Influence of linker structure on the anion binding affinity of biscyclopeptides†‡

Carsten Reyheller,[a] Benjamin P. Hay[b] and Stefan Kubik*[a]

Received (in Montpellier, France) 8th May 2007, Accepted 18th July 2007
First published as an Advance Article on the web 13th August 2007
DOI: 10.1039/b706932d

A systematic analysis is presented on the influence of the linking unit between two cyclopeptide rings on the affinity of such biscyclopeptide-based anion receptors in aqueous solvent mixtures. Although the differences in the affinity and selectivity of these receptors towards a given anion are not very pronounced, there are profound differences in the thermodynamics of anion complexation. Enthalpic and entropic contributions both (1) play a role in determining the binding affinity and (2) show significant variation as the linking structure is changed. A decrease in conformational rigidity of the linker improves the entropic advantage for complex formation, but not necessarily the overall complex stability. This effect may be due, in part, to the fact that structural constraints within more rigid linkers might prevent efficient interactions between the host and guest. The optimal linker, which exhibits both favourable enthalpic and entropic contributions, was identified using *de novo* structure-based design methods as implemented in the HostDesigner software.

Introduction

Criteria for the evaluation and comparison of synthetic receptors are selectivity and affinity in the solvent or solvent mixture in which substrate binding takes place. While affinity is expressed in terms of association constant K_a, dissociation constant K_d, or free energy of complex formation ΔG, a measure of selectivity of a receptor for substrate A over substrate B is the ratio of the binding constants $K_a(A)/K_a(B)$ of the respective complexes. Ideally, synthetic receptors should be highly selective and strong binding to the substrate can be advantageous. The design of efficient synthetic receptors is, however, not trivial. Preorganisation, a strategy pioneered by Cram,[1] is an important approach, but has its limitations because the exact positioning of converging binding sites on a rigid three-dimensional receptor scaffold complementary to the substrate is difficult. In addition, high enthalpy of binding arising from strong interactions between a well preorganised host and its substrate is often accompanied by a considerable loss of entropy thus reducing the overall gain in binding free energy (*enthalpy–entropy compensation*).[2] Optimisation of the binding properties of synthetic receptors has therefore often proceeded in incremental steps by systematically changing receptor structures. Only recently, with the advent of combinatorial techniques[3] as well as powerful molecular modelling programs,[4] have alternatives to this traditional approach to receptor optimisation emerged. Although impressive results have thus been achieved, the corresponding investigations often lack detailed insight into the factors governing the binding process that is provided by a comparison of the behaviour of structurally closely related receptors. Particularly, trend analyses using calorimetric techniques have been shown to be very useful in revealing how the finely tuned interplay between binding enthalpy and binding entropy influences receptor selectivity as well as affinity.[5] Here, we present a related study in which we have investigated the effects of structure and conformational rigidity of the linker between two cyclopeptide rings on the receptor properties of such anion binding biscyclopeptides.

The investigations are based on our finding that cyclic hexapeptide **1** (Chart 1) is able to bind halides and sulfate anions in highly competitive aqueous solvent mixtures by forming a 2 : 1 complex in which the anion is sandwiched between two cyclopeptide rings.[6a,b] By covalently linking two cyclopeptide rings together *via* an adipic acid linker we converted these 2 : 1 complexes into 1 : 1 complexes.[6c] Although a significant improvement of receptor efficiency could thus be achieved[6d] one can expect that anion affinity and selectivity of these biscyclopeptides sensitively depend on structural characteristics of the linker such as length and conformational flexibility. Indeed, by using dynamic combinatorial chemistry we identified several biscyclopeptides, for example **3a** and **3b**, with even higher anion affinity than **2a** indicating that adipic acid is by far not the best linker.[6e] Assignment of the structural factors responsible for the different affinities of **2a**, **3a** and **3b** is difficult, however, because the linkers in the three receptors are not easily comparable. We therefore decided to synthesise a series of biscyclopeptides containing structurally more closely related linkers and study the effects of the systematic change in

[a] *Technische Universität Kaiserslautern, Fachbereich Chemie-Organische Chemie, Erwin-Schrödinger-Strasse, D-67663 Kaiserslautern, Germany. E-mail: kubik@chemie.uni-kl.de; Fax: +49-631-205-3921*
[b] *Chemical Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, 37831-6119, USA*

† Dedicated to Professor George Gokel on the occasion of his 60th birthday.

‡ Electronic supplementary information (ESI) available: Experimental details of the ITC experiments, NOESY NMR spectra of receptors **2b–d** and of the sulfate complexes of these biscyclopeptides, ESI-MS spectra of the halide complexes of biscyclopeptides **2b** and **2c**. See DOI: 10.1039/b706932d

