Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 2747–2752
Bioorganic & Medicinal Chemistry Letters

Potent nonpeptide vasopressin receptor antagonists based on oxazino- and thiazinobenzodiazepine templates

Jay M. Matthews,[a,*] William J. Hoekstra,[a,†] Alexey B. Dyatkin,[a] Leonard R. Hecker,[a] Dennis J. Hlasta,[a] Brenda L. Poulter,[a] Patricia Andrade-Gordon,[a] Lawrence de Garavilla,[a] Keith T. Demarest,[b] Eric Ericson,[b] Joseph W. Gunnet,[b] William Hageman,[a] Richard Look,[b] John B. Moore,[b] Charles H. Reynolds[a] and Bruce E. Maryanoff[a,*]

[a]*Drug Discovery, Johnson & Johnson Pharmaceutical Research & Development, Spring House, PA 19477-0776, USA*
[b]*Drug Discovery, Johnson & Johnson Pharmaceutical Research & Development, Raritan, NJ 08869-0602, USA*

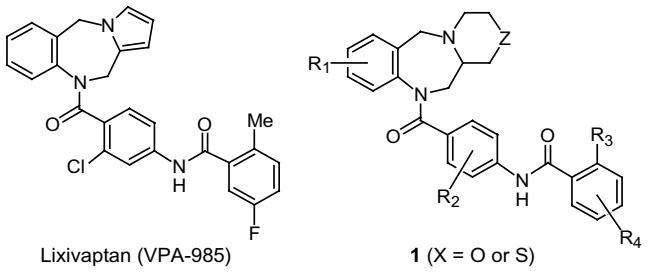
Received 10 February 2004; accepted 25 March 2004

Abstract—Vasopressin receptor antagonists can elicit ion-sparing diuretic effects (i.e., aquaresis) in vivo by blunting the action of the circulating hypophyseal hormone arginine vasopressin. We have identified two new series of basic tricyclic benzodiazepines, represented by general structure **1**, which contain compounds that bind with high affinity to human V_2 receptors. For example, (*S*)-(+)-**8** and **5** are potent and selective V_2 receptor antagonists with pronounced aquaretic activity in rats on oral administration.

1. Introduction

The nonapeptide arginine vasopressin (AVP), which is principally secreted from the posterior pituitary gland, is responsible for numerous biological actions as both a hormone and a neurotransmitter.[1] Three G-protein-coupled receptors, denoted as V_{1a}, V_{1b}, and V_2, are involved in AVP binding and cellular activation, resulting in important physiological responses such as reabsorption of water in the kidneys (V_2), contraction of bladder, uterine, and vascular smooth muscle (V_{1a}), breakdown of glycogen in the liver (V_{1a}), aggregation of platelets (V_{1a}), and release of corticotropin from the anterior pituitary gland (V_{1b}).[1] Additionally, in the central nervous system, AVP modulates aggressive, social, and sexual behavior, stress response, and memory.

The V_2 receptors on renal epithelial cells mediate AVP-induced antidiuresis to preserve normal plasma osmolality. Thus, selective, nonpeptide vasopressin V_2 receptor antagonists have received attention for their potential use in treating diseases of excessive renal reabsorption of water.[2–4] For instance, lixivaptan (VPA-985), a pyrrolobenzodiazepine that exhibits low-nanomolar binding to the V_2 receptor and 230-fold selectivity over V_{1a} receptor binding, exerts an aquaretic effect on oral administration to rats and dogs (Fig. 1).[2a,c] Tricyclic vasopressin receptor antagonists of this structural type[2–4] have physical properties that can present difficulties for oral drug development, such as elevated molecular weight (450–600 Da), high hydrophobicity ($\log P > 4$), and limited aqueous solubility (<1 mg/mL).[5] Thus, we became interested in introducing a basic amine center into the tricyclic molecular framework of lixivaptan in the context of thiomorpholine and morpholine



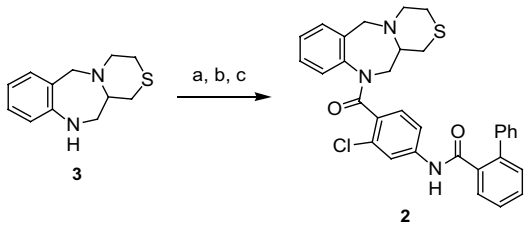
Lixivaptan (VPA-985) **1** (X = O or S)

Figure 1. Structures of lixivaptan and benzodiazepine **1**.

