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Extraction of Radio-Labelled Xanthine Derivatives by Artificial Receptors: Deep Insight into the Association Behaviour

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Dedicated to Professor Dr. Peter Mühl on the occasion of his 75th birthday

Abstract: Association constants for the interaction of almost insoluble substrates with triphenylene ketal-based receptors in toluene have been determined by means of an extraction method employing the corresponding radio-labelled substrates. Flexible and more polar receptors tend to aggregate and exhibit inferior extraction qualities. Binding constants in toluene were found to be in the range 10^5–$10^7\,M^{-1}$, which is significantly higher than in dichloromethane. X-ray analyses indicate the direct participation of a water molecule in the binding process, which may account for the surprisingly small effect of moisture in the solvent on the stability of the complexes.

Keywords: caffeine · extraction · isotopic labelling · receptors · supramolecular chemistry

Introduction

The strength of binding and the selectivity for particular substrates are fundamental characteristics of affinity systems.[1] Association constants for the interaction of such receptors with the corresponding substrates are usually determined by various spectroscopic techniques.[2] Most commonly, NMR titrations are exploited for the determination of these association constants.[3]

The sensitivity of this technique requires that the experiments are performed within a specific concentration range. If under these conditions the affinity is either extremely high or extremely low, little meaningful information may be gleaned. Therefore, this technique cannot be applied to such "extreme" systems. Furthermore, with substrates that show extraordinarily low solubility in a given solvent, the problem arises that the total amount of available substrate in solution can barely be determined.

Of special interest are systems pertaining to biologically active and technically relevant target molecules.[4] Among such molecules, caffeine has been the subject of increased attention in recent years because of the broad and emerging use of this particular alkaloid in nutrient technology, cosmetics, and pharmaceuticals.[5] In general, caffeine is considered to be harmless to adults, whereas there are severe concerns about its action on young children and pregnant women, including the risk of foetal death.[6] Several chemoreceptors capable of binding this alkaloid have been designed.[7]

Recently, we reported a novel concept for the molecular recognition of caffeine (**2**) and related compounds, employing receptors **1** with a cleft-like structure (Scheme 1). A clear picture of the binding of caffeine and related compounds was established, wherein hydrogen bonding to both carbonyl functions and the heterocyclic nitrogens of caffeine is crucial.[8] Among the known artificial caffeine receptors, the triphenylene ketal-based system exhibits the highest affinity for the substrate. Determination of the association constant of **1c**·**2** by ^{1}H NMR spectroscopy resulted in a value of $(37\,000 \pm 2000)\,M^{-1}$,[9] whereas titrations based on

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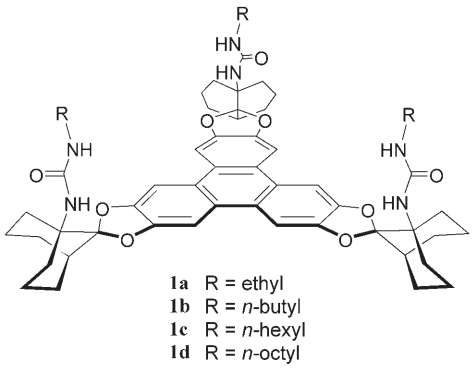
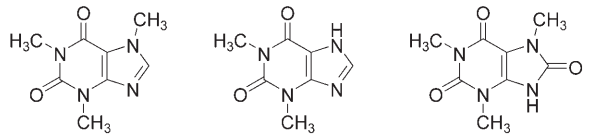




2 3 4

Scheme 1. Prototype artificial caffeine receptor **1** and some substrates: caffeine (**2**), theophylline (**3**), and 1,3,7-trimethyluric acid (**4**).

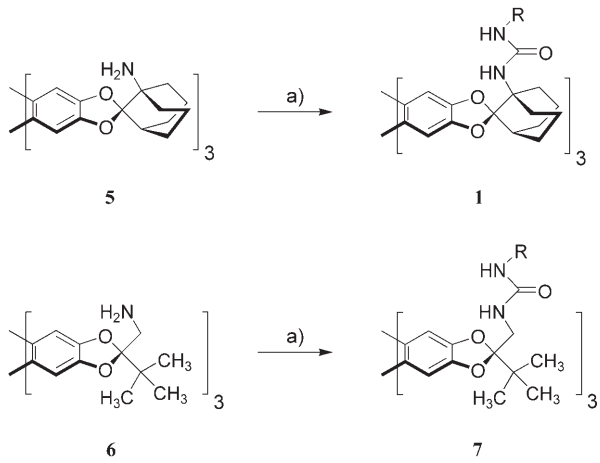
fluorescence spectroscopy revealed a slightly lower affinity, yielding an association constant of $(28000 \pm 3000)\,M^{-1}$.[10] The affinity of chirally modified receptors towards caffeine in dichloromethane has been investigated by CD spectroscopy, which yielded values of the order of $50000\,M^{-1}$.[11] As regards other substrates, none of the aforementioned protocols could be applied to 1,3,7-trimethyluric acid (**4**) due to its very limited solubility in organic media. Likewise, theophylline (**3**) also exhibits low solubility, and thus represents another challenging substrate.

A mass spectrometric approach to assessing the supramolecular interaction indicated that caffeine, its nor-derivatives and their metabolites, such as uric acid derivatives, show significantly different affinities towards **1**. However, these studies yielded only qualitative information about the strength of binding to these respective substrates.[12] Therefore, more quantitative data that would allow comparison of the affinities of such substrates are highly desirable. Liquid–liquid partition of radio-labelled substrates between aqueous/organic, two-phase systems ought to give precise and reliable quantitative data.[13] These could then be easily analysed by using established software to yield the desired thermodynamic data such as extraction and association constants.[14] Liquid scintillation counting allows precise determinations, even at low concentrations of the analyte in both phases, and should therefore yield reliable association constants.

In addition, this method provides a powerful tool for demonstrating the binding efficacy of triphenylene ketal-based receptors in challenging two-phase systems. This can be expected to yield valuable information to assist in the development of caffeine assays that might involve "immiscible" phases (e.g., test strips).

