as group means ± s.e.m.

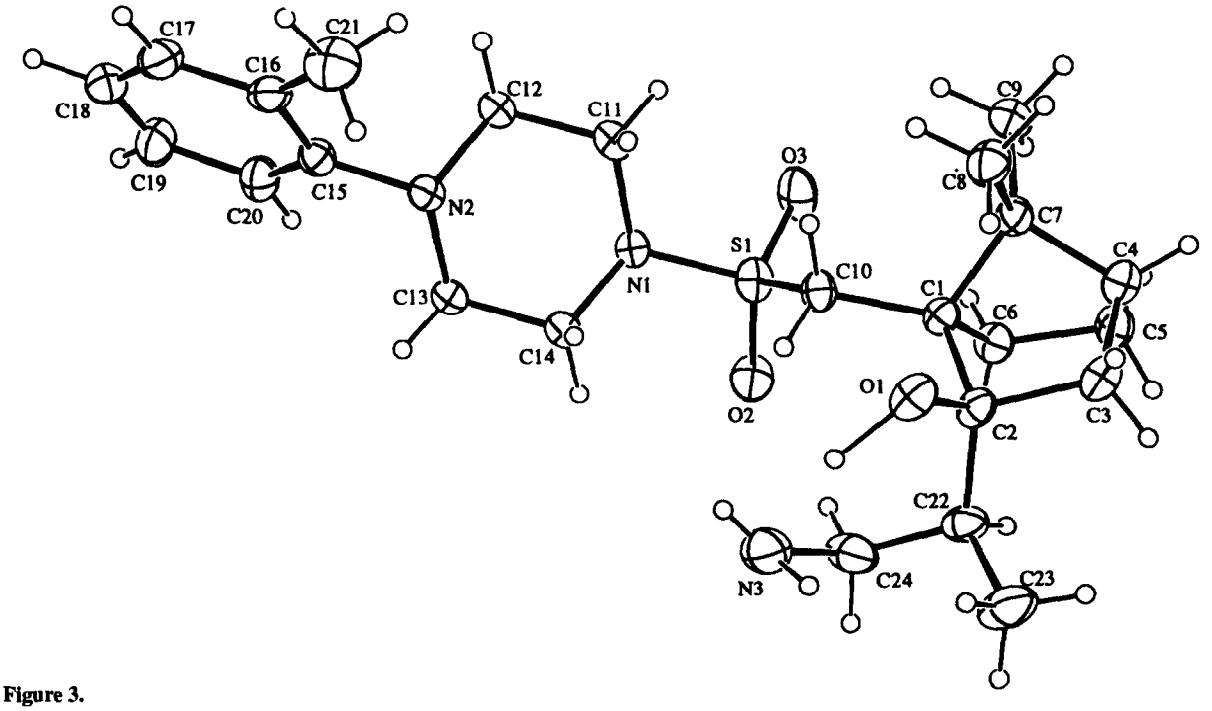


Figure 3.

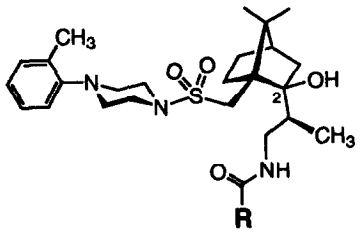
the conformational preference of the branched *endo* substituent as suggested by molecular mechanics energy calculations, together with the topological similarity of the preferred conformer to a structurally dissimilar, conformationally constrained OT receptor ligand suggest that the conformation of **6b** shown in Figure 4 may resemble its receptor-bound conformation. Such information may be useful in the design of new OT receptor ligands.

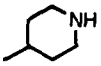
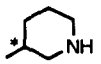
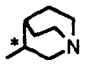
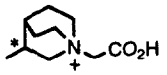
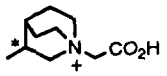
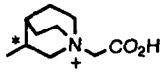
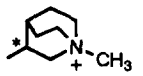
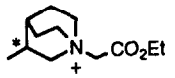
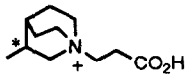
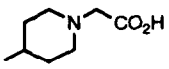
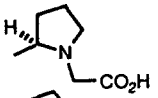
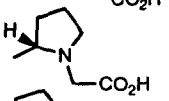
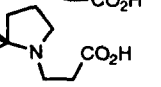
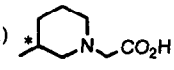
A number of amide derivatives of the branched amino alcohol **4b** were examined in which the acyl group contains various types of polar functionalities (Table 2). Compared to acetamide **5b**, reduced OT receptor affinity was observed with the acid-containing succinamide derivative **7**. Analogs containing basic groups, on the other hand, were well tolerated (**8–13**), but also exhibited significant affinity for AVP-V_{1a} receptors. A remarkable enhancement of OT receptor selectivity was observed upon derivatization of the completely non-selective quinuclidine **13** to the carboxymethyl analog **14**. With this change, OT receptor affinity was improved 3-fold, while affinity at the AVP-V_{1a} and AVP-V_2 sites was reduced 100-fold and 65-fold, respectively. The high affinity and selectivity of **14** appear to be related to the presence (**17**, **18**) and proper positioning (**15**, **16**, **19**) of the carboxylate group. These findings prompted the investigation of carboxyalkylation as a general strategy for improving OT receptor selectivity of amines **8–12**. Indeed, this type of modification proved to be successful in reducing affinity for AVP receptors, particulary at the V_{1a} site (**20–25**). With the amino acids **20**, **21**, and **25**, OT receptor affinity was also diminished by a small amount (approximately 2-fold) relative to the amine counterparts. Unlike the observations for the quinuclidine diastereomers **15** and **16**, little difference in receptor affinity was found for the proline diastereomers **22** and **23**, and in contrast with the unfavorable effect of lengthening the carboxyalkyl chain in the quinuclidine series (*c.f.* **14** and **19**), a modest improvement in OT receptor affinity and selectivity was observed with this type of modification in the L-proline series (*c.f.* **23** and **24**).

A comparison of rat and human receptor affinities for selected compounds is given in Table 3. A small difference was noted for binding to rat versus human uterine OT receptors, with affinity being 2- to 5-fold weaker at the human site. Other members from the *o*-tolylpiperazine and spiroindenylpiperidine camphorsulfonamide classes have behaved similarly.[7,16,18] Significant species differences (> 10-fold) have been noted in other structural classes for binding to AVP-V_{1a} receptors,[28,29] e.g., favoring the human site for certain OT/AVP analogs such as ORF 22164, and favoring the rodent site for dihydroquinolinone non-peptide AVP-V_1

Figure 4.

Table 2.



no.	R	Receptor Affinities (IC_{50}, nM)[a] OT	V_{1a}	V_2
7	$-(CH_2)_2CO_2H$	180	2,400	2,100
8	$-(CH_2)_2NH_2$	23	53	280
9		27 ± 4.7	39 ± 1.0	260 ± 26
10		57 ± 14	150	1,100
11		34	130	520
12	(*1:1) 	15	31	230
13	(*1:1) 	16 ± 3.4	12 ± 1.7	310 ± 26
14	(*1:1) 	5.7 ± 0.46	1,200 ± 58	2,000 ± 180
15	(*>95:5[b]) 	42 ± 5.3	1,800	3,100
16	(*8:92[b]) 	4.6 ± 0.16	800	1,500
17	(*1:1) 	13	47	200
18	(*1:1) 	11	110	470
19	(*1:1) 	84	270	1,800
20	$-(CH_2)_2NH-CH_2CO_2H$	59	1,100	1,000
21		56	1,200	1,700
22		52	1,200	1,100
23		28	1,000	1,200
24		20 ± 0.88	1,700 ± 330	1,300 ± 250
25	(*1:1) 	38	1,500	1,900

[a]Binding affinities as defined in Table 1, footnote a.
[b]Isomer ratio as determined by ^1H NMR (see text); absolute stereochemistry at the quinuclidine 3-position not determined.

