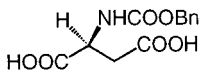
Negative Cooperativity in the Molecular Recognition of Excitatory Amino-Acid Derivatives by Synthetic Allosteric 1,1′-Binaphthalene Receptors

by **Philipp Lustenberger**, **Roger Welti**, and **François Diederich***

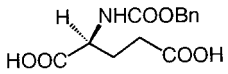
Laboratorium für Organische Chemie, Eidgenössische Technische Hochschule, ETH-Zentrum, Universitätstrasse 16, CH-8092 Zürich

The optically active allosteric receptors (−)-(*R*,*R*)-**3** and (+)-(*R*,*R*)-**4** were synthesized for the molecular recognition of the *N*-(benzyloxy)carbonyl (*N*-Cbz)-protected excitatory amino acids aspartic acid (Asp, **1**) and glutamic acid (Glu, **2**). These macrocyclic structures consist of two 1,1′-binaphthalene moieties connected by two but-2-yne-1,4-diyl (for (−)-(*R*,*R*)-**3**) or *p*-xylylene (for (+)-(*R*,*R*)-**4**) bridges between the O-atoms in the minor grooves. Each 1,1′-binaphthalene moiety contains two 2-acetamidopyridin-6-yl (CONH(py)) H-bonding sites in the major groove to bind excitatory amino-acid derivatives *via* two COOH ··· CONH(py) H-bonding arrays and additional secondary electrostatic interactions. The formation of stable complexes with 1 : 2 host-guest stoichiometry was proven by the evaluation of fluorescence binding titrations using a multiple-wavelength nonlinear least-squares curve-fitting procedure, *Job* plot analysis, and solubilization experiments. Complexation of the first excitatory amino-acid guest at binding site 1 reduces the affinity for the second guest at binding site 2. As measures for the negative cooperativity between the two sites, the ratios of the association constants for the first and second binding events, $\{K_a(1:1)/K_a(1:2)\}_{corr.}$ (corrected for the statistical preference of the 1 : 1 complex formation), were found to adopt values between 1.4 and 2.4, and the *Hill* coefficients n_H varied between 0.49 and 0.59.

1. Introduction. – In our initial development of optically active, 1,1′-binaphthalene-based H-bonding receptors for the selective recognition of excitatory amino-acid derivatives [1] such as *N*-Cbz-L-Asp (Cbz = (benzyloxy)carbonyl, Asp = aspartic acid; **1**) and *N*-Cbz-L-Glu (Glu = glutamic acid; **2**), we had observed high association strength (ΔG°) but poor binding enantioselectivities ($\Delta(\Delta G^\circ)$ = difference in stability between diastereoisomeric complexes) [2]. The origin of these particular binding characteristics was the high degree of conformational flexibility about the chirality axis through the C(1)−C(1′) bond of the 1,1′-binaphthalene moiety [3], which allowed an energetically favorable adaptation of both receptor enantiomers to the optically active guest. The binding enantioselectivity was, however, strongly improved when the 2,2′-positions of the 1,1′-binaphthalene moiety were bridged by short tethers, thereby enforcing a distinct dihedral angle preference about the chirality axis (θ in *Fig. 1,A*) [4] and enhancing the preorganization of the receptors [5].



1 *N*-Cbz-L-Asp



2 *N*-Cbz-L-Glu

The observed large effects from bridging the minor groove of 1,1′-binaphthalene receptors on both binding free enthalpy (ΔG°) and enantioselectivity ($\Delta(\Delta G^\circ)$) of recognition processes at major groove H-bonding sites [4] led us to consider the

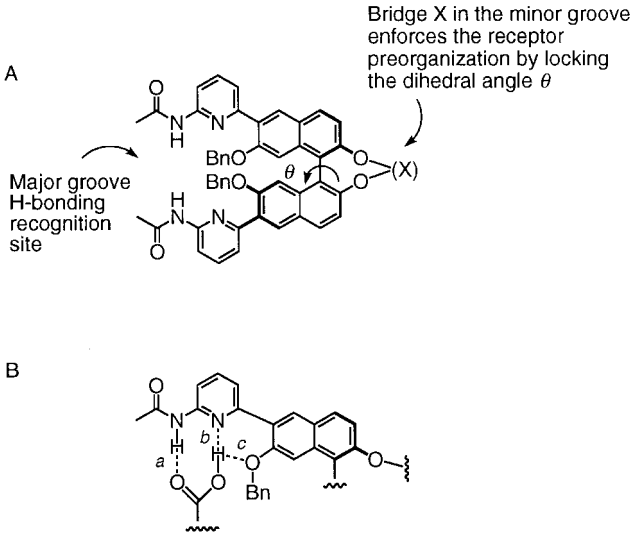
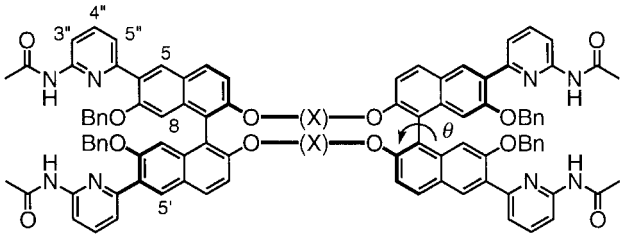


Fig. 1. A) *General shape of conformationally locked* (R)*-1,1′-binaphthalene receptors for the enantioselective molecular recognition of the excitatory amino-acid derivatives* N-*Cbz*-L-*Asp* (**1**) *and* N-*Cbz*-L-*Glu* (**2**). B) *Recognition of a carboxylic-acid residue by H-bonds* (*a* and *b*) *and secondary electrostatic interactions* (*c*).

construction of the homotropic (with identical binding sites) allosteric systems (−)-(*R*,*R*)-**3** and (+)-(*R*,*R*)-**4**. They feature macrocyclic structures composed of two 1,1′-binaphthalene receptor moieties that are connected by two but-2-yne-1,4-diyl or *p*-xylylene bridges between the O-atoms in the minor grooves. Each 1,1′-binaphthalene moiety contains two 2-acetamidopyridin-6-yl (CONH(py)) H-bonding sites in the 6,6′-positions of the major groove to bind excitatory amino-acid derivatives *via* two COOH ··· CONH(py) H-bonding arrays and additional secondary electrostatic interactions (*Fig. 1,B*) [2]. The question we intended to answer was whether the information of the binding of a first guest molecule (**1** or **2**; the effector) to recognition site 1 would be transferred by the rather rigid linkers to recognition site 2 and alter its binding capacity and selectivity. Specifically, we expected complexation of the guest at site 1 to change and enforce the dihedral angle θ at site 2. According to our previous study [4], this should alter the binding characteristics of the latter site. Allosteric binding phenomena [6][7] have received much attention in the past, and positive and negative cooperativity in artificial homotropic [8] and heterotropic (with different binding sites) [9] allosteric systems has been investigated by several research groups. Allosteric effects are ubiquitous in nature, with the large positive cooperativity in the binding of $4\,O_2$ molecules to hemoglobin perhaps providing the most prominent example [10].

