and SCHAKAL (E. Keller, Universität Freiburg, **1997**) for molecular graphics. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-155454 (**8**) and CCDC-155453 (**9**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

Towards Synthetic Adrenaline Receptors—Shape-Selective Adrenaline Recognition in Water**

Michael Herm, Oliver Molt, and Thomas Schrader*

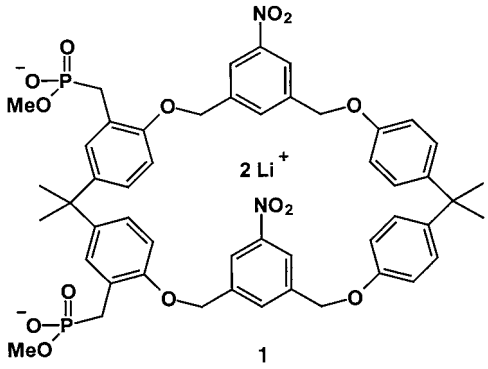
Dedicated to Professor Günter Wulff on occasion of his 66th birthday

The adrenergic receptor family is one of the most intensively investigated receptor types. Its G-protein-coupled signal transduction influences a broad range of vital body functions—from respiration to blood pressure.[1] Every year about 3000 research articles appear dealing with biochemical, medicinal, and pharmaceutical aspects of this important class of receptors. However, mainly because of the lack of X-ray crystal structures of these integral membrane proteins, their tertiary structure and mechanism of action have not been fully elucidated to date.[2] A synthetic model which imitates the postulated receptor–ligand interactions could shed new light on the efficiency of the specific combination of selected weak attractive forces. Such a model could also become a new prototype for artificial adrenaline sensors. Many attempts have been made to create synthetic receptor molecules for catecholamines. Most of these are monotopic: in some recent developments dopamine selectivity has been achieved with a pyrazol-containing podand, a homocalix[3]arene triether, as well as with a sol–gel process.[3] Enantioselective 3,4-dihydroxyphenylalanine (DOPA) recognition has been achieved by a peptide–bipyridinium cyclophane.[4] Boronic acids have been used in newer ditopic receptors for molecular recognition of the catechol ring.[5a,b] In an alternative design the catechol has been bound by a symmetric hydrophobic cavity with peripheral carboxylate groups for dopamine recognition.[5c] All these artificial host molecules, however, are far from biomimetic and not selective for catecholamino alcohols.

[*] Prof. T. Schrader, O. Molt
Fachbereich Chemie der Universität
Hans-Meerwein-Strasse, 35032 Marburg (Germany)
Fax: (+49) 6421-28-25544
E-mail: schradet@mailer.uni-marburg.de
M. Herm
Dupont
Wuppertal (Germany)

[**] Towards Synthetic Adrenaline Receptors, Part 4. This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie. Part 3: ref. [6].

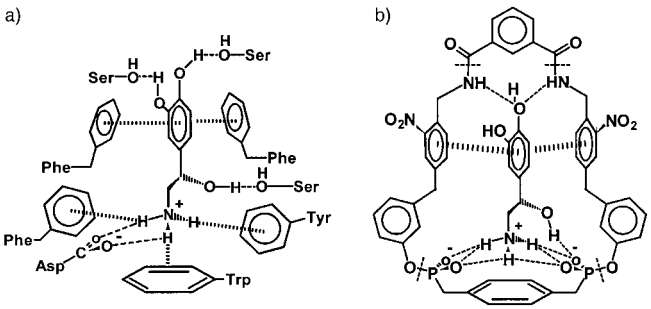
Recently, we introduced macrocyclic receptor molecule **1** with a hydrophobic cavity for the strong binding of adrenaline derivatives in methanol.[6] However, the amphiphilic host molecule undergoes strong self-association in water and is not