Table 3.

no.	Rat and Human§ Receptor Affinities[a] K_i, nM			*in vitro* pA_2 (rat)[b]		*in vivo* AD_{50}
	OT	V_{1a}	V_2	uterus[c]	caudal artery[d]	mg/kg (i.v., rat)[e]
6b	3.3 ± 0.27 15§	56 ± 7.5 63§	120 ± 12	8.68 ± 0.13 (1.16 ± 0.31)	-	<3.0
9	11 ± 1.9	12 ± 0.30	91 ± 9.1	-	7.66 ± 0.15 (1.22 ± 0.16)	-
13	7.3 ± 1.4 11 ± 1.5§	3.5 ± 0.49 98 ± 17§	110 ± 9.4	8.47 ± 0.19 (1.26 ± 0.24)	-	1.5
14	2.3 ± 0.19 9.2 ± 1.5§	360 ± 17 400 ± 43§	700 ± 63 2100 ± 1400§	8.83 ± 0.14 (1.35 ± 0.39)	-	0.080
24	8.1 ± 0.36 23 ± 2.7§	500 ± 97 1100§	630 ± 87	-	-	0.37

[a] K_i values for inhibiting binding of [^{3}H]OT to rat uterus or human uterus (OT column), [^{3}H]AVP to rat liver or human liver (V_{1a} column), and [^{3}H]AVP to rat kidney or human kidney (V_2 column) were determined form IC_{50} values as indicated in the text. The § symbol refers to results using human tissue. Compounds for which there were ≥3 separate determinations, K_i values are reported as group means ± s.e.m.
[b] pA_2 value refers to the negative logarithm of the molar concentration of test compound that produces a 2-fold rightward shift of the dose-response curve. The Schild slope is reported in parentheses. Values are reported as group means ± 95 % confidence limit.
[c] pA_2 for contraction of the isolated rat uterus with OT.
[d] pA_2 for contraction of the isolated rat tail artery with AVP (AVP-V_1 receptor mediated response).
[e] AD_{50} values refer to the dose of test compound given intravenously that reduces the contractile response of the *in situ* rat uterus to exogenous OT by 50 %.

antagonists such as OPC 21268. Species differences at the AVP-V_{1a} site within the *o*-tolylpiperazine and spiroindenylpiperidine camphorsulfonamide classes appears to be case-dependent, e.g., good correspondence was found for several compounds (≤ 2-fold difference for **6b**, **14** and **24**), but not for others (**13** has > 10-fold weaker affinity for the human site and L-366,509 has > 10-fold higher affinity for the human site[7]). However, the high selectivity for OT versus AVP-V_{1a} and AVP-V_2 receptors with compound **14** holds up across species.

To follow up on the high OT receptor affinity exhibited by several of the compounds described in the foregoing discussion, their behavior in *in vitro* and/or *in vivo* functional assays was examined (Table 3). Compounds **6b**, **13** and **14** were all found to be potent antagonists of OT-stimulated contraction of the isolated rat uterus, the rank order of which parallels their potencies in the OT receptor binding assay. The non-selective OT/AVP-V_{1a} receptor ligand, compound **9**, was found to be a potent antagonist of AVP-induced contraction of the isolated rat caudal artery, a functional assay associated with vascular AVP-V_{1a} receptors.[25] When administered intravenously to anesthetized rats, compounds **6b**, **13**, **14** and **24** antagonized the contractile effect of exogenous OT on the *in situ* uterus. The difference in rank order of potency in this assay as compared to the OT receptor binding assay most likely reflects differences in their pharmacokinetic behavior, i.e., differences in tissue distribution and clearance. When administered intraduodenally at greater than fifty times the iv AD_{50} dose, neither **16** nor **24** significantly antagonized OT-stimulated contractions of the *in situ* uterus, indicating poor bioavailability from the gut. Thus, in contrast to modifications reported previously,[16,19] the *endo* substituent modifications in the present study have not provided the level of oral bioavailability needed for a drug development candidate. The *N*-carboxymethylquinuclidinium analog **14**, however, is one of the most potent iv antagonists of OT-induced rat uterine contractions observed to date in the camphor-based OT antagonist class.

Summary

A series of new *o*-tolylpiperazine camphorsulfonamide OT antagonists was developed that contains conformationally constrained 1-acylamino-2-propyl substituents at the C2 *endo* position on camphor to arrive at high affinity OT receptor ligands. High AVP-V_{1a} receptor affinity also resulted with analogs containing basic groups in the acyl moiety of the 1-acylamino-2-propyl substituent. Carboxyalkylation of the basic amine resulted in substantially decreased AVP-V_{1a} receptor affinity with little effect on OT receptor affinity. Several high affinity compounds were found to be potent antagonists of OT-induced contraction of the isolated and/or *in situ* rat uterus. The rotational preference of the 1-acylamino-2-propyl *endo* substituent on camphor, together with the topological similarity of **6b** to a potent, conformationally constrained cyclic hexapeptide OT antagonist, suggests a receptor-bound conformation of **6b** and its analogs.

Experimental Section

Biological methods

Radioligand binding assays, and *in vitro* and *in vivo* antagonist studies were performed as described in the text.

Detailed experimental procedures for these assays have been reported previously.[6,7,25]

Molecular modeling and computational methods

Figure 2: a series of random *endo* substituent conformations for compounds **6a** and **6b** were generated using a distance geometry program.[30] The *o*-tolylpiperazine camphorsulfonamide portion of the molecule was rigidly fixed in an orientation that was shown in previous studies[19] to align well with the 2,3-dipeptide of a potent cyclic hexapeptide OT antagonist. The resulting conformers were energy-minimized using version 4.0 of the MacroModel[26] program with a distance dependent dielectric of 4. Three distinct local energy minima were observed in the energy-minimized conformers and are represented by rotamers A, B and C in Figure 2. For compound **6a**, rotamer B was the lowest energy conformation, rotamer A was 0.4 kcal/mol higher in energy than rotamer B, and rotamer C was 9.0 kcal/mol higher in energy than rotamer B. For compound **6b**, rotamer A was the lowest energy conformation, rotamer B was 4.9 kcal/mol higher in energy than rotamer A, and rotamer C was 7.6 kcal/mol higher in energy than rotamer A.

Figure 4: a set of random conformations of **6b** was generated using distance geometry.[30] Conformers within this set were energy-minimized using MMFF,[31] and each conformer was overlaid with a low energy, NOE-consistent conformer[32] of the cyclic hexapeptide L-366,948 using SQUEAL,[33] an alignment algorithm that randomly rotates and translates two rigid structures and utilizes a scoring system based on molecular volume, hydrophobicity, and electrostatics to rank the alignments. The highest scoring alignments were those in which the *o*-tolylpiperazine camphorsulfonamide moiety of **6b** overlays the D-Nal-L-Ile 2,3-dipeptide of L-366,948, with several different orientations of the camphor *endo* substituent. The high scoring alignment shown in Figure 4 utilizes a conformer of **6b** in which the *endo* substituent adopts an orientation similar to that of rotamer A in Figure 2. Atomic coordinates for each of the two molecules in Figure 4 can be obtained from the authors upon request.

