ORGANIC LETTERS

2007
Vol. 9, No. 25
5271−5274

Selective Sensing of Sulfate in Aqueous Solution Using a Fluorescent Bis(cyclopeptide)

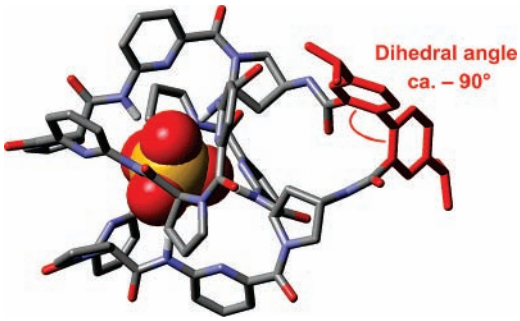
Carsten Reyheller and Stefan Kubik*

Fachbereich Chemie - Organische Chemie, Technische Universität Kaiserslautern, Erwin-Schrödinger-Strasse, D-67663 Kaiserslautern, Germany

kubik@chemie.uni-kl.de

Received October 5, 2007

ABSTRACT



Fluorescence of a bis(cyclopeptide) in which two cyclohexapeptide moieties with alternating L-proline and 6-aminopicolinic acid subunits are attached to a 4,4′-bis(dimethylamino)biphenyl linker is quenched in 1:1 (v/v) water/methanol in the presence of sulfate. Of eight other anions tested, none produced a similar effect. This bis(cyclopeptide) allows the qualitative and quantitative detection of sulfate even in the presence of an excess of chloride anions. Calculations provided insight into the causes of fluorescence quenching and anion selectivity.

Combining the binding event of a synthetic receptor with the change of an easily measurable receptor property produces a chemosensor. Chemosensors that selectively detect an analyte under conditions relevant for practical applications have the potential to find applications in areas such as medicinal diagnostics or environmental monitoring.[1] Of the several techniques that can be used to follow the binding event of a synthetic receptor, fluorescence spectroscopy is particularly attractive because it is rapidly performed, nondestructive, and highly sensitive.[2]

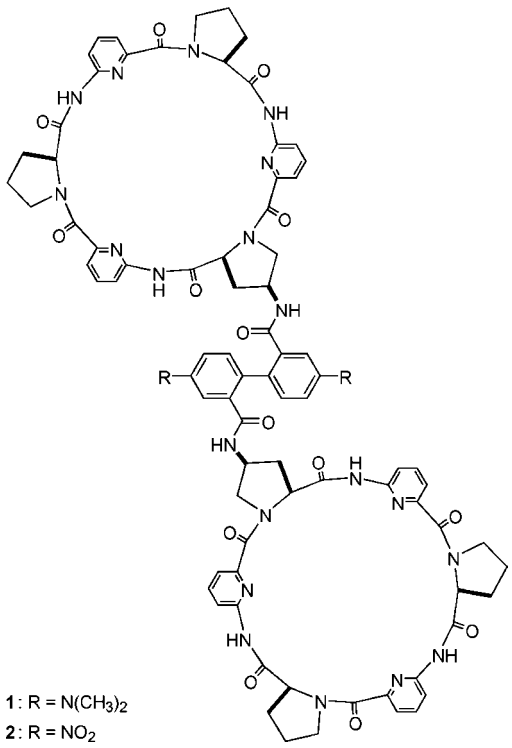
Fluorescent chemosensors contain a fluorophore as the reporter group and a subunit where substrate binding takes place. An event that can affect fluorescence of such a conjugate is conformational reorganization or rigidification during substrate binding. This can effectively alter the optical properties of the fluorophore particularly if the latter is an integral part of the sensor conformationally coupled to the binding site.

Examples for chemosensors whose mode of action is based on this principle are 2,2′-disubstituted biphenyl derivatives.[3] Fluorescence of such compounds depends sensitively on the dihedral angle between the two aryl rings. If this angle changes during interactions of appropriately placed substituents with a substrate, complex formation is reported by a change in fluorescence.

