Towards Synthetic Adrenaline Receptors Part III[+]

Towards Synthetic Adrenaline Receptors

Michael Herm, and Thomas Schrader*[a]

Dedicated to Prof. Dr. Wolfgang Steglich on occasion of his 65th birthday

Abstract: The macrocyclic bisphosphonate **2** forms complexes with amino alcohols, amines, and amino acid esters with high association constants in polar organic solvents. Exertion of solvophobic interactions inside the macrocyclic cavity in DMSO and methanol leads to specificity for guest molecules with hydrophobic moieties. Experimental evidence is presented for the insertion of the guest molecules' nonpolar groups into the macrocycle's hydrophobic cavity. NMR spectra of complexes with **2** in DMSO show a molecular imprint of the guest molecule; this gives information about its location inside the macrocycle. In aqueous solutions strong self-association of **2** occurs, which is explained by distinct structural similarities between **2** and micelle-forming phospholipids.

Keywords: bioorganic chemistry · catecholamines · hormones · host-guest chemistry · NMR titrations

Introduction

Inspite of worldwide intense research efforts and considerable recent progress in the X-ray crystallographic characterization of membrane-bound proteins,[1] the structure and mechanism of action of the natural adrenergic receptors are not yet completely elucidated.[2] For this and many other reasons a synthetic model could be helpful to understand nature. A small, microscopically well-defined model compound that imitates the natural receptor–ligand interactions would allow the systematic study of the interplay of various noncovalent interactions of the receptor with its substrate adrenaline. One obvious benefit of these studies would be the gain of knowledge for a rational design of synthetic receptor molecules for neurotransmitters with optimal binding strength and selectivity.

In our biomimetic approach we recently presented xylylene bisphosphonates as the first host molecules that are selective for the 1,2-amino alcohol functionality.[3] Like these, most other synthetic receptors for catecholamines are monotopic.[4] Binding of the amino group has been performed with crown ethers,[4a, b] ester crowns,[4d] and a cyclopeptide,[4e] but only a zinc porphyrin tweezer[4f] and our bisphosphonates[3] bind specifically to amino alcohols. The crown ether approach even has practical applications in capillary zone electrophoresis.[5] Receptors for the catechol ring contain either an aza crown[4b] or a bipyridinium moiety.[4c, g]

Simultaneous interaction with more than one part of the molecule leads to stronger binding and greater specificity.[6] In ditopic receptors the ammonium moiety is usually bound by a crown ether or aza crown ether group,[6b] which are in turn linked to a protonated aza crown,[6a] a macrotricyclic cavity,[6b] quinone,[6c] or a boronic acid[6d,e] for molecular recognition of the catechol ring by hydrogen-bond, hydrophobic, $\pi-\pi$, or even covalent interactions. A different approach has been taken by Inoue et al.,[6f] they have prepared a cyclophane host with a nonpolar cavity for the insertion of the catechol ring and peripheral carboxylate groups for molecular recognition of the ammonium moiety of dopamine. However, all these receptors are not specific for catechol amino alcohols.

In order to develop our amino alcohol recognition motif into an efficient catechol receptor we converted simple bisphosphonates into their respective bisaryl esters, which could, in principle, be involved in hydrophobic or even $\pi-\pi$ interactions[7] with the catechol ring.[3b] Unfortunately the association constants could not be increased, probably because the open chain receptors have too many rotatory degrees of freedom.[3b] Only an amino alcohol guest bearing an electron-deficient nitro arene could be bound moderately

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well. Our new approach is based on a preorganized host that retains both phosphonate groups for selective binding of the amino alcohol moiety, but also contains an additional hydrophobic binding pocket for the catechol ring. This structure should provide a binding mode much closer to the natural example than any former host molecule.

In the natural receptor, the catechol moiety lies in a deep cleft between two phenylalanine residues (Figure 1).[2] Here, hydrophobic interactions should play a prominent role, whereas $\pi-\pi$ interactions are probably weak because of low dipole–dipole attraction between the electron-rich phenylalanine arenes and the very electron-rich catechol ring. The simplest model for such a three-dimensional binding pocket is a macrocycle; in its design optimal preorganization of the binding site should be considered.

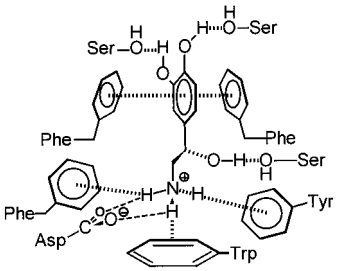

Figure 1. Assumed binding mode of noradrenaline in the β-adrenergic receptors.

Results and Discussion

In recent years many macrocyclic structures have been designed that can bind aromatic molecules, for example, benzene or toluene.[8] We have now converted such a monotopic host molecule to a ditopic receptor by combining the hydrophobic binding pocket with phosphonate groups for biomimetic binding of adrenaline.

The phosphonate groups should not be attached directly to the macrocyclic ring in order to maintain enough flexibility for an induced fit. The schematic structure in Figure 2 shows preorientation of the binding groups as well as a hydrophobic cavity, into which the catechol ring of the adrenaline molecule can be inserted. All these structural features have been incorporated into the bisphosphonate host molecule **2** (Figure 3); its macrocycle is equipped with sterically rigid building blocks to prevent internal collapse.