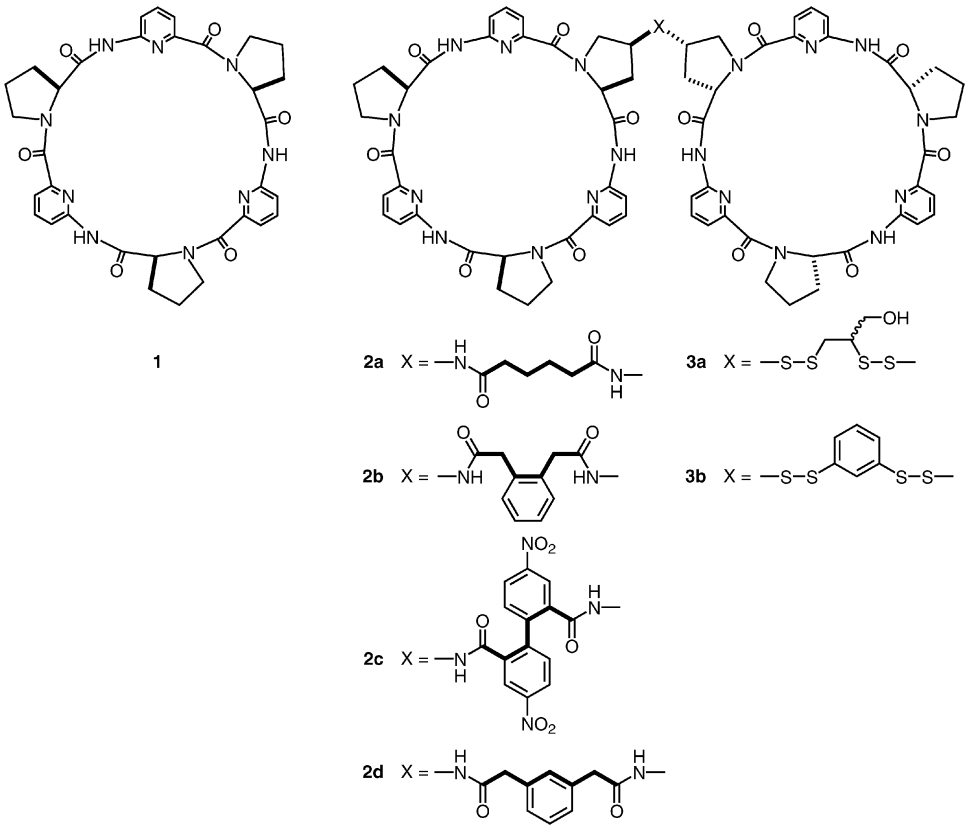


Chart 1

linker structure on receptor properties. Biscyclopeptides **2b** and **2c** have thus been prepared containing, respectively, 2,2′-(1,2-phenylene)diacetic acid and 4,4′-dinitrobiphenyl-2,2′-dicarboxylic acid. These biscyclopeptides contain the same number of atoms in the linker as **2a** but become increasingly more rigid. In addition, biscyclopeptide **2d**, which was identified with a *de novo* structure-based design approach, was included in the investigations. Spectroscopic and mass spectrometric characterisation of the anion complexes of **2b–d** demonstrated that the complexes of all three biscyclopeptides are structurally closely related to those of **2a**. Despite these similarities, a calorimetric analysis of the binding equilibria revealed substantial effects of linker structure on the thermodynamic parameters of complex formation.

Results and discussion

Structures and synthesis

Biscyclopeptides **2b** and **2c** contain, respectively, 2,2′-(1,2-phenylene)diacetic acid (oPA) and 4,4′-dinitrobiphenyl-2,2′-dicarboxylic acid (DBA) as linkers. These linkers are structurally related to adipic acid in that they contain the same number of atoms between the carboxyl groups. Whereas all bonds between the carboxyl groups in adipic acid are single bonds and thus rotatable, in oPA rotation around one bond and in DBA rotation around two bonds is prevented by phenylene rings. The linkers thus become increasingly less flexible on going from receptor **2a** to **2b** to **2c**, a property which might cause anion binding of the corresponding biscyclopeptides to become entropically more favourable in the same direction. DBA was introduced into **2c** instead of unsubstituted biphenyl-2,2′-dicarboxylic acid because **2c** proved to be significantly more soluble in the solvent mixtures used for the characterisation of binding properties than the analogue lacking the nitro groups.

The linker in receptor **2d** was identified using a *de novo* structure-based design approach as implemented in the HostDesigner software.[4b] The successful application of this computational method to design ion receptors recently has been demonstrated.[7] The starting point for the calculations performed here were the coordinates of the crystal structure of the iodide complex of **1**.[6a] Assuming that this structure represents an optimal complex geometry, HostDesigner was used to search for linking fragments taken from a database that optimally bridge the two cyclopeptide rings. This search was restricted to fragments linking the C4 of a proline residue in one cyclopeptide ring and another proline C4 of the second ring *via* amide groups. The resulting biscyclopeptides were scored based on geometric factors yielding a list of top receptor candidates containing linkers depicted in Chart 2. Of these, 2,2′-(1,3-phenylene)diacetic acid (mPA), required for the synthesis of **2d**, is commercially available and was therefore included in this study.

All receptors were synthesised by coupling two equivalents of cyclopeptide **4**[6c] to one equivalent of the respective diacid in the presence of *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) and *N*-ethyl-

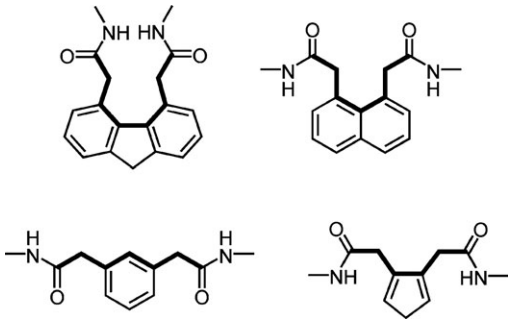


Chart 2

diisopropylamine (DIEA) in DMF (Scheme 1). After chromatographic work-up the products were isolated analytically pure in yields between 43 and 62%.

NMR spectroscopic and mass spectrometric characterisation

Anion binding of the newly synthesised receptors **2b–d** was first studied by using electrospray ionisation mass spectrometry (ESI-MS) and ^{1}H NMR spectroscopy. Because of the mild ionisation technique ESI-MS is a particularly useful method for the characterisation of non-covalently assembled complexes in solution which can yield information on composition and on stability.[8] Fig. 1 shows the ESI mass spectrum of a solution of **2d** in 1 : 1 (v/v) water–methanol in the presence of three sodium halides (negative mode).

Practically only three signals are visible in this spectrum in addition to the signal of the free receptor [**2d**-H$^+$] (1489.6) whose *m*/*z* ratios can be assigned to 1 : 1 complexes of the composition [**2d** + Cl$^-$] (1525.5), [**2d** + Br$^-$] (1571.5), and [**2d** + I$^-$] (1617.5). No signals are detectable that correspond to complexes of higher stoichiometry. The formation of complexes in which, *e.g.*, two anions bind to two molecules of **2d** can be ruled out on the basis of the isotope patterns of the signals, a result that is further corroborated by the lack of signals in the spectrum corresponding to complexes in which two or more molecules of **2d** bind *different* halides. It is therefore reasonable to assume that **2d** forms similar clam-shell-like complexes with anions in which both cyclopeptide rings are involved in complex formation as receptor **2a**.