(1) Czarnik, A. W. *Fluorescent Chemosensors for Ion and Molecule Recognition*; ACS Symposium Series 538; American Chemical Society: Washington, DC, 1993. Desvergne, J. P.; Czarnik, A. W. *Chemosensors for Ion and Molecule Recognition*; NATO Asi Series, Series C; Kluwer Academic Publishers: London, 1997.

(2) For, selected reviews, see: Czarnik, A. W. *Acc. Chem. Res.* **1994**, *27*, 302−308. Fabbrizzi, L.; Poggi, A. *Chem. Soc. Rev.* **1995**, *24*, 197−202. de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515−1566. de Silva, A. P.; Fox, D. B.; Huxley, A. J. M.; Moody, T. S. *Coord. Chem. Rev.* **2000**, *205*, 41−57. Martínez-Máñez, R.; Sancenón, F. *Chem. Rev.* **2003**, *103*, 4419−4476. Kim, J. S.; Quang, D. T. *Chem. Rev.* **2007**, *107*, 3780−3799.

10.1021/ol702386e CCC: $37.00
Published on Web 11/15/2007



Here, we present a related system **1** with two cyclohexapeptide moieties containing alternating L-proline and 6-aminopicolinic acid subunits attached to 4,4′-bis(dimethylamino)biphenyl-2,2′-dicarboxylic acid.[4] Such bis(cyclopeptides) have been shown to effectively bind anions such as halides or sulfate in aqueous solvent mixtures.[5] In the case of **1**, anion binding causes quenching of fluorescence which can easily be monitored spectroscopically. Interestingly, quenching is observed exclusively in the presence of sulfate, an anion whose monitoring is relevant in the disposal of radioactive waste,[6] making sensing of **1** very selective.

Synthesis of **1** was performed by reductive amination of dinitro derivative **2** whose synthesis has been described previously (see the Supporting Information).[5d] Compound **1** was obtained in 29% yield after chromatographic purification in sufficient amounts for further investigations.

Anion binding of **1** was studied by using various techniques. Figure 1 shows the ESI mass spectrum of a 0.1 mM solution of **1** in 1:1 (v/v) water/methanol in the presence of 5 equiv of sodium sulfate. Only one signal is visible in this spectrum whose *m*/*z* ratio of 860.3 can be assigned to a 1:1 complex between **1** and a sulfate anion.[7] Similarly, the ESI-MS spectrum of a mixture of **1** and sodium iodide contains a peak corresponding to the 1:1 complex $[\mathbf{1} + \mathrm{I}]^-$, but an additional peak is also visible in this spectrum of a 1:2 complex in which two iodide ions are bound to a receptor molecule (see the Supporting Information). ESI-MS therefore indicates that, similar to previously investigated bis(cyclopeptides), **1** can form complexes with anions in which the two cyclopeptide rings cooperatively interact with the substrate. An indication of a reduced stability of the 1:1 anion complexes of **1** with respect to previously studied systems is, however, the fact that formation of complexes in which a bis(cyclopeptide) interacts with two anions, as in the case of iodide complexation, has not been observed before.[5]

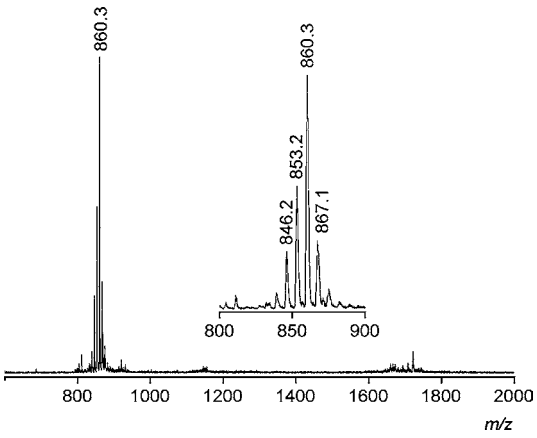


Figure 1. ESI-MS spetrum of a 0.1 mM solution of **1** in 1:1 (v/v) water/methanol in the presence of 5 equiv of Na_2SO_4.