1

selective for adrenaline derivatives. We felt, that in order to imitate the natural binding site, an artificial biomimetic adrenaline host should be able to provide—at least after an induced-fit process—a microenvironment with a shape complementary to the geometrical form of its guest. A high number of van der Waals contacts would help desolvation in water and lead to a strong hydrophobic attraction.[7] In addition, the functional groups of the artificial receptor molecule should be positioned much more precisely, so that multiple noncovalent interactions could become effective in a cooperative fashion simultaneously after docking of the substrate. Extensive modeling experiments suggested a new approach, namely incorporation of the xylylene bisphosphonate moiety in a macrocycle, which should also be able to form the sandwich arrangement found in the natural example, as well as provide binding sites for the catechol hydroxyl groups at its opposite end.

Scheme 1 shows our solution to this problem: the nitroarene groups in the macrocyclic receptor molecule **2** can undergo double π-stacking interactions with the catechol ring of adrenaline without producing any significant ring strain in the receptor molecule, while the isophthalic amide group is ideally preoriented to form hydrogen bonds to the phenolic OH groups. Molecular mechanics calculations predict high binding enthalpies resulting from the interplay of noncovalent interactions operating simultaneously.[8] Monte–Carlo simu-



Scheme 1. a) Noncovalent interactions between noradrenaline and the β-adrenergic receptor; b) energy-minimized structure of the complex formed between noradrenaline and macrocyclic bisphosphonate **2**. The dashed lines indicate retrosynthetic cuts.

  1433-7851/01/4017-3148 $ 17.50+.50/0

lations in water reproducibly find energy-minimum structures very close to the postulated conformation of the complex formed between noradrenaline and **2** in Scheme 1 (Figure 1). Even the geometry of the free host is confirmed as an open conformation ready to receive adrenaline-type guests. In the absence of a metal cation the phosphonates are rotated more or less outwards to avoid electrostatic repulsion and to gain solvation energy.

In the retrosynthetic analysis (Scheme 1b) two apparent cuts lead to an activated *p*-xylylene bisphosphonate moiety and an isophthalic acid derivative. The remaining unit is the