Here, we report the synthesis of the two allosteric receptor systems (−)-(*R*,*R*)-**3** and (+)-(*R*,*R*)-**4**, and demonstrate significant negative cooperativity between their two recognition sites for the excitatory amino acids **1** and **2**, *i.e.*, the second binding event is thermodynamically less favorable than the first one.

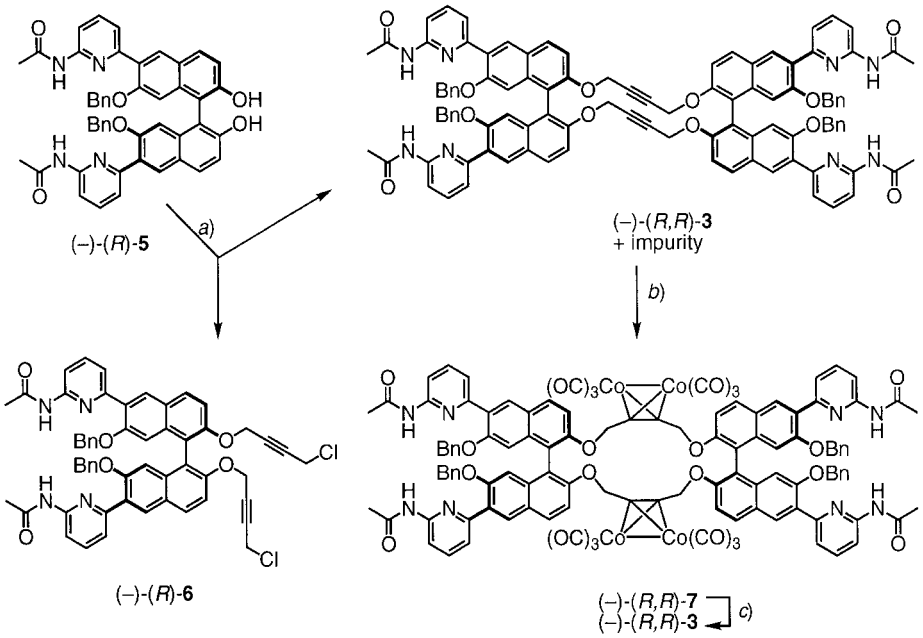
2. Results and Discussion. – 2.1. *Synthesis.* The preparation of the allosteric receptors (−)-(*R*,*R*)-**3** and (+)-(*R*,*R*)-**4** started from (−)-(*R*)-**5** [4]. Treatment of this



(–)-(*R,R*)-**3** X = CH_2–C≡C–CH_2
(+)-(*R,R*)-**4** X = CH_2–(*p*-C_6H_4)–CH_2

diol with a *ca.* 30-fold excess of 1,4-dichlorobut-2-yne and K_2CO_3 in MeCN at 80° surprisingly yielded a substantial amount of the macrocyclic receptor (–)-(*R,R*)-**3** (*ca.* 20%) together with dialkylated (–)-(*R*)-**6** (12%), which was the initially targeted product under the chosen reaction conditions (*Scheme 1*). A tenacious impurity in the (–)-(*R,R*)-**3** fraction could not be removed chromatographically; therefore, a purification *via* derivatization was chosen. Treatment with $[Co_2(CO)_8]$ in $CHCl_3$ afforded the dicobalt complex (–)-(*R,R*)-**7** (61%) [11], which could be purified by column chromatography. Oxidative cleavage of the dicobalt complex with I_2 subsequently provided pure (–)-(*R,R*)-**3** (48%), which was used in the complexation studies.

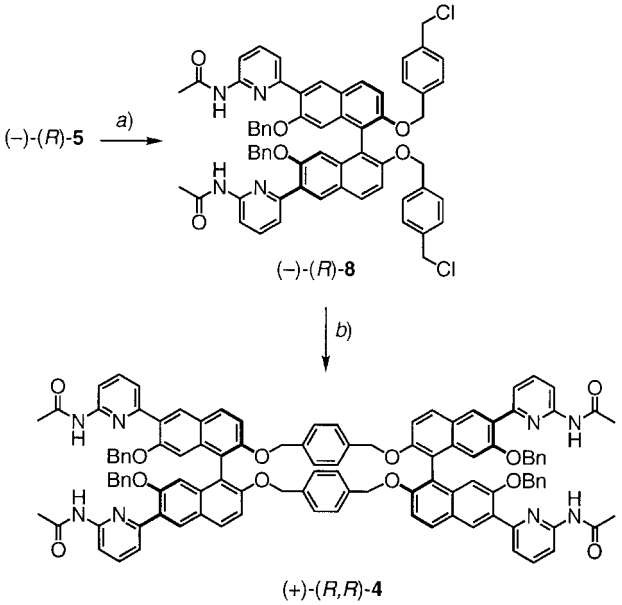
Scheme 1. *Synthesis of the Allosteric Receptor (–)-(*R,R*)-***3***



a) 1,4-Dichlorobut-2-yne, K_2CO_3, MeCN, 80°, 3 h; 12% of (–)-(*R*)-**6** and *ca.* 20% of (impure (–)-(*R,R*)-**3**). *b*) $[Co_2(CO)_8]$, $CHCl_3$, 0°, 2 h; 61%. *c*) I_2, THF, 0°, 4 h; 48%.

For the synthesis of the macrocycle (+)-(*R*,*R*)-**4**, the diol (−)-(*R*)-**5** was converted with a 30-fold excess of α,α'-dichloro-*p*-xylene and K_2CO_3 into the dichloride (−)-(*R*)-**8** (37% yield) (*Scheme 2*). In sharp contrast to the reaction of (−)-(*R*)-**5** with 1,4-dichlorobut-2-yne (see above), this transformation did not yield any significant amount of macrocyclic product. Subsequent *Williamson* macrocyclization of (−)-(*R*)-**5** with (−)-(*R*)-**8** in the presence of NaI (*Finkelstein* conditions) provided the allosteric receptor (+)-(*R*,*R*)-**4** in excellent yield (88%).

Scheme 2. *Synthesis of the Allosteric Receptor (+)-(*R,R*)-**4***

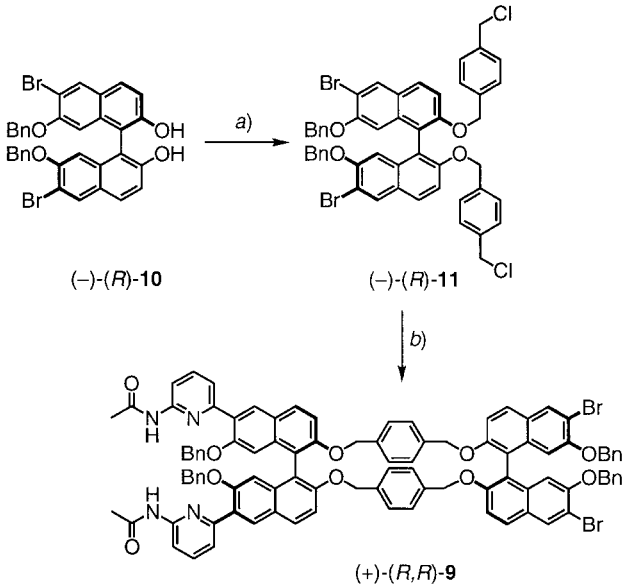


a) α,α'-Dichloro-*p*-xylene, K_2CO_3, MeCN, 80°, 14 h; 37%. *b*) (−)-(*R*)-**5**, Cs_2CO_3, NaI, MeCN, 80°, 14 h; 88%.