X-Ray crystallography

Diffraction data were collected using an Enraf–Nonius CAD4F diffractometer and Cu K_α monochromatized radiation (λ = 1.54184 Å). The data set was corrected for Lorentz, polarization and background effects and absorption.[34] No appreciable intensity decay was observed during data collection. The structure was solved using SHELXS-86[35] and refined (*Structure Determination Package Version 3*. Enraf–Nonius, Delft, The Netherlands (1985) implemented on a Sun Microsystems workstation) using full-matrix least-squares on F with a weighting scheme of $1/\sigma^2(F)$. A drawing of the molecule is presented in Figure 3 and the crystallographic coordinates have been deposited at the Cambridge Crystallographic Data Centre.

Colorless crystals of **4b** were grown from methanol. The crystal chosen for data collection has approximate dimensions 0.18 × 0.16 × 0.30 mm. The crystal data and experimental conditions are: formula = $C_{24}H_{39}N_3O_3S$, M_r = 449.66, orthorhombic space group $P2_12_12_1$, a = 14.538 (2), b = 19.120 (3), c = 8.750 (1) Å, V = 2432.2 Å^3, Z = 4, D_{calc} = 1.228 g/cm^3, μ(Cu K_α) = 1.37/mm, $F(000)$ = 976, T = 296 K. Data were collected to a 2θ limit of 150 ° yielding 2856 measured reflections. Scan type is ω with a range of 0.66 + 0.14tan(θ) ° and a variable speed of 10.1 to 3.5 deg/min. Of the 2856 reflections measured there are 2180 observed data at the $I \geq 3\sigma(I)$ level. Included in the refined parameters is a secondary extinction coefficient of 6.36 × 10^{-7}. The final agreement statistics for 281 variables are: R = 0.041, wR = 0.041, S = 2.55, $(\Delta/\sigma)_{max}$ = 0.009. There is no structural significance to the maximum peak in a final difference Fourier (0.15(3)eÅ^{-3}).

Chemical methods[20]

Melting points were determined on a Thomas–Hoover melting point apparatus and are uncorrected. ^{1}H NMR spectra were obtained at 300 MHz on a Varian XL-300 instrument or at 400 MHz on a Varian XL-400 instrument using $(CH_3)_4Si$ as an internal standard. Fast atom bombardment mass spectra (FAB-MS) were obtained on a VG-ZAB-HF spectrometer using xenon as the reagent gas. TLC was performed on Analtech "Uniplate silica gel GF" plates (25 × 100 mm, 250 microns) and chromatograms were visualized under ultraviolet light (254 nm), by dipping in ethanolic phosphomolybdic acid and heating, or by exposure to iodine vapors. Pressurized silica gel column chromatography was performed according to the method of Still and co-workers[36] using 230–400 mesh E. Merck silica gel. THF was distilled from CaH_2–$NaBH_4$ under inert atmosphere. Dioxane for lyophilization was dried and freed of peroxides by passage through a column of neutral alumina (EM Science "Aluminum Oxide 90" activity 1, 70–230 mesh). Estimation of reaction pH was accomplished by spotting an aliquot from the reaction mixture on wetted pH indicator strips (E. Merck "colorpHast" pH 0–14). *o*-Tolylpiperazine hydrochloride, (+)-10-camphorsulfonyl chloride, 4(5)-imidazole acetic acid hydrochloride, and N-Fmoc derivatives of L-proline, D-proline and 3-aminopropionic acid were obtained from commercial sources. N-Fmoc derivatives of nipecotic acid and isonipecotic acid were prepared by the method of Bolin, *et al.*[37] Quinuclidine-3-carboxylic acid was prepared by the method of Grob.[22] Analytical HPLC were run on a Spectra Physics SP4270/8800 instrument using the following conditions:

Column: Vydac reverse phase C_{18} "peptide and protein" 0.21 × 15 cm. Mobile phases: A = 0.1 % TFA in H_2O, B = 0.1 % TFA in acetonitrile. Gradient: T = 0 min, 95 % A, 5 % B; T = 15 min, 0 % A, 100 % B. Flow = 2.0 mL/min, UV detection at 215 nm.

Preparative HPLC were run on a SepTech 800B instrument using the following conditions:

Column: 5 × 30 cm C_{18} Waters DeltaPak Prep Cartridge. Mobile phases: A = 0.1 % TFA in H_2O, B = 0.1 % TFA in acetonitrile or A = 1 % HOAc in H_2O, B = 1 % HOAc in acetonitrile. Gradient: T = 0 min, 95 % A, 5 % B, T = 45 min, 0 % A, 100 % B. Flow = 40 mL/min, UV detection at 220 nm.

Detailed procedures for the preparation of compounds **3b**, **4b**, **5b**, **6b**, **11**, **13**, **14–16**, **23** and **24** are given below and serve to exemplify the methods used to prepare all of the other compounds listed in Tables 1 and 2. All new compounds were purified to homogeneity (single component by TLC, ≥ 99 % pure by reverse phase HPLC analysis) and exhibited FAB mass spectra and ^{1}H NMR spectra in keeping with the structural assignments. Non-crystalline compounds were found to retain solvent and/or H_2O even after having been dried under reduced pressure (0.1–1.0 torr) for 12–24 hours. The presence and stoichiometry of certain solvents (e.g., CH_2Cl_2, $CHCl_3$, and HOAc) were verified by ^{1}H NMR. The presence and stoichiometry of other solvents not observed directly by NMR (e.g., H_2O and TFA) were determined from the combustion analyses. Purification methods, physical data and analytical data for compounds not exemplified below are collected in Table 4.

Table 4.

cmpd	[a]purification method	form; melting point	[b]TLC R_f	[b]HPLC retention time	[b]FAB mass spec	[b]C, H, N analysis
3a	A1, B1	crystals, 138-9°C	0.56 (3:1 hexane:EtOAc)	10.77 min	432 (M^+ + H)	$C_{23}H_{33}N_3O_3S$ calc: C, 64.01; H, 7.70; N, 9.74 found: C, 64.12; H, 8.07; N, 9.75
3c	A1, B2	crystals, 174-5°C	0.33 (4:1 hexane:EtOAc)	10.65 min	460 (M^+ + H)	$C_{25}H_{37}N_3O_3S$ calc: C, 65.33; H, 8.11; N, 9.14 fou nd: C, 65.34; H, 8.20; N, 9.14
4a	A5	amorphous solid from $CHCl_3$	0.10 (95:5:0.25 CH_2Cl_2:MeOH:NH_4OH)	8.36 min	436 (M^+ + H)	$C_{23}H_{37}N_3O_3S$, 0.35 $CHCl_3$ calc: C, 58.74; H, 7.89; N, 8.80 found: C, 58.82; H, 8.20; N, 8.50
4c	B3	crystals, 167-8°C	0.57 (95:5:0.25 $CHCl_3$:MeOH:NH_4OH)	9.40 min	464 (M^+ + H)	$C_{25}H_{41}N_3O_3S$ calc: C, 64.76; H, 8.91; N, 9.06 found: C, 64.81; H, 8.79; N, 8.91
5a	A2	amorphous solid from CH_2Cl_2	0.43 (95:5:0.25 CH_2Cl_2:MeOH:NH_4OH)	9.72 min	478 (M^+ + H)	$C_{25}H_{37}N_3O_4S$, 0.33 CH_2Cl_2 calc: C, 60.16; H, 7.91; N, 8.31 found: C, 60.03; H, 7.91; N, 8.38
5c	A3	lyophilized powder from dioxane	0.25 (1:1 hexane:EtOAc)	10.21 min	506 (M^+ + H)	$C_{27}H_{43}N_3O_4S$, 1.0 H_2O calc: C, 61.92; H, 8.66; N, 8.02 found: C, 61.91; H, 8.55; N, 7.92
6a	C1	lyophilized powder from CH_3CN-H_2O-TFA	0.52 (90:10:0.5 CH_2Cl_2:MeOH:NH_4OH)	8.50 min	544 (M^+ + H)	$C_{28}H_{41}N_5O_4S$, 1.75 TFA, 0.3 H_2O calc: C, 50.53; H, 5.84; N, 9.36 found: C, 50.52; H, 5.82; N, 9.50
6c	A4	amorphous solid from $CHCl_3$	0.40 (95:5:0.25 CH_2Cl_2:MeOH:NH_4OH)	9.12 min	572 (M^+ + H)	$C_{30}H_{45}N_5O_4S$, 0.2 $CHCl_3$, 1.0 H_2O calc: C, 59.11; H, 7.75; N, 11.41 found: C, 59.11; H, 7.50; N, 11.25
7	C1	lyophilized powder from CH_3CN-H_2O-TFA	0.61 (90:10:0.2 CH_2Cl_2:MeOH:HOAc)	9.12 min	550 (M^+ + H)	$C_{28}H_{43}N_3O_6S$, 0.8 TFA calc: C, 55.46; H, 6.89; N, 6.56 found: C, 55.56; H, 6.80; N, 6.51
8	A4	amorphous solid from $CHCl_3$	0.21 (90:10:0.5 CH_2Cl_2:MeOH:NH_4OH)	8.50 min	521 (M^+ + H)	$C_{27}H_{44}N_4O_4S$, 0.25 $CHCl_3$ calc: C, 59.23; H, 8.07; N, 10.13 found: C, 59.56; H, 8.19; N, 10.14