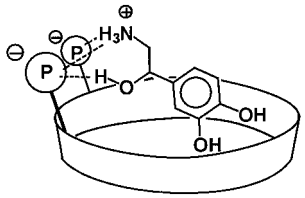

Figure 2. Schematic structure of a ditopic adrenaline receptor model; the amino alcohol moiety of noradrenaline is recognized by a peripheral bisphosphonate binding site, while the catechol ring lies in a macrocyclic hydrophobic cavity.

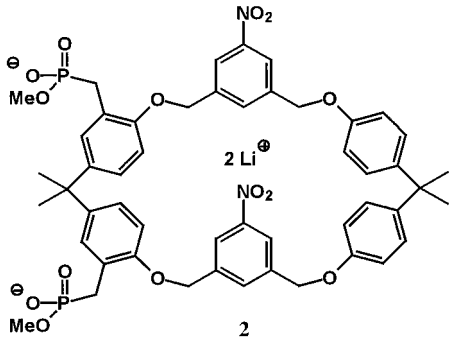

Figure 3. Macrocyclic bisphosphonate host molecule **2**.

Force-field calculations[9] for the complex formation between **2** and noradrenaline (**1**) suggest the energy-minimized structure shown in Figure 4. An arrangement of the bisphosphonate/amino alcohol chelate is produced that is very similar to the open-chain receptor complexes.[3] The catechol ring penetrates deeply into the macrocyclic cavity, but it does not lie parallel to a host arene in the cavity, so $\pi-\pi$ interactions seem improbable. However, the cavity is strongly hydrophobic. The macrocycle's conformation shown here is one of several calculated conformations with similar energies; all of them provide an open cavity for guest binding.

The preparation of **2** is depicted in Scheme 1. Care has been taken to develop a highly convergent and modular synthetic route, that also opens the path to many other related macrocycles. Phosphorus-modified bisphenol **7** can be prepared from *o*-cresol in a five-step synthesis. Bisphenol A and two equivalents of 3,5-bis(bromomethyl)nitrobenzene give the U-shaped precursor molecule **9**. The cyclization between **7** and **9** is then performed under high dilution conditions with benzene as a template, following previous work by Saigo et al.[8d,e] The resulting tetramethylbisphosphonate ester **10** is finally hydrolyzed smoothly with lithium bromide in boiling acetonitrile to give the target molecule **2**. An impurity of excess lithium bromide in the hydrolyzed product can be eliminated almost completely by multiple dialysis (2–3 times).

The resulting lithium salt **2** was used for the following NMR-titrations in different solvents, ranging from DMSO

Abstract in German: *Das macrocyclische Bisphosphonat **2** bildet Komplexe mit Aminoalkoholen, Aminen und Aminosäureestern, die sich durch hohe Assoziationskonstanten in polaren organischen Lösungsmitteln auszeichnen. Durch solvophobe Wechselwirkungen im Innern des macrocyclischen Hohlraums bindet der Wirt in DMSO und Methanol bevorzugt Gäste mit hydrophoben Gruppen. Experimentelle Belege für die Einlagerung der unpolaren Gruppen der Gastmoleküle in die hydrophobe Cavität des Macrocyclus werden präsentiert. NMR-Spektren von Komplexen mit **2** in DMSO zeigen einen molekularen Abdruck des Gastmoleküls, der Aufschluß über seine räumliche Lage im Macrocyclus gibt. In wäßriger Lösung tritt eine starke Selbstassoziation von **2** auf, die durch seine deutliche strukturelle Ähnlichkeit mit micellenbildenden Phospholipiden erklärt wird.*

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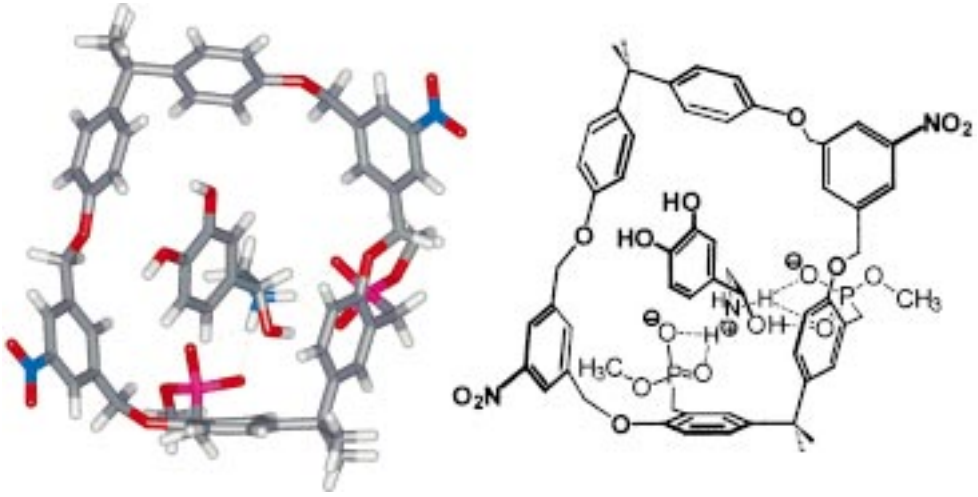
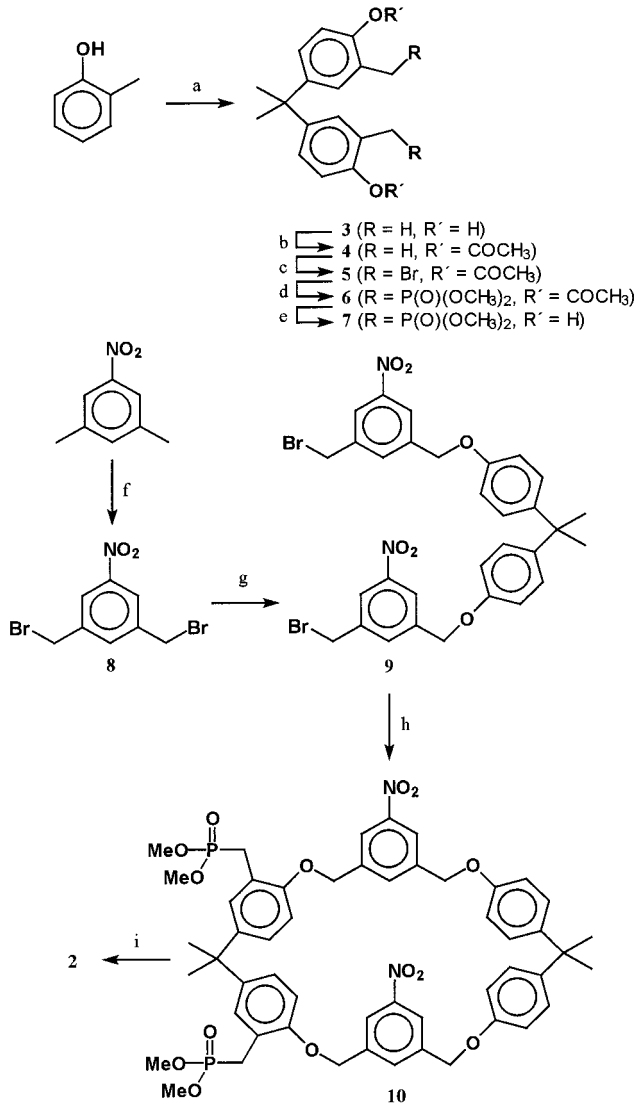
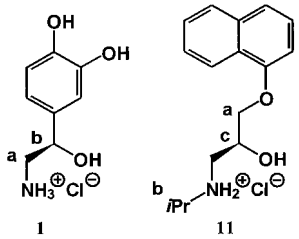


