Toward Synthetic Adrenaline Receptors: Strong, Selective, and Biomimetic Recognition of Biologically Active Amino Alcohols by Bisphosphonate Receptor Molecules†

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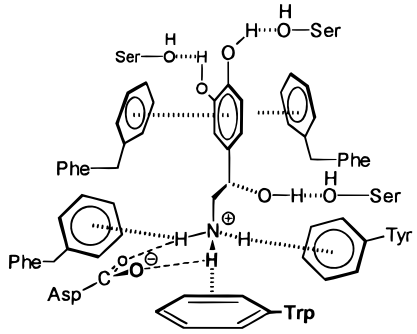
Xylylene bisphosphonates represent a new class of artificial receptor molecules for alkylammonium ions (Schrader, T. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2649−2651). Molecular recognition takes place in a 1:1 chelate-binding mode, and an almost ideal array of short, linear hydrogen bonds is created that guarantees maximum electrostatic and hydrogen-bond interactions. The host molecule, which was designed to imitate the natural adrenergic receptor, is selective for 1,2- and 1,3-amino alcohols due to formation of an additional cooperative hydrogen bond between the phosphonate anion and the hydroxyl groups. Biologically important amino alcohols such as glucosamine, 1-aminosorbitol, ephedrine, and the β-blocker propranolol are bound in DMSO with K_a values between 60 000 and 130 000 M^{-1}. Secondary amines are complexed at least as strongly as their primary counterparts. The phosphonate ester groups allow lateral recognition of the substate. This could be demonstrated for adrenaline model compounds that were recognized by phosphonates carrying extended aromatic ester groups for π,π-interactions.

Introduction

During the past decade, enormous research activities have been directed toward a better understanding of structure and function of the adrenergic receptor family,[2] notably when in 1984 the role of the G-proteins was discovered.[3] Every year, more than 3000 publications appear dealing exclusively with biochemical and medicinal aspects of research on adrenaline and noradrenaline. This highlights the great importance of adrenaline for signal transfer and the related control of biological processes in the human body. Many pharmaceuticals act as agonists or antagonists for the β-adrenergic receptor, e.g., the well-known β-blockers.[4] However, this receptor located in the membrane of adrenal gland cells has never been isolated in a pure form and has hence proven elusive to X-ray structure analysis.[5] The sequence of several human subtypes is known and, on the basis of site-directed mutagenesis experiments as well as on molecular modeling studies, some very similar proposals on the mechanism of neurotransmitter binding have evolved.[6] The main noncovalent interactions are summarized in Scheme 1. Electrostatic and hydrogen-bond interactions with an aspartate carboxylate bind the ammonium functionality; in addition, the NH_3^+ protons experience π-cation stabilization because they are surrounded by three electron-rich aromatic residues. Each of the aliphatic and catechol hydroxyls is hydrogen-bonded to a serine OH, while the aromatic ring is buried in a deep cleft flanked by two phenylalanine aromatic rings that are involved in double π-stacking. Although several structures have been synthesized that bind catecholamine neurotransmitters,[7] most of them recognize only one single characteristic part of the molecule by utilizing interactions that are far from biomimetic.[8]

Scheme 1. Noncovalent Interactions between Noradrenaline and the β-Adrenergic Receptor



It would be of great value for biochemists as well as pharmacologists to gain access to a small defined model of the adrenergic receptor that imitates the natural receptor−ligand interactions. To this end, we designed a new receptor structure for biomimetic recognition of

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† Synthetic Adrenaline Receptors. 2. For part 1, see ref 1.

⊗ Abstract published in *Advance ACS Abstracts*, December 1, 1997.

(1) Schrader, T. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2649−2651.

(2) Strader, C. D.; Fong, T. M.; Tota, M. R.; Underwood, D. *Annu. Rev. Biochem.* **1994**, *63*, 101−132.

(3) Gilman, A. G. *Cell* **1984**, *36*, 577−579. Nobel lecture: *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1406.

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(5) Recently, electron crystallography has been carried out on two-dimensional rhodopsin crystals: Schertler, G. F. X.; Villa, C.; Henderson, R. *Nature* **1993**, *362*, 770−772.

(6) Mutagenesis experiments: Ostrowski, J.; Kjelsberg, M. A.; Caron, M. C.; Lefkowitz, R. J. *Annu. Rev. Pharmacol. Toxicol.* **1992**, *32*, 167−183. Molecular modeling studies: Trumpp-Kallmeyer, S.; Hoflack, J.; Bruinvels, A.; Hibert, M. *J. Med. Chem.* **1992**, *35*, 3448−3462.

(7) (a) Behr, J.-P.; Lehn, J.-M.; Vierling, P. *Helv. Chim. Acta* **1982**, *65*, 1853−1867. (b) Bernardo, A. R.; Stoddart, J. F.; Kaifer, A. *J. Am. Chem. Soc.* **1992**, *114*, 10624−10631. (c) Ishizu, T.; Hirayama, J.; Noguchi, S. *Chem. Pharm. Bull.* **1994**, *42*, 1146−1148. (d) Campayo, L.; Bueno, J. M.; Ochoa, C.; Navarro, P.; Jimenez-Barbero, J.; Pepe, G.; Samat, A. *J. Org. Chem.* **1997**, *62*, 2684.

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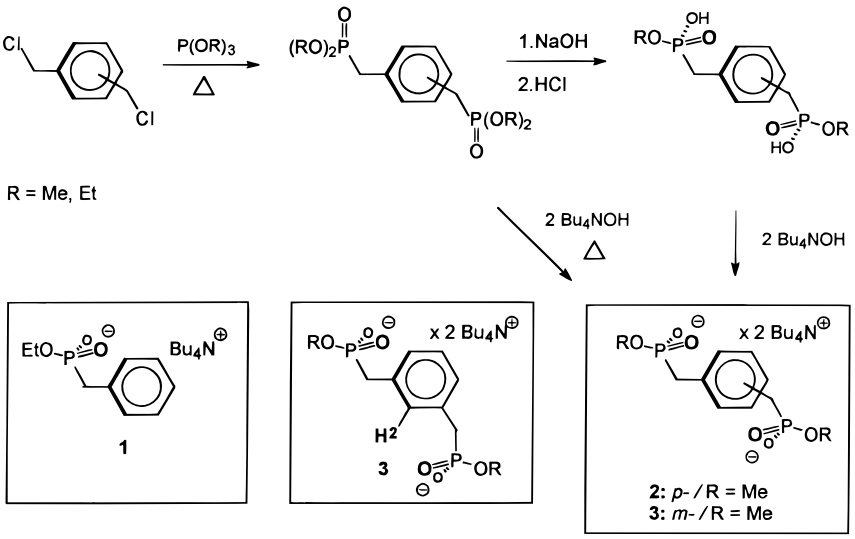
Scheme 2. Synthesis of Phosphonate Receptors 1−3