* Corresponding authors. Tel.: +1-215-628-5530; fax: +1-215-628-4985 (B.E.M.); tel.: +1-215-628-5214; fax: +1-215-628-3297 (J.M.M.); e-mail addresses: bmaryano@prdus.jnj.com; jmatthew@prdus.jnj.com
† Present address: GlaxoSmithKline, R&D, 5 Moore Drive, PO Box 13398, Research Triangle Park, NC 27709, USA.

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.03.083

rings fused to the benzodiazepine framework, as in formula **1** (Fig. 1). It was hoped that these novel chemical entities would give rise to receptor antagonists with good in vitro and in vivo potency, and favorable oral bioavailability. In this paper, we report on the biological properties of compounds of general formula **1**, some of which represent advanced V_2 receptor antagonist leads, such as **5** and (*S*)-(+)-**8**.

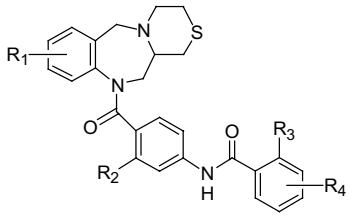


Scheme 1. Reagents and conditions: (a) 2-Cl–4-$NO_2C_6H_4C(O)Cl$, Et_3N, CH_2Cl_2 (90%); (b) Zn dust, NH_4Cl, MeOH (90%); (c) 2-$PhC_6H_4C(O)Cl$, Et_3N, CH_2Cl_2 (55%).

2. Synthetic chemistry

The thiazinobenzodiazepines (Table 1) were obtained by reacting the appropriate tricyclic secondary amine,[6] as illustrated for target **2** in Scheme 1. Racemic **3** was acylated with 2-chloro-4-nitrobenzoyl chloride and the intermediate amide was reduced with zinc dust and ammonium chloride. Acylation of the resulting aniline with 2-phenylbenzoyl chloride yielded **2**. To prepare the (*R*)-(−) enantiomer of **2**, (*R*)-(−)-**3**[7] (>97% ee) was obtained by fractional crystallization of the (*S*)-(−)-binaphthyl-2,2′-diyl hydrogen phosphate salt in ethanol,[6] and the free base was carried through the remainder of the route in Scheme 1. We generated deshalogen analogues (*R*)-(−)-**8** and (*S*)-(+)-**8** (Table 1) in an analogous fashion.[8] The other analogues in Table 1 were prepared from tricycle **3**, or its congeners, using similar chemistry. The oxazinobenzodiazepines (Table 2) were accessed from appropriate tricycles, as illus-

Table 1. Vasopressin V_{1a} and V_2 binding and functional data for thiazinobenzodiazepines



Compd[a]	R_1	R_2	R_3	R_4	V_{1a} bndg[b] K_i, nM	V_2 bndg[b] K_i, nM	V_{1a} funct[c] K_i, μM	V_2 funct[c] K_i, μM
6	H	Cl	Me	5-F	IA	60	14	0.3
7	H	Cl	Ph	5-F	IA	37	14	0.07
8	H	H	Ph	H	49	5.0	8.0	0.02
(+)-**8**[d,e]	H	H	Ph	H	84	3.2	1.4	0.015
(−)-**8**[e,f]	H	H	Ph	H	290	25	1.3	0.04
9[g]	H	H	Ph	H	IA	65	1.4	0.04
2	H	Cl	Ph	H	490	11		
(+)-**2**[e,h]	H	Cl	Ph	H	250	3.7	14	0.017
10	H	Cl	Ph	4-F	410	3.7		
11	H	F	Ph	H	IA	10		
12	H	Me	Ph	H	IA	10		
13	H	OMe	Ph	H	IA	15		
14	H	OH	Ph	H	110	3.2		
15	9-Cl	H	Ph	H	IA	10		
16	8-Me	H	Ph	H	18	8.0	14	0.023
17	8-F	H	Ph	H	IA	9.0		
18	8,9-F_2	H	Ph	H	IA	18		
VPA-985[i]					44	2.3	6.0	0.023

[a] Target compounds were purified by reverse-phase semi-prep HPLC and isolated as trifluoroacetate salts, unless noted otherwise. Purities were judged by reverse-phase HPLC/MS at 215 and 254 nm (YMC J'Sphere C-18 column, 0.4×5 cm; mobile phase: MeCN–H_2O). All compounds were characterized by ESI-MS; selected compounds were analyzed by 300-MHz ^{1}H NMR. Compounds are racemates unless noted otherwise.