Figure 4. Left: Energy-minimized structure of the complex between **2** and noradrenaline. Right: Lewis-structure of the calculated complex.



Scheme 1. Synthesis of **2**. a) acetone, MeOH, HCl, RT; b) acetic anhydride, pyridine, RT; c) NBS, CCl_4, benzoyl peroxide, 80 °C; d) $P(OMe)_3$, 140 °C; e) K_2CO_3, MeOH, RT; f) NBS, CH_2Cl_2, *hv*, 40 °C; g) bisphenol A, KOtBu, EtOH/THF, 80 °C; h) **7**, NaOMe, MeOH/THF, 50 °C; i) LiBr, acetonitrile, 80 °C.

over methanol to water.[10] Initially, complexation experiments were performed in [D_6]DMSO so that their results could be compared with those obtained before with the open-chain receptor molecules.[3] The resulting binding curves were analyzed by nonlinear regression methods and the binding constants were calculated as described earlier.[10] Scheme 2 shows the structures of representative target molecules, namely the agonist noradrenaline **1** and the β-blocker propranolol **11**, and their calculated association constants with **2** are presented in Table 1.



Scheme 2. Titration partners of **2** in DMSO.

Table 1. Association constants of **2** with noradrenaline (**1**) and propranolol (**11**) in DMSO.

	Proton a[a]		Proton b[a]		Proton c[a]	
	K_a [M^{-1}]	$\Delta\delta_{sat}$ [ppm]	K_a [M^{-1}]	$\Delta\delta_{sat}$ [ppm]	K_a [M^{-1}]	$\Delta\delta_{sat}$ [ppm]
1	19300	0.32	11100	0.17	–	–
11	22500	0.12	36000	0.15	9100	0.47

[a] Due to the strongly hygroscopic character of both titration partners the [D_6]DMSO-solution contained ~0.1% of water. Errors are standard deviations; they were estimated at ±10–20% for $10^4 M^{-1} \le K_a \le 10^5 M^{-1}$ and at ±3–10% for $\Delta\delta_{sat}$ values.

All the open-chain xylylene bisphosphonates tested previously showed a drastic difference in their association constants for these two guest molecules, because additional hydrogen-bond interactions of the catechol hydroxy groups of adrenaline and noradrenaline with the phosphonate moieties interfered with the established amino alcohol/phosphonate binding scheme. Thus, in DMSO the association constant produced for adrenaline was always five times lower than that of propranolol without aryl hydroxy groups.[3] Campayo et al. have presented calculations for crown-ether-type receptors with similar results.[4d] However, this is not the case with **2**: Both molecules are bound equally well. The elimination of this competing catechol–phosphonate interaction strongly indicates that in complexes of **2** with catecholamines, the catechol hydroxy groups are shielded because the arene has been inserted into the macrocycle's cavity.

Further examination of the NMR spectra during the host–guest titrations revealed an unexpected effect, which offers

additional structural information about the complex geometry: With increasing guest concentration distinct host arene signals show strong line broadening with a remarkable correlation; the bulkier the guest molecule, the more signals of the host are affected. On complex formation with small 2-aminoethanol, only the proton signals of the phosphonate carrying ring A become broad (Figure 5); if noradrenaline with its catechol residue is the guest molecule, an aromatic proton signal of ring B also broadens. Finally, propranolol, which has a longer carbon chain, an additional ether moiety and a larger arene system than noradrenaline, induces line broadening of proton signals in all rings A–C of the host molecule.