For comparison purposes, the macrocycle (+)-(*R*,*R*)-**9** was prepared starting from the diol (−)-(*R*)-**10** [4] (*Scheme 3*). This compound structurally resembles closely the allosteric receptor (+)-(*R*,*R*)-**4**, but can only undergo 1:1 host-guest complexation with **1** and **2**, since it possesses only a single recognition site for dicarboxylic acids. Treatment of (−)-(*R*)-**10** with a 30-fold excess of α,α'-dichloro-*p*-xylene and K_2CO_3 yielded (−)-(*R*)-**11** (91% yield), which was subjected to the macrocyclization with (−)-(*R*)-**5** to yield the monotopic receptor (+)-(*R*,*R*)-**9** in 58% yield.

2.2. *Molecular-Recognition Studies.* The complexation of *N*-Cbz-L-Asp (**1**) and *N*-Cbz-L-Glu (**2**) by receptors (−)-(*R*,*R*)-**3** and (+)-(*R*,*R*)-**4** could not be investigated by ^{1}H-NMR binding titrations (500 MHz, $T = 300$ K) in noncompetitive solvents [4], since host-guest exchange on the ^{1}H-NMR time scale led to strong signal broadening. Fortunately, both receptors were highly fluorescent which permitted the evaluation of their binding characteristics by fluorescence titrations at $T = 296$ K at constant host concentration. In these titrations, the changes in fluorescence emission of the receptors, induced by guest complexation, were monitored over the wavelength range between

Scheme 3. *Synthesis of the Monotopic Receptor (+)-(*R,R*)-***9**



a) α,α'-Dichloro-*p*-xylene, K_2CO_3, MeCN, 80°, 14 h; 91%. *b*) (−)-(*R*)-**11**, Cs_2CO_3, NaI, MeCN, 80°, 16 h; 58%.

$\lambda_{em} = 360$ and 450 nm ($\lambda_{exc} = 335$ nm for (+)-(*R*,*R*)-**4** and (+)-(*R*,*R*)-**9** and 350 nm for (−)-(*R*,*R*)-**3**) and subsequently analyzed by the multiple-wavelength nonlinear least-squares curve-fitting procedure implemented in the program 'SPECFIT' [12]. This program performs global least-squares fits which include the entire measured wavelength region. The titrations performed in dry CH_2Cl_2 produced the following results (*Table*):

i) Both ditopic receptors (−)-(*R*,*R*)-**3** and (+)-(*R*,*R*)-**4** formed stable 1:2 complexes by binding two molecules of the excitatory amino-acid derivatives at their two major groove recognition sites, whereas the monotopic receptor (+)-(*R*,*R*)-**9** expectedly only formed a 1:1 complex. The formation of host-guest complexes with 1:2 stoichiometry by the two ditopic receptors was clearly proven by *Job* plot analysis [13][14].

ii) Receptor (−)-(*R*,*R*)-**3** bound *N*-Cbz-L-Glu preferentially over *N*-Cbz-L-Asp, whereas (+)-(*R*,*R*)-**4** displayed a slight selectivity for *N*-Cbz-L-Asp.

iii) In all runs with the ditopic receptors, the association constants $K_a(1:2)$ for the complexation of the second guest were lower than those calculated for the first binding event ($K_a(1:1)$). The binding of the first excitatory amino-acid derivative at site 1 clearly reduces the affinity for the second guest at site 2. As a measure for cooperativity effects, we calculated the ratio $\{K_a(1:1)/K_a(1:2)\}_{corr.}$ (*Table*) which includes a correction factor of 0.25 for the statistical preference of the first over the second binding event [14]. This way, a 1.4- to 2.4-fold negative cooperativity was determined. Negative cooperativity is also expressed by *Hill* coefficients n_H in the range between 0 and 1, and these coefficients were calculated according to $n_H = 2/\{1 + [K_a(1:1)/K_a(1:2)]^{0.5}\}$ [14][15] to adopt values in the range between 0.49 and 0.59.

Table. *Association Constants* $K_a(1:1)$ *and* $K_a(1:2)$ [l mol^{-1}] *and Complexation Free Enthalpies* $\Delta G^\circ(1:1)$ *and* $\Delta G^\circ(1:2)$ [kcal mol^{-1}] *for the Complexes of Receptors (−)-(*R,R*)-***3**, *(+)-(*R,R*)-***4**, *and (+)-(*R,R*)-***9** *with* N-*Cbz*-L-*Asp* (**1**) *and* N-*Cbz*-L-*Glu* (**2**) *in* CH_2Cl_2 (296 K), *Determined by Fluorescence Titrations*

Entry	Receptor[a])	Guest	$K_a(1:1)$ $K_a(1:2)$ [l mol^{-1}]	$\Delta G^\circ(1:1)$[b]) $\Delta G^\circ(1:2)$[b]) [kcal mol^{-1}]	$\{K_a(1:1)/K_a(1:2)\}_{corr.}$[c])	n_H[d])
1	(−)-(*R*,*R*)-**3**	**1**	19770	− 5.82		
			3460	− 4.79	1.4	0.59
2	(+)-(*R*,*R*)-**4**	**1**	57900	− 6.45		
			6110	− 5.13	2.4	0.49
3	(+)-(*R*,*R*)-**9**	**1**	17580	− 5.75	–	–
4	(−)-(*R*,*R*)-**3**	**2**	31970	− 6.10		
			3570	− 4.81	2.2	0.50
5	(+)-(*R*,*R*)-**4**	**2**	46600	− 6.32		
			4920	− 4.99	2.4	0.49

[a]) $[Host]_0 = 8 \times 10^{-6}$ M, $[guest]_0 = 1 \times 10^{-5}$ to 5×10^{-4} M. [b]) Uncertainty in ΔG°: ± 0.1 kcal mol^{-1}. [c]) $\{K_a(1:1)/K_a(1:2)\}_{corr.} = 0.25 \times [K_a(1:1)/K_a(1:2)]$; ratio for determining cooperativity including a correction for the statistical preference of 1:1 over 1:2 host-guest complex formation. [d]) *Hill* coefficient $n_H = 2/\{1 + [K_a(1:1)/K_a(1:2)]^{0.5}\}$ [14].