Marked downfield shifts of the proline H(α) signals are observed in the ^{1}H NMR spectrum of **1** in 1:1 (v/v) D_2O/CD_3OD after the addition of sodium sulfate (Figure 2). In addition, signals in the aromatic region of the spectrum sharpen and experience distinct shifts. Similar effects have been observed for other bis(cyclopeptides) where the shifts of the H(α) signals were attributed to the spatial proximity of the H(α) protons and the anionic guest in the complex and those of the signals in the aromatic region to a change

(3) (a) McFarland, S. A.; Finney, N. S. *J. Am. Chem. Soc.* **2001**, *123*, 1260−1261. (b) Lee, D. H.; Im, J. H.; Lee, J. H.; Hong, J. I. *Tetrahedron Lett.* **2002**, *43*, 9637−9640. (c) Costero, A. M.; Andreu, R.; Monrabal, E.; Martínez-Mañez, R.; Sancenón, F.; Soto, J. *J. Chem. Soc., Dalton Trans.* **2002**, 1769−1775. (d) Cody, J.; Fahrni, C. J. *Tetrahedron* **2004**, *60*, 11099−11107. (e) Costero, A. M.; Gil, S.; Sanchis, J.; Peransí, S.; Sanz, V.; Williams, J. A. G. *Tetrahedron* **2004**, *60*, 6327−6334. (f) Costero, A. M.; Sanchis, J.; Gil, S.; Sanz, V.; Williams, J. A. G. *J. Mater. Chem.* **2005**, *15*, 2848−2853. (g) Costero, A. M.; Bañuls, M. J.; Aurell, M. J.; Ochando, L. E.; Domenech, A. *Tetrahedron* **2005**, *61*, 10309−10320.

(4) For recent examples of other cyclopeptide-based chemosensors, see: Leipert, D.; Nopper, D.; Bauser, M.; Gauglitz, G.; Jung, G. *Angew. Chem.* **1998**, *110*, 3503−3505; *Angew. Chem., Int. Ed.* **1998**, *37*, 3311−3314. Ngu-Schwemlein, M.; Butko, P.; Cook, B.; Whigham, T. *J. Pept. Res. Suppl. 1* **2005**, *66*, 72−81. McDonough, M. J.; Reynolds, A. J.; Lee, W. Y. G.; Jolliffe, K. A. *Chem. Commun.* **2006**, 2971−2973. Zhang, Y. H.; Yin, Z. M.; He, J. Q.; Cheng, J. P. *Tetrahedron Lett.* **2007**, *48*, 6039−6043.

(5) (a) Kubik, S.; Kirchner, R.; Nolting, D.; Seidel, J. *J. Am. Chem. Soc.* **2002**, *124*, 12752−12760. (b) Otto, S.; Kubik, S. *J. Am. Chem. Soc.* **2003**, *125*, 7804−7805. (d) Rodriguez-Docampo, Z.; Pascu, S. I.; Kubik, S.; Otto, S. *J. Am. Chem. Soc.* **2006**, *128*, 11206−11210. (d) Reyheller, C.; Hay, B. P.; Kubik, S. *New J. Chem.* **2007**, *31*, in press (http://dx.doi.org/10.1039/b706932d).

(6) Eller, L. R.; Stepien, M.; Fowler, C. J.; Lee, J. T.; Sessler, J. L.; Moyer, B. A. *J. Am. Chem. Soc.* **2007**, *129*, 11020−11021.

(7) Additional peaks are visible in the spectrum around the peak of the 1:1 sulfate complex evenly spaced by exactly 14 mass units. These peaks could correspond to sulfate complexes containing derivatives of **1** with different numbers of methyl groups in the biphenyl substituents. As HPLC and NMR spectroscopic characterization gave no indication of major impurities in the sample used for the measurement we believe that partial decomposition of **1** occurs during ionization.