[b] Inhibition of [^{3}H]-AVP binding to recombinant human vasopressin V_{1a} or V_2 receptors ($N = 1$–3). IA = inactive, that is, <30% inhibition of radioligand binding at a concentration of 100 nM.

[c] Inhibition of AVP-induced effects on cells expressing either human V_{1a} or human V_2 receptors. K_i values were determined by using cells treated with 1 nM AVP to stimulate calcium mobilization for V_{1a} receptors or cAMP accumulation for V_2 receptors ($N = 1$–4).

[d] (*S*)-(+) enantiomer; $[\alpha]_D^{25}$ +583.3 (*c* 0.03, MeOH).

[e] HCl salt.

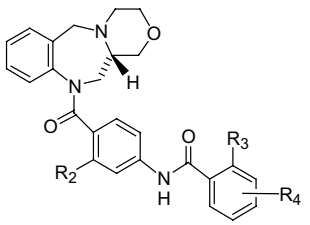
[f] (*R*)-(−) enantiomer; $[\alpha]_D^{25}$ −1045 (*c* 0.06, MeOH).

[g] *S*,*S*-Dioxide (i.e., a sulfone).

[h] (*S*)-(+) enantiomer; $[\alpha]_D^{25}$ +173.4 (*c* 0.15, MeOH).

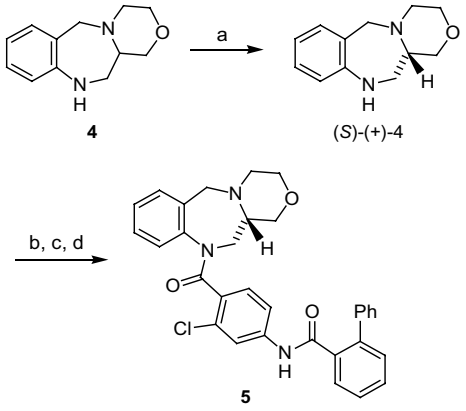
[i] Reference standard. IC_{50} values (left to right): 150, 5.0 nM; 12.5, 0.091 μM.

Table 2. Vasopressin V_{1a} and V_2 binding and functional data for oxazinobenzodiazepines



Compd[a]	R_2	R_3	R_4	V_{1a} bndg[b] K_i, nM	V_2 bndg[b] K_i, nM	V_{1a} funct[c] K_i, μM	V_2 funct[c] K_i, μM
19	Cl	Me	5-F	100	2.8	2.0	0.012
20	Cl	Ph	5-F	ca. 300[d]	11	6.4	0.012
21[e]	H	Ph	H	ca. 30[f]	3.7		0.016
5[g]	Cl	Ph	H	24	0.90	0.60	0.004
22[h]	Cl	Ph	H	640	15	14	0.17
23	Cl	4-MeOPh	H	IA	2.8	1.9	0.003
24	Cl	3-MeOPh	H	IA	3.7	3.4	0.002
25	Cl	4-OH–Ph	H	IA	4.2	4.2	0.011
26	Cl	3-OH–Ph	H	IA	5.0	11	0.02
27	Cl	Ph	4-F	IA	1.9	0.70	0.002
28	Cl	Ph	4-OMe	IA	4.2	1.3	0.005
29	Cl	Ph	5-OMe	IA	17		
30	Cl	Ph	4-OH	ca. 30	1.4	1.6	0.006
31	Cl	Ph	5-OH	IA	13	1.9	0.07
VPA-985[i]				44	2.3	6.0	0.023