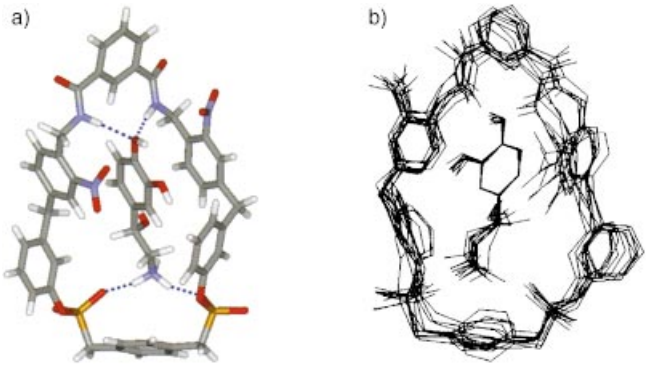


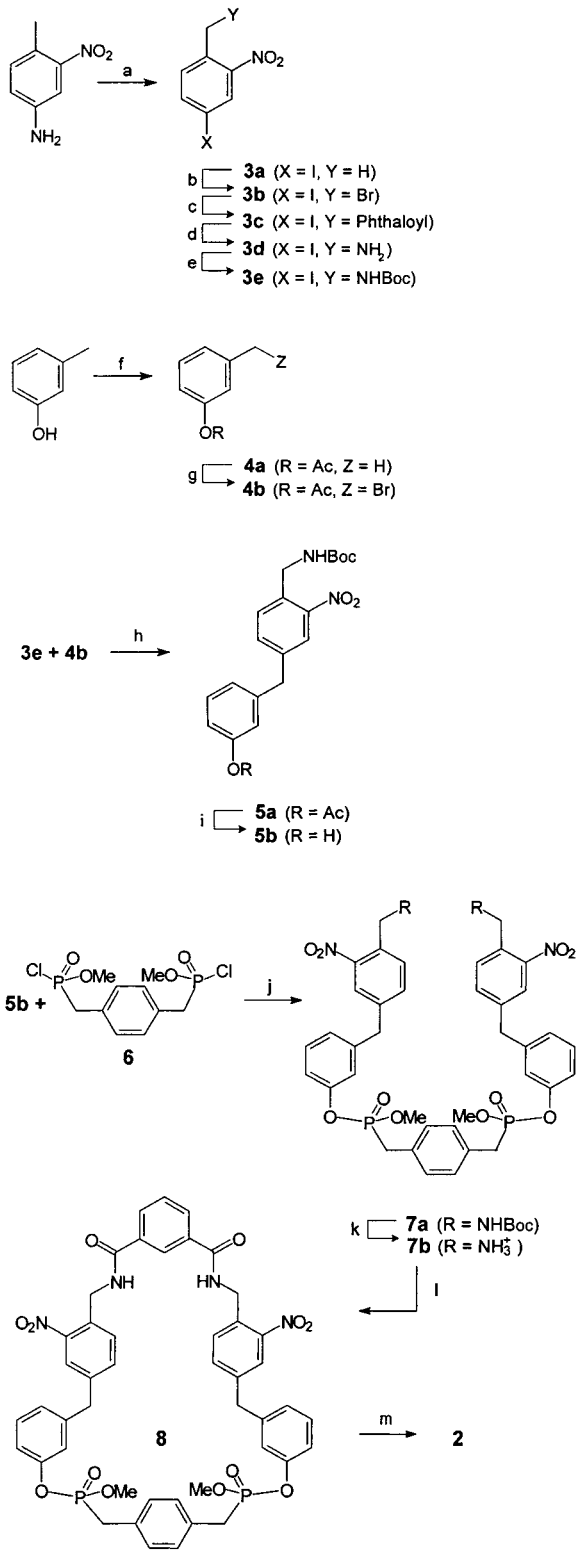
Figure 1. a) Structure of the complex formed between noradrenaline and macrocyclic bisphosphonate **2** according to Monte–Carlo simulations in water; b) superimposed snapshots of the subsequent molecular dynamics calculation.

central highly functionalized diphenylmethane building block with an unsymmetrical substitution pattern.

This target molecule can be effectively synthesized by a modified Negishi coupling, that is, a Pd-catalyzed cross-coupling reaction of aromatic iodide **3e** with the benzylzinc reagent obtained from **4b** (Scheme 2).[9] Deprotection of the *O*-acetyl groups in **5a**, followed by treatment with *p*-xylylene bisphosphonic acid ester chloride **6** to form a double ester furnishes the U-type predecessor **7a**. After cleavage of the Boc protecting groups under mild conditions, the critical macrocyclization step is carried out with isophthalic acid dichloride under high dilution conditions. Finally the methyl phosphonate is selectively cleaved with an equimolar amount of LiBr as a mild nucleophile. Receptor molecule **2** is obtained as a colorless hygroscopic solid that is soluble in a wide range of polar solvents (from DMSO to water).

Initial binding experiments were carried out with **2** and noradrenaline hydrochloride in DMSO, methanol, and water. A Job plot (Figure 2a) proved unambiguously that in all solvents a 1:1 complex is formed between host and guest molecule.[10] The electrospray ionization (ESI) spectrum shows a strong molecular ion peak for the 1:1 complex formed between noradrenaline and **2** (*m*/*z* 1046). Molecular ion peaks of higher mass could not be detected. For additional structural information NOESY NMR spectra were recorded in DMSO of both individual complexation partners as well as the complex itself.