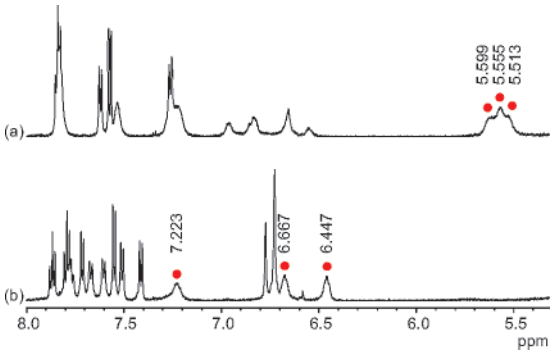


Figure 2. ^{1}H NMR spectrum of a 0.25 mM solution of **1** in 1:1 (v/v) D_2O/CD_3OD in the absence (a) and in the presence (b) of 5 equiv of Na_2SO_4. The signals of H(α) protons are marked.

in electron density of the aminopicolinic acid π-systems caused by interaction of the anion with the NH groups.[5] NMR spectroscopy indicates that the mode of anion binding of **1** is similar.

Sulfate and iodide affinity was determined quantitatively using isothermal titration calorimetry (ITC). The results of these measurements are summarized in Table 1, in which

Table 1. Association Constants log K_a, Gibbs Energies ΔG, Enthalpies ΔH, and Entropies $T\Delta S$ of Binding of NaI and Na_2SO_4 to Bis(cyclopeptides) **1** and **2**[a]

		log K_a	ΔG	ΔH	$T\Delta S$
iodide	**1**	2.68 (0.03)	−15.3 (0.2)	−6.7 (3.1)	8.6 (3.0)
	2[b]	3.61 (0.07)	−20.6 (0.4)	−4.2 (1.5)	15.4 (3.4)
sulfate	**1**	3.87 (0.02)	−22.2 (0.1)	−14.0 (0.6)	8.2 (0.8)
	2[b]	5.32 (0.06)	−30.3 (0.2)	−8.6 (0.5)	21.7 (0.7)

[a] Recorded in 1:1 (v/v) water/methanol at 298 K by at least three independent measurements; standard deviations are specified in parentheses; energies are given in kJ·mol^{-1}. [b] Taken from ref 5d.

previously obtained thermodynamic parameters for the binding of **2** are also included.[5d]

Table 1 shows that binding of **1** to both anions is weaker by at least 1 order of magnitude than that of **2**.[8] This decrease is due to a significant reduction of the entropic contribution to binding which is not fully compensated by a more favorable enthalpic term.

Similar to other 4,4′-bis(dimethylamino)biphenyl derivatives, **1** fluoresces upon excitation at 300 nm.[3f] Whether anion complexation is reported by a change in fluorescence was studied by comparing the fluorescence spectrum of **1** with spectra recorded in the presence of 10 equiv of potential anionic substrates. These investigations revealed that the emission band of **1** at 505 nm is practically unaffected by the presence of the sodium salts of iodide, bromide, chloride, nitrate, perrhenate, perchlorate, and hydrogen phosphate. Sodium selenate produces a slight decrease in fluorescence intensity (see the Supporting Information); the presence of 10 equiv of sodium sulfate, however, almost completely quenches fluorescence (Figure 3). This decrease allowed us

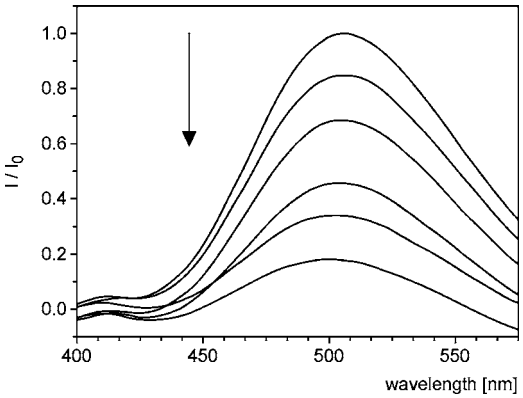


Figure 3. Fluorescence spectra of a 12.5 μM solution of **1** in 1:1 (v/v) water/methanol in the presence of 0, 0.5, 1.0, 2.5, 5.0, and 10.0 equiv of Na_2SO_4. Excitation wavelength 300 nm.

to determine sulfate complex stability also by fluorescence titration (see the Supporting Information). A log K_a of 4.15 ± 0.02 has thus been determined, which is in reasonable agreement with the result of the ITC measurement.