[a] Same as in Table 1, except compounds are (*S*)-(+) enantiomers (as depicted in the structural formula), unless noted otherwise.
[b] Same as in Table 1.
[c] Same as in Table 1, except $N = 1$–9.
[d] 62% inhibition @ 1000 nM.
[e] Racemic mixture.
[f] 69% inhibition @ 100 nM.
[g] $C_{32}H_{28}ClN_3O_3 \cdot HCl \cdot 1.3H_2O$ (correct microanalysis for C/H/N/H_2O); mp 210 °C (dec), $[\alpha]_D^{25}$ +215.5 (*c* 0.28, MeOH); 98.7% enantiomeric purity by chiral HPLC (Chiralcel AS column, 0.46×5 cm; mobile phase: 90:10 hexanes/*i*-PrOH, with 0.1% Et_2NH).
[h] HCl salt; (*R*)-(−) enantiomer related to **5**, $[\alpha]_D^{25}$ −200.7 (*c* 0.28, MeOH).
[i] Reference standard. IC_{50} values (left to right): 150, 5.0 nM; 12.5, 0.091 μM.



Scheme 2. Reagents and conditions: (a) Ref. 6 (35%); (b) 2-Cl–4-$NO_2C_6H_4C(O)Cl$, Et_3N, CH_2Cl_2 (52%); (c) Zn dust, NH_4Cl, MeOH (97%); (d) 2-$PhC_6H_4C(O)Cl$, Et_3N, CH_2Cl_2 (52%).

trated in Scheme 2 for the (*S*)-(+) enantiomeric series. We prepared tricycle **4** according to literature procedures.[6,9] To obtain **5**, and the corresponding (*R*)-(−) enantiomer, **22** (Table 2), we resolved racemate **4** into its enantiomers by fractional crystallization through acid-addition salts involving the opposite enantiomers of di-*p*-toluoyltartaric acid (methanol-ether).[6,10] Other chiral analogues (Table 2) were prepared from (*S*)-(+)-**4** in a similar manner.

3. Results and discussion

The thiazinobenzodiazepines were evaluated for their binding to human V_{1a} and V_2 receptors (Table 1).[12] The direct analogue of VPA-985, **6**, exhibited weaker binding to both receptors than the reference agent, although the V_2 K_i value of 60 nM is still respectable. Introduction of an *o*-phenyl group, as in **7**, enhanced V_2 receptor affinity ($K_i = 37$ nM), with good V_2 selectivity. Thus, we adopted the *o*-phenyl substituent for other analogues.[13] Deshalogen parent **8** showed notable V_2 affinity ($K_i = 5.0$ nM) with modest selectivity ($V_{1a}/V_2 = 10$). Each of its enantiomers, (*S*)-(+)-**8** and (*R*)-(−)-**8**, surprisingly has significant V_2 receptor affinity ($K_i = 3.2$ and 25 nM, respectively). The mere 8-fold difference in V_2 affinity for these two enantiomers suggests that the geometry around this portion of the ligand is not very critical for V_2 binding interactions. The excellent V_2 affinity of (*S*)-(+)-**8**, coupled with the 26-fold selectivity for V_2 over V_{1a}, was viewed as encouraging. Subsequent variation of R_2 on the 4-aminobenzamide ring provided

potent, reasonably V_2-selective analogues (cf. **8** with **2** and **10**–**14**). Overall, notable V_2 affinities were obtained with (*S*)-(+)-**2** ($K_i = 3.7$ nM), (*S*)-(+)-**8** ($K_i = 3.2$ nM), **10** ($K_i = 3.7$ nM), and **14** ($K_i = 3.2$ nM). *S*,*S*-Dioxide **9** had a 13-fold lower affinity than that for sulfide **8**. The results for **15**–**18** indicate that R_1 substitution is well tolerated, although this modification can enhance V_{1a} affinity, as observed for **16** (V_{1a} $K_i = 18$ nM).

To follow up on these results, we studied several compounds in cell-based functional assays involving human V_{1a} and V_2 receptors.[14] Compounds (+)-**2**, (*S*)-(+)-**8**, (*R*)-(−)-**8**, **9**, **16**, and VPA-985 potently antagonized the effects of AVP on human V_2 receptors ($K_i = 0.015$–0.04 μM), whereas they had just weak potency against human V_{1a} receptors ($K_i = 1.3$–14 μM).[14] The reduced V_2 functional potency for **6** ($K_i = 300$ nM) versus VPA-985 is consistent with the V_2 binding result. Enantiomer (*S*)-(+)-**28** gave functional V_{1a} and V_2 K_i values of 1400 and 15 nM, for ca. 90-fold V_2 selectivity, and (*S*)-(+)-**30** gave functional V_{1a} and V_2 K_i values of 14,000 and 17 nM, for an impressive V_2 selectivity of ca. 820-fold. The potency of each enantiomer of **28** in the V_2 receptor functional assay ($K_i = 15$–40 nM) is reasonably consistent with the binding data.