2.3. *Solid-Liquid Extraction and Solubilization Experiments.* To further confirm the formation of stable complexes with 1:2 host-guest stoichiometry by the ditopic receptors (−)-(*R*,*R*)-**3** and (+)-(*R*,*R*)-**4**, solubilization studies were undertaken using $CDCl_3/CCl_4$ 1:3 as the solvent. This particular solvent mixture was chosen, since it readily dissolves the two receptors, whereas the pure excitatory amino-acid derivatives remain insoluble [16]. When solutions of (*R*,*R*)-**3** or (*R*,*R*)-**4** ($c \approx 1$ mM) containing a large excess of solid *N*-Cbz-L-Asp (**1**) or *N*-Cbz-L-Glu (**2**) were sonicated for 5 min, in all cases 1.95 ± 0.1 equiv. of the excitatory amino acid were solubilized. Under the same conditions, the monotopic receptor (+)-(*R*,*R*)-**9** only extracted 1.0 ± 0.1 equiv. of solid **1** into the liquid phase. The solubilization studies, therefore, confirm the binding stoichiometries determined in homogenous solution by fluorescence binding titrations and *Job* plot analyses.

A comparison of the ^{1}H-NMR spectrum (300 MHz) of pure (−)-(*R*,*R*)-**3** in $CDCl_3/CCl_4$ 1:3 with that of the solution obtained by solid-liquid extraction of *N*-Cbz-L-Glu (**2**) revealed large, specific complexation-induced changes in chemical shift of the aromatic receptor protons (*Fig. 2*). Thus, the pyridine resonances H−C(3″) and H−C(4″) move downfield ($\Delta\delta = 0.21$ and 0.12 ppm, resp.) as a result of the H-bonding to the pyridine N-atoms. In contrast, the signals of H−C(5″) and H−C(5) move significantly upfield ($\Delta\delta = -0.25$ and -0.27 ppm, resp.), which is indicative of complexation-induced conformational changes by rotation about the C−C bonds between naphthyl and pyridyl rings. On the other hand, the resonance of H−C(8), which is quite sensitive to changes in the dihedral angle θ about the chirality axis through the C(1)−C(1′) bond of the 1,1′-binaphthalene moiety, is only weakly upfield shifted ($\Delta\delta = -0.01$ ppm), suggesting that this angle adopts a similar value in the free receptor and its 1:2 complex.

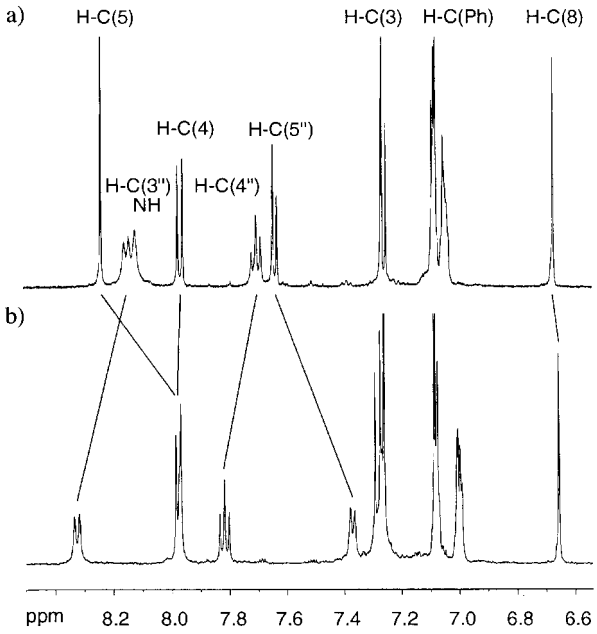


Fig. 2. *Aromatic region in the ^{1}H-NMR spectra* (300 MHz, $CDCl_3/CCl_4$ 1:3) a) *of pure (−)-(*R,R*)-***3** *and* b) *of the solution obtained by solid-liquid extraction of* N-*Cbz*-L-*Glu* (**2**) *with a solution of the ditopic receptor*

3. Conclusions. – The two allosteric receptors (−)-(*R*,*R*)-**3** and (+)-(*R*,*R*)-**4** were prepared for the complexation of the excitatory amino-acid derivatives *N*-Cbz-L-Asp (**1**) and *N*-Cbz-L-Glu (**2**) by H-bonding in noncompetitive solvents. The formation of stable complexes with 1:2 host-guest stoichiometry was clearly established by the evaluation of fluorescence binding titrations using a multiple-wavelength nonlinear least-squares curve-fitting procedure, *Job* plot analysis, and solubilization experiments. Complexation of the first excitatory amino-acid guest at binding site 1 clearly reduces the affinity for the second guest at binding site 2. As measures for the negative cooperativity between the two sites, the ratios between the association constants for the first and second binding events, $\{K_a(1:1)/K_a(1:2)\}_{corr.}$, corrected for the statistical preference of the 1:1 complex formation, were found to adopt values between 1.4 and 2.4, and *Hill* coefficients n_H varied between 0.49 and 0.59. We can, therefore, conclude that the bridges, which link two 1,1′-binaphthalene sub-receptors by their minor grooves, are efficient in transmitting binding information from one major-groove recognition site to the second one. This study provides yet another example of how bridging the minor groove of a 1,1′-binaphthalene spacer controls its conformation and changes the binding affinity of a major-groove recognition site [4].

Experimental Part

General. All reactions were carried out under Ar. Solvents and reagents were reagent-grade commercials and were used without further purification unless otherwise stated. THF and Et_2O were freshly distilled from sodium benzophenone ketyl. MeCN was stored over molecular sieves (3 Å). Evaporation *in vacuo* was

conducted at H_2O aspirator pressure. Column chromatography (CC): SiO_2 *60* (230–400 mesh, 0.040–0.063 mm) from *Fluka* and *N*-Alox (neutral Al_2O_3, Act. 1) from *Woelm*. M.p.: *Büchi SMP-20*; uncorrected. IR Spectra [cm^{-1}]: *Perkin-Elmer 1600-FT*. NMR Spectra: *Bruker AMX 500* and *Varian Gemini 300* or *200* at 296 or 300 K, with solvent peak as reference. MS (*m*/*z* (%)): FAB: *VG ZAB2-SEQ* spectrometer with 3-nitrobenzyl alcohol (3-NOBA) as matrix. HR-ESI-MS: *Finnigan New Star FT/MS* with 7-T magnet. Elemental analyses were performed by the Mikrolabor at the Laboratorium für Organische Chemie, ETH-Zürich.

Fluorescence Binding Titrations. Quant. binding data ($K_a(1:1)$, $K_a(1:2)$, $\Delta G^\circ(1:1)$, and $\Delta G^\circ(1:2)$) were determined by multiple-wavelength nonlinear least-squares curve-fitting of the emission data recorded in the wavelength region between 360 and 450 nm [12]. The excitation wavelength was set at 350 nm in binding studies with (−)-(*R*,*R*)-**3**, and at 335 nm in the studies with (+)-(*R*,*R*)-**4** and (+)-(*R*,*R*)-**9**. All titrations were done in dry CH_2Cl_2, which was stored over K_2CO_3 and filtered through a pad of *N*-Alox before use. Commercially available **1** and **2** (*Sigma*) were used without further treatment. The receptor concentration was kept constant at 8×10^{-6} M, and the guest concentration was varied (1×10^{-5} to 5×10^{-4} M) by adding a soln. of guest (containing 8×10^{-6} M of host) in portions *via* microsyringe to the septum-capped fluorescence cuvette containing the host soln. and a magnetic stirring bar. After each addition, a fluorescence emission spectrum was taken.