For practical applications it is often necessary to detect an analyte in the presence a large excess of competing substrates. We therefore also performed a fluorescence titration in the presence of 100 equiv of sodium chloride. Under these conditions, a log K_a of 4.12 ± 0.02 was determined, showing that quantitative determination of sulfate is not affected by the presence of chloride ions.

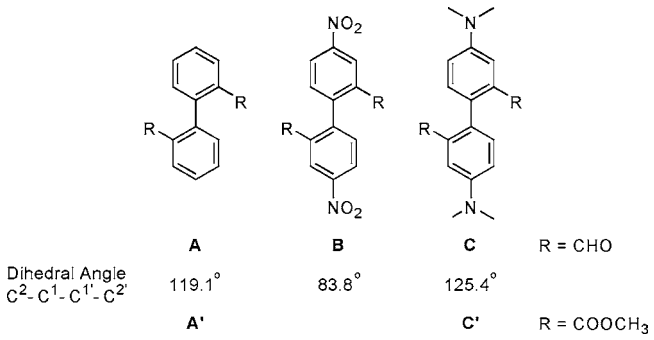
Previous investigations have shown that anion affinity of our bis(cyclopeptides) decreases in the order sulfate > iodide > bromide > chloride, nitrate.[5] Thus, under the conditions of fluorescence spectroscopy, **1** only allows detection of the best substrate while other anions are most probably bound too weakly to induce an optical response. Selectivity of **1** in anion sensing can therefore best be rationalized by the correct balance between sulfate affinity and affinity to other anions.

The strong effect on anion affinity of substituents in the biphenyl linker of **1** and **2** was unexpected. One explanation could be that the substituents in the linker have an effect on receptor conformation and, as a consequence, on binding properties. To test this assumption we performed DFT calculations with the B3LYP/6-31G* method on model compounds **A**, **B**, and **C**.[9]

According to these calculations, the unsubstituted dialdehyde **A** prefers a dihedral angle of 119.1° in the most stable

(8) The binding isotherms obtained by the ITC titrations were all fitted by using the model of substrate binding to receptors with a single set of identical binding sites although formation of higher complexes in the case of iodide complexation cannot be ruled out.

(9) Calculations were performed by using MacSpartan '04 (Wavefunction Inc.) on the *P* enantiomers of **A**, **B**, and **C** (positive dihedral angle) with *anti*-oriented formyl groups. The biphenyl linker in the sulfate complex of **1** adopts the *M* conformation (negative dihedral angle).



conformer. The angle is reduced to 83.8° in the dinitro derivative **B** probably because the nitro groups weaken conjugation of the two aryl rings allowing them to adopt an arrangement with reduced steric strain. Conjugation of the dimethylamino groups in **C** with the carbonyl groups in the 2- and 2′-positions, on the other hand, increases the order of the bond between the aryl rings. This conjugation is presumably responsible for the large angle of 125.4°.

Crystal structures of structurally related biphenyl derivatives **A′** and **C′** are described. The dihedral angle in the unsubstituted derivative **A′** amounts to 89.8°, thus deviating from the calculated value for **A**.[10] Compound **C′** adopts a conformation in the crystal with syn-oriented methyl ester groups and a $C^2-C^1-C^{1'}-C^{2'}$ dihedral angle of 55.6°.[11] The corresponding $C^2-C^1-C^{1'}-C^{6'}$ dihedral angle of 124.4° thus compares favorably with the calculated angle in **C**. Although effects of crystal packing can affect molecular structure in the solid state, the results of our calculations in combination with the crystal structures of **A′** and **C′** indicate that substituents in 4-position in conjunction with electron-withdrawing substituents in 2-position have an effect on biphenyl conformation.