Results and Discussion

Syntheses: The employed receptors were assembled according to known protocols. Triphenylene ketals with a rigid backbone were synthesised in a ten-step sequence that has been described previously.[15] The final, divergent transformation involves treating the triamino platform **5** with the appropriate isocyanates so as to generate the corresponding threefold functionalised systems **1** bearing urea moieties (Scheme 2, Table 1). The more flexible, and therefore less pre-organised host molecules **7** were obtained by a similar sequence.[16]



5 1

6 7

Scheme 2. a) Isocyanate (6 equiv), NEt_3, CH_2Cl_2 (42–87%).

Table 1. Synthesised receptors based on triphenylene ketals.

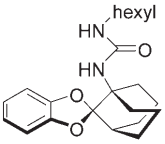
Entry	Receptor	R-	Yield [%]
1	**1a**	H_3C-CH_2-	62[a]
2	**1b**	$H_3C(-CH_2)_2$-CH_2-	60
3	**1c**	$H_3C(-CH_2)_4$-CH_2-	48[9]
4	**1d**	$H_3C(-CH_2)_6$-CH_2-	56
5	**1e**	phenethyl-	79[8]
6	**1f**	menthyl-	63[8]
7	**1g**	8-methylmenthyl-	47[8]
8	**1h**	8-phenylmenthyl-	87[8]
9	**1i**	4-bromophenyl-	62[a]
10	**7c**	$H_3C(-CH_2)_4$-CH_2-	46
11	**7d**	$H_3C(-CH_2)_6$-CH_2-	42

[a] Purified by crystallisation from ethyl acetate.

It was found that these host molecules could be most efficiently purified by column chromatography on silica employing mixtures of ethyl acetate and cyclohexane as eluents. Because of their strong hydrogen-donor qualities, these systems are almost invariably associated with solvent molecules. Consequently, no reproducible elemental analysis could be obtained. The major analytical tools for confirming the structures were NMR spectroscopy and HRMS.

The rigid receptors bearing simple alkyl substituents on their urea moieties (**1a**–**1d**) were obtained as colourless solids upon concentration of solutions in ethyl acetate or by crystallisation from ethanol. In an analogous manner, the 4-

bromophenyl derivative **1i** was synthesised. The preparation of chiral receptors **1e**–**1h**, based on the rigid platform, has been described in detail elsewhere.[9,15] To underline the importance of appropriately arranged multiple binding sites, a corresponding subunit **8** of **1c** was prepared as previously reported and was also employed in the affinity testing.[15]



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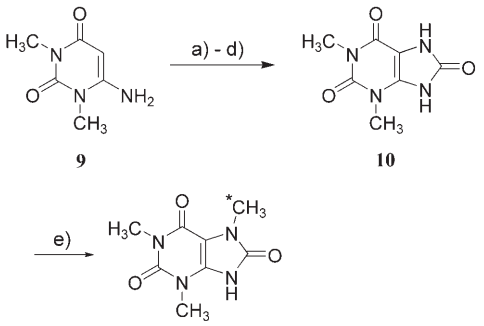
Applying the synthetic protocol to the more flexible platform **6** yielded the host molecules **7**, which are less lipophilic than the rigid congeners (Scheme 2).

The polarity of the homologues **1a**–**1d** decreases tremendously with increasing length of the alkyl chain. More elaborate and bulkier substituents attached to the urea groups shield the polar recognition site of the receptor from the top. Thus, receptors **1e**–**1h** are freely soluble in organic media. The rigid nature of all of the constituent moieties of **1i** renders this particular host poorly soluble in most organic solvents.

^{14}C-labelled caffeine and ^{3}H-labelled theophylline are commercially available. ^{14}C-labelled 1,3,7-trimethyluric acid was synthesised by methylation of 1,3-dimethyluric acid using ^{14}C-labelled methyl iodide (Scheme 3). 1,3-Dimethyluric acid (**10**) was obtained by way of a four-step sequence from commercially available **9**, as described in the literature.[17] Radio-labelled **11** was purified by column chromatography to yield a product with a specific activity of 3.5 mCi mg^{-1}.



Scheme 3. a) KNO_2, CH_3COOH, 95%; b) $Na_2S_2O_4$, NH_3, 89%; c) ClCOOEt, H_2O, 99%; d) 235 °C, neat, 89%; e) NaOH, $^{14}CH_3I$, acetone/H_2O, 22%.

Liquid–liquid extraction: The artificial receptors were evaluated for their extraction abilities towards the substrates caffeine (**2**), theophylline (**3**), and 1,3,7-trimethyluric acid (**4**) in aqueous/organic systems (substrate/buffer/H_2O//receptor/organic solvent). Several organic solvents were tested for use in the distribution experiments. The use of chlorinated solvents such as chloroform and dichloromethane resulted in predominant extraction of caffeine by the solvent itself. This would have led to a significant error in the calculation of association constants of complexes formed in the organic phase had these solvents been used.[18] Next, we considered a 1:1 mixture of hexane and ethyl acetate, but most of the studied receptors proved to be insufficiently soluble in this medium to perform the desired experiments.[19] Toluene provided a good compromise, in that it readily dissolved the supramolecular hosts and resulted in well-defined phase separation. The simple overall equilibrium for the extraction of xanthine substrates by a neutral supramolecular receptor is illustrated schematically in Figure 1.

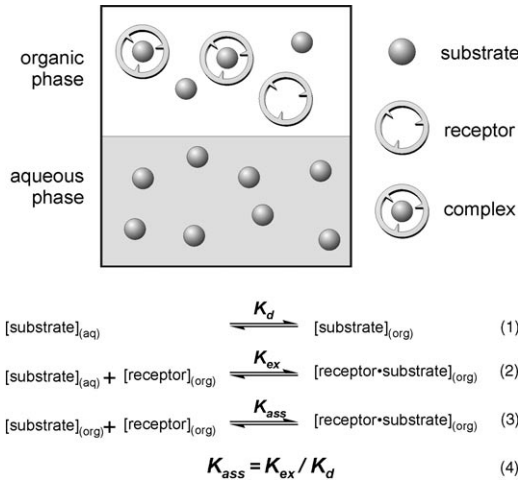


Figure 1. Determination of association constants by extraction methods.

The distribution constant K_d for the substrate alone in the absence of the receptor [Eq. (1)] and the extraction constant K_{ex} [Eq. (2)] in the presence of the receptor can easily be derived by measuring the concentration of the substrate in each phase by using radiolabelled xanthine derivatives.[13] The association constant, K_{ass}, which describes the binding of xanthine derivatives to the receptor in the organic phase [Eq. (3)] can then be calculated according to Equation (4).