Table 4. *Continued.*

cmpd	[a]purification method	form; melting point	[b]TLC R_f	[b]HPLC retention time	[b]FAB mass spec	[b]C, H, N analysis
9	C1	lyophilized powder from CH_3CN-H_2O-TFA	0.10 (90:10:1 $CHCl_3$:MeOH:NH_4OH)	8.33 min	561 (M^+ + H)	$C_{30}H_{48}N_4O_4S$, 1.95 TFA, 0.05 H_2O calc: C, 51.93; H, 6.43; N, 7.15 found: C, 51.93; H, 6.36; N, 7.28
10	C1	lyophilized powder from CH_3CN-H_2O-TFA	0.48 (90:10:0.5 CH_2Cl_2:MeOH:NH_4OH)	8.58 min	547 (M^+ + H)	$C_{29}H_{46}N_4O_4S$, 1.9 TFA, 0.1 H_2O calc: C, 51.48; H, 6.34; N, 7.32 found: C, 51.46; H, 6.16; N, 7.51
12	A2	amorphous solid from $CHCl_3$	0.40 (90:10:1 $CHCl_3$:MeOH:NH_4OH)	8.67 min	561 (M^+ + H)	$C_{30}H_{48}N_4O_4S$, 0.8 $CHCl_3$ calc: C, 56.37; H, 7.49; N, 8.54 found: C, 56.49; H, 7.44; N, 8.50
17	C1	lyophilized powder from CH_3CN-H_2O-TFA	c	8.79 min	601 (M^+)	$C_{33}H_{53}N_4O_4S^+$ $CF_3CO_2^-$, 1.0 TFA calc: C, 53.61; H, 6.57; N, 6.76 found: C, 53.26; H, 6.58; N, 6.86
18	C2	lyophilized powder from CH_3CN-H_2O-HOAc	c	9.02 min	673 (M^+)	$C_{36}H_{57}N_4O_6S^+$ Br^-, 0.85 HOAc, 0.1 H_2O calc: C, 56.25; H, 7.56; N, 6.96 found: C, 56.24; H, 7.27; N, 6.76
19	C1	lyophilized powder from CH_3CN-H_2O-TFA	c	8.74 min	659 (M^+)	$C_{35}H_{55}N_4O_6S^+$ $CF_3CO_2^-$, 0.85 TFA, 0.1 H_2O calc: C, 53.26; H, 6.57; N, 6.42 found: C, 53.26; H, 6.41; N, 6.61
20	C1	lyophilized powder from CH_3CN-H_2O-TFA	0.25 (80:20:1 CH_2Cl_2:MeOH:NH_4OH)	8.80 min	579 (M^+ + H)	$C_{29}H_{46}N_4O_6S$, 1.9 TFA, 1.5 H_2O calc: C, 47.64; H, 6.26; N, 6.77 found: C, 47.51; H, 6.35; N, 6.75
21	C1	lyophilized powder from CH_3CN-H_2O-TFA	0.27 (80:20:1 CH_2Cl_2:MeOH:NH_4OH)	8.59 min	619 (M^+ + H)	$C_{32}H_{50}N_4O_6S$, 1.3 TFA, 0.75 H_2O calc: C, 53.23; H, 6.82; N, 7.18 found: C, 53.20; H, 6.81; N, 7.18
22	C1	lyophilized powder from CH_3CN-H_2O-TFA	0.35 (80:20:1 CH_2Cl_2:MeOH:NH_4OH)	8.81 min	605 (M^+ + H)	$C_{31}H_{48}N_4O_6S$, 1.5 TFA calc: C, 52.64; H, 6.43; N, 7.22 found: C, 52.59; H, 6.69; N, 7.25
25	D	amorphous solid from THF-H_2O	0.55, 0.61 (85:15 $CHCl_3$:MeOH)	8.77 min	619 (M^+ + H)	$C_{32}H_{50}N_4O_6S$, 1.2 H_2O calc: C, 60.01; H, 8.25; N, 8.75 found: C, 59.95; H, 8.02; N, 8.88

[a]purifications were performed as described in the Experminental Section: A = pressurized silica gel column chromatography using as eluant: 1 = 1:4 EtOAc:hexanes, 2 = 95:5:0.5 $CHCl_3$:MeOH:NH_4OH, 3 = 3:1 EtOAc: hexanes, 4 = 90:10:1 $CHCl_3$:MeOH:NH_4OH; B = crystallization from: 1 = ether-hexanes, 2 = EtOAc, 3 = MeOH; C = preparative reverse phase HPLC using a H_2O-CH_3CN gradient containing: 1 = 0.1 % TFA, 2 = 1 % HOAc; D = precipitation from H_2O-THF.

[b]TLC, analytical reverse phase HPLC, FAB mass spectra, and C, H, N analyses were obtained as described in the Expermiental Section.

[c]This compound streaked in TLC systems eluted with 80:20:2 $CHCl_3$:MeOH:NH_4OH or 80:20:0.5 $CHCl_3$:MeOH:HOAC.

General procedure for preparation of β-hydroxy nitriles ***3a–c***

*2S-(1S-Cyanoethyl)-7,7-dimethyl-2S-hydroxy-1S-(((4-(2-methylphenyl)piperazinyl)sulfonyl)methyl)-bicyclo[2.2.1.]-heptane (**3b**).* To a stirred solution of diisopropylamine (21.0 mL, 150 mmol) in THF (350 mL) at –78 °C was added *n*-butyllithium (60 mL of a 2.5 M solution in hexane, 150 mmol). The solution was warmed to 0 °C for 15 min, then cooled to –78 °C. A solution of propionitrile (10.1 mL, 141 mmol) in THF (75 mL) was added dropwise, and the resulting solution was stirred at –78 °C for 45 min. A solution of ketone **2**[19] (50.0 g, 128 mmol) in THF (350 mL) at –78 °C was added via cannula, and the resulting solution was stirred at –78 °C for 5 min. A solution of 5:1 THF:water (100 mL) was added and the mixture was warmed to ambient temperature. The mixture was diluted with EtOAc (500 mL) and washed with 5 % aqueous citric acid (2 x 500 mL) and brine (250 mL). The organic phase was dried ($MgSO_4$), filtered, and the solvents were removed under reduced pressure. The crude product was dissolved in ether and **3b** crystallized out in pure form, mp 163–165 °C (29 g, 51 %). The faster eluting minor β-hydroxynitrile diastereomers and small amount of starting ketone were removed from the filtrate residue by pressurized silica gel column chromatography using 80:20 hexanes:EtOAc as eluant to obtain more **3b** (17 g, 30 %).