This assumption is supported by our NMR spectroscopic results. In the ^{1}H NMR spectrum of a solution of **2d** in 1 : 1 (v/v) D_2O–CD_3OH marked downfield shifts are visible of the

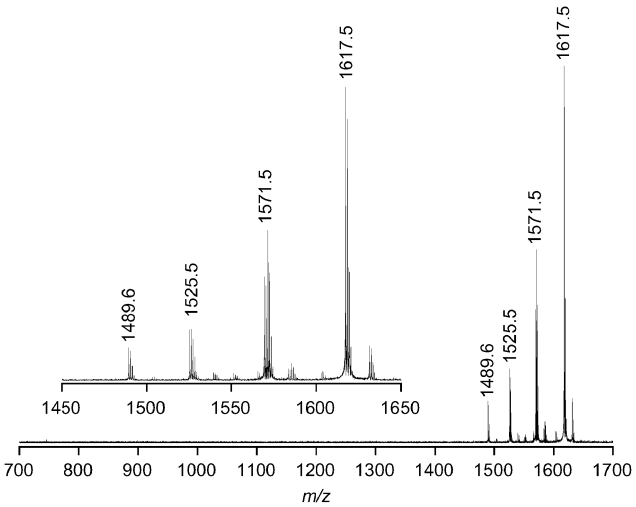
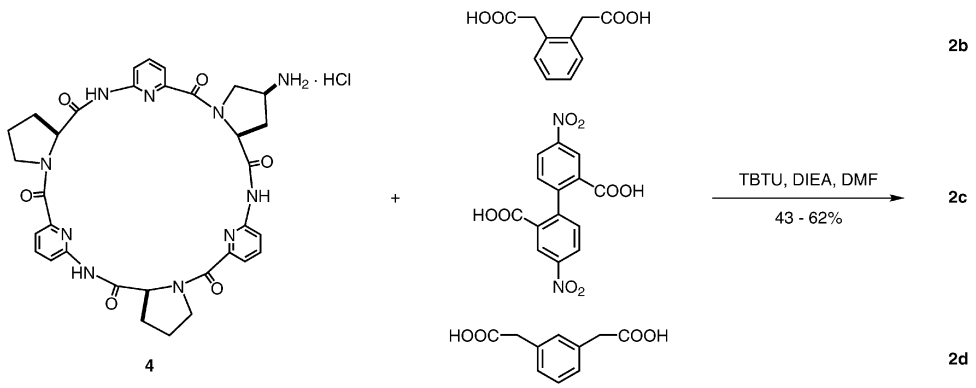


Fig. 1 ESI mass spectrum of **2d** in 1 : 1 (v/v) water–methanol (0.1 mM) after the addition of 0.33 equivalents of each NaCl, NaBr, and NaI.

receptor's H(α) signals in the presence of anions. As an example, the spectrum of free receptor and the one of its sulfate complex are depicted in Fig. 2. A similar deshielding of the H(α) protons upon anion binding, which is due to the spatial proximity of these protons to the anionic guest in the complex has been observed in the ^{1}H NMR spectrum of **2a**.[6c] It is therefore reasonable to assume that the mode of inclusion of anions in the cavities of receptors **2a** and **2d** is comparable.

Finally, in the NOESY NMR spectrum of the sulfate complex of **2d** a crosspeak is visible between the H(α) signal of a substituted and an unsubstituted proline unit that indicates a close contact of the corresponding protons in the complex (see ESI‡). For biscyclopeptide **2a**, whose NOESY NMR spectrum of the sulfate complex contains an analogous crosspeak, it was shown that this contact can only be realised if the receptor adopts a folded conformation with the two cyclopeptide rings approaching each other.[6c] With no anion included between the cyclopeptide rings **2a** and **2d** on average adopt more open conformations as evidenced by the absence of a crosspeak between the H(α) signals in the spectrum. A theoretical structure for the iodide complex of **2d**, presented in Fig. 3, is fully consistent with the experimental evidence.



Scheme 1

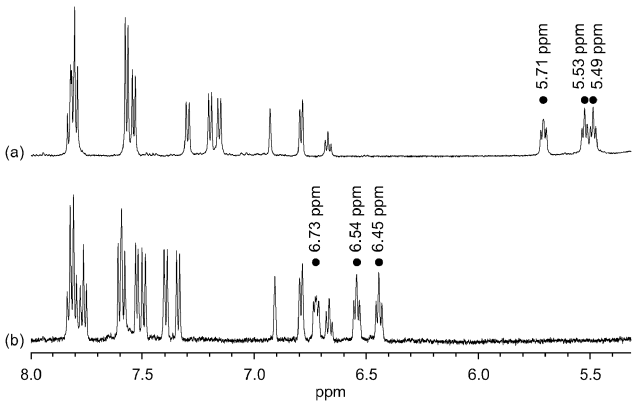


Fig. 2 ^{1}H NMR spectrum of **2d** in 1 : 1 (v/v) D_2O–CD_3OD (1 mM) before (a) and after (b) the addition of 1 equivalent of Na_2SO_4. The signals of the H(α) protons are marked with black dots.

Similar results have been obtained for receptors **2b** and **2c** (see ESI‡) and it can therefore be concluded that the mode of anion binding of all four compounds **2a–d** is comparable.

ITC measurements

Isothermal titration calorimetry (ITC) was used to quantitatively evaluate the binding properties of biscyclopeptides **2b–d** in 1 : 1 (v/v) water–methanol. One major advantage of this technique is that a single titration yields all thermodynamic parameters of complex formation simultaneously, the stability constant of the complex K_a (and in turn the free energy of complex formation ΔG), the binding enthalpy ΔH as well as the binding entropy ΔS.[9] In principle, variations in the stability of different host/guest systems can thus be traced to whether they are of enthalpic or of entropic origin. It has to be considered, however, that the heats measured during an ITC titration are produced by various processes occurring in solution simultaneously of which complex formation is only one. ITC therefore does not directly provide structural information, but a careful comparison of results obtained for structurally closely related complexes can provide a correlation between complex structure and the thermodynamic signature of its formation.[5]

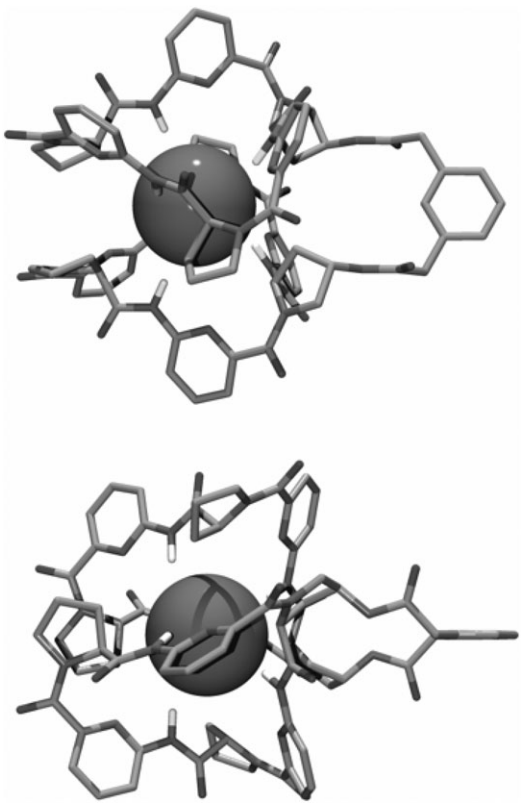


Fig. 3 Calculated geometry for the iodide complex of **2d** obtained with the MMFF94 force field as implemented in PCModel, Serena Software, Inc. Hydrogen atoms at the carbon atoms of the receptor have been omitted for clarity.