For the oxazinobenzodiazepines, we generally studied the (*S*)-(+) enantiomeric series because it has better V_2 receptor binding (Table 2). In comparing **5** and **22**, each enantiomer has a significant V_2 affinity ($K_i = 0.9$ and 15 nM, respectively), but there is a 17-fold preference for **5**. Interestingly, **5** also has high affinity for the V_{1a} receptor ($K_i = 24$ nM), which accounts for a 26-fold V_2 binding selectivity. The direct VPA-985 analogue, **19**, exhibited excellent V_2 receptor affinity, as well as modest V_{1a} affinity, in contradistinction to related thiazinobenzodiazepine **6**. Given our success with the *o*-phenyl group for the thiazinobenzodiazepine series, we incorporated it here, as well.[13] In general, this substitution resulted in good-to-excellent V_2 affinity. Indeed, several oxazino analogues had single-digit nanomolar V_2 receptor binding with good selectivity versus V_{1a}, such as **5**, **21**, **23**–**25**, **27**, **28**, and **30**. The most potent compounds possessed 2-phenyl (**5**, V_2 $K_i = 0.9$ nM), 2-phenyl-4-fluoro (**27**, V_2 $K_i = 1.9$ nM), and 2-phenyl-4-hydroxy (**30**, V_2 $K_i = 1.4$ nM) groups. In the V_2 functional assay, **5**, **19**, **20**, **23**–**28**, and **30** were very potent in antagonizing the effects of AVP ($K_i = 0.002$–0.02 μM), whereas they were weak in the V_{1a} functional assay ($K_i = 0.6$–14 μM).[14] In the cell-based assays, **5** exhibited K_i values of 600 and 4 nM for V_{1a} and V_2, reflecting 150-fold V_2 selectivity. The (*R*)-(−) enantiomer **22** showed comparatively less functional potency than did **5** ($K_i = 14,000$ and 170 nM), in contrast to (*S*)-(+)-**8** and (*R*)-(−)-**8**.

Oral administration of (*S*)-(+)-**8** to Sprague-Dawley rats elicited a dose-dependent aquaretic effect. At a dose of 10 mg/kg, po, urine output ($N = 8$) was increased 300% over untreated controls ($N = 8$), with a reduction in urine osmolality of 70%.[15] Oral administration of **5** to Sprague-Dawley rats produced a dose-dependent aquaretic effect with remarkable potency. A dose of just 1 mg/kg, po, caused a 700% increase of urine output ($N = 10$) over untreated controls ($N = 18$), with a 60% reduction of urine osmolality.[15]

The structure of **5**, as a tosylate salt, was determined by X-ray diffraction (Crystalytics).[16] The tricycle adopts a chair-like conformation for the seven-membered ring with its pendant amide carbonyl (C13) in an axial orientation. The carbonyl oxygen of this amide, O2, is *anti* to the fused benzene ring (Fig. 2). This arrangement is analogous to that observed for related *N*-acyltetrahydrobenzazepines in solution.[17] We carried out a Monte Carlo conformational search on protonated **5** with the OPLS-AA force field and GB/SA water model.[18] The global energy minimum contains a *trans*-fused oxazinobenzodiazepine with a chair-like seven-membered ring, an axial pendant amide, and an amide carbonyl *anti* to the fused benzene ring (Fig. 3). The

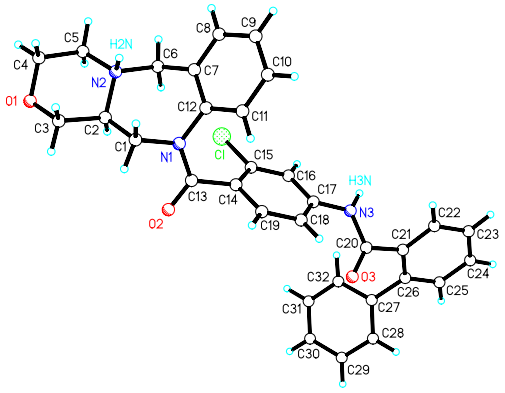


Figure 2. Perspective drawing of the solid-state structure of **5**·TsOH [(*S*)-(+) isomer], showing the cationic subunit, with its atom-numbering scheme (standard atom color code; H = cyan).