First, the distribution constants for caffeine, theophylline, and 1,3,7-trimethyluric acid in the system toluene//water were determined (Table 2).

The distribution constants clearly reveal that the xanthine derivatives preferentially reside in the aqueous layer. In particular, 1,3,7-trimethyluric acid remains almost completely in the aqueous phase.

Extraction experiments using the lipophilic receptor subunit **8** in toluene indicated no transfer of xanthine derivatives into the organic phase, even at high concentrations of this ligand.[20] This reveals that no extraction is possible by the

Table 2. Distribution constants K_d of caffeine, theophylline, and 1,3,7-trimethyluric acid for the system toluene//water ($c_{xanthine} = 1 \times 10^{-4}$ M in HEPES/NaOH buffer, pH 7.4).

Entry	Substrate	K_d[a]
1	caffeine	$5.38 \times 10^{-1} \pm 1.6 \times 10^{-2}$
2	theophylline	$1.32 \times 10^{-2} \pm 1.6 \times 10^{-3}$
3	1,3,7-trimethyluric acid	$2.38 \times 10^{-4} \pm 3.1 \times 10^{-5}$

[a] Mean values from ten independent distribution experiments.

self-assembly of three subunits **8** around a substrate molecule. Similar results were obtained when Z-protected **5** and the *anti*,*anti*,*syn*-congener of **1c** were employed.[15] Host molecules bearing ethyl groups on their urea moieties (**1a**) were found to self-aggregate, leading to precipitation during extraction experiments. Consequently, the determination of an association constant was not possible. However, as depicted in Figures 2–4, the extraction behaviour of all of the other receptors investigated was found to correspond quite well with the formation of 1:1 complexes with caffeine, theophylline, and 1,3,7-trimethyluric acid, respectively. This is reflected by the respective $\log D$ versus $\log c_{\text{receptor}}$ plots, which show lines with a uniform slope of unity.[21]

The receptors bearing the bulky 8-methylmenthyl (**1g**) and phenethyl moieties (**1e**) showed the highest extraction constants for the xanthine substrates used. Increasing alkyl chain length led to a slight increase in the extraction efficiency (**1d** > **1c** > **1b**). Receptor **1h** displayed a significantly lower affinity as compared to the other systems examined. Simple molecular modelling investigations indicate that the phenyl substituents of **1h** occupy substantial amounts of the space inside the cavity and thus impede the formation of the complex.[15] For all of the other receptors **1**, the enthalpic contribution associated with the formation of the hydrogen bonds should be comparable in each case. Therefore, solvation and changes in the molecular entropy of the host can be expected to have critical effects on the binding efficiencies.[22] Additionally, self-aggregation of the receptors may interfere with the binding. Branched or highly flexible side chains may reduce the tendency for this self-assembly.

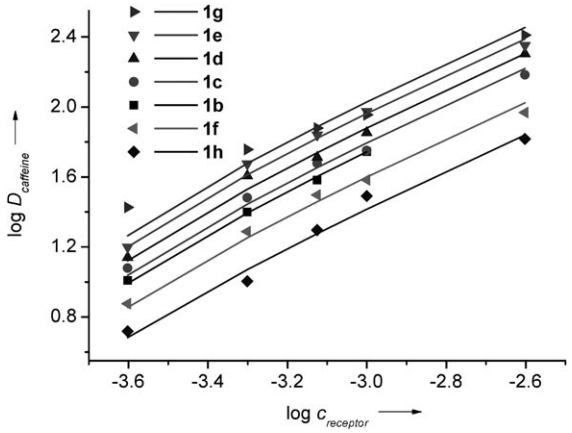


Figure 2. Variation of $\log D$ with receptor concentration for the extraction of ^{14}C-labelled caffeine (**2**) with **1b**–**1h** at ambient temperature in toluene; $c_{\text{caffeine}} = 1 \times 10^{-4}$ M, pH 7.4 (HEPES/NaOH), $t_{\text{extraction}} = 30$ min.

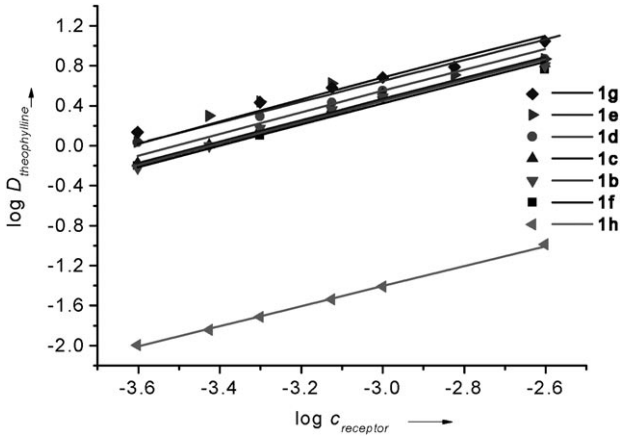


Figure 3. Variation of $\log D$ with receptor concentration for the extraction of ^{3}H-labelled theophylline (**3**) with **1b**–**1h** at ambient temperature in toluene; $c_{\text{theophylline}} = 1 \times 10^{-4}$ M, pH 7.4 (HEPES/NaOH), $t_{\text{extraction}} = 30$ min.

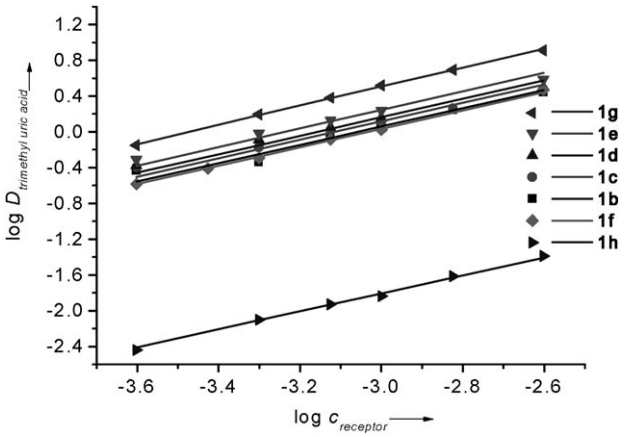


Figure 4. Variation of $\log D$ with receptor concentration for the extraction of ^{14}C-labelled 1,3,7-trimethyluric acid (**4**) with **1b**–**1h** at ambient temperature in toluene; $c_{\text{trimethyluric acid}} = 1 \times 10^{-4}$ M, pH 7.4 (HEPES/NaOH), $t_{\text{extraction}} = 30$ min.