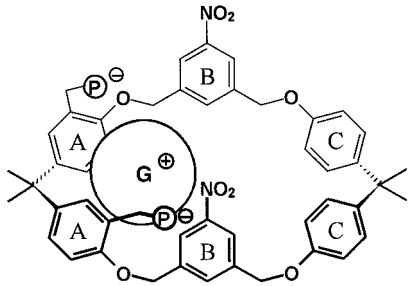


Figure 5. Schematic drawing of complexes of macrocycle **2** with simplified guest molecules **G**: the molecular imprint effect. The arene code refers to considerable line broadening of respective host signals in the ^{1}H NMR spectra of the complexes.

This is a dynamic effect, as evidenced by measurements at different NMR frequencies and temperatures. It can be explained by restricted rotation around certain single bonds that determine the cyclophane's conformation (C_{Ar}–O, C_{Bn}–O, C_{Ar}–C_{Bn}) with the host molecule penetrating into the macrocyclic cavity. Whitlock and others have observed similar effects with their arene binding hosts.[8] Evidently NMR spectra of complexes with **2** in DMSO reveal molecular imprints of the guest molecules and give information about the guests' steric demand as well as its exact location inside the cavity. In a variable temperature NMR experiment (Figure 6) with a 2:1 mixture of propranolol (**11**) and **2** the broad signals become sharp at temperatures above 300 K. The signals of protons that are further away from the polar binding site (rings B and C, benzyl, isopropylidene, and CH_2P groups) appear sharp at 320 K, whereas those of ring A close to the polar binding site become sharp at 340 K. This can also be observed with a few guest molecules' signals; the CH_2O protons of propranolol appear sharp at 320 K, those of the isopropyl group, which is attached to the positively charged nitrogen atom, become sharp only at 340 K. Unfortunately, the coalescence temperature could not be determined because no measurements can be carried out below room temperature in DMSO; however, it can be considered to be close to room temperature because of the strong signal broadening at 300 K.

If the solvent is changed from DMSO to the much more polar methanol, electrostatic and hydrogen-bond interactions are greatly weakened by solvation; in the case of complexes with **2**, however, this weakening should be compensated, at least in part, by stronger solvophobic effects. We know from experiments with the open-chain xylylene bisphosphonates, and many other related examples from the literature, that the binding constants for amino alcohols decrease by two orders of magnitude upon changing the solvent from DMSO to methanol in the absence of hydrophobic interactions. By contrast, in complexes with macrocycle **2** they decrease only 20-fold if the guests carry lipophilic groups capable of participating in hydrophobic interactions as is the case with noradrenaline (**1**, see Table 2). The association constants in Table 2 represent the highest K_a values for substituted amines ever obtained with a bisphosphonate host.

Table 2. Association constants of **2** with various guest molecules in pure methanol.[10]

	Proton a[a]		Proton b[a]		Proton c[a]	
	K_a [M^{-1}]	$\Delta\delta_{sat}$ [ppm]	K_a [M^{-1}]	$\Delta\delta_{sat}$ [ppm]	K_a [M^{-1}]	$\Delta\delta_{sat}$ [ppm]
1	1250	0.12	1000	0.07	1020	0.08
11	1250	0.17	530	0.09	1210	0.12
12	1030	0.17	920	0.16	1080	0.07
2-aminoethanol	560	0.09	570	0.06	–	–
13	960	0.17	940	0.12	930	0.12
14	1230	0.14	1110	0.09	1010	0.14
15	2020	0.04	1490	0.05	1787	0.06
Tyr-OMe · HCl	1660	0.07	2420	0.15	1450	0.05
Trp-OMe · HCl	1970	0.07	2020	0.20	1170	0.12
Ala-OMe · HCl	1080	0.05	1300	0.13	1230	0.06

[a] Due to the strongly hygroscopic character of both titration partners the [D_4]methanol-solution contained $\sim$0.1% of water. Errors are standard deviations; they were estimated at $\pm$5–10% for 10^3 M$^{-1} \le K_a \le 10^4$ M^{-1} and at $\pm$3–10% for $\Delta\delta_{sat}$ values.

Propranolol (**11**) binds to the host as well as adrenaline (**12**) and noradrenaline (**1**) despite their additional phenolic OH groups; this corresponds well with the results obtained in DMSO. On the other hand, 2-aminoethanol, which does not contain a bulky substituent and thus cannot exert hydrophobic interactions, is bound much more weakly. Surprisingly, the simple amines **13** and **14** (Scheme 3) are bound with almost the same association constants as adrenaline. The selectivity of the bisphosphonate binding site between amino alcohols and amines[3a] is evidently weakened in the highly competitive methanol.

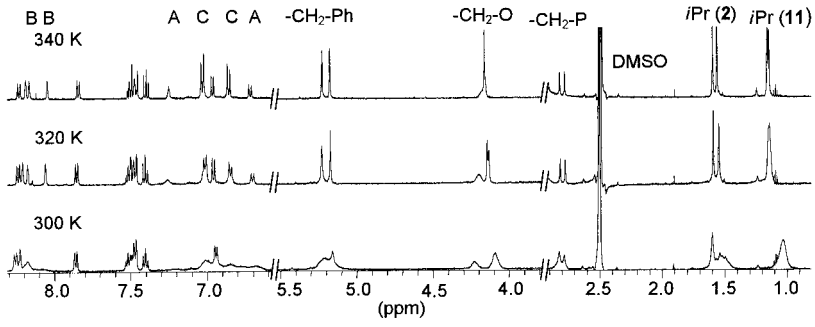


Figure 6. Proton NMR spectra of a 2:1-mixture of **2** and **11** at 300 K, 320 K, and 340 K. A indicates protons of ring A, B those of ring B and C the corresponding ring C (for nomenclature of the rings see Figure 5).