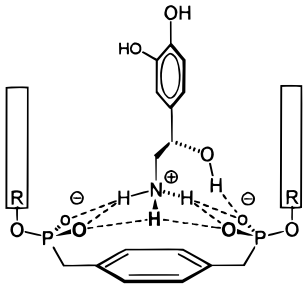


Figure 1. Complex of *p*-xylylene bisphosphonates with noradrenaline according to force-field calculations.[11]

alkylammonium ions. As stated above, the new host should be capable of a triple binding mode, i.e., exert attraction for the guest by means of electrostatic, hydrogen-bond, and π-cation interactions. One of the simplest solutions to this problem is a *p*-xylylene bisphosphonate that not only imitates the ammonium-aspartate interaction but also amplifies it due to its bidentate character (Figure 1).[9] The great advantage of the phosphonate over a carboxylate stems from the fact that at neutral pH phosphonates are fully dissociated due to their much lower pK_a values (1.8 vs 4.8), a prerequisite for molecular recognition at physiological conditions.[10] Another convenient feature of the phosphonate is the additional ester functionality that can at a later stage serve to introduce substituents for lateral recognition of the catechol. Furthermore, chiral ester alcohols may lead to discrimination between enantiomers of optically active catecholamines. According to molecular modeling, complexation of alkylammonium ions by the *p*-xylylene phosphonate **2** leads to a chelate structure with very strong electrostatic interactions, enhanced by a network of short and almost linear hydrogen bonds (Figure 1).[11] Each of the three positively polarized ammonium hydrogen atoms is located exactly above the xylylene phenyl ring at a calculated distance of 3.5 Å—very similar to the situation in the natural receptor, where three electron-rich aromatic residues are actively involved in π-cation stabilization.[12]

Results and Discussion

The phosphonates can be prepared by the classic route employing the Michaelis−Arbuzow reaction of xylylene halides with trialkyl phosphites (Scheme 2).[13] Basic hydrolysis of the obtained tetraalkyl bisphosphonates affords the respective bisphosphonic acids, which are subsequently converted into their tetrabutylammonium salts. We developed a shortcut for the direct transformation of alkylphosphonates into their tetrabutylammonium salts. An aqueous solution of equimolar amounts of tetrabutylammonium hydroxide and a dialkyl phosphonate are heated for 1 week at ~100 °C. During this time, the equilibrium is shifted slowly but steadily toward the product side because the resulting phosphonate anion is resonance-stabilized. After 1 week, spectroscopically pure product is obtained in quantitative yield (Scheme 2).

In the ^{1}H NMR spectra of equimolar mixtures of aliphatic primary ammonium chlorides with phosphonate receptors **2** and **3**, large downfield shifts of 0.5−0.8 ppm

(8) Exceptions are some recent ditopic receptors for dopamine: (a) Kimura, E.; Fujioka, H.; Kodama, M. *J. Chem. Soc., Chem. Commun.* **1986**, 1158−1159. (b) Schmidtchen, F. P. *Z. Naturforsch., C. Biosci.* **1987**, *42*, 476−485. (c) Hayakawa, K.; Kido, K.; Kanematsu, K. *J. Chem. Soc., Perkin Trans. 1* **1988**, 511−519. (d) Paugam, M.-F.; Valencia, L. S.; Bogess, B.; Smith, B. D. *J. Am. Chem. Soc.* **1994**, *116*, 11203−1120. Paugam, M.-F.; Biens, J. T.; Smith, B. D.; Christoffels, A. J.; de Jong, F.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1996**, *118*, 9820−9825.

(9) A bisphosphonate and a tetraphosphate have recently been used for strong glucoside binding in acetonitrile: (a) Das, G.; Hamilton, A. D. *J. Am. Chem. Soc.* **1994**, *116*, 11139−11140. Das, G.; Hamilton, A. D. *Tetrahedron Lett.* **1997**, *38*, 3675−3678. (b) Anderson, S.; Neidlein, U.; Gramlich, V.; Diederich, F. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1596−1600. Neidlein, U.; Diederich, F. *J. Chem. Soc., Chem. Commun.* **1996**, 1493−1494. (c) Recently a neutral phosphonate−calix[4]arene was presented that utilizes P═O···HN$^+$ hydrogen bonds for complexation of simple amines: Delangle, P.; Dutasta, J.-P. *Tetrahedron Lett.* **1995**, *36*, 9325. (d) Polyammonium compounds have been reported as artificial phosphate receptors: Furuta, H.; Magda, D.; Sessler, J. L. *J. Am. Chem. Soc.* **1991**, *113*, 978−985. Hosseini, M. W.; Lehn, J.-M.; Mertes, M. P. *Helv. Chim. Acta* **1983**, *66*, 2454−2466.

(10) (a) Natural examples for phosphate−ammonium interactions are found in many enzymes that recognize phosphates, e.g., the inositol 1,4,5-trisphosphate receptor: Potter, B. V. L.; Lampe, D.; *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1933. (b) Polyammonium cations are known to complex the phosphate groups of DNA and RNA and thereby stabilize their tertiary structure: Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984; pp 343−346.

(11) Molecular Modeling Program: CERIUS^2 from Molecular Simulations, Inc. Force field: Dreiding 2.21.

(12) Dougherty, D. A. *Science* **1996**, *271*, 163−167.

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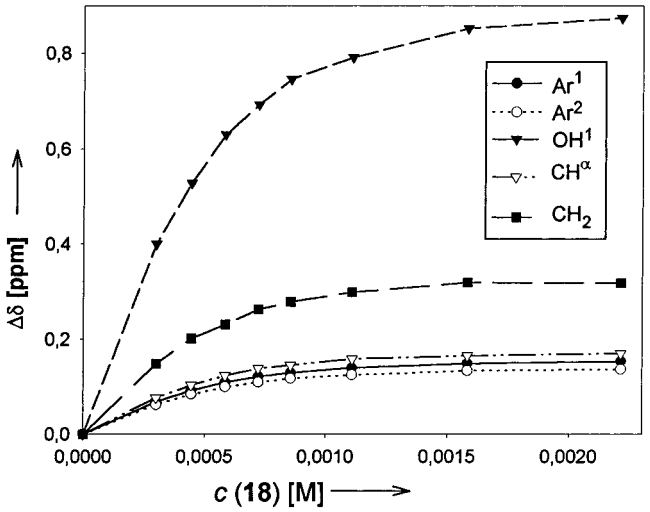


Figure 2. Dependence of the change in chemical shift of five signals of **17** (c = 0.5 mM) on the concentration of bisphosphonate **18** in DMSO-d_6 at 20 °C.

Table 1. Association Constants ($K_{1:1}$) [M^{-1}] from NMR Titrations in DMSO at 20 °C[a]

receptor	benzylamine 10^3 [M^{-1}]	(±)-**4** 10^3 [M^{-1}]
1	0.2	
2	2.8	15.5
3	7.4	55.0

[a] Because of the strongly hygroscopic character of both titration partners the DMSO-d_6-solution contained about 0.1% of water. Errors in K_a are standard deviations; they were estimated at ≤20% for $K \leq 10^4$ M^{-1} and at ≤40% for $K \leq 10^5$ M^{-1}.

and line broadening of the NH_3^+ signals demonstrate their hydrogen bonding to the phosphonate anions. As a consequence, the α-methylene protons are markedly shifted upfield in the range of 0.3−1.0 ppm. On the other hand, small but distinct complexation-induced shifts (CIS) of 0.1−0.2 ppm are also observed at the receptor molecule, namely downfield shifts of the α-methylene protons and upfield shifts of the methyl esters. Finally, at a 1:1 stoichiometry a downfield shift of 1−3 ppm is consistently observed for the host's ^{31}P NMR signal.[14] A second hint for interaction was found when at higher concentrations (>10^{-2} M) the 2:1 complexes of various alkylammonium chlorides with *p*-xylylene bisphosphonate **2** precipitated from DMSO solution.