The results of our ITC measurements are summarised in Table 1. The numbers given in the table represent averages over at least three independent measurements. Standard deviations are specified in brackets. Sulfate, iodide, bromide, and chloride were used as guests, with each anion in the form of its sodium salt. For comparison, Table 1 also contains the thermodynamic parameters for the corresponding complexes of biscyclopeptide **2a**, which have been determined previously.[6c]

With the exception of the chloride complex of receptor **2c**, exothermic complex formation was observed in all cases accompanied by a favourable positive entropic contribution. The heat generated during the titration of **2c** with NaCl was too small to allow for a quantitative estimation of complex stability. Although small enthalpic changes do not necessarily imply weak binding the results obtained for the other complexes suggest that the chloride complex of **2c** is indeed the least stable one.

Complex formation of a number of host/guest combinations is dominated by enthalpy with entropy contributing to a smaller but decisive extent to the overall free energy of binding. In other cases, the share of entropy is similar or even larger than that of enthalpy. Trends in the dependence of enthalpy and entropy of binding on receptor structure can thus best be analysed by using the graphical representations in Fig. 4. Graphs for the chloride complexes are not included in this Figure due to the lack of data for the complex of **2c**.

Table 1 and Fig. 4(a) show that anion affinities of all four biscyclopeptides decrease in the order sulfate > iodide > bromide > chloride. This order differs from expectations based on intrinsic hydrogen bonding strength, which would predict sulfate > chloride > bromide > iodide. As we have noted in our earlier study,[6c] the fact that iodide is better bound than the smaller halides whose interactions with amide NH groups should be stronger indicates that the larger iodide anion better fits into the cavity between the two receptor halves.

Clearly the best anion receptor in the series of biscyclopeptides investigated is the one identified through computer-aided molecular design, namely **2d**, which possesses the highest affinity for all anions studied. Stability of the sulfate complex, for example, amounts to an appreciable log K_a of almost 6.0 in 1 : 1 (v/v) water–methanol which makes **2d** one of the most potent neutral synthetic receptors for sulfate in aqueous solution.[10] Direct comparison of the receptor properties of **2d** with those of **3a** and **3b** is not possible on the basis of the

Table 1 Association constants, Gibbs energies, enthalpies and entropies of binding of Na_2SO_4, NaI, NaBr, and NaCl to receptors **2a–d**[a]

		2a[b]	**2b**	**2c**	**2d**
Sulfate	$\log K_a$	5.28 (0.10)	5.10 (0.05)	5.32 (0.06)	5.97 (0.02)
	ΔG	−30.2 (0.2)	−29.2 (0.3)	−30.3 (0.2)	−34.1 (0.4)
	ΔH	−15.5 (0.4)	−13.5 (0.3)	−8.6 (0.5)	−13.2 (1.1)
	$T\Delta S$	14.7 (0.6)	15.7 (0.5)	21.7 (0.7)	20.9 (1.2)
Iodide	$\log K_a$	3.79 (0.26)	4.00 (0.03)	3.61 (0.07)	4.43 (0.11)
	ΔG	−21.6 (2.5)	−22.7 (0.3)	−20.6 (0.4)	−25.2 (1.1)
	ΔH	−13.2 (1.0)	−9.1 (0.2)	−4.2 (1.5)	−15.4 (0.7)
	$T\Delta S$	8.4 (2.5)	13.6 (0.2)	15.4 (3.4)	9.8 (1.2)
Bromide	$\log K_a$	3.45 (0.39)	3.30 (0.04)	3.03 (0.14)	4.01 (0.03)
	ΔG	−19.7 (2.2)	−19.0 (0.2)	−17.3 (0.8)	−22.9 (0.2)
	ΔH	−11.2 (0.9)	−6.2 (0.6)	−4.0 (0.4)	−12.1 (1.7)
	$T\Delta S$	8.5 (3.1)	12.8 (0.8)	13.3 (1.0)	10.8 (1.9)
Chloride	$\log K_a$	2.51 (0.86)	1.86 (0.14)	n.d.[c]	3.39 (0.08)
	ΔG	−14.2 (4.9)	−10.6 (0.8)		−19.3 (0.5)
	ΔH	−10.5 (1.5)	−3.4 (1.1)		−4.1 (1.2)
	$T\Delta S$	3.8 (6.4)	7.2 (1.5)		15.2 (1.5)

[a] Recorded in 1 : 1 (v/v) water–methanol at 298 K; K_a in M^{-1} and energies in kJ mol^{-1}. [b] ref. 6*c*. [c] n.d., not detectable.

data collected in Table 1 as complex formation of the latter two receptors has been evaluated in 2 : 1 (v/v) acetonitrile–water using potassium salts.[6e] We therefore also characterized sulfate and iodide binding of **2d** under these conditions. The results of these measurements are summarized in Table 2.

Table 2 shows that **2d** is a significantly better sulfate and iodide receptor than **2a** also in 2 : 1 (v/v) acetonitrile–water. Anion affinity is still lower than that of **3a** and **3b**, however, showing that dynamic combinatorial chemistry has furnished more potent receptors than molecular modelling. It is also evident that the reason for the superior properties of **3a** and **3b** is in the more favourable (in the case of sulfate binding the less unfavourable) enthalpic contribution to complex formation, which might be caused by a better mutual arrangement of the receptor subunits allowing strong interactions with the guests.