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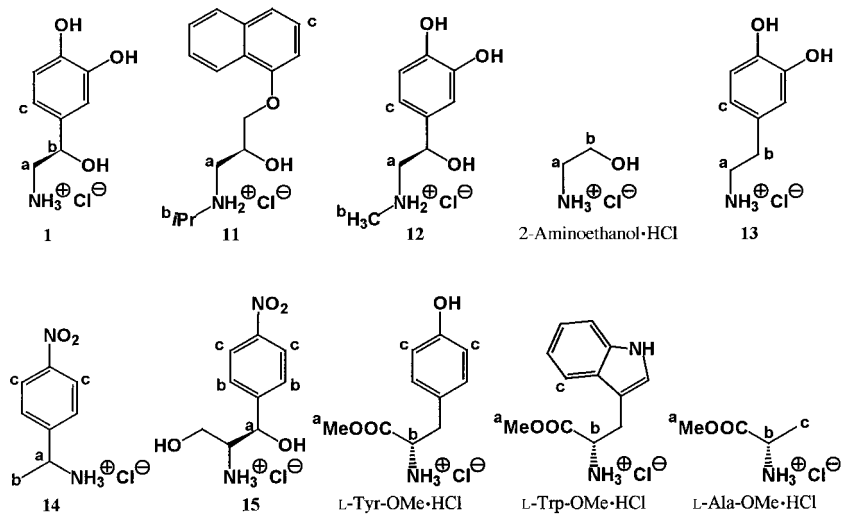

Scheme 3. Titration partners of **2** in methanol.[10]

Enhanced binding affinity for substrates with hydrophobic residues was also found with ANP (**15**), a chloroamphenicol precursor molecule, and the aromatic amino acids tyrosine and tryptophane, with large π faces as opposed to alanine (all amino acids were used as their methyl esters). The assumed 1:1-stoichiometry of the noradrenaline–**2** complex could be confirmed in methanol by Job's method of continuous variations (Figure 7).[11]

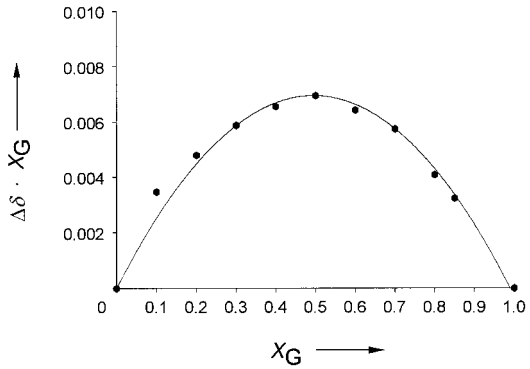

Figure 7. Job plot of noradrenaline with **2** in methanol.

The three-dimensional structure of the complex with noradrenaline, which is shown in Figure 4, could be supported by a two-dimensional NOESY ^{1}H NMR experiment (Figure 8). No intramolecular NOEs were observed that would indicate an internal collapse of the quite flexible macrocycle. Only weak interactions could be measured between the CH_2P protons and the aromatic host proton 3. This conformational preference corresponds well with a chelate-type complexation of the ammonium moieties by the phosphonate groups. In the guest molecule a strong Overhauser effect of the CH_2N protons to proton 2 of the catechol group and a weak one to proton 6 confirm the shown conformation of the guest molecule. We could observe intermolecular NOEs between the CH_2N group of the guest and the host's CH_2P and OCH_3 moieties; this indicates the close proximity of the binding partners' polar groups. An additional NOE between proton 6 of ring A and the guest's proton 5 shows that the hydrophobic catechol group is positioned on the macrocycle's "upper" side opposite to the polar binding group (see Figure 8). All these NOEs from different sides of the macrocycle to distinct moieties of the guest clearly show that the guest molecule is inserted into the cavity.

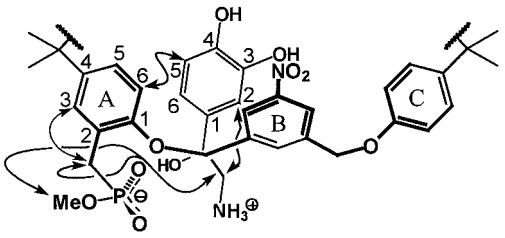

Figure 8. Illustration of intramolecular and intermolecular NOESY peaks in the spectrum of the complex between noradrenaline and **2**. Only the NOEs discussed in the text are shown (thin line: weak NOE).

To take the next step towards binding in physiological solution, we carried out titrations in a 1:1-mixture of methanol and water. With noradrenaline as guest molecule, the fitting of the titration curve resulted in a high association constant ($\approx 1500\,\text{M}^{-1}$), but the standard deviation was unacceptably high. Furthermore, NMR signals of the pure host shifted strongly when measured at different concentrations. This points to self-association in highly polar solvents; in dilution experiments we obtained a self-association constant of $270\,\text{L mol}^{-1}$ for methanol/water 1:1. The self-association of cyclophane **2** is even stronger in pure water: a titration with noradrenaline resulted in an even higher association constant ($\approx 3000\,\text{M}^{-1}$), but with an extraordinarily high standard deviation of 200% and very strong signal broadening of all protons in **2**. Identical results have been obtained in a phosphate-buffer solution, which maintained a pH of 7. A dilution experiment of the host molecule in pure water gave sharp signals only at concentrations below $10^{-4}\,\text{M}$, so a self-association constant could not be calculated from the spectra, but very strong self-association of **2** can be assumed (Figure 9, left).[12] Careful comparison of the macrocycle's structure with that of micelle-forming phospholipids reveals distinct structural similarities: both molecules are amphiphilic, carrying a short polar phosph(on)ate head group and long nonpolar tails (in the case of **2** connected to a ring, see Figure 9).