Of the three substrates examined, the least lipophilic, 1,3,7-trimethyluric acid, displayed the largest boost in its transfer into the organic phase when a receptor was present. Association constants (K_{ass}) for caffeine, theophylline, and 1,3,7-trimethyluric acid with the respective receptors **1b**–**1h** in toluene, calculated from the corresponding extraction constants K_{ex} and distribution constants K_{d}, are summarised in Table 3 and are plotted in Figure 5.

Surprisingly, the association constants for the binding of caffeine by the receptor **1c** determined by the distribution experiments indicate a much higher affinity than the data derived from ^{1}H NMR titration experiments performed in

Table 3. Association constants of caffeine, theophylline, and 1,3,7-trimethyluric acid with various hosts in toluene, as determined by extraction studies.

Entry	Receptor	Association constant/$\log K_{\text{ass}}$		
		caffeine (**3**)	theophylline (**4**)	trimethyluric acid (**5**)
1	**1b**	5.05 ± 0.01	5.34 ± 0.02	6.71 ± 0.03
2	**1c**	5.07 ± 0.02	5.38 ± 0.01	6.77 ± 0.02
3	**1d**	5.19 ± 0.01	5.46 ± 0.04	6.81 ± 0.03
4	**1e**	5.28 ± 0.01	5.47 ± 0.03	6.90 ± 0.02
5	**1f**	4.91 ± 0.02	5.36 ± 0.02	6.68 ± 0.01
6	**1g**	5.34 ± 0.04	5.60 ± 0.02	7.17 ± 0.01
7	**1h**	4.72 ± 0.02	3.48 ± 0.01	4.82 ± 0.01

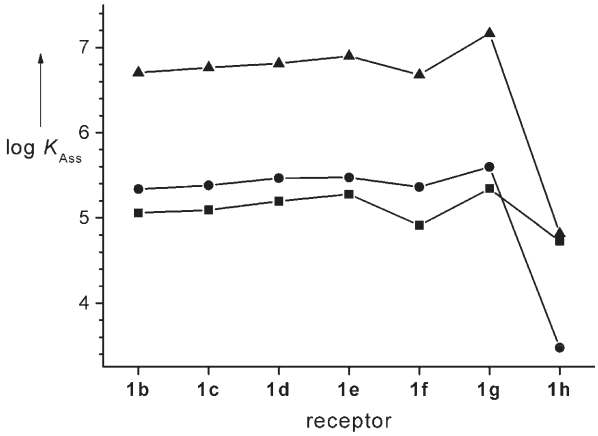


Figure 5. Graphical representation of the binding constants involving caffeine (**2**, ■), theophylline (**3**, ●), and 1,3,7-trimethyluric acid (**4**, ▲).

CD_2Cl_2. This surprising result prompted us to also determine the binding constants for receptors **1c** and **1d** with caffeine by our recently reported fluorescence approach.[10] To obtain controlled conditions, anhydrous toluene was used as solvent. The concentration of the receptors was 1 μM. The observed affinities ($\log K$) under these conditions were 5.9 ± 0.1 and 5.8 ± 0.1 for receptors **1c** and **1d**, respectively. These values are even higher than those derived from the extraction experiments. It seems reasonable to attribute this difference to the amount of water dissolved in the organic phase. Titration of **1c** with caffeine in aqueous toluene gave a slightly lower association constant of 5.6 ± 0.1. However, the influence of water on the stability of this complex proved surprisingly low. Entropic and solvation effects are the subject of ongoing investigations. Nevertheless, the molecular structures of several of the complexes reveal a more intimate relationship between water and the complex, which may explain the unexpected stability even in aqueous toluene.

The molecular structure of the complex consisting of **1c** and caffeine revealed that the cavity of the receptor is slightly too large for this substrate.[9] Co-crystallisation of caffeine (**2**) and the 4-bromophenyl-substituted receptor (**1i**) in the presence of water yielded crystals suitable for X-ray analysis. The molecular structure reveals that an additional water molecule is bound beside the caffeine (Figure 6). Compared to the solid-state structure of a water-free caffeine–receptor complex,[9] the guest is shifted laterally and the resulting gap is filled by the water molecule. To accommodate these two guest molecules within the cavity, the three arms of the receptor need to open up. The formation of the complex should thus be favoured, since this opening of the arms relieves torsional strain in the side groups.[23] Through this rearrangement, both the distal and the proximal N-H functions of the urea moiety become geometrically capable of hydrogen bonding, which should further contribute to the stability of the complex. The carbonyl groups of **2** establish direct interactions with these urea moieties. Moreover, N-9 of caffeine binds to the proton of the water molecule, which in turn is involved in hydrogen bonding with the receptor. All of these enthalpic contributions may compensate the entropic costs associated with the formation of a ternary complex and therefore lead to significant stability.

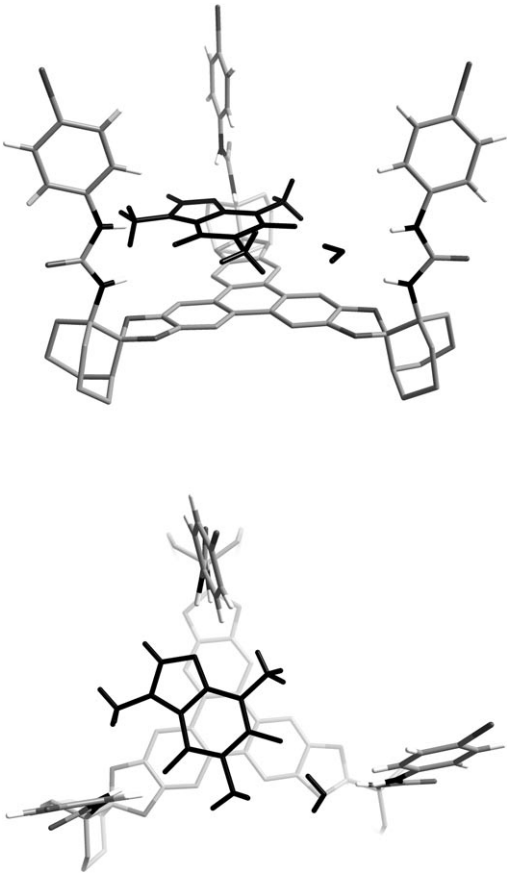


Figure 6. X-ray structure of 4-bromophenyl-substituted receptor **1i** with water and caffeine (both in black); top: side view; bottom: top view.