Calculations were also performed on the sulfate complex of **1** to gain insight into the structure of the complexes of this receptor. It can be assumed that linker conformation is in this case largely determined by the arrangement of the two cyclopeptide rings and not by electronic effects of biphenyl substituents. Depending on the method used for optimization, the biphenyl dihedral angle amounts to −93.9° (force-field, MMFF) or −86.6° (semiempirical, AM1) (see the Abstract graphic).[9] The absolute value of this angle is close to the preferred one in a 4,4′-dinitro-substituted biphenyl derivative but significantly different from the angle in a biphenyl with dimethylamino substituents. Anion complexation of **1** thus requires the linker to adopt an energetically unfavorable conformation, whereas linker conformation in the complexes of **2** does not deviate significantly from the most stable arrangement.

The calculations thus provide a rationale for the lower anion affinity of **1**. Since quantum yields of fluorescence have been shown to be higher in biphenyl derivatives that can adopt a planar or near planar conformation in the excited S_1 state, the calculated structure of the sulfate complex also explains why fluorescence intensity of **1** decreases upon sulfate binding.[12] A similar argument explains why **2** is not a suitable chemosensor.

Reexamination of the results of the ITC titrations on the basis of these considerations is less straightforward. The more favorable binding entropy of **2** could indicate that, because the linker induces a mutual orientation of the two cyclopeptide rings in uncomplexed **2** close to that in the complex, this receptor is better preorganized for anion binding. The more negative binding enthalpy of **1** cannot be explained so easily, however. Considering that the linker adopts an energetically unfavorable conformation in the complexes of **1** one should expect binding enthalpy to be less favorable than that of **2**. This interpretation might be oversimplified, however, as it neglects solvation effects which should differ for receptors that adopt different conformations in the absence of substrates. Thermodynamic contributions of solvent reorganization during complex formation superimpose those of direct substrate receptor interactions in ITC titrations making the interpretation of the binding data of receptors **1** and **2** difficult.

In summary, linking two cyclopeptide rings via a 4,4′-bis(dimethylamino)-substituted biphenyl derivative has furnished a highly selective sulfate sensor, which allows qualitative and quantitative sulfate detection in aqueous solution even in the presence of an excess of chloride anions. Calculations provided insights into the causes for fluorescence quenching and anion selectivity. The detailed information thus obtained should now allow further optimization of the system or its implementation in practical applications.

Acknowledgment. S.K. thanks the Deutsche Forschungsgemeinschaft for generous funding. C.R. thanks the Cusanuswerk for a fellowship. The support and sponsorship concerted by COST action D31 are also kindly acknowledged.

Supporting Information Available: Synthesis and spectroscopic characterization of **1**. Experimental details and results of the binding studies (ESI-mass spectra, fluorescence spectra, fluorescence titrations). This material is available free of charge via the Internet at http://pubs.acs.org.

OL702386E

(10) Benmenni, L.; Alilou, E. H.; Giorgi, M.; Pierrot, M.; Reglier, M. *J. Chem. Cryst.* **1994**, *24*, 345−352.

(11) Costero, A. M.; Andreu, C.; Martínez-Mañez, R.; Soto, J.; Ochando, L. E.; Amígo, J. M. *Tetrahedron* **1998**, *54*, 8159−8170.

(12) Nijegorodov, N. I.; Downey, W. S. *J. Phys. Chem.* **1994**, *98*, 5639−5643. Berlman, I. B. *J. Phys. Chem.* **1970**, *74*, 3085−3093. Berlman, I. B. *J. Chem. Phys.* **1970**, *52*, 5616−5621. For a recent related study, see: Allen, B. D.; Benniston, A. C.; Harriman, A.; Llarena, I.; Sams, C. A. *J. Phys. Chem. A* **2007**, *111*, 2641−2649.