Because solutions of **2** also show a tyndall effect and foam on shaking, we assumed that the host molecule **2** forms micellar structures in aqueous solution; proton NMR signal broadening observed in our case has also been observed with micelles in which slow molecular motion occurs.[13] Therefore we intend to incorporate the molecule into membranes, for example, in artificial liposomes.[14] Such a membrane immo-

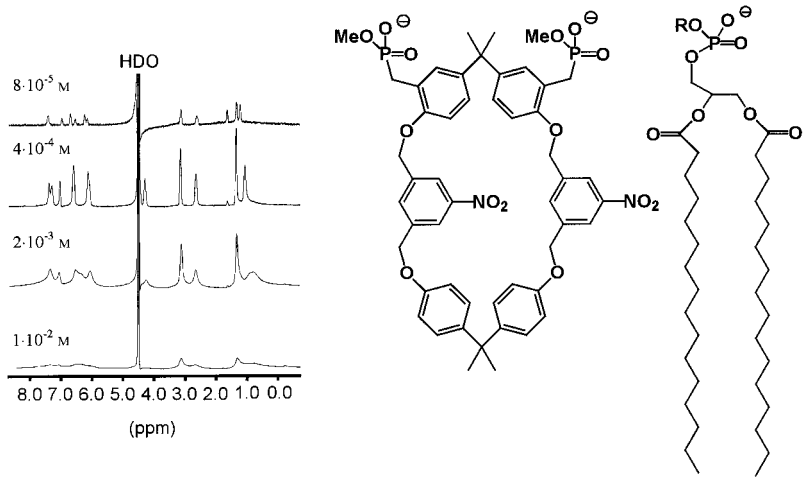

Figure 9. Left: ^{1}H NMR spectra (500 MHz) of pure **2** in D_2O at different concentrations. Right: Structural similarity between the macrocyclic bisphosphonate (**2**, left) and phospholipids (right).

bilization would imitate the chemical surroundings in the natural receptor and could be the first step towards imitation of the natural signal-transduction process.

In summary, we have designed a new host molecule for substrates of the adrenergic receptor; this molecule has a flexible bisphosphonate as chelating unit for the amino alcohol part and an electron-poor nonpolar macrocyclic cavity for the catechol ring. This approach imitates the electrostatic and hydrogen-bond interactions in the natural receptor as well as the deep aromatic cleft, which receives the catechol moiety. The suggested geometry of the complex from molecular dynamics calculations was confirmed by experiments in DMSO and methanol. In DMSO a molecular imprint of the guest molecule is obtained; this indicates that rotation of one or more aromatic rings of the host is hindered. In methanol the highest binding constants for amino alcohols ever measured with a bisphosphonate have been found; removal of the nonpolar substituent, as in 2-aminoethanol, causes a marked drop in binding energy. Strong self-association of the new host molecule in water along with its structural similarity to phospholipids suggests that it can be incorporated into membranes to imitate the chemical environment of the natural receptor.

Experimental Section

2,2-Bis(4′-acetoxy-3′-dimethoxyphosphorylmethylphenyl)propane (6): 2,2-Bis(4′-acetoxy-3′-methylphenyl)propane[15] (**4**, 8.50 g, 25.0 mmol) was dissolved in tetrachloromethane (25 mL) and refluxed with *N*-bromosuccinimide (NBS, 8.98 g, 50.4 mmol) and catalytic amounts of benzoyl peroxide until all NBS had been consumed (ca. 4 h). Filtration and evaporation of the solvent gave the oily raw product of the bromination (**5**, 11.5 g), which was refluxed with trimethyl phosphite (13.6 mL, 115.4 mmol) for 3 hours without any further purification. After removal of excess trimethyl phosphite and dimethyl methylphosphonate by distillation, an oily product was obtained, which was purified by column chromatography (silica gel 60) with acetone as eluent ($R_f = 0.30$, 6.25 g, 11.2 mmol, 45 %). ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): $\delta = 1.65$ (s, 6H; CH_3), 2.30 (s, 6H; CH_3), 3.08 (d, $^2J(H,P) = 21.8$ Hz, 4H; CH_2), 3.57 (d, $^3J(H,P) = 10.8$ Hz, 12H; CH_3), 7.00 (d, $^3J(H,H) = 8.5$ Hz, 2H; CH), 7.12 (ddd, $^3J(H,H) = 8.5$ Hz, $^4J(H,H) = 2.3$ Hz, $^6J(H,P) = 2.3$ Hz, 2H; CH), 7.18 (dd, $^3J(H,H) = 2.3$ Hz, $^4J(H,P) = 2.3$ Hz, 2H; CH); ^{13}C NMR (126 MHz, $CDCl_3$, 25 °C): $\delta = 21.0$ (s), 27.3 (d, $^1J(C,P) = 140.5$ Hz), 30.6 (s), 42.3 (s), 52.8 (d, $^2J(C,P) = 7.3$ Hz), 122.2 (d, $^4J(C,P) = 2.4$ Hz), 122.9 (d, $^2J(C,P) = 9.7$ Hz), 126.4 (d, $^5J(C,P) = 3.6$ Hz), 129.9 (d, $^3J(C,P) = 6.1$ Hz), 146.7 (d, $^3J(C,P) = 7.3$ Hz), 147.7 (d, $^4J(C,P) = 3.6$ Hz), 169.0 (s); ^{31}P NMR (202 MHz, $CDCl_3$, 25 °C): $\delta = 29.0$ (s); $C_{25}H_{34}O_{10}P_2$ (556.5): calcd C 53.96, H 6.16; found C 53.85, H 6.20.