1,3,7-Trimethyluric acid bears three carbonyl functions as hydrogen-bond acceptors in a slightly different arrangement. Two of these carbonyl functions are positioned close to the long "axis" of the purine backbone, resulting in a larger distance between them. This places at least two of the binding units in a rather upright, strain-free orientation. As discussed above, this reorientation makes the proximal N-H available for hydrogen bonding, thereby increasing the stability. Earlier investigations of the binding behaviour of **4** by means of NMR competition experiments in dichloromethane indicated binding constants in excess of $200000\,M^{-1}$.[9] However, it was not possible to determine exact values for this affinity. The extraction experiments revealed extremely strong binding of **4** to the receptors **1b**–**1g**, with association constants ranging from $5000000\,M^{-1}$ to $14700000\,M^{-1}$. Due to the insolubility of **4** in toluene, the application of fluorescence spectroscopy for further investigations was not feasible. Therefore, the contribution of a damp environment to these extreme association constants could not be determined. However, a crystal suitable for X-ray analysis could only be obtained under damp conditions. Once again, the

molecular structure revealed a ternary complex incorporating a water molecule. In this case, the water molecule is bound to the carbonyl function that is closer to the short "axis" of the backbone. As a result, the hydrogen-bond acceptors that interact with the urea moieties are positioned in an almost regular triangle, which matches the C_3 symmetry of the receptor (Figure 7).

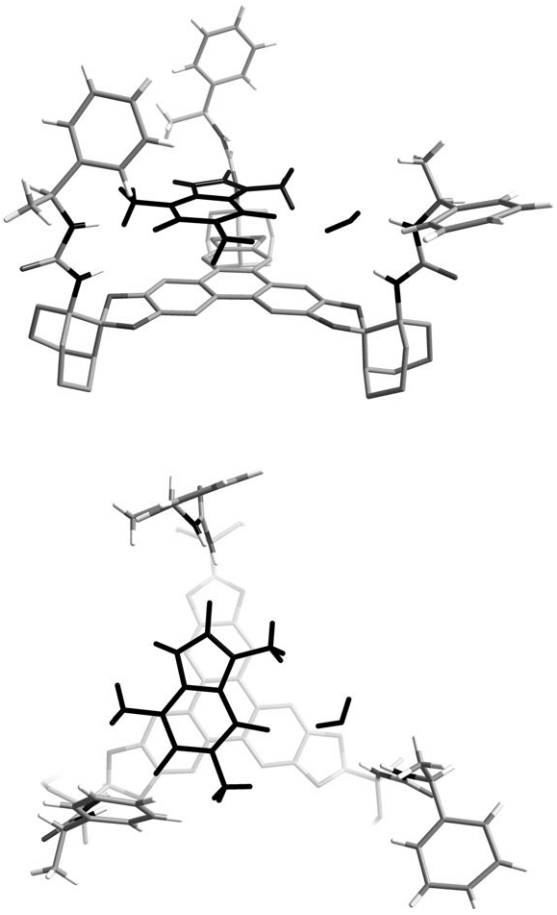


Figure 7. X-ray structure of **1e** with water and 1,3,7-trimethyluric acid (both in black); top: side view; bottom: top view.

It is known that bound water can stabilise supramolecular aggregates by hydrogen bonding, by filling up empty spaces, or by screening off electrostatic repulsions. Therefore, cobound water controls the stability, structure, dynamics, affinities, and functions of biological systems.[24] In some cases, the presence of water enhances the affinity of supramolecular systems. In particular, the binding of sugars to porphyrins[25] or to artificial C_3-symmetric receptors is strongly supported under damp conditions.[26]

However, the water is not usually detected. In some cases, it can be identified by NMR techniques,[27] as demonstrated by Davis et al. for a water-mediated ion-pair receptor.[28] According to the concept of Adrian and Wilcox, rigid receptors based on triphenylene ketals **1a**–**1i** may be considered as non-closable receptors.[29] Consequently, their binding should be affected by environmental changes. Apparently, the presence of water does not suppress the affinity for the corresponding substrate.

The involvement of the water molecule in the binding process could not be verified by ^{1}H NMR techniques. Only broad signals were recorded in damp solvents ($CDCl_3$, CH_2Cl_2 or C_6D_6). Switching to lower temperatures in order to avoid dynamic processes on the time scale of ^{1}H NMR resulted in freezing out of the water, whereupon the resulting spectra resembled those obtained in previous investigations conducted under anhydrous conditions.

Distribution studies were also carried out with the more flexible receptors **7c** and **7d**. The corresponding $\log D_{\text{caffeine}}$ versus $\log c_{\text{receptor}}$ plots show a slope much lower than unity, which indicates an aggregation of the receptor. In addition, the absolute extraction of the caffeine is much lower than with the rigid systems.[30] Therefore, the determination of an association constant by extraction experiments would be very costly and complicated. As is evident from the crystal structure of the more flexible receptor **7c**, the urea functions protrude laterally from the side arms of the system (Figure 8). This is in sharp contrast to receptors **1a**–**1h**, which exhibit highly pre-organised urea moieties that point towards the centre of the cavity. The C–N bond close to the platform in receptor **7c** is rotated through about 90°, creating a receptor geometry that supports the formation of aggregates.

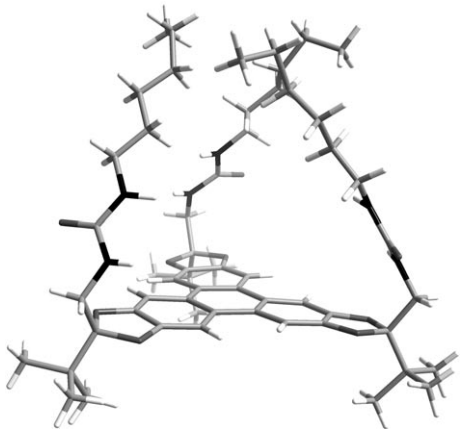


Figure 8. Molecular structure of receptor **7c** determined by X-ray analysis.