We performed NMR titrations of benzylammonium chloride with phosphonate receptors **1**−**3** in DMSO-d_6. We measured the CIS of the α-C*H*N^+ proton and analyzed the binding curves by nonlinear regression methods (Figure 2).[15] The calculated association constants are summarized in Table 1. The simple monophosphonate/ammonium interaction is comparatively weak; binding constants of this kind are typically in the 10^2 M^{-1} range. By contrast, the *p*-xylylene bisphosphonate binds to the benzylammonium ion 14 times stronger—a very clear indication for the postulated chelate effect. No change is observed for the *p*-xylylene aromatic proton at 7.0 ppm,

(14) No self-association of the simple phosphonates **1**−**3** could be detected by simple dilution experiments.

(15) a) Schneider, H. J.; Kramer, R.; Simova, S.; Schneider, U. *J. Am. Chem. Soc.* **1988**, *110*, 6442. (b) Wilcox, C. S. In *Frontiers in Supramolecular Chemistry and Photochemistry*; Schneider, H.-J., Dürr, H., Eds.; VCH: Weinheim, 1991; p 123. We thank Prof. Schneider for his program to evaluate 1:1 complexation.

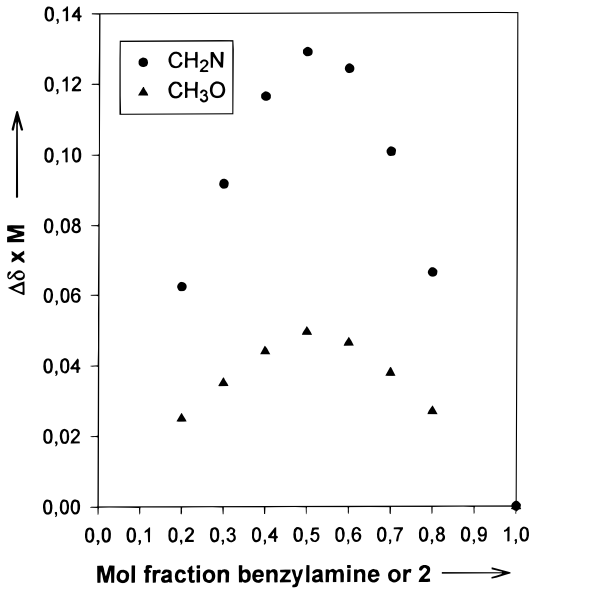


Figure 3. Job plot for complex formation between *p*-xylylene bisphosphonate **2** and benzylamine (c = 10 mM).

Table 2. Association Constants for Amines vs Amino Alcohols ($K_{1:1}$) [M^{-1}] from NMR Titrations with 3 in DMSO at 20 °C[a]

amines	10^3 [M^{-1}]	amino alcohols	10^3 [M^{-1}]
benzylamine	7	(±)-**4**	55
L-alanine methyl ester	10	α-D-glucosamine **10**	59
2-phenylethylamine	12	(1*S*,2*R*)-norephedrine **6**	62
N-benzyl-*N*-methylamine	19	(*R*)-propranolol **11**	66

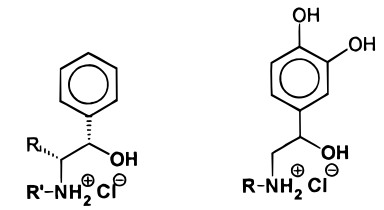
[a] Because of the strongly hygroscopic character of both titration partners the DMSO-d_6-solution contained about 0.1% of water. Errors in K_a are standard deviations; they were estimated at ≤20% for $K \leq 10^4$ M^{-1} and at ≤40% for $K \leq 10^5$ M^{-1}.

so that the existence of π-cation interactions remains speculative. Perhaps the aromatic ring has to be located in such a way that the $H^{\delta+}$ points directly toward the center of electron density, as is the case in the natural receptor. In comparison, the *m*-diphosphonate **3** binds even stronger, although here such a π-cation stabilization is not possible because the NH_3^+ group no longer lies exactly above the xylene ring. The advantage of **3** over **2** (i.e., of a *m*- vs *p*-xylylene core) according to force-field calculations is the decreased $PO^-\cdots HN^+$ distance, which strengthens both electrostatic attractions and hydrogen bonds.[10] Another indicator for the chelate-binding mode is the 0.2 ppm downfield shift of the *m*-xylylene bisphosphonate's lone aromatic proton C*H*-2 (Scheme 2), which shows that the receptor conformation is changing when the alkylammonium ion approaches (induced fit).[9a]

Job's method of continuous variations[16] confirms the postulated 1:1 stoichiometry. Maxima in the range of M = 0.5−0.55 are observed for guest as well as for host signals (Figure 3).

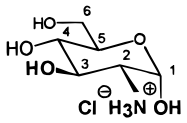
When 2-phenyl-2-hydroxyethylamine hydrochloride **4** (a model for the amino alcohol moiety in adrenaline derivatives) was treated with the bisphosphonate receptor molecules, the O*H*-signal immediately broadened and strongly shifted downfield by ~1.0 ppm. The β-C*H* signal shifted upfield, but only by 0.2−0.3 ppm. Obviously, an

(16) (a) Job, P. *Compt. Rend.* **1925**, *180*, 928. (b) Blanda, M. T.; Horner, J. H.; Newcomb, M. *J. Org. Chem.* **1989**, *54*, 4626.



4: R = H, R' = H
5: R = H, R' = Me
6: R = Me, R' = H
7: R = Me, R' = Me

8: R = H, **9:** R = Me



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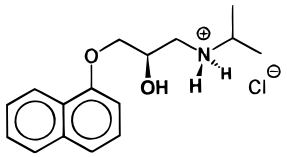


11

additional hydrogen bond is formed between the phosphonate and the alcohol moiety of **4**. This single additional hydrogen bond results in a drastic 5−7.5-fold increase of the binding constant over benzylamine's K_a value, reaching 55 000 M^{-1} for **3** (Table 1). We examined other biologically important 1,2-amino alcohols (e.g., glucosamine **10** as a model for neuraminic acid, ephedrine **7** as an agonist of the adrenergic receptor, and propranolol **11** as a typical β-blocker) and compared them to the respective amines. The results are summarized in Table 2.

(a) All amino alcohols are bound much stronger than their simple amine counterparts. An average estimate of the association constants for the amino alcohols of 60 000 M^{-1} is five times higher than the average estimate for simple amines of 12 000 M^{-1}.

(b) NMR experiments provide further structural information and confirm the strong hydroxyl binding: In the free glucosamine **10** only the OH-1 and OH-3 adjacent to the ammonium functionality shift during the titration, whereas OH-4 and OH-6 are pointing away from the receptor and thus remain untouched (Figure 4). Propranolol **11** has a diastereotopic *O*-methylene group, which is normally conformationally flexible. On addition of small amounts of the phosphonate, however, these methylene protons immediately turn chemically nonequivalent because the OH group is conformationally locked. The same effect is observed even for the NH_2^+ protons. Obviously, each NH is pointing to a different phosphonate moiety, with only one of the latter being involved in additional OH binding (Figure 5).