Solid-Liquid Extraction and Solubilization Experiments. To solid receptor (0.8 mg, *ca.* 4×10^{-7} mol) and guest (4.0 mg, *ca.* 2×10^{-5} mol) was added $CCl_4/CDCl_3$ 3:1 (0.45 ml), and the mixture was sonicated at r.t. for 5 min. The slurry was filtered and the solvent evaporated *in vacuo*. The formed complex was dissociated by adding $(CD_3)_2SO$ (0.5 ml) and the host-guest ratio determined by ^{1}H-NMR, comparing the integral for the PhCH_2 protons of the guest (*s* at 5.00 ppm) with that for H–C(8) of the host (*s* at 6.55 to 6.73 ppm, depending on the host) taken as internal standard.

*(−)-(*R*)-*N,N′*-{[7,7′-Bis(benzyloxy)-2,2′-bis(4-chlorobut-2-ynoxy)-1,1′-binaphthalene-6,6′-diyl]di(pyridine-6,2-diyl)}bis(acetamide)* ((−)-(*R*)-**6**) *and (−)-(*R*,R)-N,N′,N″,N‴-{[5,24,31,50-Tetrakis(benzyloxy)-12,17,38,43-tetraoxanonacyclo[42.8.0.0^{2,11}.0^{3,8}.0^{18,27}.0^{21,26}.0^{28,37}.0^{29,34}.0^{47,52}]dopentaconta-1(52),2,4,6,8,10,18,20,22,24,26,28,30,32,34,36,44,46,48,50-icosaene-14,40-diyne-6,23,32,49-tetrayl]tetrakis(pyridine-6,2-diyl)}tetrakis(acetamide)* ((−)-(*R*)-**3**). To a degassed slurry of (−)-(*R*)-**5** (150 mg, 0.2 mmol) [4] and K_2CO_3 (213 mg, 1.56 mmol) in dry MeCN (45 ml) was added under Ar at 80° 1,4-dichlorobut-2-yne (0.57 ml, 5.85 mmol), and the mixture was stirred for 1 h. After addition of a second portion of 1,4-dichlorobut-2-yne (0.57 ml, 5.85 mmol) and Cs_2CO_3 (510 mg, 1.56 mmol), the mixture was stirred for another 2 h at reflux. The mixture was cooled to r.t., filtered over *Celite*, evaporated *in vacuo*, and the residue purified by CC (SiO_2, $CH_2Cl_2 \rightarrow CH_2Cl_2$/AcOEt 9:1) to provide pure (−)-(*R*)-**6** (22 mg, 12%) and (−)-(*R*,*R*)-**3** (50 mg, *ca.* 20%) contaminated with an inseparable impurity.

*(−)-(*R*)-**6***: Yellowish solid. M.p. >124° (dec.). $[\alpha]_D^{23} = -194.8$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3390 (br.), 1693*m*, 1624*s*, 1572*s*, 1472*m*, 1263*w*, 1219*m*, 1044*w*, 809*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 2.20 (*s*, 6 H); 4.05 (*s*, 4 H); 4.54 (*m*, 4 H); 4.68 (*d*, *AB*, $J = 12.1$, 2 H); 4.74 (*d*, *AB*, $J = 12.1$, 2 H); 6.53 (*s*, 2 H); 6.90–7.20 (*m*, 10 H); 7.39 (*d*, $J = 8.7$, 2 H); 7.60–7.80 (*m*, 4 H); 7.97 (*d*, $J = 8.7$, 2 H); 8.02 (*s*, 2 H); 8.09 (*d*, $J = 8.1$, 2 H); 8.24 (*s*, 2 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 24.77; 30.25; 57.58; 69.99; 81.88; 82.32; 105.97; 112.28; 114.39; 119.46; 121.58; 125.45; 127.19; 127.75; 128.52; 130.10; 131.16; 135.41; 136.77; 138.41; 151.22; 154.50; 154.96; 168.95. FAB-MS: 938.9 (100, MH$^+$). HR-FAB-MS: 939.2721 (MH$^+$, $C_{56}H_{45}N_4O_6Cl_2^+$; calc. 939.2716).

*(−)-(*R*,R)-Bis(hexacarbonyl-μ-η)-(*N,N′,N″,N‴*-{[5,24,31,50-tetrakis(benzyloxy)-12,17,38,43-tetraoxanonacyclo[42.8.0.0^{2,11}.0^{3,8}.0^{18,27}.0^{21,26}.0^{28,37}.0^{29,34}.0^{47,52}]dopentaconta-1(52),2,4,6,8,10,18,20,22,24,26,28,30,32,34,36,44,46,48,50-icosaene-14,40-diyne-6,23,32,49-tetrayl]tetrakis(pyridine-6,2-diyl)}tetrakis(acetamide)bis[(dicobalt) (Co–Co)]* ((−)-(*R*,*R*)-**7**). A soln. of impure (−)-(*R*,*R*)-**3** (60 mg, *ca.* 36.7 μmol) and $[Co_2(CO)_8]$ (75 mg, 220 μmol) in $CHCl_3$ (5 ml) was stirred at 0° for 2 h. CC (SiO_2, $CHCl_3$) gave (−)-(*R*,*R*)-**7** (50 mg, 61%). Red film. $[\alpha]_D^{23} = -57.3$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3399 (br.), 2093*s*, 2052*s*, 2024*s*, 1699*m*, 1623*m*, 1571*m*, 1473*m*, 1216*m*, 1188*m*. ^{1}H-NMR (200 MHz, $CDCl_3$): 2.18 (*s*, 12 H); 4.57 (*d*, *AB*, $J = 12.1$, 4 H); 4.72 (*d*, *AB*, $J = 12.1$, 4 H); 5.31 (*d*, *AB*, $J = 14.3$, 4 H); 5.59 (*d*, *AB*, $J = 14.3$, 4 H); 6.51 (*s*, 4 H); 6.90–7.20 (*m*, 20 H); 7.41 (*d*, $J = 8.5$, 4 H); 7.60–7.80 (*m*, 8 H); 7.96 (*d*, $J = 8.5$, 4 H); 8.14 (*d*, $J = 7.8$, 4 H); 8.20 (*s*, 4 H); 8.25 (*s*, 4 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 24.74; 69.92; 71.78; 90.45; 105.84; 112.28; 116.53; 120.67; 121.64; 125.80; 127.24; 127.76; 128.47; 128.93; 130.56; 131.26; 135.56; 136.56; 138.34; 151.25; 154.31; 154.89; 155.31; 168.92; 199.94. FAB-MS: 2205.8 (100, MH$^+$).