Intramolecular NOE effects for the guest molecule confirmed that noradrenaline is bound in its thermodynamically favorable, bioactive conformation. Two of the critical flexible



Scheme 2. Modular and convergent synthesis of host **2** from 4-amino-2-nitrotoluene, *m*-cresol, and *p*-xylylene bisphosphonic acid dimethyl ester dichloride; a) 1. $NaNO_2$, 2. KI (61%); b) NBS, CCl_4 (46%); c) potassium phthalimide, [18]crown-6, toluene (94%); d) N_2H_4, ethanol (65%); e) Boc_2O, CH_2Cl_2 (95%); f) Ac_2O (89%); g) NBS, CCl_4 (47%); h) 1. Zn, reflux, 2. $[Pd(PPh_3)_4]$/DIBAL-H (58%); i) K_2CO_3, methanol, RT (94%); j) CH_2Cl_2, Et_3N (57%); k) TFA, CH_2Cl_2, 0°C (99%); l) isophthaloyl chloride, Et_3N, THF, benzene, RT (38%); m) LiBr, acetonitrile, 80°C >(66%). NBS = *N*-bromosuccinimide, Boc = *tert*-butoxycarbonyl, DIBAL-H = diisobutylaluminum hydride, RT = room temperature, TFA = trifluoroacetic acid.

  1433-7851/01/4017-3149 $ 17.50+.50/0

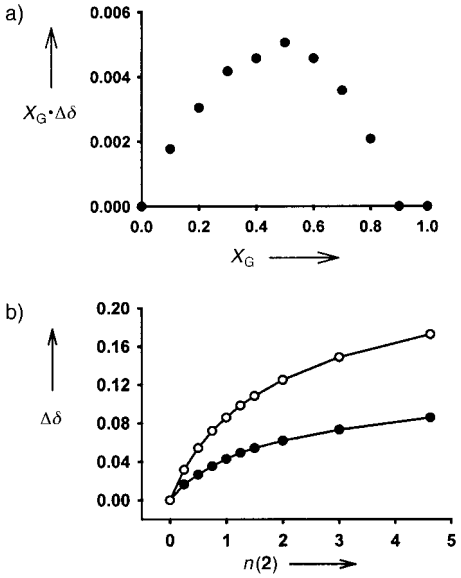
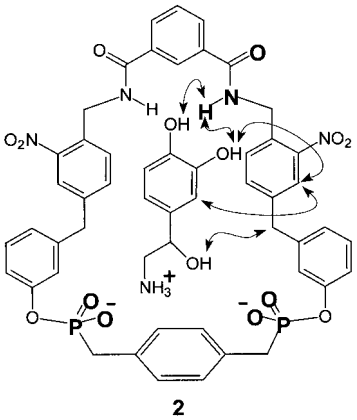


Figure 2. a) Job plot for the formation of a complex between host **2** and the guest (G) noradrenaline hydrochloride (CH-N proton) in D_2O:methanol (1:1); b) ^1H NMR titration curve showing the complexation-induced shifts (CIS) of the CHN (○) and CHO protons (●) on formation of a complex between host **2** (*n* equiv) and noradrenaline hydrochloride in methanol.

host units, namely the *p*-xylylene bisphosphonate and the diphenylmethane centerpiece, show a strong tendency for optimal preorganization, as evidenced from numerous NOE effects observed. In the complex, none of the intramolecular NOE effects of the host and guest are significantly altered, which demonstrates that again there is a good preorientation of the host structure. We were pleased to find a number of intermolecular NOE effects, which indicate the mutual orientation of both complexation partners. The result is exactly the same as predicted by molecular modeling studies (Scheme 3): The amino alcohol binds to the bisphosphonate, the catechol comes close to the nitroarenes, and even the catechol phenolic hydroxyl groups show NOE contacts to the isophtaloylamidic protons.[11]

Another indication for the complex geometry came from the FT-IR spectra. In the complex of **2** with noradrenaline the phosphonate P=O valence bands as well as those of the amide carbonyl groups were shifted towards lower values. Both effects can be explained by strong hydrogen bonds operating in the recognition motif between the amino alcohol and the catechol hydroxyl groups.