Anal. calcd for $C_{24}H_{35}N_3O_3S$: C, 64.69; H, 7.92; N, 9.43; found: C, 64.74; H, 7.99; N, 9.35. TLC: R_f 0.31 (75:25 hexane:EtOAc). HPLC: retention time 10.20 min. FAB-MS: m/z 446 ($M^+ + H$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.19 (m, 2H), 3.70 (d, J = 15 Hz, 1H), 3.68 (s, 1H), 3.49 (m, 4H), 3.38 (d, J = 15 Hz, H), 2.75 (q, J = 7 Hz, 1H), 2.30 (s, 2H), 2.05 (m, 2H), 1.7–1.9 (m, 3H), 1.47 (d, J = 7 Hz, 3H), 1.41 (d, J = 12 Hz, 1H), 1.40 (s, 3H), 1.15 (s, 3H), 1.04 (m, 1H).

General procedure for preparation of amino alcohols ***4a–c***

*2S-(2S-(1-Amino) propyl)-7,7-dimethyl-2S-hydroxy-1S-(((4-(2-methylphenyl)piperazinyl)sulfonyl) methyl)-bicyclo-[2.2.1.]heptane (**4b**).* To a stirred solution of **3b** (25.0 g, 56.2 mmol) in THF (350 mL) at –78 °C was added dropwise a 1.0 M solution of LAH in THF (170 mL, 170 mmol). The resulting solution was stirred at –78 °C for 1 h, and then warmed to 0 °C for 3 h. Ether (300 mL) was added, followed by the slow dropwise addition of 5 M NaOH solution (35 mL; caution! foaming occurs). The resulting suspension was warmed to ambient temperature and stirred for 1 h. EtOAc (250 mL) was added and stirring was continued for 30 min. The solids were removed by filtration through Celite and the filtercake was washed with EtOAc. The filtrate solvents were removed under reduced pressure and **4b** was obtained by crystallization from MeOH, mp 172–174 °C (17.2 g, 68 %). The filtrate was purified by pressurized silica gel column chromatography using 97:3:0.3 $CHCl_3$:MeOH:NH_4OH as eluant to obtain more **4b** (3.8 g, 15 %).

Anal. calcd for $C_{24}H_{39}N_3O_3S$: C, 64.11; H, 8.74; N, 9.35; found: C, 64.09; H, 8.88; N, 9.31. TLC: R_f 0.50 (95:5:0.5 $CHCl_3$:MeOH:NH_4OH). HPLC: retention time 9.80 min. FAB-MS: m/z 450 ($M^+ + H$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.20 (m, 2H), 7.05 (m, 2H), 2.32 (s, 3H), 1.13 (d, J = 6 Hz, 3H), 1.11 (s, 3H), 1.02 (s, 3H).

General procedure for preparation of amino amides ***8–12*** *from N-Fmoc amino acids*

*2S-(2S-(1-(L-Prolyl)amino)propyl)-7,7-dimethyl-2S-hydroxy-1S-(((4-(2-methylphenyl) piperazinyl)sulfonyl)methyl)-bicyclo[2.2.1.]heptane (**11**).* To a stirred solution of **4b** (2.00 g, 4.45 mmol) in DMF (30 mL) was added N-Fmoc-L-proline (1.58 g, 4.68 mmol), BOP (2.17 g, 4.90 mmol), and DIEA (1.71 mL, 9.80 mmol). After 16 h, diethylamine (6 mL) was added and the solution was stirred at ambient temperature for 3 h. The solvents were removed under reduced pressure and the residue was purified by preparative reverse phase HPLC using a H_2O–CH_3CN gradient containing 0.1 % TFA. The TFA salt of **11** was obtained as an amorphous powder by lyophilization (2.8 g, 85 %).

Anal. calcd for $C_{29}H_{46}N_4O_4S$, 1.7 TFA, 0.05 H_2O: C, 52.48; H, 6.50; N, 7.56; found: C, 52.46; H, 6.50; N, 7.69. TLC: R_f 0.45 (90:10:1 $CHCl_3$:MeOH:NH_4OH). HPLC: retention time 8.60 min. FAB-MS: m/z 547 ($M^+ + H$). ^1H NMR (400 MHz, $CDCl_3$): δ 7.55 (br t, 1H), 7.18 (m, 2H), 7.03 (m, 2H), 2.31 (s, 3H), 1.14 (s, 3H), 1.02 (s, 3H), 0.99 (d, J = 7 Hz, 3H).

General procedure for the preparation of amides ***6a–c*** *and* ***13***

*2S-(2S-(1-((4(5)-Imidazolylmethyl) carbonyl) amino)-propyl) -7,7- dimethyl -2S- hydroxy -1S- (((4-(2-methyl-phenyl)piperazinyl)sulfonyl)methyl)-bicyclo[2.2.1.]heptane (**6b**).* To a stirred solution of **4b** (1.50 g, 3.34 mmol) in DMF (15 mL) was added 4(5)-imidazole acetic acid hydrochloride (679 mg, 4.18 mmol), BOP (1.85 g, 4.18 mmol), and DIEA (2.18 mL, 12.5 mmol). After 16 h, the solvent was removed under reduced pressure. The residue was dissolved in EtOAc (100 mL) and washed with saturated aqueous $NaHCO_3$ (2 × 50 mL) and water (2 × 50 mL). The organic phase was dried ($MgSO_4$), filtered, and the solvent was removed under reduced pressure. The residue was purified by pressurized silica gel column chromatography using 92:8:0.8 $CHCl_3$:MeOH:NH_4OH as eluant to obtain a foam that crystallized from EtOAc to give **6b** , mp 159–163 °C (1.56 g, 84 %).

Anal. calcd for $C_{29}H_{43}N_5O_4S$: C, 62.45; H, 7.77; N, 12.56; found: C, 62.88; H, 7.68; N, 12.79. TLC: R_f 0.44 (90:10:1 $CHCl_3$:MeOH:NH_4OH). HPLC: retention time 8.72 min. FAB-MS: m/z 558 ($M^+ + H$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.57 (s, 1H), 7.2 (m, 3H), 7.0 (m, 2H), 6.88 (s, 1H), 3.55 (m, 2H), 3.4 (m, 5H), 2.95 (m, 4H), 2.87 (d, J = 15 Hz, 1H), 2.31 (s, 3H), 1.71 (t, J = 4 Hz, 1H), 1.52 (d, J = 13 Hz, 1H), 1.15 (s, 3H), 1.03 (s, 3H), 0.97 (d, J = 6 Hz, 3H).

2S-(2S-(1-((Quinuclidin-3R,S-yl)carbonyl)amino)propyl)-7,7-dimethyl-2S-hydroxy-1S-(((4-(2-methylphenyl)-piperazinyl)sulfonyl)methyl)-bicyclo[2.2.1.]heptane (13). To a stirred solution of **4b** (2.00 g, 4.45 mmol) in DMF (50 mL) was added quinuclidine-3-carboxylic acid hydrochloride[22] (938 mg, 4.90 mmol), BOP (2.17 g, 4.90 mmol), and DIEA (2.56 mL, 14.7 mmol). After 16 h, the solvent was removed under reduced pressure. The residue was purified by preparative reverse phase HPLC using a H_2O–CH_3CN gradient containing 1 % HOAc. The acetate salt of **16** was obtained as an amorphous powder by lyophilization (2.60 g, 86 %). The presence of two diastereomers was not evidenced chromatographically by TLC or HPLC, but was seen in the ^1H NMR spectrum of the free base in $CDCl_3$ solution with the presence of two pairs of closely spaced methyl singlets (camphor methyls) and two closely spaced methyl doublets (aminopropyl methyls) of equal intensity.