(c) Secondary amines seem to be bound even stronger than primary amines (see Table 2: benzylamine vs benzylmethylamine; see also propranolol **11** with its sterically demanding isopropyl group vs the primary amino alcohols). We checked this hypothesis for several pairs of biologically important amino alcohols that differ only in the extra methyl group of the respective secondary amine. The results are given in Table 3. With the exception of **4**/**5**, the secondary amines are indeed complexed at least as strongly as their primary counterparts. This may indicate that electrostatic interactions contribute most to the stabilization energy in the complex, so that loss of one hydrogen bond can be overcompensated by other factors such as hydrophobic interactions with the additional alkyl group. Contrary to all artificial ditopic catecholamine receptors known to date, our

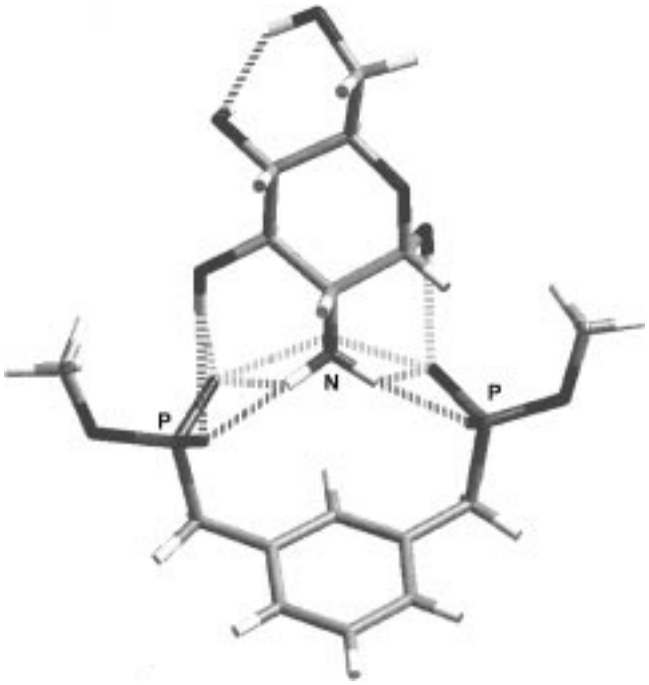


Figure 4. Energy-minimized complex of glucosamine with bisphosphonate **3**.[11]

Table 3. Association Constants ($K_{1:1}$) [M^{-1}] for Primary vs the Corresponding *N*-Methyl Substituted Secondary Amines from NMR Titrations with 3 in DMSO at 20 °C[a]

primary amines	10^3 [M^{-1}]	secondary amines	10^3 [M^{-1}]
benzylamine	7	*N*-methyl-*N*-benzylamine	19
(±)-noradrenaline **8**	18	(±)-adrenaline **9**	16
(±)-**4**	55	(±)-halostachine **5**	26
(1*S*,2*R*)-norephedrine **6**	62	(1*S*,2*R*)-ephedrine **7**	71
		(*R*)-propranolol **11**	66

[a] Because of the strongly hygroscopic character of both titration partners the DMSO-d_6-solution contained about 0.1% of water. Errors in K_a are standard deviations; they were estimated at ≤20% for $K \leq 10^4$ M^{-1} and at ≤40% for $K \leq 10^5$ M^{-1}.

bisphosphonates can be expected to bind especially well to adrenaline itself as well as to the respective β-blockers.

The moderate binding constant for adrenaline **9** can be rationalized by *inter*molecular competition of the catechol OH groups, which also bind to the receptor molecule. On phosphonate addition, large shifts of both relatively acidic phenol protons indicate a second superimposed equilibrium, so that evaluation based on a simple 1:1 stoichiometry of the complex must give misleading results. Therefore, adrenaline is assumed to bind to **3** as strongly as the other amino alcohols. This competition of several groups in the guest for binding sites on the host indicates that by introduction of a properly positioned functional group for catechol recognition the overall binding constant should increase markedly.

The question arises how far the capability of the bisphosphonate reaches to establish cooperative hydrogen bonds with remote hydroxyl groups in a substrate. We studied the binding behavior of **3** with 1,*n*-amino alcohols systematically. The results are summarized in Table 4. While 1,2- and 1,3-amino alcohols are bound very strongly, a marked drop in binding energy is observed for 1,4- and 1,5-amino alcohols, although even in the latter case a strong downfield shift of the OH proton accompanied with

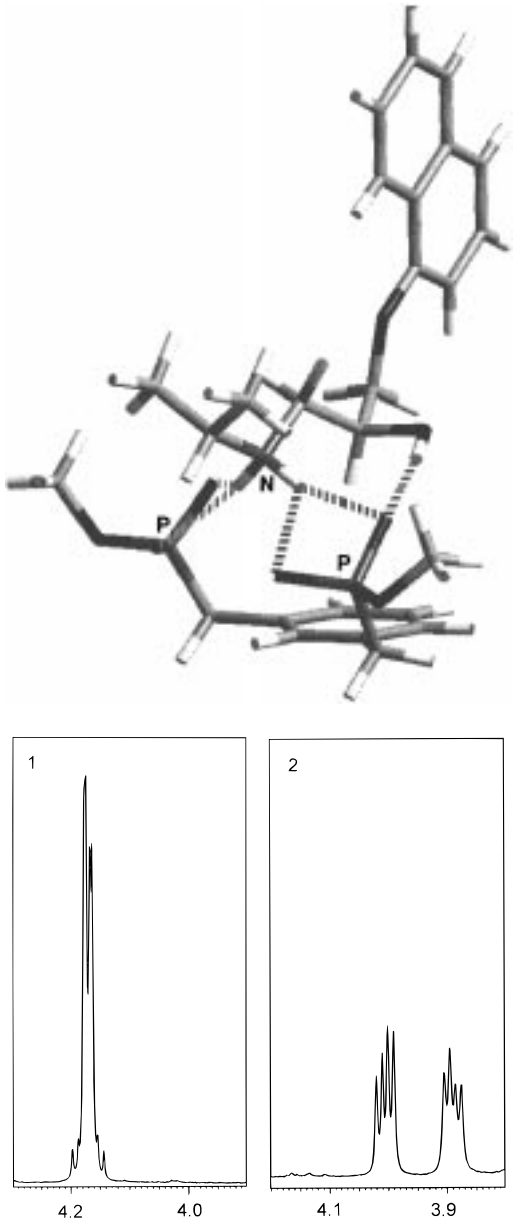


Figure 5. (Top) energy-minimized complex of (*R*)-propranolol with bisphosphonate **3**.[11] (Bottom) ^{1}H NMR signal of the diastereotopic methylene protons (OCH_2) in (*R*)-propranolol (1) before addition of **3** and (2) with 1 equiv of **3**.