2,2-Bis(4′-hydroxy-3′-dimethoxyphosphorylmethylphenyl)propane (7): A solution of **6** (6.0 g, 10.8 mmol) in methanol (120 mL) was added to a solution of potassium carbonate (2.98 g, 21.6 mmol) in methanol (500 mL). The mixture was stirred at room temperature for 2 h, then HCl (1N, 43.1 mL) was added. The product was extracted with dichloromethane, the solution was dried with magnesium sulfate, and the solvent removed in vacuo. A white solid was obtained, which was sensitive to light (4.4 g, 9.3 mmol, 86 %). M.p. 180–181 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): $\delta = 1.60$ (s, 6H; CH_3), 3.18 (d, $^2J(H,P) = 20.8$ Hz, 4H; CH_2), 3.61 (d, $^3J(H,P) = 10.7$ Hz, 12H; CH_3), 6.77 (d, $^2J(H,H) = 8.8$ Hz, 2H; CH), 6.94 (ddd, $^2J(H,H) = 8.8$ Hz, $^4J(H,H) = 2.3$ Hz, $^6J(H,P) = 2.3$ Hz, 2H; CH), 7.10 (dd, $^4J(H,H) = 2.3$ Hz, $^4J(H,P) = 2.3$ Hz; CH), 9.40 (s, 2H; OH); ^{13}C NMR (126 MHz, [D$_6$]DMSO, 25 °C): $\delta = 25.1$ (d, $^1J(C,P) = 136.8$ Hz), 31.0 (s), 41.1 (s), 52.5 (d, $^2J(C,P) = 6.0$ Hz), 114.7 (s), 117.2 (d, $^2J(C,P) = 8.5$ Hz), 125.9 (s), 129.3 (d, $^3J(C,P) = 4.9$ Hz), 141.1 (s), 152.9 (d, $^3J(C,P) = 6.1$ Hz); ^{31}P NMR (202 MHz, [D$_6$]DMSO, 25 °C): $\delta = 30.7$ (s); $C_{21}H_{30}O_8P_2$ (472.4): calcd C 53.39, H 6.40; found C 53.16, H 6.35.

2,2-Bis[4′-(3″-nitro-5″-bromomethylbenzyl)oxyphenyl]propane (9): 3,5-Bis(bromomethyl)nitrobenzene[16] (**8**, 10.00 g, 32.37 mmol) was refluxed in THF (35 mL). Over one hour a solution of 2,2-bis(4′-hydroxyphenyl)-propane (1.23 g, 5.39 mmol) and potassium *tert*-butylate (1.21 g, 10.79 mmol) in ethanol (95 mL) was added; then the mixture was refluxed for 3 hours. After evaporation of the solvent, the residue was treated with a mixture of dichloromethane and water, and the organic layer was separated, dried with sodium sulfate, and the solvent was removed in vacuo. Excess bis(bromomethyl)nitrobenzene was obtained in pure form by column chromatography (silica gel 60) with a 2:1 mixture of dichloromethane and *n*-hexane as eluent, and the solid product was isolated by column chromatography with a 5:1 mixture of dichloromethane and *n*-hexane as eluent ($R_f = 0.45$, 1.65 g, 2.41 mmol, 45 %). M.p. 117–119 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): $\delta = 1.66$ (s, 6H; CH_3), 4.54 (s, 4H; CH_2), 5.12 (s, 4H, CH_2), 6.88 (d, $^3J(H,H) = 8.8$ Hz, 4H; CH), 7.18 (d, $^3J(H,H) = 8.8$ Hz, 4H; CH), 7.79 (s, 2H; CH), 8.21 (s, 2H; CH), 8.23 (s, 2H; CH); ^{13}C NMR (126 MHz, [D$_6$]DMSO, 25 °C): $\delta = 31.0$, 41.8, 68.4, 114.2, 121.9, 123.2, 127.9, 133.4, 140.0, 140.2, 144.0, 148.6, 155.9; $C_{31}H_{28}N_2O_6Br_2$: C 54.41, H 4.12, N 4.09; found C 54.16, H 4.04, N 3.92.