Anal. calcd for $C_{32}H_{50}N_4O_4S$, 0.8 HOAc, 1.85 H_2O: C, 60.39; H, 8.58; N, 8.39; found: C, 60.41; H, 8.19; N, 8.58. TLC: R_f 0.65 (80:20:2 $CHCl_3$:MeOH:NH_4OH). HPLC: retention time 8.68 min. FAB-MS: m/z 587 (M^+ + H). ^1H NMR (free base, 300 MHz, $CDCl_3$): δ 7.19 (m, 2H), 7.02 (m, 2H), 6.26, 6.15 (br triplets, 1H), 2.30 (s, 3H), 1.15, 1.16 (two singlets, 3H), 1.05, 1.04 (two singlets, 3H), 1.02 and 1.00 (two closely spaced doublets, 3H).

*General procedure for the preparation of amides **5a-c**, and **7** from anhydrides*

*2S-(2S-(1-(Acetyl)amino)propyl)-7,7-dimethyl-2S-hydroxy-1S-(((4-(2-methylphenyl) piperazinyl) sulfonyl)methyl)-bicyclo[2.2.1.]heptane (**5b**).* To a stirred solution of **4b** (0.50 g, 1.1 mmol) in CH_2Cl_2 (15 mL) was added acetic anhydride (0.12 mL, 1.3 mmol). After 16 h, the solvent was removed under reduced pressure. The residue was purified by pressurized silica gel column chromatography using a gradient elution of 1:1 to 2:1 EtOAc:hexanes. Concentration of the product-containing fractions under reduced pressure gave **5b** as an amorphous white powder (0.48 g, 88 %).

Anal. calcd for $C_{26}H_{41}N_3O_4S$: C, 63.51; H, 8.41; N, 8.55; found: C, 63.75; H, 8.45; N, 8.39. TLC: R_f 0.48 (95:5:0.25 CH_2Cl_2:MeOH:NH_4OH). HPLC: retention time 9.52 min. FAB-MS: m/z 492 (M^+ + H). ^1H NMR (300 MHz, $CDCl_3$): δ 7.19 (m, 2H), 7.01 (m, 2H), 6.38 (br t, 1H), 3.61 (m, 1H), 3.4 (m, 6H), 3.0 (m, 4H), 2.88 (d, J = 15 Hz, 1H), 2.31 (s, 3H), 1.97 (s, 3H), 1.58 (d, J = 13 Hz, 1H), 1.16 (s, 3H), 1.05 (s, 3H), 1.02 (d, J = 6 Hz, 3H).

*General procedure for the preparation of carboxymethylamino amides **20–23** and **25***

*2S-(2S-(1-(N-Carboxymethyl-L-prolyl)amino)propyl)-7,7-dimethyl-2S-hydroxy-1S-(((4-(2-methylphenyl) piperazinyl)sulfonyl)methyl)-bicyclo[2.2.1.]heptane (**23**).* To a stirred solution of the free base of **11** (0.20 g, 0.37 mmol) in DMF (5 mL) was added ethyl bromoacetate (0.045 mL, 0.40 mmol) and DIEA (0.071 mL, 0.41 mmol). After 24 h at ambient temperature, the solvent was removed under reduced pressure and the residue was purified by preparative reverse phase HPLC using a H_2O–CH_3CN gradient containing 0.1 % TFA. The TFA salt of the ethyl ester of **23** was obtained as an amorphous powder by lyophilization (0.22 g, 76 %; anal. calcd for $C_{33}H_{52}N_4O_6S$, 1.4 TFA: C, 54.25; H, 6.79; N, 7.07; found: C, 54.25; H, 6.78; N, 7.02; TLC R_f = 0.50 (1:1 EtOAc:$CHCl_3$); HPLC retention time 9.68 min; FAB-MS m/z 633 (M^+ + H)). To a stirred solution of the ethyl ester (0.20 g, 0.32 mmol) in THF (5 mL) was added 1 M NaOH until a pH 10 solution persisted for 1 h. The solvent was removed under reduced pressure and the residue was purified by preparative reverse phase HPLC using a H_2O–CH_3CN gradient containing 0.1 % TFA. The TFA salt of **23** was obtained as an amorphous powder by lyophilization (0.21 g, 84 %). Anal. calcd for $C_{31}H_{48}N_4O_6S$, 1.5 TFA: C, 52.64; H, 6.43; N, 7.22; found: C, 52.49; H, 6.51; N, 7.22. TLC: R_f 0.40 (80:20:2 $CHCl_3$:MeOH:NH_4OH). HPLC: retention time 8.79 min. FAB-MS: m/z 605 (M^+ + H). ^1H NMR (400 MHz, CD_3OD): δ 7.17 (m, 2H), 7.07 (d J = 5 Hz, 1H), 6.99 (t, J = 5 Hz, 1H), 4.30 (dd, J = 4, 5 Hz, 1H), 4.21 (d, J = 14 Hz, 1H), 4.04 (d, J = 14 Hz, 1H), 2.32 (s, 3H), 1.18 (s, 3H), 1.03 (s, 3H), 1.01 (d, J = 7 Hz, 3H).

*Carboxyethylation of **11***

*2S-(2S-(1-(N-Carboxyethyl-L-prolyl) amino) propyl)-7,7-dimethyl-2S-hydroxy-1S-(((4-(2-methylphenyl) piperazinyl)sulfonyl)methyl)-bicyclo[2.2.1.]heptane (**24**).* To a stirred solution of the free base of **11** (1.50 g, 2.74 mmol) in MeOH (15 mL) was added methyl acrylate (0.310 mL, 3.43 mmol). After 72 h at ambient temperature, the solvent was removed under reduced pressure and the residue was purified by preparative reverse phase HPLC using a H_2O–CH_3CN gradient containing 0.1 % TFA. The TFA salt of the methyl ester of **24** was obtained as an amorphous powder by lyophilization (1.96 g, 88 %; anal. calcd for $C_{33}H_{52}N_4O_6S$, 1.65 TFA: C, 53.10; H, 6.59; N, 6.82; found: C, 53.09; H, 6.58; N, 6.88; TLC R_f = 0.55 (95:5 $CHCl_3$:MeOH); HPLC retention time 9.45 min; FAB-MS m/z 633 (M^+ + H)). To a stirred solution of the methyl ester (1.00 g, 1.22 mmol) in THF (15 mL) was added 1 M NaOH until a pH 10 solution persisted for 1 h. The solution was evaporated under reduced pressure and the residue was purified by preparative reverse phase HPLC using a H_2O–CH_3CN gradient containing 0.1 % TFA. The TFA salt **24** was obtained as an amorphous powder by lyophilization (872 mg, 87 %).

Anal. calcd for $C_{32}H_{50}N_4O_6S$, 1.8 TFA: C, 51.88; H, 6.34; N, 6.80; found: C, 51.87; H, 6.28; N, 6.82. TLC: R_f 0.40 (80:20:2 $CHCl_3$:MeOH:NH_4OH). HPLC: retention time 8.88 min. FAB-MS: m/z 619 (M^+ + H). ^1H NMR (400 MHz, $CDCl_3$): δ 8.50 (br s, 1H), 7.20 (m, 2H), 7.05 (m, 2H), 2.33 (s, 3H), 1.12 (s, 3H), 1.03 (s, 3H), 0.99 (d, J = 6 Hz, 3H).