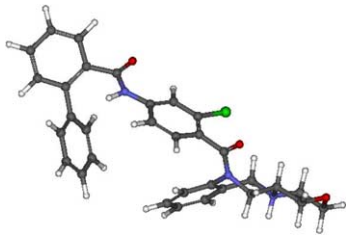


Figure 3. Structure of the global minimum-energy conformation of protonated **5** (standard atom color code).

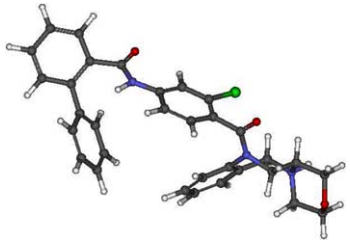


Figure 4. Structure of the next-higher-energy conformation of protonated **5** (+1.7 kcal/mol) (standard atom color code).

tricyclic nucleus in this structure was closely superimposable on that of the X-ray structure. The next-higher-energy structure is 1.7 kcal/mol (7.1 kJ/mol) less stable than the global minimum. It has a *cis*-fused tricycle, resulting from inversion of the ammonium center (NH^+), a chair-like seven-membered ring, an axial amide, and an amide carbonyl *anti* to the fused benzene (Fig. 4). This structural information could be helpful in developing a pharmacophore model for V_2 receptor binding.[19]

4. Conclusion

We have identified two noteworthy series of nonpeptide vasopressin receptor antagonists containing thiazino- and oxazinobenzodiazepine core structures. There were several analogues with low-nanomolar V_2 receptor affinity and at least 20-fold selectivity for V_2 over V_{1a} receptors. In the thiazino class, (*S*)-(+)-**8** has excellent V_2 affinity ($K_i = 3.2$ nM), moderate binding selectivity ($V_{1a}/V_2 = 26$), good functional selectivity ($V_{1a}/V_2 = 93$), and oral efficacy as an aquaretic agent in rats. Also, (*S*)-(+)-**2** has excellent V_2 affinity ($K_i = 3.7$ nM) and notable V_2 selectivity (binding $V_{1a}/V_2 = 68$; functional $V_{1a}/V_2 = 823$). In the oxazino class, (*S*)-(+) enantiomer **5** has excellent V_2 receptor affinity ($K_i = 0.9$ nM), moderate binding selectivity ($V_{1a}/V_2 = 27$), good functional selectivity ($V_{1a}/V_2 = 150$), and impressive oral potency as an aquaretic agent in rats. Specific compounds from these two novel series have potential for the treatment of edematous conditions in patients. On the basis of an extensive array of pre-clinical data, oxazinobenzodiazepine **5** was advanced into human clinical studies.[20]

Acknowledgements

We are grateful to Diane Gauthier and Patrick Sasso for chemical and biological support, and thank Ralph A. Rivero for helpful discussions and support.