Comparison of the anion affinity of biscyclopeptides **2a–c** in 1 : 1 (v/v) water/methanol shows that **2b** is the weakest sulfate binder while the halides are bound the least strongly by **2c**. Although the differences in the affinity of these receptors towards a given anion are not very pronounced, the enthalpic and entropic signatures in anion complex formation of biscyclopeptides **2a–c** differ profoundly, as illustrated by Fig. 4(b) and (c). Thus, a pronounced decrease in the enthalpy of anion binding is observed in the order **2a** > **2b** > **2c** (Fig. 4(b)) while the entropic contribution to complex formation becomes more favourable in the same direction (Fig. 4(c)). A possible reason for the reduced binding enthalpy of the more rigid biscyclopeptides could be that these linkers prevent the two receptor subunits from achieving optimal contacts with the bound guests. Flexible linkers as in **2a**, on the other hand, allow the biscyclopeptides more easily to adapt to the size and structure of the substrate. The stronger interactions resulting from this better fit in turn cause a considerable loss of conformational mobility in the complex, explaining why entropy of binding is least favourable for receptor **2a**. In contrast, anions are bound more loosely in the cavities of **2b** and **2c** which, together with the progressive reduction of rotational degrees of freedom in the linkers of these receptors, cause more favourable entropy terms. The comparable binding strengths observed for receptors **2a–c** can therefore best be rationalized on the basis of enthalpy–entropy compensation.

In light of the trends observed for the thermodynamic parameters of complex formation of receptors **2a–c** it is interesting to analyse how enthalpy and entropy of binding of **2d** compares with these results. Fig. 4(a) clearly shows that complex formation of **2d** is associated with large enthalpic contributions similar or even larger than those observed for complex formation of biscyclopeptide **2b** containing the

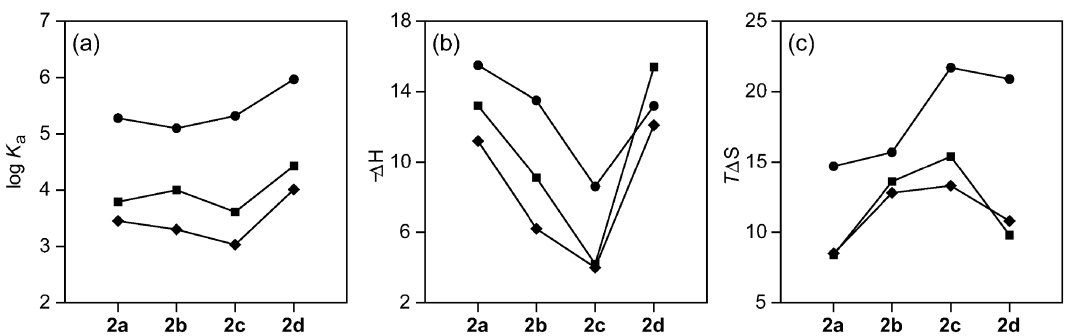


Fig. 4 Graphical representation of the dependence of the thermodynamic parameters $\log K_a$ (a), ΔH (b), and $T\Delta S$ (c) of the sulfate (circles), iodide (squares), and bromide (diamonds) complexes of biscyclopeptides **2a–d** on receptor structure.

Table 2 Association constants, Gibbs energies, enthalpies and entropies of binding of K_2SO_4 and KI to receptors **2a**, **2d**, **3a** and **3b**[a]

		2a[b]	**2d**	**3a**[b]	**3b**[b]
Sulfate	$\log K_a$	5.30	5.75 (0.06)	6.73	6.83
	ΔG	−30.2	−32.8 (0.4)	−38.4	−39.0
	ΔH	10.7	9.7 (1.4)	1.8	3.7
	$T\Delta S$	41.0	42.5 (1.6)	40.1	42.7
Iodide	$\log K_a$	3.52	4.05 (0.17)	4.46	4.75
	ΔG	−20.0	−23.1 (1.0)	−25.5	−27.1
	ΔH	−4.3	−8.3 (1.0)	−20.7	−13.4
	$T\Delta S$	15.7	14.8 (1.8)	4.8	13.7

[a] Recorded in 2 : 1 (v/v) acetonitrile–water at 298 K; K_a in M^{-1} and energies in kJ mol^{-1}. [b] Ref. 6*e*.

structurally related oPA linker. Despite the reduced flexibility of **2d** with respect to **2a**, the structure of the linker obviously also allows this biscyclopeptide to efficiently bind to the anionic guests. Particularly noteworthy is the large negative enthalpy observed for the formation of the iodide complex of **2b** which could reflect the fact that the calculations which led to the identification of the mPA linker in **2d** were based on the coordinates of the iodide complex of **1**. More investigations are needed to confirm this interpretation, however.

In terms of absolute contribution of entropy, complex formation of **2d** is less favourable than that of the rigid receptor **2c** but more favourable than that of **2a** and **2b** (Fig. 4(c)). Thus, the high sulfate affinity of **2d** with respect to the other receptors is essentially due to the entropic term, which accounts for 61% of the binding, as binding enthalpy is only of the same order of magnitude as that of **2b**. The entropy component of halide complexation of **2d** (four rotatable bonds), on the other hand, lies between that of **2a** (five rotatable bonds) and **2b** (four rotatable bonds). As a consequence, receptor **2d** is a better halide binder than **2b** and **2c** because of a more favourable binding enthalpy while halide affinity of **2d** is higher than that of **2a** because of a combination of both entropic as well as enthalpic factors. In this case, high binding enthalpy is obviously not compensated by an unfavourable entropy term resulting in large overall complex stability. Interestingly, the higher anion affinities of biscyclopeptides **3a** and **3b** with respect to **2a** proved to be due to binding enthalpy only (Table 2).[6e] Thus, anion complexation of our biscyclopeptides indeed sensitively depend on linker structure. The linker in **2d** seems to combine good conformational rigidity with the ability to permit a mutual arrangement of the biscyclopeptide subunits ideal for efficient interactions between host and guest thus causing a remarkably high affinity of the corresponding receptor.

Conclusion

In conclusion, we have shown that control of receptor properties is possible by varying the linker between the two cyclopeptide rings of our anion binding biscyclopeptides. Although the differences in the affinity and selectivity of these receptors towards a given anion are not very pronounced, there are profound differences in the thermodynamics of anion complexation. Both enthalpic and entropic contributions (1) play a role in determining the binding affinity and (2) show significant variation as the linker is changed. We found that a decrease in conformational rigidity of the linker improves the entropic advantage for complex formation, but not necessarily the overall complex stability. This effect may be due, in part, to the fact that structural constraints within more rigid linkers might prevent efficient interactions between the host and guest. The optimal linker in receptor **2d** exhibits both favourable enthalpic and entropic contributions. Interestingly, this linker was identified by molecular modelling illustrating the increasing importance that computational methods have in the identification of new synthetic receptors.