*General procedure for the preparation of the quinuclidinium derivatives **14** and **17–19***

2S-(2S-(1-((N-Carboxymethylquinuclidin-3R,S-yl)-carbonyl)amino)propyl)-7,7-dimethyl-2S-hydroxy-1S-(((4-

(2-methylphenyl) piperazinyl)sulfonyl) methyl)-bicyclo-[2.2.1.]heptane (14). To a stirred solution of the acetate salt of **13** (1.50 g, formula wt = 668, 2.25 mmol) in DMF (30 mL) was added iodoacetic acid (543 mg, 2.92 mmol) and DIEA (0.43 mL, 2.48 mmol). After 16 h, TLC showed complete consumption of **13**. The solvent was removed under reduced pressure and the residue was purified by preparative reverse phase HPLC using a H_2O–CH_3CN gradient containing 1 % HOAc. **14** was obtained as an amorphous powder by lyophilization (1.29 g, 82 %). The presence of two diastereomers was not evidenced chromatographically by TLC or HPLC, but was seen in the ^1H NMR spectrum in CD_3OD solution with the presence of two closely spaced methyl doublets (aminopropyl methyls) at δ 0.98 and 0.97 ppm of equal intensity.
Anal. calcd for $C_{34}H_{52}N_4O_6S$, 0.55 HOAc, 0.95 H_2O: C, 60.52; H, 8.18; N, 8.04; found: C, 60.52; H, 7.98; N, 8.15. TLC: R_f 0.20 (80:20:2 $CHCl_3$:MeOH:NH_4OH). HPLC: retention time 8.73 min. FAB-MS: m/z 644 (M^+). ^1H NMR (acetate salt; 300 MHz, CD_3OD): δ 7.1–7.2 (m, 2H), 7.05 (d, J = 6.5 Hz, 1H), 6.98 (t, J = 6.5 Hz, 2H), 2.31 (s, 3H), 1.69 (t, J = 4.3 Hz, 1H), 1.53 (d, J = 13 Hz), 1.17 (s, 3H), 1.04 (s, 3H), 0.98 and 0.97 (overlapping doublets, J = 6.5 and 6.6 Hz, 3H total integration).

Fractional crystallization of 14 to 15 and 16

The acetate salt of **14** and 1.1 equivalents of aqueous 1 % HCl were lyophilized from H_2O-CH_3CN. The resulting chloride salt of **14** (0.25 g) was dissolved in $CHCl_3$ (10 mL) and allowed to evaporate slowly to about one half the original volume over 2 days, whereupon crystal formation occurred. The mixture was cooled to 10 °C overnight and the crystals were removed by filtration (0.12 g). ^1H NMR Analysis of the crystalline solid in CD_3OD solution showed a single isomer as judged by the presence of a single methyl doublet at 0.978 ppm. The filtrate was evaporated and lyophilized from 5 % aqueous acetic acid to give a powder (0.14 g), analysis of which by ^1H NMR in CD_3OD solution showed a mixture of isomers in a ratio of about 92:8 as judged by integration of the two methyl doublets at 0.978 ppm (minor) and 0.968 ppm (major).

15: Anal. calcd for $C_{34}H_{52}N_4O_6S$, HCl: C, 59.93; H, 7.84; N, 8.22; found: C, 60.12; H, 7.55; N, 8.10. TLC: R_f 0.20 (80:20:2 $CHCl_3$:MeOH:NH_4OH). HPLC: retention time 8.73 min. FAB-MS: m/z 644 (M^+). ^1H NMR (300 MHz, CD_3OD): δ 7.1–7.2 (m, 2H), 7.05 (d, J = 6.5 Hz, 1H), 6.98 (t, J = 6.5 Hz, 2H), 2.31 (s, 3H), 1.69 (t, J = 4.3 Hz, 1H), 1.53 (d, J = 13 Hz), 1.17 (s, 3H), 1.04 (s, 3H), 0.98 (d, J = 6.5 Hz, 3H).

15: Anal. calcd for $C_{34}H_{52}N_4O_6S$, HCl, 1.75 HOAc, 0.1 H_2O: C, 57.14; H, 7.70; N, 7.11; found: C, 57.14; H, 7.42; N, 7.11. TLC: R_f 0.20 (80:20:2 $CHCl_3$:MeOH: NH_4OH). HPLC: retention time 8.73 min. FAB-MS: m/z 644 (M^+). ^1H NMR (300 MHz, CD_3OD): δ 7.1–7.2 (m, 2H), 7.05 (d, J = 6.5 Hz, 1H), 6.98 (t, J = 6.5 Hz, 2H), 2.31 (s, 3H), 1.69 (t, J = 4.3 Hz, 1H), 1.53 (d, J = 13 Hz), 1.17 (s, 3H), 1.04 (s, 3H), 0.97 (d, J = 6.6 Hz, 3H).

Acknowledgments

We gratefully acknowledge the expert technical assistance and enthusiasm of Carla J. Woyden and Maribeth Guidotti (radioligand binding assays), Edward V. Lis, Jr (*in vitro* OT antagonist studies), Duane R. Reiss (*in vitro* AVP antagonist and *in vivo* OT antagonist studies), as well as the invaluable contributions of John Moreau (C, H, N analyses), Harri Ramjit and Arthur C. Coddington (mass spectra), and Jean Kaysen (manuscript preparation). We also thank Dr Paul S. Anderson for encouragement and support of this work.

References and Notes

1. Plattner, J. J.; Norbeck, D. W. In *Drug Discovery Technologies*, pp. 92–126, Clark, C. R.; Moos, W. H., Eds; Ellis Horwood; Chichester, England, 1990.

2. Freidinger, R. M. In *Prog. Drug Res.*, Volume 40, pp. 33–98, Jucker, E., Ed.; Birkhauser Verlag; Boston, 1993.

3. For an example from the cholecystokinin area: Horwell, D. C.; Hughes, J.; Hunter, J. C.; Pritchard, M. C.; Richardson, R. S.; Roberts, E.; Woodruff, G. N. *J. Med. Chem.* **1991**, *34*, 404.

4. For an example of ligand design based on the X-ray crystal structure of the HIV protease: Lam, P. Y. S.; Jadhav, P. K.; Eyermann, C. J.; Hodge, C. N.; Ru, Y.; Bacheler, L. T.; Meek, J. L.; Otto, M. J.; Rayner, M. M.; Wong, Y. N.; Chang, C. H.; Weber, P. C.; Jackson, D. A.; Sharpe, T. R.; Erickson-Viitanen, S. *Science* **1994**, *263*, 380.

5. Wiley, R. A.; Rich, D. H. *Med. Res. Rev.* **1993**, *13*, 327.

6. Pettibone, D. J.; Clineschmidt, B. V.; Anderson, P. S.; Freidinger, R. M.; Lundell, G. F.; Koupal, L. R.; Schwartz, C. D.; Williamson, J. M.; Goetz, M. A.; Hensens, O. D.; Liesch, J. M.; Springer, J. P. *Endocrinology* **1989**, *125*, 217.

7. Pettibone, D. J.; Clineschmidt, B. V.; Kishel, M. T.; Lis, E. V.; Reiss, D. R.; Woyden, C. J.; Evans, B. E.; Freidinger, R. M.; Veber, D. F.; Cook, M. J.; Haluska, G. J.; Novy, M. J.; Lowensohn, R. I. *J. Pharm. Exp. Ther.* **1993**, *264*, 308.