*(−)-(*R*,R)-**3***. To (−)-(*R*,*R*)-**7** (71 mg, 32 μmol) in abs. THF (20 ml) was added at 0° under Ar and exclusion of light a soln. of I_2 (294 mg, 1.15 mmol) in abs. THF (12 ml), and the mixture was stirred at 0° for 4 h. The mixture was quenched with 1M $Na_2S_2O_3$ (30 ml) and extracted with CH_2Cl_2 (3 × 50 ml). The combined org. layers were washed with sat. aq. NH_4Cl soln., dried ($MgSO_4$), and evaporated *in vacuo*. CC (SiO_2, CH_2Cl_2/AcOEt 19:1 → 9:1) yielded (−)-(*R*,*R*)-**3** (25 mg, 48%). Cream-colored powder. M.p. >127° (dec.). $[\alpha]_D^{23} = -68.5$ ($c = 0.5$, $CHCl_3$). IR (KBr): 3407 (br.), 1687*m*, 1626*m*, 1570*m*, 1442*s*, 1181*w*, 1003*w*. ^{1}H-NMR (200 MHz,

$CDCl_3$): 2.19 (*s*, 12 H); 4.49 (*d*, *AB*, *J* = 14.1, 4 H); 4.58 (*d*, *AB*, *J* = 14.1, 4 H); 4.74 (*d*, *AB*, *J* = 11.8, 4 H); 4.89 (*d*, *AB*, *J* = 11.8, 4 H); 6.73 (*s*, 4 H); 7.00–7.20 (*m*, 20 H); 7.60–7.80 (*m*, 12 H); 8.02 (*d*, *J* = 8.7, 4 H); 8.16 (*d*, *J* = 8.3, 4 H); 8.21 (*s*, 4 H); 8.26 (*s*, 4 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 24.60; 62.19; 69.88; 88.11; 106.68; 112.19; 119.38; 121.47; 126.57; 126.65; 127.29; 127.60; 128.31; 129.57; 130.58; 130.90; 135.20; 136.47; 138.20; 151.06; 153.69; 154.21; 154.83; 168.79. FAB-MS: 1633.3 (2, MH^+), 817.1 (100, MH^{++}).

*(−)-(*R*)-N,N′-{[7,7′-Bis(benzyloxy)-2,2′-bis{[4-(chloromethyl)phenyl]methoxy}-1,1′-binaphthalene-6,6′-diyl]-di(pyridine-6,2-diyl)}bis(acetamide)* ((−)-(*R*)-**8**). To a suspension of (−)-(*R*)-**5** (100 mg, 0.13 mmol) and K_2CO_3 (144 mg, 104 mmol) in MeCN (30 ml) was added under Ar at 80° α,α′-dichloro-*p*-xylene (680 mg, 3.9 mmol), and the mixture was stirred at 80° for 14 h. The mixture was cooled to r.t., filtered over *Celite*, and evaporated *in vacuo*. CC (SiO_2, $CH_2Cl_2 \rightarrow CH_2Cl_2$/AcOEt 20:1) yielded (−)-(*R*)-**8** (47 mg, 37%). White solid. M.p. >117° (dec.). $[\alpha]_D^{23} = -25.4$ (*c* = 0.5, $CHCl_3$). IR (KBr): 3422 (br.), 3033*w*, 2922*w*, 2855*w*, 2355*w*, 1694*m*, 1623*s*, 1572*m*, 1444*s*, 1222*m*, 1011*m*, 811*w*. ^{1}H-NMR (200 MHz, $CDCl_3$): 2.21 (*s*, 6 H); 4.45 (*s*, 4 H); 4.67 (*s*, 4 H); 4.90 (*d*, *AB*, *J* = 12.9, 2 H); 4.97 (*d*, *AB*, *J* = 12.9, 2 H); 6.56 (*s*, 2 H); 6.90–7.30 (*m*, 18 H); 7.27 (*d*, *J* = 8.9, 2 H); 7.60–7.80 (*m*, 4 H); 7.96 (*d*, *J* = 8.9, 2 H); 8.09 (*s*, 2 H); 8.15 (*d*, *J* = 8.0, 2 H); 8.26 (*s*, 2 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 24.64; 45.88; 69.68; 70.43; 105.65; 112.02; 113.86; 119.23; 121.45; 124.93; 126.95; 127.00; 127.50; 128.28; 128.35; 128.49; 129.83; 130.98; 135.39; 136.58; 136.66; 137.87; 138.21; 151.04; 154.44; 154.68; 155.04; 168.76. FAB-MS: 1043.3 (100, MH^+). Anal. calc. for $C_{64}H_{52}Cl_2N_4O_6$ (1044.04): C 73.63, H 5.02, N 5.37; found: C 73.44, H 5.03, N 5.11.

*(+)-(*R,R*)-N,N′,N″,N‴-{[10,17,38,45-Tetrakis(benzyloxy)-3,24,31,52-tetraoxaundecacyclo[52.2.2.2^{26,29}.0^{4,13}.0^{7,12}.0^{14,23}.0^{15,20}.0^{32,41}.0^{35,40}.0^{42,51}.0^{43,48}]hexaconta-1(56),4,6,8,10,12,14,16,18,20,22,26,28,32,34,36,38,40,42,44,46,48,50,54,57,59-hexacosaene-9,18,37,46-tetrayl]tetrakis(pyridine-6,2-diyl)}tetrakis(acetamide)* ((+)-(*R,R*)-**4**). A suspension of (−)-(*R*)-**8** (41 mg, 39.2 μmol), (−)-(*R*)-**5** (30 mg, 39.2 μmol), Cs_2CO_3 (102 mg, 0.314 mmol), and NaI (29 mg, 0.196 mmol) in MeCN (50 ml) was stirred at 80° for 14 h, then cooled to r.t. CH_2Cl_2 (40 ml) was added, and the mixture filtered over *Celite*. Evaporation *in vacuo* and CC (SiO_2, CH_2Cl_2/AcOEt 9:1 → 4:1) provided (+)-(*R,R*)-**4** (63 mg, 88%). White powder. M.p. >153° (dec.). $[\alpha]_D^{23} = +84.4$ (*c* = 1.0, $CHCl_3$). IR (KBr): 3420 (br.), 1696*m*, 1624*s*, 1572*s*, 1466*m*, 1438*s*, 1216*m*, 1017*w*, 808*w*. ^{1}H-NMR (200 MHz, $CDCl_3$): 2.21 (*s*, 12 H); 4.73 (*s*, 8 H); 4.84 (*d*, *AB*, *J* = 13.3, 4 H); 4.94 (*d*, *AB*, *J* = 13.3, 4 H); 6.57 (*s*, 4 H); 6.73 (*s*, 8 H); 6.90–7.20 (*m*, 20 H); 7.60–7.80 (*m*, 12 H); 7.88 (*d*, *J* = 8.7, 4 H); 8.17 (*d*, *J* = 7.0, 4 H); 8.22 (*s*, 4 H); 8.25 (*s*, 4 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 24.74; 69.89; 70.70; 105.89; 112.18; 114.06; 119.09; 121.60; 124.98; 126.74; 127.23; 127.64; 128.23; 128.50; 129.77; 131.17; 135.52; 136.78; 136.90; 138.37; 151.27; 154.66; 155.44; 168.92. FAB-MS: 1738.2 (100, MH^+). HR-ESI-MS: 869.3347 (MH_2^{++}, $C_{112}H_{90}N_8O_{12}^{++}$; calc. 869.3334).