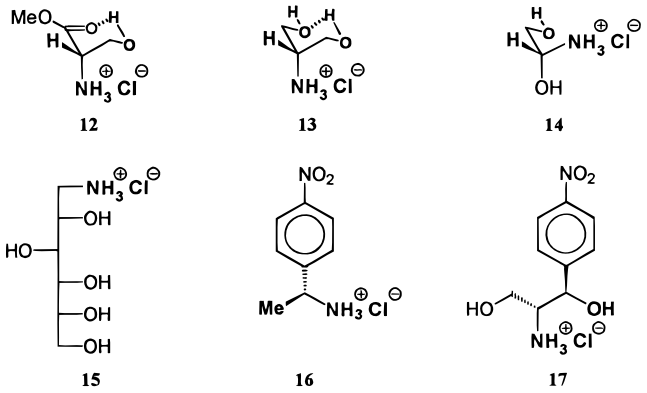
Table 4. Association Constants ($K_{1:1}$) [M^{-1}] for 1,*n*-Amino Alcohols from NMR Titrations with 3 in DMSO at 20 °C[a]

1,*n*-amino alcohol	10^3 [M^{-1}]	1,*n*,*m*-amino alcohol	10^3 [M^{-1}]
1,2-aminoethanol	73	serine methyl ester **12**	6
1,3-aminopropanol	66	serinol **13**	16
1,4-aminobutanol	21	3-amino-1,2-propanediol **14**	99
1,5-aminopentanol	27	1-aminosorbitol **15**	127

[a] Because of the strongly hygroscopic character of both titration partners the DMSO-d_6-solution contained about 0.1% of water. Errors in K_a are standard deviations; they were estimated at ≤20% for $K \leq 10^4$ M^{-1} and at ≤40% for $K \leq 10^5$ M^{-1}.

a distinct upfield shift of the corresponding OC*H* proton proves hydrogen bonding to the phosphonate anion. We conclude that due to smaller entropy losses 1,2- and 1,3-amino alcohols are much better bound by the *m*-xylylene bisphosphonate than their higher homologs. Since the association constants of 1,4- and 1,5-amino alcohols are still well above those found for simple amines (cf. Table 2), there is ample evidence for the fact that cooperative hydrogen bonds are formed even at a 1,5-distance of the binding sites. This is in full agreement with the highly selective binding of *N*-amide-protected arginine derivatives by similar bisphosphonate tweezer molecules.[17] These strongly bind to the guanidinium cation and simultaneously form a cooperative hydrogen bond to the arginine's amide-NH five atoms away from the guanidine. The additional hydrogen bond leads to an increase in the binding constant by 1 order of magnitude and demonstrates together with the above-discussed examples that high degrees of organization can be achieved in a complex by means of a single cooperative hydrogen bond to a relatively acidic XH functionality.

However, additional hydrogen bonds can also be counterproductive if they are competitive, as comparison of the amino diols and the related serine derivative shows (they represent other biologically important 1,2-amino alcohols, e.g., the amino acid derivative serine methyl ester **12** and serinol **13** as a model for sphingosine, Table 4). The 1,3-functionalities in serine methyl ester and



serinol form an internal hydrogen bond producing a six-membered ring, which has to be broken before OH binding can occur. Thus, binding to the bisphosphonate receptor remains moderate. By contrast, 3-amino-2-hydroxypropanol can form two cooperative hydrogen bonds with both OH groups, and the binding constant is even much higher than for the 1,2- or the 1,3-amino alcohol. Extension of this concept leads to the complexation of amino sugar alcohols. When 1-aminosorbitol was titrated with **3**, all OC*H* protons shifted upfield and the binding constant reached 127 000 M^{-1}. Force-field calculations suggest that all OH groups may be involved in hydrogen bonding if the aminosorbitol chain winds itself around the receptor (Figure 6).[11] It is tempting to draw parallels to nucleosomes in which the DNA is wound around histones interacting mainly with its polyphosphate anions.[18]

The above results provide ample evidence for the fact that **2** and especially **3** represent indeed highly efficient biomimetic receptor molecules for primary and secondary amines and that they are selective for 1,2- and 1,3-amino alcohols. To our surprise, very little synthetic work has been done on this field of supramolecular chemistry:

(17) Schrader, T. *Chem. Eur. J.* **1997**, *3*, 1537−1541.
(18) For the structure of nucleosomes, see: Darnell, J.; Lodish, H.; Baltimore, B. *Molecular Cell Biology*; Scientific American Books: New York, 1990.

Scheme 3. Synthesis of Aryl Bisphosphonate Receptors 18−20

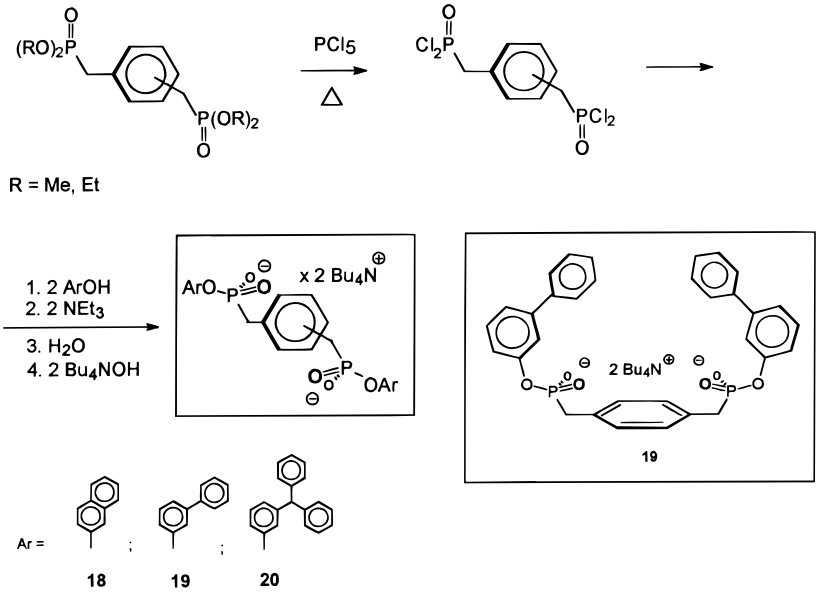


Figure 6. Energy-minimized structure of the 1-aminosorbitol/**3** complex.[11]

Reetz reported about an artificial receptor for amines *and* alcohols, based on a crown ether boronate; however, this system works only in dry nonpolar dichloromethane.[19a] Gellman achieved strong binding of amino sugars in methanolic chloroform with his neutral P=O/S=O-"bowls"; however, for solubility reasons, binding is also restricted to organic solvents.[19b] The above-discussed NMR titrations with our bisphosphonate receptor molecules have been carried out in DMSO with small amounts of water—for such a competitive solvent binding constants up to 130 000 M^{-1} are remarkably high, especially in view of the important role of hydrogen bonding. In methanol, *m*-xylylene bisphosphonate **3** binds the noradrenaline model compound **4** with a K_a of 620 M^{-1} and glucosamine with a K_a of 490 M^{-1}; even in water weak binding in observed, indicated by a 0.1 ppm upfield shift of the C*H*N proton.

(19) (a) Reetz, M. T.; Niemeyer, C. M.; Harms, K. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1474. (b) Savage, P. B.; Gellman, S. H. *J. Am. Chem. Soc.* **1993**, *115*, 10448−10449.

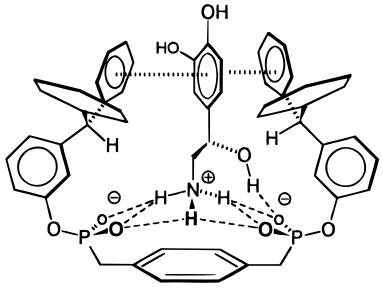
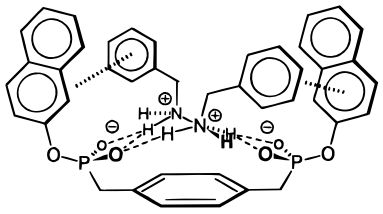
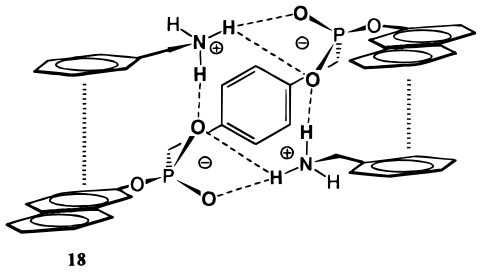


Figure 7. Energy-minimized structure of the noradrenaline/**20** complex.[11]



18



18

Figure 8. Alternating network of charges and hydrogen bonds in the 2:1 complex between benzylamine and **18**; formation of an aromatic hydrophobic cluster.