By careful optimization of linker structure it is therefore possible to obtain neutral receptors for anions on the basis of our biscyclopeptides with remarkably high anion affinity in protic solvents. The introduction of more than one linker between the two cyclopeptide rings should also provide a means to improve binding while introduction of hydrophilic substituents into the linkers could serve to improve water solubility ultimately allowing direct comparison of the binding properties of our biscyclopeptides with those of natural anion binders. Control of anion affinity is therefore much more straightforward than control of selectivity the latter of which most probably requires completely different strategies. Possible approaches are currently being investigated in our group.

Experimental

General details

Analyses were carried out as follows: melting points, Müller SPM-X 300; NMR, Bruker Avance 600; MALDI-TOF-MS, Bruker Ultraflex TOF/TOF; ESI-MS, Bruker APEX III FT-ICR-MS with Apollo ESI-Source; elemental analysis, Perkin Elmer Elemental Analyser 2400 CHN; RP chromatography, MERCK LiChroprep RP-8 (40–63 μm) prepacked column size B (310–25); ITC, Microcal VP-ITC. The following abbreviations are used: DIEA, *N*-ethyldiisopropylamine; TBTU, *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate; Pro, L-proline; Apro, 4*S*-amino-L-proline; APA, 6-aminopicolinic acid; AP, adipic acid; oPA, 2,2′-(1,2-phenylene)diacetic acid; mPA, 2,2′-(1,3-phenylene)-diacetic acid, BPA, 4,4′-dinitrobiphenyl-2,2′-dicarboxylic acid. *J* values in the NMR data are given in Hz.

ITC measurements

In a typical experiment, a solution of the guest in 1 : 1 (v/v) water–methanol were injected stepwise at a rate of 0.5 μL s^{-1} to a solution of a biscyclopeptide in the same solvent mixture. Experimental details regarding the concentrations of biscyclopeptide and salt in each titration are provided in the Supporting Information. A volume of 2 μL was used in the first injection step and 10 μL in the following 24 injections. Each new step was started after reaching chemical and thermal equilibrium. The measured heat flows were recorded as function of time and converted into enthalpies by integration of the appropriate reaction peaks. Dilution effects were corrected by subtracting the results of a blank experiment with a solution of 1 : 1 (v/v) water–methanol in place of the receptor solution

under identical experimental conditions. The association parameters were evaluated by means of the Origin 7.0 software package provided by the instrument manufacturer neglecting the result of the first injection.

Syntheses

DMF p.A. was purchased and was used without further purification. TBTU, oPA, and mPA are commercially available. BPA was synthesised according to the literature procedure.[11] The syntheses of receptor **2a** and cyclopeptide **4** are described elsewhere.[6c]

Procedure for the synthesis of receptors 2b–d

Cyclopeptide **4** HCl (280 mg, 0.4 mmol) and the respective linker (0.2 mmol) were dissolved in DMF (30 mL). In succession, TBTU (141 mg, 0.44 mmol) and DIEA (220 μL, 1.28 mmol) were added, and the resulting mixture was stirred 3 h at room temperature. Afterward, the solvent was evaporated *in vacuo*, and the product was isolated from the residue by chromatographic workup. For this, the residue was dissolved in a small amount of DMF. The resulting solution was applied to a RP-8 column conditioned with 1,4-dioxane–H_2O, 1 : 10, and the eluent composition was gradually changed to 1,4-dioxane–H_2O, 1 : 1, with which pure product eluted. The material recovered was dissolved in a small amount of hot methanol. Hot water was slowly added until product precipitated. The solution was kept at room temperature overnight, the product was filtered off, washed with water, and dried.

Receptor 2b

Yield 0.15 g (50%); mp >300 °C; ^{1}H NMR (600 MHz, [d_6]DMSO, 25 °C, TMS) δ 1.75–1.91 (m, 8H; ProC(γ)H), 1.95–2.09 (m, 6H; ProC(β)H + AproC(β)H), 2.52–2.61 (m, 4H; ProC(β)CH), 2.78–2.85 (m, 2H; AproC(β)H), 3.31 (s, br, 4H; oPACH_2), 3.44–3.52 (m, 2H; AproC(δ)H), 3.53–3.61 (m, 4H; ProC(δ)H), 3.66–3.74 (m, 4H; ProC(δ)H), 3.82–3.90 (m, 2H; AproC(δ)H), 4.22–4.32 (m, 2H; AproC(γ)H), 5.50–5.60 (m, 6H; ProC(α)H + AproC(α)H), 6.79–6.94 (m, 4H; oPAH(3) + oPAH(4) + oPAH(5) + oPAH(6)), 7.17 (d, ^3J = 8.2, 2H; APAH(3)), 7.20–7.28 (m, 4H; APAH(3)), 7.38–7.49 (m, 6H; APAH(5)), 7.70–7.80 (m, 6H; APAH(4)), 8.19 (d, ^3J = 6.2, 2H; oPANH), 9.52 (s, 4H; APANH), 9.60 (s, 2H; APANH); ^{13}C NMR (151 MHz, [d_6]DMSO, 25 °C, TMS) δ 22.7 + 22.8 (ProC(γ)), 31.1 (ProC(β)), 32.8 (oPACH_2), 36.1 + 37.8 (AproC(β)), 46.7 (AproC(γ)), 48.4 (ProC(δ)), 52.3 (AproC(δ)), 60.8 (AproC(α)), 61.8 (ProC(α)), 116.3 (APAC(3)), 120.0 (APAC(5)), 126.7 (oPAC(4) + oPAC(5)), 130.0 (oPAC(3) + oPAC(6)), 135.3 (oPAC(1) + oPAC(2)), 139.3 (APAC(4)), 148.8 (APAC(2)), 152.2 (APAC(6)), 166.2 + 166.3 (APACO), 170.7 + 170.9 (oPACO), 171.3 + 171.4 (AproCO/ProCO); Calc. for $C_{76}H_{74}N_{20}O_{14} \cdot 8H_2O$ (1635.7): C 55.81, H 5.55, N 17.13; found C 56.05, H 5.73, N 16.92%; MALDI-TOF-MS: *m*/*z* (relative intensity): 1491.5 (82) [M + H^+], 1513.6 (100) [M + Na^+], 1529.5 (61) [M + K^+].