*(−)-(*R*)-7,7′-Bis(benzyloxy)-6,6′-dibromo-2,2′-bis{[4-(chloromethyl)phenyl]methoxy}-1,1′-binaphthalene* ((−)-(*R*)-**11**). To a suspension of (−)-(*R*)-**10** (100 mg, 0.152 mmol) [4] and K_2CO_3 (168 mg, 1.22 mmol) in MeCN (30 ml) was added under Ar at 80° α,α′-dichloro-*p*-xylene (800 mg, 4.57 mmol). The mixture was stirred at 80° for 14 h, then cooled to r.t. CH_2Cl_2 (30 ml) was added, and the mixture filtered over *Celite*. Evaporation *in vacuo* and CC (SiO_2, CH_2Cl_2/hexane 1:1) gave (−)-(*R*)-**11** (123 mg, 91%). White foam. M.p. 65–68°. $[\alpha]_D^{23} = -22.4$ (*c* = 0.5, $CHCl_3$). IR (KBr): 3022*w*, 2933*w*, 2355*w*, 1613*m*, 1492*s*, 1221 (br.), 1016*m*, 733*m*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 4.46 (*s*, 4 H); 4.57 (*d*, *AB*, *J* = 13.0, 2 H); 4.69 (*d*, *AB*, *J* = 13.0, 2 H); 4.86 (*d*, *AB*, *J* = 12.9, 2 H); 4.94 (*d*, *AB*, *J* = 12.9, 2 H); 6.24 (*s*, 2 H); 6.80–7.20 (*m*, 18 H); 7.25 (*d*, *J* = 9.2, 2 H); 7.78 (*d*, *J* = 9.2, 2 H); 8.10 (*s*, 2 H). ^{13}C-NMR ($CDCl_3$, 75 MHz): 46.01; 70.21; 70.75; 106.45; 111.98; 114.49; 119.61; 125.75; 126.82; 127.24; 127.77; 128.39; 128.57; 128.63; 132.42; 134.31; 136.35; 136.93; 137.79; 152.99; 154.84. FAB-MS: 931.9 (100, M^+, $C_{50}H_{38}{}^{81}Br^{79}BrCl_2O_4^+$). HR-FAB-MS: 930.0510 ($M^+$, $C_{50}H_{38}{}^{79}Br_2Cl_2O_4^+$; calc. 930.0515).

*(+)-(*R,R*)-N,N′,N″,N‴-{[37,46-Dibromo-10,17,38,45-tetrakis(benzyloxy)-3,24,31,52-tetraoxaundecacyclo-[52.2.2.2^{26,29}.0^{4,13}.0^{7,12}.0^{14,23}.0^{15,20}.0^{32,41}.0^{35,40}.0^{42,51}.0^{43,48}]hexaconta-1(56),4,6,8,10,12,14,16,18,20,22,26,28,32,34,36,38,40,42,44,46,48,50,54,57,59-hexacosaene-9,18-diyl]bis(pyridine-6,2-diyl)}bis(acetamide)* ((+)-(*R,R*)-**9**). A suspension of (−)-(*R*)-**5** (50 mg, 65.2 μmol), (−)-(*R*)-**11** (61 mg, 65.2 μmol), Cs_2CO_3 (169 mg, 521 μmol), and NaI (48 mg, 326 μmol) in MeCN (80 ml) was stirred under Ar at 80° for 16 h, then cooled to r.t. CH_2Cl_2 (30 ml) was added and the mixture filtered over *Celite*. Evaporation *in vacuo* and CC (SiO_2, CH_2Cl_2/AcOEt 9:1) yielded (+)-(*R,R*)-**9** (62 mg, 58%). White powder. M.p. >162° (dec.). $[\alpha]_D^{23} = +64.1$ (*c* = 0.5, $CHCl_3$). IR (KBr): 3400 (br.), 2933*w*, 2844*w*, 1699*m*, 1616*s*, 1573*s*, 1445*s*, 1221*s*, 1022*m*, 800*m*. ^{1}H-NMR ($CDCl_3$, 200 MHz): 2.22 (*s*, 6 H); 4.60–5.00 (*m*, 16 H); 6.27 (*s*, 2 H); 6.55 (*s*, 2 H); 6.61–6.76 (*m*, 8 H); 6.90–7.20 (*m*, 22 H); 7.60–7.80 (*m*, 8 H); 7.79–7.92 (*m*, 2 H); 8.07–8.19 (*m*, 6 H); 8.23 (*s*, 2 H). ^{13}C-NMR ($CDCl_3$, 75 MHz): 24.68; 69.70; 70.03; 70.54; 105.70; 106.28; 111.59; 112.00; 123.88; 114.19; 118.82; 118.91; 121.46; 124.80; 125.42; 126.53; 126.60; 126.69; 127.03; 127.49; 127.57; 127.97; 128.07; 128.34; 128.41; 129.62; 130.96; 132.24; 134.04; 135.33; 136.22; 136.34; 136.71; 138.21; 151.03; 152.68; 154.49; 154.55; 154.83; 155.26; 168.7. FAB-MS: 1627.4 (100, MH^+,

$C_{98}H_{75}{}^{81}Br^{79}BrN_4O_{10}^+$). Anal. calc. for $C_{98}H_{74}Br_2N_4O_{10}\cdot CH_2Cl_2$ (1712.44): C1 1 69.44, H 4.47, N 3.27; found: C 69.66, H 4.64, N 3.21.

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REFERENCES

[1] 'Frontiers in Excitatory Amino Acid Research', Eds. E. A. Cavalheiro, J. Lehmann, and L. Turski, Alan R. Liss, Inc., New York, 1988; 'The NMDA Receptor', Eds. J. C. Watkins and G. L. Collingridge, Oxford University Press, New York, 1989; 'Excitatory Amino Acid Receptors: Design of Agonists and Antagonists', Eds. P. Krogsgaard-Larsen and J. J. Hansen, Ellis Horwood, New York, 1992.

[2] E. Martinborough, T. Mordasini Denti, P. P. Castro, T. B. Wyman, C. B. Knobler, F. Diederich, *Helv. Chim. Acta* **1995**, *78*, 1037.

[3] R. E. Carter, T. Liljefors, *Tetrahedron* **1976**, *32*, 2915; W. R. Busing, *J. Am. Chem. Soc.* **1982**, *104*, 4829; M. Kranz, T. Clark, P. v. R. Schleyer, *J. Org. Chem.* **1993**, *58*, 3317; D. J. Cram, K. N. Trueblood, *Topics Curr. Chem.* **1981**, *98*, 43.

[4] P. Lustenberger, E. Martinborough, T. Mordasini Denti, F. Diederich, *J. Chem. Soc., Perkin Trans. 2* **1998**, 747.

[5] D. J. Cram, *Angew. Chem.* **1988**, *100*, 1041; *ibid., Int. Ed. Engl.* **1988**, *27*, 1009; D. J. Cram, *Angew. Chem.* **1986**, *98*, 1041; *ibid., Int. Ed. Engl.* **1986**, *25*, 1039.