Since rigid receptors facilitate good extraction of xanthines into the organic phase, the stability of the supramolecular interaction can be easily investigated. We studied the stability of the receptor **1c** and its caffeine complex towards acidic media. Under acidic conditions, cleavage of the ketals and therefore loss of the binding units is expected. Since the monomeric subunit **8** proved inactive in the extraction assays, the extraction constant is a good criterion for monitoring the integrity of the receptor. In the presence of half-concentrated hydrochloric acid (6 M), a $t_{1/2}$ of 1 h was determined for the hydrolytic decomposition of **1c**·**2** at ambient temperature. This reveals the robust nature of the

complex and especially of the triphenylene ketal-based receptors, despite the ketal moieties involved.

In conclusion, extraction of radio-labelled neutral substrates by host molecules represents not only an alternative access to association constants, but also provides a good insight into the binding mode. In particular, the affinities of substrates that are virtually insoluble in organic solvents can be determined in a reliable manner. The extraction experiments are very sensitive to aggregational phenomena. As indicated by two X-ray analyses, water seems to play a specific role in the binding of caffeine or 1,3,7-trimethyluric acid with the triphenylene ketals **1**. Furthermore, it has been demonstrated that these receptors are capable of extracting oxopurines from aqueous media in a biphasic system with very high affinities. This is a key prerequisite for the further development of caffeine detection systems based on a dipstick model.

Experimental Section

General remarks: All reagents used were of analytical grade. Solvents were desiccated by standard methods as necessary. ^{14}C-labelled caffeine and ^{3}H-labelled theophylline were purchased from Biotrend Chemikalien GmbH, Cologne. $^{14}CH_3I$ was supplied by Amersham Biosciences. NMR spectra were recorded at 25 °C on a Bruker DMX 300 or DMX 400 spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) relative to traces of [D$_5$]DMSO or $CHCl_3$ in the corresponding deuterated solvents. Mass spectra, including HRMS (positive mode), were obtained on a Quattro LC (Waters-Micromass) or FT-ICR spectrometer (Bruker APEX IV) employing ESI.

Synthesis

General procedure for the synthesis of the receptors: A thoroughly dried 100-mL flask with an argon inlet inserted through a septum was charged with the all-*syn* triamine (**5** or **6**; 1 mmol) and dichloromethane (40 mL). The solution was stirred at 0 °C for 10 min, after which triethylamine (3 mmol) and the appropriate alkyl isocyanate (6 mmol) were added dropwise. The resulting mixture was stirred at 0 °C for 1.5 h. The reaction was then quenched by partitioning the mixture between 0.1 M hydrochloric acid (100 mL) and ethyl acetate (50 mL) at 0 °C. The separated aqueous layer was extracted with ethyl acetate (3×50 mL). The combined organic layers were washed with water (3×50 mL) and brine (2×50 mL) and dried over $MgSO_4$. After evaporation of the volatile components, the crude product was purified by column chromatography or crystallisation from ethanol.

Ethyl-substituted receptor 1a: The compound was obtained as a colourless solid from triamine **5** (62 %). ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25 °C, TMS): δ = 0.85 (t, ^3J(H,H) = 7 Hz, 6H; CH_3), 1.59–1.69 (m, 12H; bicycle), 1.99–2.19 (m, 21H; bicycle), 2.83–2.90 (m, 12H; bicycle and CH_2), 5.67 (s, 3H; N-H proximal), 6.03 (t, ^3J(H,H) = 5 Hz, 3H; N-H distal), 8.17 ppm (s, 6H; triphenylene); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 25 °C, TMS): δ = 15.29, 20.47, 27.81, 33.55, 34.15, 37.20, 55.78, 102.20, 120.89, 124.26, 147.15, 157.13 ppm; HRMS: calcd for $C_{54}H_{67}N_6O_9^+$ 946.4964 [M+H$^+$]; found 943.4962; calcd for $C_{54}H_{66}N_6NaO_9^+$ 965.4783 [M+Na$^+$]; found 965.4784; calcd for $C_{54}H_{67}KN_6O_9^+$ 981.4523 [M+K$^+$]; found 981.4526.

Butyl-substituted receptor 1b: The compound was obtained as a colourless solid from triamine **5** (60 %). ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25 °C, TMS): δ = 0.77 (t, ^3J(H,H) = 7 Hz, 6H; CH_3), 1.09–1.27 (m, 3H; bicycle), 1.50–1.78 (m, 12H; bicycle), 1.90–2.15 (m, 18H; bicycle), 2.18 (s, 3H), 2.81–2.95 (m, 12H; bicycle), 5.69 (s, 3H; N-H proximal), 6.06 ppm (t, ^3J(H,H) = 5 Hz, 3H; N-H distal); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 25 °C, TMS): δ = 13.54, 19.47, 20.46, 27.80, 31.88, 34.15, 37.12, 38.28, 38.39, 55.70, 102.28, 120.89, 124.29, 147.12, 157.24 ppm; HRMS: calcd for $C_{60}H_{79}N_6O_9^+$ 1027.5903 [M+H$^+$]; found 1027.5904; calcd for $C_{60}H_{78}N_6NaO_9^+$ 1049.5722 [M+Na$^+$]; found 1049.5736; calcd for $C_{60}H_{78}KN_6O_9^+$ 1065.5462 [M+K$^+$]; found 1065.5458.

Octyl-substituted receptor 1d: The compound was obtained as a colourless solid from triamine **5** (56 %). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 0.83 (t, ^3J(H,H) = 7 Hz, 9H; CH_3), 1.12–1.25 (m, 36H), 1.70–1.73 (m, 12H), 2.13–2.30 (m, 21H; bicycle), 2.84–2.93 (m, 12H), 4.38 (br, 3H; N-H distal), 4.59 (s, 3H; N-H proximal), 7.78 ppm (s, 6H; triphenylene); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C, TMS): δ = 14.04, 21.00, 22.59, 26.87, 28.30, 29.16, 30.06, 31.74, 34.32, 38.08, 40.37, 57.60, 101.62, 122.04, 124.04, 124.59, 147.46, 157.57 ppm; HRMS: calcd for $C_{72}H_{103}N_6O_9^+$ 1195.7781 [M+H$^+$]; found 1195.7777; calcd for $C_{72}H_{102}N_6NaO_9^+$ 1217.7600 [M+Na$^+$]; found 1217.7606; calcd for $C_{72}H_{102}KN_6O_9^+$ 1233.7326 [M+K$^+$]; found 1233.7340.