Macrocycle 10: Compund **9** (414.4 mg, 0.88 mmol) and sodium methoxide (94.8 mg, 1.75 mmol) were dissolved in a mixture of methanol (40 mL) and dichloromethane (40 mL). This solution and a solution of compound **7** (600.3 mg, 0.88 mmol) in THF (40 mL) were added dropwise to a mixture of methanol (75 mL), THF (75 mL), and benzene (18 mL) at 50 °C. After complete addition the reaction mixture was refluxed for 1 hour. The solvent was removed in vacuo, and the residue was purified by column chromatography (silica gel 60) with acetone as eluent ($R_f = 0.42$). A white, crystalline product was obtained (250 mg, 0.251 mmol, 29 %). M.p. 100–102 °C; ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): $\delta = 1.60$ (s, 6H; CH_3), 1.63 (s, 6H; CH_3), 3.20 (d, $^2J(H,P) = 22.1$ Hz, 4H; CH_2), 3.53 (d, $^3J(H,P) = 10.7$ Hz, 12H; CH_3), 5.15 (s, 4H), 5.18 (s, 4H), 6.63 (d, $^3J(H,H) = 8.2$ Hz, 2H; CH), 6.79 (d, $^3J(H,H) = 8.8$ Hz, 4H; CH), 6.95 (ddd, $^3J(H,H) = 8.2$ Hz, $^4J(H,H) = 2.4$ Hz, $^6J(H,P) = 2.4$ Hz, 2H; CH), 7.09 (d, $^3J(H,H) = 8.8$ Hz, 4H; CH), 7.15 (dd, $^4J(H,H) = 2.4$ Hz, $^4J(H,P) = 2.4$ Hz, 2H; CH), 7.81 (s, 2H, CH), 8.19 (s, 2H; CH), 8.20 (s, 2H; CH); ^{13}C NMR (126 MHz, $CDCl_3$, 25 °C): $\delta = 26.4$ (d, $^1J(C,P) = 139.5$ Hz), 30.4 (s), 30.5 (s), 41.4 (s), 41.5 (s), 52.6 (d, $^2J(C,P) = 6.3$ Hz), 68.6 (s), 69.1 (s), 111.5 (s), 114.2 (s), 119.7 (d,

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^2J(C,P) = 9.7 Hz), 120.8 (s), 121.0 (s), 126.2 (s), 126.3 (s), 127.7 (s), 130.1 (d, ^3J(C,P) = 5.2 Hz), 131.1 (s), 140.0 (s), 140.3 (s), 143.5 (d, ^4J(C,P) = 3.4 Hz), 143.9 (s), 148.6 (s), 153.6 (d, ^3J(C,P) = 6.7 Hz), 155.8 (s); ^{31}P NMR (202 MHz, $CDCl_3$, 25 °C): δ = 30.2 (s); MS (CI, NH_3, 200 °C): *m/z*: 994 $[M]^-$; $C_{52}H_{56}N_2O_{14}P_2$ (995.0): calcd C 62.77, H 5.67, N 2.82; found C 62.63, H 5.80, N 2.69.

Macrocycle 2: Macrocycle **10** (201.8 mg, 0.203 mmol) and lithium bromide (40.5 mg, 0.47 mmol) were dissolved in acetonitrile (3 mL) and refluxed for 24 hours. A white solid precipitated, which was filtrated and washed with cold acetonitrile (158 mg, 0.162 mmol, 80 %) The product could be purified from excess LiBr by multiple dialysis in methanol (cellulose ester membrane, MWCO 500); the given data (esp. elementary analysis) refer to the nondialyzed product. M.p. 257–259 °C; ^{1}H NMR (500 MHz, CD_3OD, 25 °C): δ = 1.59 (s, 6H; CH_3), 1.67 (s, 6H; CH_3), 3.09 (d, ^2J(H,P) = 20.8 Hz, 4H; CH_2), 3.44 (d, ^3J(H,H) = 10.1 Hz, 6H; CH_3), 5.15 (s, 4H, CH_2), 5.17 (s, 4H; CH_2), 6.58 (d, ^3J(H,H) = 8.2 Hz, 2H; CH), 6.75 (d, ^3J(H,H) = 8.8 Hz, 4H; CH), 6.81 (d, ^3J(H,H) = 8.2 Hz, 2H; CH), 7.02 (d, ^3J(H,H) = 8.8 Hz, 4H; CH), 7.39 (s, 2H; CH), 7.83 (s, 2H; CH), 8.11 (s, 2H; CH), 8.16 (s, 2H; CH); ^{13}C NMR (126 MHz, CD_3OD, 25 °C): δ = 29.2 (d, ^1J(C,P) = 134.4 Hz), 31.4 (s), 31.5 (s), 42.8 (s), 49.6 (s), 52.6 (d, ^2J(C,P) = 6.1 Hz), 69.7 (s), 70.4 (s), 113.4 (s), 115.9 (s), 122.0 (d, ^2J(C,P) = 3.0 Hz), 125.3 (s), 127.2 (s), 127.6 (s), 129.1 (s), 130.9 (d, ^3J(C,P) = 4.8 Hz), 132.8 (s), 142.1 (s), 142.6 (s), 145.0 (d, ^3J(C,P) = 2.4 Hz), 145.3 (s), 150.2 (s), 155.2 (s), 157.5 (s); ^{31}P NMR (202 MHz, CD_3OD, 25 °C): δ = 23.4 (s); $C_{50}H_{50}N_2O_{14}P_2Li_2$ (978.8): calcd C 61.36, H 5.51, N 2.86; found C 58.64, H 4.41, N 2.62.

NMR titrations: Ten NMR tubes were each filled with 0.8 mL of a solution of the guest compound (c_{guest} = 0.5–4 mM) in a deuterated solvent. The host compound (1.525 equiv with respect to the guest) was dissolved in the same solvent (0.61 mL); the resulting solution was added, through a microsyringe and with increasing volume, to the guest solution in nine of the ten NMR tubes. The resulting ten solutions with host/guest ratios in the range from 0 to 5:1 were used for the NMR experiments (500 MHz). Volume and concentration changes were taken into account during analysis. The obtained binding curves were analyzed by nonlinear regression methods and the association constants were calculated as described earlier.[10]

Job plot: Equimolar solutions (4 mM) of host **2** and noradrenaline were prepared and 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.[9]

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