To achieve biomimetic recognition of the catechol by π-stacking interactions, we synthesized the three bisaryl phosphonates **18**−**20** (Scheme 3). The tetraalkyl esters were converted into the corresponding tetrachlorides with PCl_5 or milder $SOCl_2$ and a catalytic amount of DMF. Subsequent alcoholysis with phenol derivatives furnished the bismonoesters,[20] which were subjected to pH titration

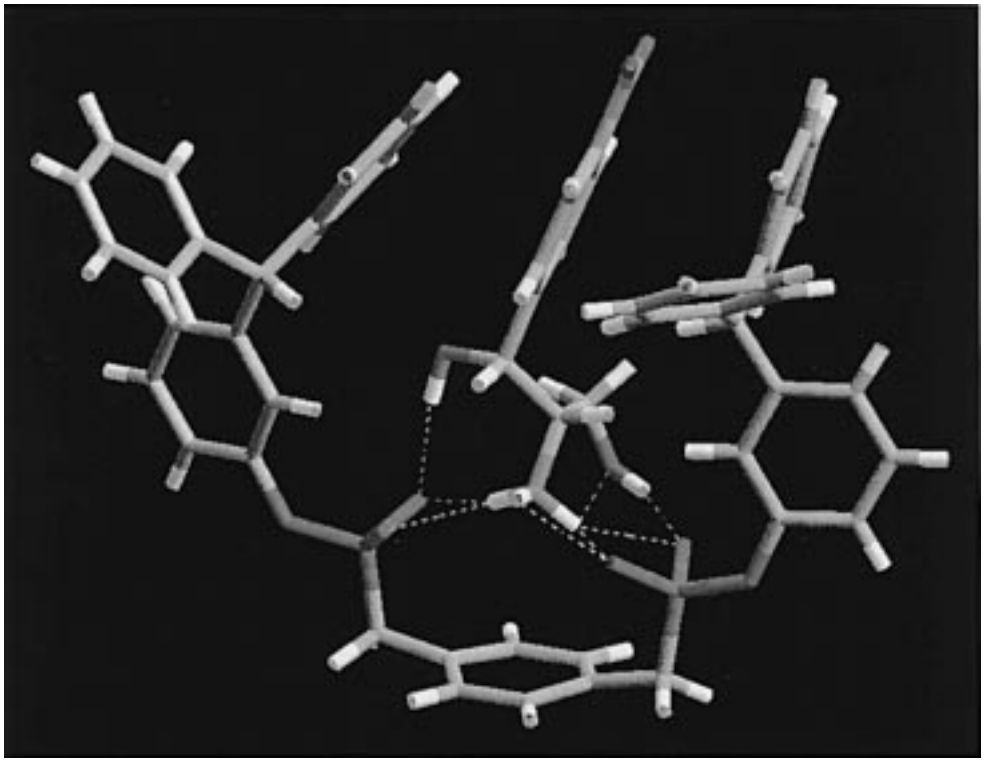


Figure 9. Computer-calculated structure of the complex between **17** and aryl bisphosphonate **20**.[11]

Table 5. Association Constants for the Aromatic Bisphosphonates ($K_{1:1}$) [M^{-1}] from NMR Titrations in DMSO at 20 °C[a]

receptor	benzylamine 10^3 [M^{-1}]	(*R*)-**16**	(±)-**4** 10^3 [M^{-1}]	(−)-**17**
18	2.0	→ 8.9	7.0	→ 10.2
19	1.1		3.4	
20	4.2		6.0	→ 18.9

[a] Because of the strongly hygroscopic character of both titration partners the DMSO-d_6-solution contained about 0.1% of water. Errors in K_a are standard deviations; they were estimated at ≤20% for $K \leq 10^4$ M^{-1} and at ≤30% for $K \geq 10^4$ M^{-1}.

to give the bis(tetrabutylammonium) salts. From molecular modeling studies, we expected that the aromatic residues should be capable of increased π-stacking in the order of **18** (β-naphthyl), **19** (*m*-biphenylyl), and **20** [*m*-(diphenylmethyl)phenyl]. With **20**, we hoped to mimic the double sandwich-type π-stacking interactions present in the natural adrenergic receptor; furthermore, we hoped to achieve complete solvent exclusion from the inner sphere of the complex by means of the two additional protecting phenyl rings (solvophobic effect, Figure 7).[21] For each receptor molecule, we checked its binding behavior toward benzylamine and 2-phenyl-2-hydroxyethylamine **4** (Table 5).

Each receptor confirmed the considerably stronger binding of amino alcohols by means of an additional hydrogen bond. However, complexes with **18** showed marked differences to those with **19** and **20**. Addition of various primary alkylammonium chlorides including the above-mentioned to a solution of the β-naphthylphosphonate **18** even in water resulted in spontaneous precipitation of the analytically pure 2:1 complex. On the basis of force-field calculations, we assume that a very stable alternating network of NH_3^+ and PO_2^- groups is formed because this array guarantees maximum electrostatic as well as hydrogen-bond interactions.[9] It may also explain the extremely low solubility of these complexes, for all charges and protons are directed inward while the aromatic rings can form a hydrophobic cluster that suppresses solvation by polar solvents (Figure 8). Highly diluted DMSO-d_6 solutions of the complexes still show a significant upfield shift of the CH_2N^+ proton of ca. 0.3 ppm, but the NMR titration with benzylamine or **4** gave only moderate K_a's (Table 5).

Primary ammonium complexes with **19** and **20** are very well soluble, but also for these hosts the binding constants remain lower than for the simple *p*-xylylene bisphosphonate. Highfield shifts are not detectable in the aromatic region of the modified receptors or of their guests. This rules out the presence of π-stacking interactions. Perhaps the low values of K_a originate from competing self-association of the extended aromatic arms of the receptor molecules. In addition, free rotation of the twisted biphenyl and trityl moieties may hinder the access of guest molecules. In general, π,π-interactions of electron-rich phenol esters with electron-rich phenyl rings are relatively weak. Therefore, it will be necessary to design phosphonate hosts with electron-poor aromatic moieties in order to achieve a maximum effect for binding of the catechol by means of a CT complex. To check the validity of this concept, we compared the association constants between **18** or **20** and benzylamine or **4** with those obtained for the related *p*-nitroaromatic compounds (*R*)-(α)-methyl-4-nitrobenzylamine **16** or D-(−)-*threo*-2-amino-1-(4-nitrophenyl)-1,3-propanediol (**17**) (Table 5). In all cases, small but distinct upfield shifts for both protons of the nitroarene were observed. Table 5 shows that the association constants for the electron poor aromatic

(20) The parent phenol for **20** was conventionally synthesized from ethyl *m*-hydroxybenzoate and phenylmagnesium bromide followed by reduction with zinc/acetic acid: Baeyer, A. *Liebigs Ann. Chem.* **1907**, *354*, 167−171.

(21) Schneider, H. J. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1419.

amino alcohols are consistently higher than those obtained for the simple phenyl-substituted compounds by a factor of 1.5−4.5 (Figure 9). From this, we conclude that π-stacking interactions between an electron-rich and an electron-poor binding partner are indeed a valuable means to substantially enlarge the affinity for an adrenaline-type guest even of an open-chain receptor molecule. We are currently incorporating the xylylene bisphosphonate moiety into macrocylic ring systems with a high degree of preorientation of all functional groups for strong, selective, *and* biomimetic adrenaline recognition.