Receptor 2c

Yield 0.20 g (62%); mp >300 °C; ^{1}H NMR (600 MHz, [d_6]DMSO, 25 °C, TMS) δ 1.75–1.92 (m, 8H; ProC(γ)H), 1.95–2.09 (m, 6H; ProC(β)CH + AproC(β)H), 2.53–2.61 (m, 4H; ProC(β)CH), 2.75–2.84 (m, 2H, AproC(β)H), 3.52–3.61 (m, 6H; ProC(δ)H + AproC(δ)H), 3.65–3.72 (m, 6H; ProC(δ)H), 3.73–3.79 (m, 2H; AproC(δ)H), 4.21–4.30 (m, 2H; AproC(γ)H), 5.40–5.69 (m, 6H; ProC(α)H + AproC(α)H), 7.09–7.29 (m, 6H; APAH(3)), 7.30–7.57 (m, 8H; APAH(5) + BPAH(6)), 7.64–7.79 (m, 6H; APAH(4)), 8.19–8.41 (m, 4H; BPAH(3) + BPAH(5)), 8.70 (s, br, 2H; BPANH), 9.55 (s, br, 4H; APANH), 9.71 (s, br, 2H; APANH); ^{13}C NMR (151 MHz, [d_6]DMSO, 25 °C, TMS) δ 22.8 (ProC(γ)), 33.0 (ProC(β)), 37.5 (AproC(β)), 47.4 (AproC(γ)), 48.6 (ProC(δ)), 52.1 (AproC(δ)), 60.8 (AproC(α)), 61.9 (ProC(α)), 116.1 (APAC(3)), 120.2 (APAC(5)), 122.7 (BPAC(3)), 124.9 (BPAC(5)), 131.7 BPAC(6)), 139.5 + 139.6 (APAC(4)), 147.0 (BPAC(1)), 147.3 (BPAC(4)), 149.1 (APAC(2)), 152.0 (BPAC(4)), 152.4 + 152.5 (APAC(6)), 166.0 (APACO), 171.1 (BPACO), 171.4 + 171.5 (AproCO/ProCO); Calc. for $C_{80}H_{72}N_{22}O_{18} \cdot 5H_2O$ (1719.6): C 55.88, H 4.81, N 17.92; found C 55.91, H 4.78, N 17.82%; MALDI-TOF-MS: *m*/*z* (relative intensity): 1629.4 (100) [M + H^+].

Receptor 2d

Yield 0.14 g (43%); mp >300 °C; ^{1}H NMR (600 MHz, [d_6]DMSO, 25 °C, TMS) δ 1.75–1.91 (m, 8H; ProC(γ)H), 1.98–2.11 (m, 6H; ProC(β)H + AproC(β)H), 2.53–2.62 (m, 4H; ProC(β)CH), 2.81–2.92 (m, 2H; AproC(β)H), 3.27 (s, 4H; mPACH_2), 3.44–3.53 (m, 2H; AproC(δ)H), 3.54–3.63 (m, 4H; ProC(δ)H), 3.66–3.76 (m, 4H; AproC(δ)H), 3.83–3.91 (m, 2H; AproC(δ)H), 4.26–4.35 (m, 2H; AproC(γ)H), 5.50–5.67 (m, 6H; ProC(α)H + AproC(α)H), 6.78–6.83 (m, 1H; mPAH(5)), 6.87 (d, ^3J = 7.6, 2H; mPAH(4) + mPAH(6)), 6.98 (s, 1H, mPAH(2)), 7.18 (d, ^3J = 8.2, APAH(3)), 7.25 (m, 4H; APAH(3)), 7.38–7.49 (m, 6H; APAH(5)), 7.70–7.80 (m, 6H; APAH(4)), 8.13 (d, ^3J = 6.2, 2H; mPANH), 9.55 (s, 2H; APANH), 9.64 (s, 4H; APANH); ^{13}C NMR (151 MHz, [d_6]DMSO, 25 °C, TMS) δ 22.8 + 22.9 (ProC(γ)), 32.9 + 33.0 (ProC(β)), 37.8 (AproC(β)), 42.5 (mPACH_2), 46.8 (AproC(γ)), 48.5 (ProC(δ)), 52.4 (AproC(δ)), 60.9 (AproC(α)), 61.9 + 62.0 (ProC(α)), 116.1 + 116.4 (APAC(3)), 120.2 (APAC(5)), 127.2 (mPAC(4) + mPAC(6)), 128.3 (mPAC(5)), 130.4 (mPAC(2)), 136.1 (mPAC(1) + mPAC(3)), 139.4 + 139.5 + 139.6 (APAC(4)), 148.9 (APAC(2)), 152.0 + 152.4 + 152.5 (APAC(6)), 166.3 + 166.5 (APACO), 170.7 (mPACO), 171.0 + 171.4 + 171.5 (AproCO/ProCO); Calc. for $C_{76}H_{74}N_{20}O_{14} \cdot 5.5H_2O$ (1590.6): C 57.39, H 5.39, N 17.61; found C 57.41, H 5.52, N 17.32%; MALDI-TOF-MS: *m*/*z* (relative intensity): 1491.6 (38) [M + H^+], 1513.6 (100) [M + Na^+], 1529.6 (56) [M + K^+].

Acknowledgements

S. K. thanks the Deutsche Forschungsgemeinschaft for generous funding. C. R. thanks the Cusanus-Stiftung for a fellowship. The support and sponsorship concerted by COST action D31 on "Organising Non-Covalent Chemical Systems with Selected Functions" are also kindly acknowledged. B. P. H. was sponsored by the Division of Chemical Sciences, Geosciences, and Biosciences, Office of Basic Energy Sciences, US Department of Energy, under contract number DE-AC05-

00OR22725 with Oak Ridge National Laboratory (managed by UT-Battelle, LLC). We also thank Mrs A. Lagutschenkov for measuring the ESI-MS spectra.

References

1 (*a*) D. J. Cram, *Angew. Chem.*, 1986, **98**, 1041–1060 (*Angew. Chem., Int. Ed.*, 1986, **25**, 1039–1057); (*b*) D. J. Cram, *Angew. Chem.*, 1988, **100**, 1041–1052 (*Angew. Chem., Int. Ed.*, 1988, **27**, 1009–1020).