8. Fuchs, A.-R.; Fuchs, F.; Husslein, P.; Soloff, M.; Fernstrom, M. J. *Science* **1982**, *215*, 1396.

9. Chard, T. *Am. J. Perinat.* **1989**, *6*, 145.

10. Chibbar, R.; Miller, F. D.; Mitchell, B. F. *J. Clin. Invest.* **1993**, *91*, 185.

11. Akerlund, M.; Stromberg, P.; Hauksson, A.; Andersen, L. F.; Lyndrup, J.; Trojnar, J.; Melin, P. *Br. J. Obstet. Gynaecol.* **1987**, *94*, 1040.

12. Anderson, L. F.; Lyndrup, J.; Akerlund, M.; Melin, P. *Am. J. Perinat.* **1989**, *6*, 196.

13. Goodwin, T. M.; Paul, R. H.; Silver, H.; Parsons, M.; Chez, R.; Spellacy, R. W.; Hayashi, R.; North, L.; Merriman, R. *Am. J. Obstet. Gynecol.* **1992**, *166*, 359.

14. Melin, P. *Bailliere's Clinical Obstetrics and Gynaecology* **1993**, *7*, 577.

15. Evans, B. E.; Leighton, J. L.; Rittle, K. E.; Gilbert, K. F.; Lundell, G. F.; Gould, N. P.; Hobbs, D. W.; DiPardo, R. M.; Veber, D. F.; Pettibone, D. J.; Clineschmidt, B. V.; Anderson, P. S.; Freidinger, R. M. *J. Med. Chem.* **1992**, *35*, 3919.

16. Evans, B. E.; Lundell, G. F.; Gilbert, K. F.; Bock, M. G.; Rittle, K. E.; Carroll, L. A.; Williams, P. D.; Pawluczyk, J. M.; Leighton, J. L.; Young, M. B.; Erb, J. M.; Hobbs, D. W.; Gould, N. P.; DiPardo, R. M.; Hoffman, J. B.; Perlow, D. S.; Whitter, W. L.; Veber, D. F.; Pettibone, D. J.; Clineschmidt, B. V.; Anderson, P. S.; Freidinger, R. M. *J. Med. Chem.* **1993**, *36*, 3993.

17. Bock, M. G.; Carroll, L. A.; DiPardo, R. M.; Evans, B. E.; Leighton, J. L.; Rittle, K. E.; Gilbert, K. F.; Lundell, G. F.; Gould, N. P.; Whitter, W. L.; Williams, P. D.; Perlow, D. S.; Pettibone, D. J.; Clineschmidt, B. V.; Veber, D. F.; Freidinger, R. M. In *Vasopressin*, pp. 539–547, Gross, P.; Richter, D.; Robertson, G., Ed.; John Libby Eurotext Ltd; Paris, 1993.

18. Pettibone, D. J.; Clineschmidt, B. V.; Guidotti, M. T.; Lis, E. V.; Reiss, D. R.; Woyden, C. J.; Bock, M. G.; Evans, B. E.; Freidinger, R. M.; Hobbs, D. J.; Veber, D. F.; Williams, P. D.; Chiu, S.-H. L.; Thompson, K. L.; Kaufman, M. J.; Cukierski, M. A.; Haluska, G. J.; Cook, M. J.; Novy, M. J. *Drug Devel. Res.* **1993**, *30*, 129.

19. Williams, P. D.; Anderson, P. S.; Ball, R. G.; Bock, M. G.; Carroll, L. A.; Chiu, S. L.; Clineschmidt, B. V.; Culberson, J. C.; Erb, J. M.; Evans, B. E.; Fitzpatrick, S. L.; Freidinger, R. M.; Kaufman, M. J.; Lundell, G. F.; Murphy, J. S.; Pawluczyk, J. M.; Perlow, D. S.; Pettibone, D. J.; Pitzenberger, S. M.; Thompson, K. L.; Veber, D. F. *J. Med. Chem.* **1994**, *37*, 565.

20. Abbreviations: AVP = arginine vasopressin, Boc = *tert*-butyloxycarbonyl, BOP = benzotriazolyl-*N*-oxy-tris(dimethylamino)phosphonium hexafluorophosphate, DES = diethylstilbestrol dipropionate, DIEA = diisopropylethylamine, DMF = dimethylformamide, DMSO = dimethylsulfoxide, EtOAc = ethyl acetate, EtOH = ethanol, FAB-MS = fast-atom bombardment mass spectrum, Fmoc = (9-fluorenyl)methoxycarbonyl, HOAc = acetic acid, LAH = lithium aluminum hydride, MeOH = methanol, Nal = 2-naphthylalanine, OT = oxytocin, TFA = trifluoroacetic acid, THF = tetrahydrofuran.

21. Castro, B.; Dormoy, J. R.; Evin, G.; Selve, C. *Tetrahedron Lett.* **1975**, *16*, 1219.

22. Grob, C. A.; Renk, E. *Helv. Chim. Acta* **1954**, *37*, 1689.

23. Cheng, Y.-C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.

24. Arunlakshana, O.; Schild, H. O. *Br. J. Pharm.* **1959**, *14*, 48.

25. Clineschmidt, B. V.; Lis, E. V. *Arch. Int. Pharmacodyn. Ther.* **1986**, *284*, 72.

26. Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 44.

27. Williams, P. D.; Bock, M. G.; Tung, R. D.; Garsky, V. M.; Perlow, D. S.; Erb, J. M.; Lundell, G. F.; Gould, N. P.; Whitter, W. L.; Hoffman, J. B.; Kaufman, M. J.; Clineschmidt, B. V.; Pettibone, D. J.; Freidinger, R. M.; Veber, D. F. *J. Med. Chem.* **1992**, *35*, 3905.

28. Howl, J.; Ismail, T.; Strain, A. J.; Kirk, C. J.; Anderson, D.; Wheatley, M. *Biochem. J.* **1991**, *276*, 189.

29. Pettibone, D. J.; Kishel, M. T.; Woyden, C. J.; Clineschmidt, B. V.; Bock, M. G.; Freidinger, R. M.; Veber, D. F.; Williams, P. D. *Life Sciences* **1992**, *50*, 1953.

30. An unpublished distance geometry program, JIGGLE, was used: Kearsley, S. K.; Merck Research Laboratories, Rahway, NJ.

31. MMFF (Merck Molecular Force Field) is an unpublished force field that is very similar to the Allinger force field (Allinger, N. L. *J. Am. Chem. Soc.* **1977**, *99*, 8127): Halgren, T. A.; Merck Research Laboratories, Rahway, NJ.

32. Full details of the cyclic hexapeptide molecular modeling and NMR studies will be published separately. Atomic coordinates for the the two molecules in Figure 4 can be obtained from the authors upon request.

33. SQUEAL (Structural and Qualitative Electrostatic Alignment) is an unpublished variation of the molecular alignment algorithm, SEAL (Kearsley, S. K.; Smith, G. M. *Tetrahedron Computer Methodology* **1990**, *3*, 615): Miller, M. D.; Merck Research Laboratories, West Point, PA.

34. Walker, N.; Stuart, D. *Acta Crystallogr.* **1983**, *A39*, 158.

35. Sheldrick, G. M., SHELXS-86. *Crystallographic Computing 3*, pp. 175–189, Sheldrick, G. M.; Krüger, C.; Goddard, R., Eds; Oxford University Press; 1985.

36. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

37. Bolin, D. R.; Sytwu, I.-I.; Humiec, F.; Meienhofer, J. *Int. J. Peptide Protein Res.* **1989**, *33*, 353.

(*Received* 17 *June* 1994)