References and notes

1. (a) Albright, J. D.; Chan, P. S. *Curr. Pharm. Design* **1997**, *3*, 615; (b) Thibonnier, M.; Conarty, D. M.; Preston, J. A.; Wilkins, P. L.; Berti-Mattera, L. N.; Mattera, R. *Adv. Exp. Med. Biol.* **1998**, *449*, 251.
2. (a) Albright, J. D.; Reich, M. F.; Santos, E. G. D.; Dusza, J. P.; Sum, F. W.; Venkatesan, A. M.; Coupet, J.; Chan, P. S.; Ru, X.; Mazandarani, H.; Bailey, T. *J. Med. Chem.* **1998**, *41*, 2442; (b) Aranapakam, V.; Albright, J. D.; Grosu, G. T.; Chan, P. S.; Coupet, J.; Saunders, T.; Ru, X.; Mazandarani, H. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1733; (c) Martinez-Castelao, A. *Curr. Opin. Invest. Drugs* **2001**, *2*, 525; (d) Albright, J. D.; Santos, E. G. D.; Dusza, J. P.; Chan, P. S.; Coupet, J.; Ru, X.; Mazandarani, H. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 695; (e) Ashwell, M. A.; Bagli, J. F.; Caggiano, T. J.; Chan, P. S.; Molinari, A. J.; Palka, C.; Park, C. H.; Rogers, J. F.; Sherman, M.; Trybulski, E. J.; Williams, D. K. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 783.
3. (a) Ogawa, H.; Yamashita, H.; Kondo, K.; Yamamura, Y.; Miyamoto, H.; Kan, K.; Kitano, K.; Tanaka, M.; Nakaya, K.; Nakamura, S.; Mori, T.; Tominaga, M.; Yabuuchi, Y. *J. Med. Chem.* **1996**, *39*, 3547; (b) Matsuhisa, A.; Taniguchi, N.; Koshio, H.; Yatsu, T.; Tanaka, A. *Chem. Pharm. Bull.* **2000**, *48*, 21.
4. Trybulski, E. J. *Annu. Rep. Med. Chem.* **2001**, *36*, 159.
5. (a) Oprea, T. I. *J. Comput.-Aided Mol. Design* **2000**, *14*, 251; (b) Walters, W.; Patrick, A.; Murcko, M. A. *Curr. Opin. Chem. Biol.* **1999**, *3*, 384.
6. Matthews, J. M.; Dyatkin, A. B.; Evangelisto, M.; Gauthier, D. A.; Hecker, L. R.; Hoekstra, W. J.; Poulter, B. L.; Maryanoff, B. E. *Tetrahedron: Asymmetry* **2004**, *15*, in press.
7. The absolute configuration of intermediate (*R*)-(−)-**3** was assigned by X-ray crystallography of its (*S*)-(−)-binaphthyl-2,2′-diyl hydrogen phosphate salt.[6]
8. (*S*)-(+)-**8** was derived from (*S*)-(+)-**3** (95% ee), which was obtained by fractional crystallization of its (*R*)-(+)-binaphthyl-2,2′-diyl hydrogen phosphate salt (from EtOH).[6]
9. Kogami, Y.; Okawa, K. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 2963.
10. The absolute configuration of intermediate (*R*)-(−)-**4** was initially assigned by comparing the sign of its optical rotation to that of (*R*)-(−)-**3**. This assignment was confirmed by a vibrational circular dichroism (VCD) study on (*S*)-(+)-**4**.[11]
11. Dyatkin, A. B.; Freedman, T. B.; Cao, X.; Dukor, R. K.; Maryanoff, B. E.; Maryanoff, C. A.; Matthews, J. M.; Shah, R. D.; Nafie, L. A. *Chirality* **2002**, *14*, 215.
12. Receptor binding studies were performed by using recombinant human V_{1a} or V_2 receptor preparations derived from the membranes of transfected HEK-293 cells. Compounds were evaluated for their ability to displace [^{3}H]-AVP.
13. An *o*-phenylbenzoyl group is present in the vasopressin receptor antagonist conivaptan.[3b] The *o*-phenylbenzoyl analogue of lixivaptan was reported to have potent human V_2 receptor binding ($IC_{50} = 2.7$ nM), with 140-fold selectivity relative to human V_{1a} binding.[2d]
14. Inhibition of receptor activation caused by AVP was quantitated in HEK-293 cells expressing human V_{1a} or V_2 receptors; changes in intracellular Ca^{2+} (V_{1a}) or cAMP (V_2) concentrations were measured.
15. For a comparison, at doses of 10 and 1 mg/kg (po), OPC-31260 is reported to give the following values for urine output/osmolality, respectively: +500%/−65% and 0%/−10%;[3a] lixivaptan is reported to give the following values for urine output/osmolality: +450%/−70% and +200%/−50%.[2a]
16. Single crystals of **5**·TsOH, $[C_{32}H_{29}N_3O_3Cl][C_7H_7O_3S]$, from EtOAc/MeOH (mp > 240 °C), are (at −80 ± 2 °C) monoclinic [space group $P2_1-C_2^2$ (No. 4)] with $a = 8.001(1)$ Å, $b = 18.231(2)$ Å, $c = 12.067(1)$ Å, $\beta = 91.395(2)°$, $V = 1759.7(3)$ Å^3, and $Z = 2$ formula units [$d_{calcd} = 1.340$ g cm^{-3}; μ_a(MoKα = 0.220 mm^{-1}]. A full hemisphere of diffracted intensities (ω-scan width = 0.30°) was measured by using graphite-monochromated MoKα radiation ($\lambda = 0.71073$ Å) on a Bruker SMART CCD Single Crystal Diffraction System. Lattice constants were determined with the Bruker SAINT software by using the peak centers for 2093 reflections. A total of 18,635 integrated intensities with 2θ(MoKα) <61.01° were produced by using SAINT, 9886 of which were independent and gave $R_{int} = 0.056$. The Bruker SHELXTL-PC software package (Version 5) was used to solve the structure via 'direct methods' techniques. All