2 (*a*) A. F. D. de Namor, M.-C. Ritt, M.-J. Schwing-Weill, F. Arnaud-Neu and D. F. V. Lewis, *J. Chem. Soc., Faraday Trans.*, 1991, **87**, 3231–3239; (*b*) E. Grunwald and C. Steel, *J. Am. Chem. Soc.*, 1995, **117**, 5687–5692; (*c*) D. H. Williams and M. S. Westwell, *Chem. Soc. Rev.*, 1998, **27**, 57–63; (*d*) L. Liu and Q.-X. Guo, *Chem. Rev.*, 2001, **101**, 673–696; (*e*) E. A. Meyer, R. K. Castellano and F. Diederich, *Angew. Chem.*, 2003, **115**, 1244–1287 (*Angew. Chem., Int. Ed.*, 2003, **42**, 1210–1250); (*f*) K. N. Houk, A. G. Leach, S. P. Kim and X. Zhang, *Angew. Chem.*, 2003, **115**, 5020–5046 (*Angew. Chem., Int. Ed.*, 2003, **42**, 4872–4897).

3 (*a*) C. Gennari, H. P. Nestler, U. Piarulli and B. Salom, *Liebigs Ann./Recueil*, 1997, 637–647; (*b*) C. Schmuck and P. Wich, *New J. Chem.*, 2006, **30**, 1377–1385; (*c*) P. T. Corbett, J. Leclaire, L. Vial, K. R. West, J.-L. Wietor, J. K. M. Sanders and S. Otto, *Chem. Rev.*, 2006, **106**, 3652–3711.

4 (*a*) W. Yang, H. He and D. G. Drueckhammer, *Angew. Chem.*, 2001, **113**, 1764–1768 (*Angew. Chem., Int. Ed.*, 2001, **40**, 1714–1718); (*b*) B. P. Hay and T. K. Firman, *Inorg. Chem.*, 2002, **41**, 5502–5512; (*c*) H. D. Huang and D. G. Drueckhammer, *Chem. Commun.*, 2005, 5196–5198; (*d*) C. Lin and D. G. Drueckhammer, *New J. Chem.*, 2006, **30**, 1725–1730; (*e*) H. D. Huang and D. G. Drueckhammer, *Chem. Commun.*, 2006, 2995–2997; (*f*) A. Ragusa, J. M. Hayes, M. E. Light and J. D. Kilburn, *Chem.–Eur. J.*, 2007, **13**, 2717–2728.

5 (*a*) M. Haj-Zaroubi and F. P. Schmidtchen, *ChemPhysChem*, 2005, **6**, 1181–1186; (*b*) V. D. Jadhav and F. P. Schmidtchen, *Org. Lett.*, 2005, **7**, 3311–3314; (*c*) F. P. Schmidtchen, *Coord. Chem. Rev.*, 2006, **250**, 2918–2928; (*d*) V. D. Jadhav and F. P. Schmidtchen, *Org. Lett.*, 2006, **8**, 2329–2332.

6 (*a*) S. Kubik, R. Goddard, R. Kirchner, D. Nolting and J. Seidel, *Angew. Chem.*, 2001, **113**, 2722–2725 (*Angew. Chem., Int. Ed.*, 2001, **40**, 2648–2651); (*b*) S. Kubik and R. Goddard, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 5127–5132; (*c*) S. Kubik, R. Kirchner, D. Nolting and J. Seidel, *J. Am. Chem. Soc.*, 2002, **124**, 12752–12760; (*d*) S. Kubik, R. Goddard, S. Otto, S. Pohl, C. Reyheller and S. Stüwe, *Biosens. Bioelectron.*, 2005, **20**, 2364–2375; (*e*) S. Otto and S. Kubik, *J. Am. Chem. Soc.*, 2003, **125**, 7804–7805.

7 (*a*) V. S. Bryantsev and B. P. Hay, *J. Am. Chem. Soc.*, 2006, **128**, 2035–2042; (*b*) B. P. Hay, A. A. Oliferenko, J. Uddin, C. G. Zhang and T. K. Firman, *J. Am. Chem. Soc.*, 2005, **127**, 17043–17053.

8 (*a*) J. M. Daniel, S. D. Friess, S. Rajagopalan, S. Wendt and R. Zenobi, *Int. J. Mass Spectrom.*, 2002, **216**, 1–27; (*b*) B. Baytekin, H. T. Baytekin and C. A. Schalley, *Org. Biomol. Chem.*, 2006, **4**, 2825–2841.

9 (*a*) F. P. Schmidtchen, in *Analytical Methods in Supramolecular Chemistry*, ed. C. A. Schalley, Wiley-VCH, Weinheim, 2006, pp. 55–78; (*b*) I. Wadsö, *Chem. Soc. Rev.*, 1997, **26**, 79–86.

10 (*a*) F. P. Schmidtchen and M. Berger, *Chem. Rev.*, 1997, **97**, 1609–1646; (*b*) A. Bianchi, K. Bowman-James and E. García-España, *Supramolecular Chemistry of Anions*, Wiley-VCH, New York, 1997; (*c*) C. R. Bondy and S. J. Loeb, *Coord. Chem. Rev.*, 2003, **240**, 77–99; (*d*) K. Choi and A. D. Hamilton, *Coord. Chem. Rev.*, 2003, **240**, 101–110; (*e*) S. Kubik, C. Reyheller and S. Stüwe, *J. Inclusion Phenom. Macrocyclic Chem.*, 2005, **52**, 137–187; (*f*) E. A. Katayev, Y. A. Ustynyuk and J. L. Sessler, *Coord. Chem. Rev.*, 2006, **250**, 3004–3037; (*g*) S. O. Kang, R. A. Begum and K. Bowman-James, *Angew. Chem.*, 2006, **118**, 8048–8061 (*Angew. Chem., Int. Ed.*, 2006, **45**, 7882–7894); (*h*) J. L. Sessler, P. A. Gale and W.-S. Cho, *Anion Receptor Chemistry*, RSC, Cambridge, 2006.

11 J. J. Ge, C. Y. Li, G. Xue, I. K. Mann, D. Zhang, S.-Y. Wang, F. W. Harris, S. Z. D. Cheng, S.-C. Hong, X. Zhuang and Y. R. Shen, *J. Am. Chem. Soc.*, 2001, **123**, 5768–5776.