4-Bromophenyl-substituted receptor 1i: The compound was obtained as a light-yellow solid from triamine **5** (62 %). ^{1}H NMR (300 MHz, [D$_6$]DMSO, 25 °C, TMS): δ = 1.64–1.71 (m, 12H; bicycle), 2.08–2.23 (m, 21H; bicycle), 2.92–2.95 (m, 6H; bicycle), 6.23 (s, 3H; N-H proximal), 7.23 (d, ^3J(H,H) = 9 Hz, 6H), 7.32 (d, ^3J(H,H) = 9 Hz, 6H), 8.26 ppm (s, 6H; triphenylene), 8.73 (s, 3H; N-H distal); ^{13}C NMR (75 MHz, [D$_6$]DMSO, 25 °C, TMS): δ = 20.39, 27.69, 33.84, 36.99, 56.14, 102.54, 112.06, 119.22, 120.67, 124.43, 131.25, 139.71, 147.09, 153.97 ppm; HRMS: calcd for $C_{66}H_{63}Br_3N_6O_9$ 1321.2279 [M+H$^+$]; found 1321.2278; calcd for $C_{66}H_{63}Br_3N_6NaO_9^+$ 1343.2099 [M+Na$^+$]; found 1343.2064; calcd for $C_{66}H_{63}Br_3KN_6O_9^+$ 1359.1838 [M+K$^+$]; found 1359.1803.

Hexyl-substituted receptor 7c: The compound was obtained as a light-yellow solid from triamine **6** (46 %). ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C, TMS): δ = 0.75 (t, ^3J(H,H) = 7 Hz, 6H), 1.07–1.19 (m, 45H), 2.84 (dt, ^3J(H,H) = 6 Hz, ^3J(H,H) = 7 Hz, 6H), 3.72 (d, ^3J(H,H) = 6 Hz, 6H), 5.74 (t, ^3J(H,H) = 6 Hz, 3H), 5.78 (t, ^3J(H,H) = 7 Hz, 3H), 7.95 ppm (s, triphenylene; 6H); ^{13}C NMR (500 MHz, [D$_6$]DMSO, 25 °C, TMS): δ = 13.71, 21.90, 24.18, 25.83, 29.66, 30.82, 39.78, 40.12, 41.36, 100.05, 122.21, 123.77, 148.67, 157.67 ppm; HRMS: calcd for $C_{57}H_{85}N_6O_9^+$ 997.6373 [M+H$^+$]; found 997.6370; calcd for $C_{57}H_{84}N_6NaO_9^+$ 1019.6192 [M+Na$^+$]; found 1019.6192; calcd for $C_{57}H_{84}KN_6O_9^+$ 1035.5931 [M+K$^+$]; found 1035.5930.

Octyl-substituted receptor 7d: The compound was obtained as a light-yellow solid from triamine **6** (42 %). ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C, TMS): δ = 0.80 (t, ^3J(H,H) = 7 Hz, 6H), 1.07–1.24 (m, 63H), 2.84 (dt, ^3J(H,H) = 6 Hz, ^3J(H,H) = 7 Hz, 6H), 3.72 (d, ^3J(H,H) = 6 Hz, 6H), 5.69 (t, ^3J(H,H) = 6 Hz, 3H), 5.77 (t, ^3J(H,H) = 7 Hz, 3H), 7.94 ppm (s, triphenylene; 6H); ^{13}C NMR (500 MHz, [D$_6$]DMSO, 25 °C, TMS): δ = 13.79, 21.95, 22.00, 24.18, 26.19, 28.53, 29.71, 31.15, 39.90, 40.10, 41.40, 100.08, 122.24, 124.00, 148.71, 157.72 ppm; HRMS: calcd for $C_{63}H_{96}N_6NaO_9^+$ 1103.7131 [M+Na$^+$]; found 1103.7117; calcd for $C_{63}H_{96}KN_6O_9^+$ 1119.6870 [M+K$^+$]; found 1119.6859.

Liquid–liquid extraction procedure: Extraction studies were performed at 25±1 °C in 2 cm^3 microcentrifuge tubes by mechanical shaking. The phase volume ratio $V_{(org)}$:$V_{(w)}$ was 1:1 (0.5 cm^3 each). The shaking period was 30 min, within which the extraction equilibrium was achieved. All samples were centrifuged after extraction. The concentrations of caffeine, theophylline, and 1,3,7-trimethyluric acid in both phases were determined radiometrically by measuring the β-radiation of the radio-labelled substrates (^{3}H, ^{14}C; Beckman LS 6000 LL liquid scintillation counter). The aqueous solution was adjusted to pH 7.4 using 0.05 M 2-(4-(2-hydroxyethyl)-1-piperazine)ethanesulfonic acid (HEPES)/NaOH.

Fluorescence titrations: Receptor solutions were prepared at a concentration of 1.0 μM in anhydrous or aqueous toluene. Aliquots (2 mL) of these solutions were placed in a 1 cm^2 quartz cell (Hellma, Mülheim, Germany) and held at (25.0±0.1) °C. Caffeine was dissolved in anhydrous toluene (c ≈0.1 mM) and this solution was added to the receptor solution by means of a 10-μL glass syringe (Hamilton, Bonaduz, Switzerland). Fluorescence spectra were recorded using an Aminco-Bowman AB2 spectrofluorimeter (Thermo Fisher Scientific, Waltham, USA). The excitation wavelength was 352 nm, and emission was recorded in the region 370–430 nm. Factor analysis and mathematical treatment of the data were performed using *Specfit 2000* (Spectrum Software Associates).[31]

X-ray crystallographic data

Suitable crystals were obtained upon diffusion of pentane into a damp equimolar solution of **1** and **2** in CH_2Cl_2.