We then performed NMR titrations (Figure 2b) and calculated the association constants by nonlinear regression.[12] The decrease of binding constants along the series DMSO (ca. $10^4\,M^{-1}$) to methanol (ca. $10^3\,M^{-1}$) to water (ca. $10^2\,M^{-1}$) is 100-fold smaller than in the case of the open chain *p*-xylylene bisphosphonate receptors, which rely almost exclusively on electrostatic and hydrogen-bond interactions with the substrate. This result is a clear indication of the presence of additional attractive forces operating especially effectively in water. The weak self-



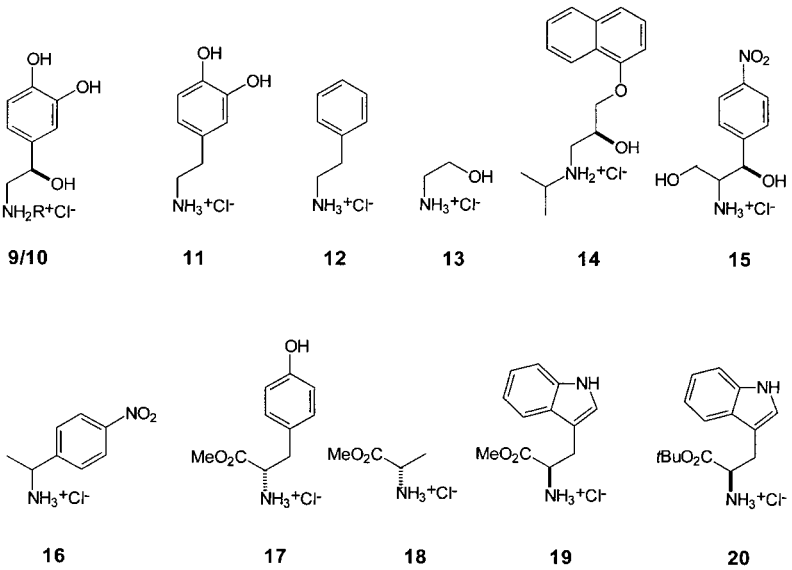
2

Scheme 3. Intermolecular NOE interactions in the complex formed between host **2** and noradrenaline. The carbon-bound hydrogen atoms have been omitted for clarity. Bold characters show IR-sensitive functional groups involved in hydrogen-bond formation.

association of the free host in pure water ($270\,M^{-1}$) could be completely suppressed in a 1:1 mixture of water and methanol, which was used for almost all of the following investigations.

We examined a number of closely related biogenic amines and amino alcohols to check the selectivity of the new host molecule. We systematically varied the guest structure by deleting selected functional groups from the target molecule adrenaline; this should allow us to estimate their contribution to the overall free binding enthalpy. The investigated guest molecules are presented in Scheme 4; the respective binding constants and ΔG values are summarized in Table 1.

The most striking effect is the small K_a value of ethanolamine which is half an order of magnitude below that of noradrenaline: the receptor molecule clearly recognizes the hormone's catechol ring (**13** versus **10**). This effect is also evidenced by the large upfield shift of the aromatic protons in many guest molecules with a 2-phenylethylamine skeleton (up to almost 0.5 ppm). Such aromatic upfield shifts are usually



9/10 **11** **12** **13** **14** **15**

16 **17** **18** **19** **20**

Scheme 4. Guest molecules for binding experiments with **2**. The structure of adrenaline has been systematically truncated to establish the contribution of specific noncovalent interactions.

  1433-7851/01/4017-3150 $ 17.50+.50/0

Table 1. Binding constants in complexes formed between host **2** and various guest molecules without (**9**–**15**) and with an alkyl or aryl substitutent α to the N atom (**16**–**20**) as determined by NMR titrations in D_2O:MeOD (1:1).