[6] J. Monod, J. Wyman, J.-P. Changeux, *J. Mol. Biol.* **1965**, *12*, 88; D. E. Koshland Jr., G. Némethy, D. Filmer, *Biochemistry* **1966**, *5*, 365; B. Perlmutter-Hayman, *Acc. Chem. Res.* **1986**, *19*, 90.

[7] J. Rebek, Jr., *Acc. Chem. Res.* **1984**, *17*, 258; J. M. Lehn, *Angew. Chem.* **1988**, *100*, 91; *ibid., Int. Ed. Engl.* **1988**, *27*, 89.

[8] J. Rebek Jr., R. V. Wattley, T. Costello, R. Gadwood, L. Marshall, *Angew. Chem.* **1981**, *93*, 584; *ibid., Int. Ed. Engl.* **1981**, *20*, 605; J. Rebek, T. Costello, L. Marshall, R. Wattley, R. C. Gadwood, K. Onan, *J. Am. Chem. Soc.* **1985**, *107*, 7481; I. Tabushi, S. Kugimiya, M. G. Kinnaird, T. Sasaki, *ibid.* **1985**, *107*, 4192; I. Tabushi, S. Kugimiya, T. Sasaki, *ibid.* **1985**, *107*, 5159; V. J. Gatto, K. Arnold, A. M. Viscariello, S. R. Miller, G. W. Gokel, *Tetrahedron Lett.* **1986**, *27*, 327; I. Tabushi, *Pure Appl. Chem.* **1988**, *60*, 581; I. Tabushi, *Mol. Struct. Energ.* **1988**, *10*, 195; S. Kugimiya, S. Shinichi, T. Sasaki, I. Tabushi, *Nippon Kagaku Kaishi* **1988**, *4*, 473; C. A. Hunter, M. N. Meah, J. K. M. Sanders, *J. Chem. Soc., Chem. Commun.* **1988**, 694; H.-J. Schneider, D. Güttes, U. Schneider, *J. Am. Chem. Soc.* **1988**, *110*, 6449; P. D. Beer, A. Rothin, *J. Chem. Soc., Chem. Commun.* **1988**, 52; F. Ebmeyer, J. Rebek, *Angew. Chem.* **1990**, *102*, 1191; *ibid., Int. Ed. Engl.* **1990**, *29*, 1148; C. D'Silva, D. Green, *J. Chem. Soc., Chem. Commun.* **1991**, 227; Y. Kikuchi, Y. Tanaka, S. Sutarto, K. Kobayashi, H. Toi, Y. Aoyama, *J. Am. Chem. Soc.* **1992**, *114*, 10302; T. Kato, A. Hiraide, M. Suda, T. Kanbe, T. Nozoe, *Bull. Chem. Soc. Jpn.* **1995**, *68*, 3145; M. Takeuchi, T. Imada, S. Shinkai, *J. Am. Chem. Soc.* **1996**, *118*, 10658; M. Takeuchi, T. Imada, S. Shinkai, *Angew. Chem.* **1998**, *110*, 2242; *ibid., Int. Ed. Engl.* **1998**, *37*, 2096.

[9] I. Willner, Z. Goren, *J. Chem. Soc., Chem. Commun.* **1983**, 1469; A. D. Hamilton, P. Kazanjian, *Tetrahedron Lett.* **1985**, *26*, 5735; C. J. van Staveren, D. E. Fenton, D. N. Reinhoudt, J. van Eerden, S. Harkema, *J. Am. Chem. Soc.* **1987**, *109*, 3456; J. H. Griffin, P. B. Dervan, *ibid.* **1987**, *109*, 6840; H.-J. Schneider, D. Ruf, *Angew. Chem.* **1990**, *102*, 1192; *ibid., Int. Ed. Engl.* **1990**, *29*, 1159; M. T. Reetz, C. M. Niemeyer, K. Harms, *Angew. Chem.* **1991**, *103*, 1515; *ibid., Int. Ed. Engl.* **1991**, *30*, 1472; P. Scrimin, P. Tecilla, U. Tonellato, N. Vignaga, *J. Chem. Soc., Chem. Commun.* **1991**, 449; R. P. Sijbesma, R. J. M. Nolte, *J. Am. Chem. Soc.* **1991**, *113*, 6695; G. Gagnaire, A. Jeunet, J.-L. Pierre, *Tetrahedron Lett.* **1991**, *32*, 2021; R. P. Sijbesma, R. J. M. Nolte, *J. Phys. Org. Chem.* **1992**, *5*, 649; H.-J. Schneider, F. Werner, *J. Chem. Soc., Chem. Commun.* **1992**, 490; C. A. Hunter, D. H. Purvis, *Angew. Chem.* **1992**, *104*, 779; *ibid., Int. Ed. Engl.* **1992**, *31*, 792; Y. Kobuke, Y. Satoh, *J. Am. Chem. Soc.* **1992**, *114*, 789; J.-L. Pierre, G. Gagnaire, P. Chautemps, *Tetrahedron Lett.* **1992**, *33*, 217; M. Inouye, T. Konishi, K. Isagawa, *J. Am. Chem. Soc.* **1993**, *115*, 8091; T. Nabeshima, T. Inaba, N. Furukawa, T. Hosoya, Y. Yano, *Inorg. Chem.* **1993**, *32*, 1407; C. A. Gleave, I. O. Sutherland, *J. Chem. Soc., Chem. Commun.* **1994**, 1873; Y. Wang, A. Ueno, F. Toda, *Chem. Lett.* **1994**, 167; A. M. Costero, M. Pitarch, *J. Org. Chem.* **1994**, *59*, 2939; M. Saadioui, Z. Asfari, J. Vicens, *Tetrahedron Lett.* **1997**, *38*, 1187.

[10] M. F. Perutz, *Ann. Rev. Biochem.* **1979**, *48*, 327; M. F. Perutz, G. Fermi, B. Luisi, B. Shaanan, R. C. Liddington, *Acc. Chem. Res.* **1987**, *20*, 309.

[11] R. S. Dickson, P. J. Fraser, *Adv. Organomet. Chem.* **1974**, *12*, 323; P. Magnus, P. Carter, J. Elliott, R. Lewis, J. Harling, T. Pitterna, W. E. Bauta, S. Fortt, *J. Am. Chem. Soc*. **1992**, *114*, 2544; F. Diederich, Y. Rubin, O. L. Chapman, N. S. Goroff, *Helv. Chim. Acta* **1994**, *77*, 1441.
[12] R. A. Binstead, A. D. Hungerbühler, 'SPECFIT v. 2.10', Spectrum Software Associates, Chapel Hill, NC 27515-4494, 1997.
[13] M. Blanda, J. Horner, M. Newcomb, *J. Org. Chem.* **1989**, *54*, 4626.
[14] K. A. Connors, 'Binding Constants: The Measurement of Molecular Complex Stability', Wiley, New York, 1987.
[15] L. D. Byers, *J. Chem. Educ.* **1977**, *54*, 352.
[16] R. J. Pieters, J. Cuntze, M. Bonnet, F. Diederich, *J. Chem. Soc., Perkin Trans. 2* **1997**, 1891.

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