X-ray crystal structure analysis for **1i·2·H$_2$O**: formula $C_{66}H_{63}N_6O_9Br_3\cdot C_8H_{10}N_4O_2\cdot H_2O\cdot 2\,CH_2Cl_2$, $M_r=1760.02$, colourless crystal $0.25\times0.15\times0.05$ mm, $a=14.781(1)$, $b=17.179(1)$, $c=17.510(1)$ Å, $\alpha=86.37(1)$, $\beta=72.93(1)$, $\gamma=64.67(1)°$, $V=3831.7(4)$ Å^3, $\rho_{calcd}=1.479$ g cm^{-3}, $\mu=1.786$ mm^{-1}, empirical absorption correction ($0.664\le T\le0.916$), $Z=2$, triclinic, space group $P\bar{1}$ (no. 2), $\lambda=0.71073$ Å, $T=198$ K, ω and ϕ scans, 33861 reflections collected ($\pm h$, $\pm k$, $\pm l$), $[(\sin\theta)/\lambda]=0.59$ Å^{-1}, 13449 independent ($R_{int}=0.083$) and 6367 observed reflections [$I\ge2\sigma(I)$], 957 refined parameters, $R=0.093$, $wR^2=0.291$, max. residual electron density 0.98 (-0.82) e Å^{-3}, hydrogen atoms at O50 from difference map; others calculated and refined as riding atoms.

Crystallisation occurred upon solvent diffusion into a solution of **1e** and **4** in CH_2Cl_2 when it was covered with cyclohexane.

X-ray crystal structure analysis for **1e·4·H$_2$O**: formula $C_{72}H_{78}N_6O_9\cdot C_8H_{10}N_4O_3\cdot H_2O\cdot CH_2Cl_2\cdot 2\,C_6H_{12}$, $M_r=1652.86$, light-yellow crystal $0.50\times0.50\times0.10$ mm, $a=14.259(1)$, $c=36.255(1)$ Å, $V=6383.8(7)$ Å^3, $\rho_{calcd}=1.290$ g cm^{-3}, $\mu=1.250$ mm^{-1}, empirical absorption correction ($0.574\le T\le0.885$), $Z=3$, trigonal, space group $P3_2$ (no. 145), $\lambda=1.54178$ Å, $T=223$ K, ω and ϕ scans, 22882 reflections collected ($\pm h$, $\pm k$, $\pm l$), $[(\sin\theta)/\lambda]=0.59$ Å^{-1}, 9353 independent ($R_{int}=0.044$) and 7141 observed reflections [$I\ge2\sigma(I)$], 995 refined parameters, $R=0.072$, $wR^2=0.203$, max. residual electron density 0.55 (-0.59) e Å^{-3}, Flack parameter 0.09(7), CH_2Cl_2 and cyclohexane refined with isotropic thermal parameters, the latter also with geometrical restraints; hydrogen atoms at O101 from difference map; others calculated and refined as riding atoms.

Crystallisation occurred upon solvent diffusion into a solution of **7c** in CH_2Cl_2 and methanol when it was covered with *n*-heptane.

X-ray crystal structure analysis for **7c·3MeOH**: formula $C_{57}H_{84}N_6O_9\cdot 3\,CH_3OH\cdot 0.25\,H_2O$, $M_r=1097.93$, colourless crystal $0.50\times0.40\times0.40$ mm, $a=17.103(1)$, $c=12.938(1)$ Å, $V=3277.5(4)$ Å^3, $\rho_{calcd}=1.113$ g cm^{-3}, $\mu=0.624$ mm^{-1}, empirical absorption correction ($0.746\le T\le0.789$), $Z=2$, trigonal, space group $P\bar{3}$ (no. 147), $\lambda=1.54178$ Å, $T=223$ K, ω and ϕ scans, 17262 reflections collected ($\pm h$, $\pm k$, $\pm l$), $[(\sin\theta)/\lambda]=0.59$ Å^{-1}, 3356 independent ($R_{int}=0.051$) and 2058 observed reflections [$I\ge2\sigma(I)$], 248 refined parameters, $R=0.076$, $wR^2=0.244$, max. residual electron density 0.53 (-0.24) e Å^{-3}; the hydrogen atoms of the water molecule were not located; others were calculated and refined as riding atoms.

Data sets were collected with Nonius KappaCCD diffractometers, in the case of Mo-radiation equipped with a rotating anode generator. Programs used: data collection: COLLECT (Nonius B.V., 1998), data reduction: Denzo-SMN (Z. Otwinowski, W. Minor, *Methods in Enzymology* **1997**, *276*, 307–326), absorption correction: SORTAV (R. H. Blessing, *Acta Crystallogr. Sect. A* **1995**, *51*, 33–37; R. H. Blessing, *J. Appl. Cryst.* **1997**, *30*, 421–426) and Denzo (Z. Otwinowski, D. Borek, W. Majewski, W. Minor, *Acta Crystallogr Sect. A* **2003**, *A59*, 228–234), structure solution: SHELXS-97 (G. M. Sheldrick, *Acta Crystallogr. Sect. A.* **1990**, *46*, 467–473), structure refinement SHELXL-97 (G. M. Sheldrick, Universität Göttingen, 1997), graphics: DIAMOND 3.0d (Crystal Impact GbR, Bonn, Germany).

CCDC-618021–CCDC-618023 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge rom the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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[18] 95.3% ($CHCl_3$) and 89.7% (CH_2Cl_2) of caffeine (1×10^{-4} M in HEPES/NaOH buffer, pH 7.4) was extracted into the organic phase.
[19] A solubility of the individual receptor in the organic solvent of at least 0.0025 M is required.
[20] $c_{ligand}=0.005$ M in toluene; $c_{xanthine}=0.0001$ M in aqueous solution (pH 7.4).
[21] *D* is defined as the quotient of the analytical concentrations of xanthine derivatives in the organic and aqueous phases; points in Figures 2–4 represent the experimental data; lines were calculated for 1:1 complex formation using a nonlinear regression program.[13a]
[22] Determination of binding constants for the system **2·1c** in the temperature range 25–65 °C and subsequent van't Hoff analysis yields $\Delta H_{ass}=(-76\pm9)$ kJ mol^{-1} and $\Delta S_{ass}=(-140\pm30)$ J K^{-1} mol^{-1}. Therefore, the complex formation is definitely enthalpy driven. However, the differences between the receptors will also have entropic contributions. These are a matter of detailed ongoing investigations and will be further discussed in due course.
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