stages of weighted full-matrix least-squares refinement were conducted by using F_o^2 data. The final agreement factors at convergence are: R_1(unweighted, based on F) = 0.054 for 5039 independent reflections with $2\theta(\text{MoK}\alpha) < 61.01°$ and $I > 2\sigma(I)$; R_1(unweighted, based on F) = 0.109 and wR_2 (weighted, based on F^2) = 0.078 for all 9886 independent reflections with $2\theta(\text{MoK}\alpha) < 61.01°$. The absolute configuration of the cation was confirmed as *S* by using the anomalous dispersion technique,[10] with the 'Flack' absolute structure parameter for this solution refined to a final value of −0.09(5). The structural model incorporated anisotropic thermal parameters for all non-hydrogen atoms and isotropic thermal parameters for all hydrogen atoms. Amine and amide hydrogen atoms (H_{2N} and H_{3N}) were located from a difference Fourier synthesis and refined as independent isotropic atoms. The tosylate methyl group (C_{47} + 3H's; not shown) was refined as a rigid rotor (idealized sp^3-hybridization and C–H bond length of 0.98 Å) that was allowed to rotate about its C–C bond in least-squares cycles. The remaining hydrogen atoms were included in the structure factor calculations as idealized atoms (sp^2- or sp^3-hybridized carbon atoms and C–H bond lengths of 0.95–1.00 Å). The isotropic thermal parameters for H_{2N} and H_{3N} refined to final U_{iso} values of 0.03(1) and 0.02(1) Å^2, respectively. The isotropic thermal parameters of the remaining hydrogens were fixed at values 1.2 (nonmethyl) or 1.5 (methyl) times the equivalent isotropic thermal parameter of the attached carbon atom. Atomic coordinates for this structure are available from the authors on request.

17. Hassner, A.; Amit, B.; Marks, V.; Gottlieb, H. E. *J. Org. Chem.* **2003**, *68*, 6853.
18. (a) Maestro, 5.1 ed., Schrodinger Inc. (Portland, OR), 2003; (b) Jorgensen, W. L.; Tirado-Rives, J. *J. Am. Chem. Soc.* **1988**, *110*, 1657; (c) Qui, D.; Shenkin, P. S.; Hollinger, F. P.; Still, W. C. *J. Phys. Chem. A* **1997**, *101*, 3005; (d) Chang, G.; Guida, W. C.; Still, W. C. *J. Am. Chem. Soc.* **1989**, *111*, 4379.
19. For some molecular modeling studies, see: Gieldon, A.; Kazmierkiewicz, R.; Slusarz, R.; Ciarkowski, J. *J. Comput.-Aided Mol. Design* **2001**, *15*, 1085.
20. (a) For comparative purposes, here are some molecular parameters for **5** and lixivaptan. Compound **5**: MW = 538 Da; log *P* = 3.63; log *D* (pH 3) = 2.01; pK_a = 4.75/12.7. Lixivaptan: MW = 474 Da; calcd log *P* = 6.1; calcd pK_a = 11.9 (SciFinder, Advanced Chemistry Development software, Solaris v4.67); (b) Compound **5** showed good pharmacokinetics in rats and dogs. In rats (30 mg/kg, po, 3 mg/kg, iv; $N = 3$), the oral bioavailability was very good ($F = 68\%$), with an oral $t_{1/2}$ of 3.7 h.