Guest[a]	K_a (1:1) [M^{-1}][b]	ΔG [kJ mol^{-1}][b]	$\Delta\delta_{sat}$[b,c]	Stoichiom.[d]
adrenaline **9** (R = Me)	153 ± 14 %	12.5	0.17 ± 10 %	1:1
noradrenaline **10** (R = H)	215 ± 12 %	13.3	0.12 ± 8 %	1:1
dopamine **11**	246 ± 38 %	13.6	0.20 ± 26 %	1:1
2-phenylethylamine **12**	102 ± 14 %	11.5	0.41 ± 11 %	1:1
ethanolamine **13**	54 ± 45 %	9.9	0.07 ± 34 %	1:1
propranolol **14**	204 ± 5 %	13.2	0.23 ± 3 %	1:1
D-*threo*-2-amino-1-(4-nitrophenyl)-1,3-propanediol (ANP) **15**	137 ± 7 %	12.1	0.36 ± 6 %	1:1
α-methyl-4-nitrobenzylamine **16**	weak binding[e]	–	no saturation	complex
L-tyrosine methyl ester **17**	weak binding	–	no saturation	complex
L-alanine methyl ester **18**	weak binding	–	no saturation	complex
D-tryptophan methyl ester **19**	weak binding	–	no saturation	complex
D-tryptophan *tert*-butyl ester **20**	weak binding	–	no saturation	complex

[a] As hydrochloride salts. [b] Errors [%] are calculated as standard deviations from the nonlinear regression. [c] Bound shift at 100 % complexation, obtained from the curve fitting (selected CH protons). [d] From Job plots and curve-fitting of the titration curves. [e] Maximum observed chemical shifts $\Delta\delta = 0.03$; upper limits for K_a are estimated at $< 10\,M^{-1}$.

indicative of π-stacking interactions, which are necessary for the postulated sandwich-type binding mode of host **2**.

Another interesting feature is the decrease in binding energy when the phenolic hydroxyl groups are deleted from the guest structure (**11** versus **12**). This effect makes **2** selective for catecholamines. Free phenolic OH groups do not significantly enhance the electron-rich character of a benzene ring because of their large negative inductive effect. Hence, the observed decrease in binding energy must be explained by loss of hydrogen bonds between the catechol hydroxyl groups and the isophthalamide head group of the host molecule. A control experiment in DMSO revealed a large downfield shift of one of the hydroxyl proton signals in noradrenaline on formation of a complex with **2**, and is another strong indicator for the formation of hydrogen bonds. The contribution of hydrogen bonds to the catechol hydroxyl groups can be estimated in the 1:1 mixture of water and methanol to be about 2.1 kJ mol^{-1}, whereas formation of π stacks with the catechol arene is worth about 1.6 kJ mol^{-1}.

Table 1 demonstrates a remarkable shape selectivity of the new adrenaline receptor molecule: in general the slim dopamine skeleton is a favorable binding motif for **2** (**9**–**15**). By contrast, the introduction of an additional substituent α to the ammonium ion, such as in amino acids, leads to weak binding and higher stoichiometries. Clearly, these guests are not included in the interior of the new host (**16**–**20**). This situation is in sharp contrast to host **1**, which, as a result of its large cavity, could not distinguish between amino acids and adrenaline derivatives. All the effects discussed above confirm that macrocyclic host **2** recognizes adrenaline derivatives in mixtures of water and methanol (1:1) by multiple noncovalent interactions including electrostatic attraction, hydrogen bonds, π stacking, and hydrophobic forces.

In summary, we have designed a shape-selective adrenaline host for dopamine with a binding constant in water which is three orders of magnitude lower than that of the natural example ($10^5\,M^{-1}$). We are currently optimizing the host structure by implementing elements of much higher rigidity to achieve an even more effective preorganization and desolvation.

Received: March 26, 2001 [Z16850]

[1] T. P. Iisma, T. J. Biden, J. Shine, *G Protein-Coupled Receptors*, Springer, Heidelberg, **1995**.