Experimental Section

General Methods. DMSO-d_6 was purchased from Aldrich in 99.8% purity. Thin-layer chromatography (TLC) analyses were performed on silica gel 60 F-254 with a 0.2 mm layer thickness. Preparative chromatography columns were packed with Kieselgel 60 (70−230 mesh) from Macherey & Nagel. All solvents were dried and freshly disitilled before use.

^{1}H NMR Titrations. A solution of the phosphonate receptor (10 equiv in 0.4 mL of DMSO-d_6) was added in portions via microsyringe to a solution of the primary or secondary amine hydrochloride (∼1−20 mM, 1 equiv in 0.7 mL of DMSO-d_6) in a capped NMR tube. The amine solution contained ∼0.05−1% water; due to its strong hygroscopic effect the tetrabutylammonium phosphonate solution contained ∼0.3−0.6% water. Volume and concentration changes were taken into account during analysis.[14]

Job Plot. Equimolar solutions (10^{-2} M) of receptor **2** and benzylamine hydrochloride were mixed in various amounts. ^{1}H NMR spectra of the mixtures were recorded, and the chemical shifts were analyzed by Job's method modified for NMR results.[15]

Synthesis of Phosphonic Acid Mono- and Diesters by Base Hydrolysis. Benzylphosphonic acid monoethylester, *p*-xylylenediphosphonic acid dimethyl ester, and *m*-xylylenediphosphonic acid dimethyl ester were prepared by base hydrolysis of the respective di- and tetraesters with 5 N aqueous sodium hydroxide, followed by acidification with 1 N HCl.

Benzylphosphonic acid monoethyl ester: yield 96%; mp 66 °C; ^{1}H NMR (90 MHz, DMSO-d_6) δ 1.25 (t, 3H, J = 7 Hz), 3.0 (d, 2H, J = 22 Hz), 3.85 (dq, 2H, J = 7 Hz), 7.25 (m, 5 H), 9.8 (s, br, 1H); ^{31}P NMR (DMSO-d_6) δ 24.74 (s). Anal. Calcd for $C_9H_{13}O_3P$: C, 53.98; H, 6.55. Found: C, 53.82; H, 6.55.

***p*-Xylylenediphosphonic acid *P,P′*-dimethyl ester:** yield 77%; mp 209−10 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.05 (d, 4H, J = 20 Hz), 3.50 (d, 6H, J = 10.5 Hz), 7.20 (s, 4 H), 8.20 (s, br, 2H); ^{31}P NMR (DMSO-d_6) δ 26.00 (s). Anal. Calcd for $C_{10}H_{16}O_6P_2$: C, 40.81; H, 5.48. Found: C, 40.56; H, 5.44.

***m*-Xylylenediphosphonic acid *P,P′*-dimethyl ester:** yield 79%; mp 99−100 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.05 (d, 4H, J = 21.6 Hz), 3.52 (d, 6H, J = 10.8 Hz), 7.10−7.24 (m, 4 H), 9.80 (s, br, 2H); ^{31}P NMR (DMSO-d_6) δ 25.86 (s).

Synthesis of the Tetrabutylammonium Salts 1, 2, and 18−20. A typical pH-titration experiment was carried out with the above-described phosphonic acid mono- and diesters and 1.50 M tetrabutylammonium hydroxide in water. When the equivalence point was reached (normally after addition of exactly 1 or 2 molar equiv of aqueous tetrabutylammonium hydroxide), the reaction mixture was evaporated to dryness and extracted with dry chloroform. After drying over magnesiumsulfate and filtration, the solvent was removed and the receptor was further dried at 10^{-3} mbar over P_2O_5.

Tetrabutylammonium ethyl benzylphosponate (1): yield 98%; mp 48 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.92 (t, 12H, J = 7.3 Hz), 1.01 (t, 3H, J = 7.0 Hz), 1.29 (tq, 8H, J = 7.3 Hz), 1.55 (m, 8H), 2.65 (d, 2H, J = 22 Hz), 3.20 (m, 8H), 3.60 (dq, 2H, J = 7.0/7.3 Hz), 7.03 (t, 1 H, J = 7.4 Hz), 7.14 (t, 2 H, J = 7.4 Hz), 7.21 (d, 2 H, J = 7.4 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.5 (s, 4 C), 16.9 (d, 1 C), 19.2 (s, 4 C), 23.1 (s, 4 C), 36.3 (d, J = 122.9 Hz, 1 C), 57.4 (m, 4 C), 58.4 (d, J = 6.2 Hz, 1 C), 124.2 (d, J = 2.3 Hz, 1 C), 127.1 (d, J = 2.2 Hz, 2 C), 129.4 (d, J = 5.6 Hz, 2 C), 138.9 (m, 1C); ^{31}P NMR δ 14.89 (s). Anal. Calcd for $C_{25}H_{48}O_3P$: C, 67.98; H, 10.96; N, 3.17. Found: C, 67.93; H, 11.19; N, 3.40.

Bis(tetrabutylammonium)dimethyl *p*-xylylenediphosphonate (2): yield 98%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.93 (t, 24H, J = 7.3 Hz), 1.30 (tq, 16H, J = 7.3 Hz), 1.57 (m, 16H), 2.54 (d, 4H, J = 18.5 Hz), 3.18 (m, 16H), 3.22 (d, 6H, J = 9.8 Hz), 7.00 (s, 4 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.5 (s, 8C), 19.2 (s, 8C), 23.1 (s, 8C), 35.1 (d, J = 123.7 Hz, 2 C), 50.5 (m, 2 C), 57.4 (m, 8 C), 128.4 (m, 4C), 134.5 (m, 2C); ^{31}P NMR δ 16.86 (s). Anal. Calcd for $C_{42}H_{86}N_2O_6P_2\cdot 2H_2O$: C, 61.88; H, 11.27; N, 3.81. Found: C, 62.04; H, 11.16; N, 3.45.

Bis(tetrabutylammonium)bis(2-naphthyl) *p*-xylylenediphosphonate (18): yield 93%; mp 159−160 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.91 (t, 24H, J = 7.3 Hz), 1.28 (tq, 16H, J = 7.3 Hz), 1.54 (m, 16H), 2.77 (d, 4H, J = 18.7 Hz), 3.14 (m, 16H), 7.06 (s, 4 H), 7.25−7.45 (2t, 1d, 6 H), 7.60 (s, 2H), 7.7−7.8 (3d, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.7 (s, 8 C), 19.6 (s, 8 C), 23.8 (s, 8 C), 35.8 (d, J = 127.5 Hz, 2 C), 58.4 (m, 8 C), 115.8/122.5/123.7/125.6/127.2/127.4/128.6/129.5/133.9/134.4/152.1 (m, 26 C); ^{31}P NMR δ 16.65 (s). Anal. Calcd for $C_{60}H_{94}N_2O_6P_2\cdot 4H_2O$: C, 67.12; H, 9.58; N, 2.61. Found: C, 66.95; H, 9.28; N, 3.20.

Bis(tetrabutylammonium)bis(*m*-biphenylyl) *p*-xylylenediphosphonate (19): yield 92%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.92 (t, 24H, J = 7.3 Hz), 1.29 (tq, 16H, J = 7.3 Hz), 1.55 (m, 16H), 2.72 (d, 4H, J = 18.8 Hz), 3.15 (m, 16H), 7.04 (s, 4 H), 7.05−7.60 (m, 18H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.7 (s, 8 C), 19.6 (s, 8 C, 23.9 (s, 8 C), 36.04 (d, J = 127.6 Hz, 2 C), 58.4 (m, 8 C), 119.4/119.8/120.2/121.0/126.6/127.0/127.5/128.6/129.1/129.6/134.1/141.1/141.7/154.8 (m, 30 C); ^{31}P NMR δ 14.23 (s).

Bis(tetrabutylammonium)bis[*m*-(diphenylmethyl)phenyl] *p*-xylylenediphosphonate (20): yield 89%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.93 (t, 24H, J = 7.3 Hz), 1.30 (tq, 16H, J = 7.3 Hz), 1.55 (m, 16H), 2.58 (d, 4H, J = 18.6 Hz), 3.16 (m, 16H), 5.51 (s, 2H), 6.63 (d, J = 7.5 Hz), 6.79 (s, 2 H), 6.87 (s, 4 H), 7.00−7.32 (m, 24H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.7 (s, 8 C), 19.6 (s, 8 C), 23.9 (s, 8 C), 35.8 (d, J = 127.6 Hz, 2 C), 56.7 (s, 2 C), 58.5 (m, 8 C), 118.6/121.8/122.6/126.1/127.4/128.1/128.3/128.6/128.9/129.5/133.9/144.1/144.5/154.5 (m, 42 C); ^{31}P NMR δ 14.05 (s). Anal. Calcd for $C_{78}H_{110}N_2O_6P_2\cdot 4H_2O$: C, 71.73; H, 9.11; N, 2.15. Found: C, 71.68; H, 8.03; N, 1.99.

Direct Synthesis of Tetrabutylammonium Bisphosphonate 3. *m*-Xylylenediphosphonic acid tetramethyl ester was treated with 2.0 equiv of aqueous tetrabutylammonium hydroxide and heated to reflux for 1 week. After evaporation to dryness, the crude product was extracted with chloroform, dried over magnesium sulfate, filtered, and again evaporated to dryness. The receptor was further dried at 10^{-3} mbar over P_2O_5.

Bis(tetrabutylammonium)dimethyl *m*-xylylenediphosphonate 3: yield 92%; mp ∼40 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.94 (t, 24H, J = 7.3 Hz), 1.31 (tq, 16H, J = 7.3 Hz), 1.57 (m, 16H), 2.53 (d, 4H, J = 18.5 Hz), 3.18 (m, 16H), 3.21 (d, 6H, J = 9.8 Hz), 6.92−6.95 (m, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.8 (s, 8 C), 19.7 (s, 8 C), 24.1 (s, 8 C), 35.7 (d, J = 124.0 Hz, 2 C), 51.61 (m, 2 C), 58.46 (m, 8 C), 126.5/126.9/131.6 (m, 4CH), 137.7 (m, 2C); ^{31}P NMR δ 16.48 (s). Anal. Calcd for $C_{42}H_{86}N_2O_6P_2\cdot 3H_2O$: C, 60.69; H, 11.16; N, 3.37. Found: C, 60.54; H, 11.33; N, 3.35.

Synthesis of the Diphosphonic Acid Tetrachlorides. In a dry argon atmosphere the tetraesters were mixed with 2 equiv of PCl_5 or with 3−4 equiv of $SOCl_2$ and a catalytic amount of DMF. After the mixture was heated to 140 °C for 2 h, $POCl_3$ or remaining $SOCl_2$ was distilled off, and the crude product was recrystallized twice from toluene.

***p*-Xylylenediphosphonic acid tetrachloride:** yield 55%; mp 171−173 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 3.95 (d, 2H, J = 15 Hz), 7.35 (m, 4H); ^{31}P NMR($CDCl_3$) δ 44.85 (s).

***m*-Xylylenediphosphonic acid tetrachloride:** yield 65%; mp 148−149 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 3.90 (d, 2H, J =

15 Hz), 7.30 (m, 4H); ^{31}P NMR ($CDCl_3$) δ 44.50 (s). Anal. Calcd for $C_8H_8O_2P_2Cl_4$: C, 28.27; H, 2.37. Found: C, 28.28; H, 2.27.

Synthesis of the *p*-Xylylenediphosphonic Acid Diaryl Esters. Under argon, *p*-xylylenediphosphonic acid tetrachloride was dissolved in dry tetrahydrofuran, and 2 equiv of the phenol was added, followed at 0 °C by 2 equiv of triethylamine. The reaction mixture was stirred for 30 min at 0 °C, warmed to room temperature, stirred for another 2 h, and filtered. Water and hexane were then added, and the resulting two-layer system was stirred vigorously overnight. The precipitated product was filtered off, washed, and recrystallized from methanol.

***p*-Xylylenediphosphonic acid *P,P′*-bis(2-naphthyl ester):** yield 41%; mp 279 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6) insoluble. Anal. Calcd for $C_{28}H_{24}O_6P_2$: C, 64.87; H, 4.67. Found: C, 62.64; H, 4.48.

***p*-Xylylenediphosphonic acid *P,P′*-bis(*m*-biphenylyl ester):** yield 36%; mp 205−6 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.28 (d, 4H, J = 20.1 Hz), 7.10−7.64 (m, 20H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 33.4 (d, J = 135.6 Hz), 118.6, 119.4 (s, 4 C), 120.8 (d, 2 C), 122.4, 126.6, 127.7, 128.9, 129.7, 130.0, 139.2, 141.7, (s, 24 C), 151.3 (m, 2 C); ^{31}P NMR ($CDCl_3$) δ 22.19 (s). Anal. Calcd for $C_{32}H_{28}O_6P_2$: C, 67.35; H, 4.95. Found: C, 67.30; H, 5.06.

***p*-Xylylenediphosphonic acid *P,P′*-bis[*m*-(diphenylmethyl)phenyl ester]:** yield 64%; mp 164 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.91 (d, 4H, J = 19.7 Hz), 5.53 (s, 2H), 6.70−7.40 (m, 32H), 7.97 (s, br, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 32.7 (d, J = 138.0 Hz, 2 C), 56.48 (s, 1C), 116.5, 121.6, 126.0, 126.4, 128.4, 129.4, 129.6, 130.2, 143.3, 145.9 (s, 42 C), 150.2 (m, 2 C); ^{31}P NMR ($CDCl_3$) δ 24.72 (s). Anal. Calcd for $C_{46}H_{40}O_6P_2$: C, 73.59; H, 5.37. Found: C, 73.49; H, 5.13.

Isolated 2:1 complex between 18 and benzylammonium chloride: Bis(benzylammonium)bis(2-naphthyl) *p*-xylylenediphosphonate: yield 95%; mp 195−200 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6): 2.83 (d, 4H, J = 18.5 Hz), 3.83 (s, 4H), 7.06 (s, 4 H), 7.30−7.50 (m, 16 H), 7.65 (d, J = 1.7 Hz, 2H), 7.75−7.85 (m, 6H), 8.20 (s, br, 6H); ^{31}P NMR (CD_3OD) δ 19.71 (s). Anal. Calcd for $C_{42}H_{42}N_2O_6P_2$: C, 68.83; H, 5.78; N, 3.82. Found: C, 68.69; H, 5.65; N, 3.62.

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