[2] Recent GPCR crystal structures: a) E. Pebay-Peyroula, G. Rummel, J.-P. Rosenbusch, E. M. Landau, *Science* **1997**, *277*, 1676; b) D. Doyle, J. M. Cabral, R. A. Pfuetzner, A. Kuo, J. M. Gulbis, S. L. Cohen, B. T. Chait, R. Mackinnon, *Science* **1998**, *280*, 69; c) K. Palczewski, T. Kumasaka, T. Hori, C. A. Behnke, H. Motoshima, B. A. Fox, I. Le Trong, D. C. Teller, T. Okada, R. O. Stenkamp, M. Yamamoto, M. Miyano, *Science* **2000**, *289*, 739–745.

[3] a) M. I. Rodriguez-Franco, P. San Lorenzo, A. Martinez, P. Navarro, *Tetrahedron* **1999**, *55*, 2763; b) K. Odashima, K. Yagi, K. Tohda, Y. Umezawa, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2375; c) R. R. Makote, M. M. Collinson, *Chem. Mater.* **1998**, *10*, 2440.

[4] J. A. Gavin, M. E. Garcia, A. J. Benesi, T. E. Mallouk, *J. Org. Chem.* **1998**, *63*, 7663–7669.

[5] a) M.-F. Paugam, L. S. Valencia, B. Bogess, B. D. Smith, *J. Am. Chem. Soc.* **1994**, *116*, 11203; b) M.-F. Paugam, J. T. Biens, B. D. Smith, A. J. Christoffels, F. de Jong, D. N. Reinhoudt, *J. Am. Chem. Soc.* **1996**, *118*, 9820–9825; c) M. B. Inoue, E. F. Velazquez, M. Inoue, Q. Fernando, *J. Chem. Soc. Perkin Trans. 2* **1997**, 2113–2118.

[6] T. Schrader, M. Herm, *Chem. Eur. J.* **2000**, *6*, 47–53.

[7] In the natural β-adrenergic receptor the binding site is formed by a cyclic array of seven membrane-spanning α-helices which produce a rather hydophobic surrounding of the adrenaline guest: S. Trumpp-Kallmeyer, J. Hoflack, A. Bruinvels, M. Hibert, *J. Med. Chem.* **1992**, *35*, 3448–3462.

[8] Molecular mechanics calculations: Cerius^2, Molecular Simulations Inc., force-field: Dreiding 2.21. Monte–Carlo Simulations and Molecular Dynamics: Macromodel 7.0, Schrödinger Inc., **2000**; force-field: Amber*. A 3000-step Monte–Carlo simulation was carried out, followed by a molecular dynamics calculation for 10 ps at 300 K.

[9] a) E. Negishi, A. O. King, N. Okukado, *J. Org. Chem.* **1977**, *42*, 1821–1823; b) reviews: *Organozinc Reagents* (Eds.: P. Knochel, P. Jones), Oxford University Press, Oxford, UK, **1999**, pp. 213–243; *Metal-Catalyzed Cross Coupling Reactions* (Eds.: F. Diederich, P. J. Stang), Wiley-VCH, Weinheim, **1998**, pp. 1–47.

[10] a) P. Job, *C. R. Hebd. Seances Acad. Sci.* **1925**, *180*, 928; b) M. T. Blanda, J. H. Horner, M. Newcomb, *J. Org. Chem.* **1989**, *54*, 4626.

[11] Since both the NOE interactions between OH and NH protons as well as the diagonal are negative, we cannot a priori exclude exchange phenomena. However, no cross-peak was found between water and the NH proton.

[12] a) H. J. Schneider, R. Kramer, S. Simova, U. Schneider, *J. Am. Chem. Soc.* **1988**, *110*, 6442; b) C. S. Wilcox in *Frontiers in Supramolecular Chemistry* (Ed.: H. J. Schneider), VCH, Weinheim, **1991**, p. 123.

  1433-7851/01/4017-3151 $ 17.50+.50/0