ORGANIC LETTERS

2011
Vol. 13, No. 14
3632–3635

Allosteric P=O-Based Receptors for Dicarboxylic Acids

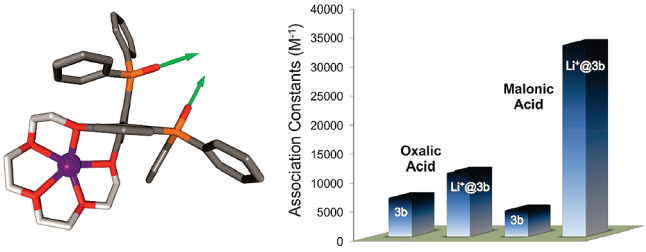
D. Amilan Jose,[†] Ignasi Mon,[†] Héctor Fernández-Pérez,[†] Eduardo C. Escudero-Adán,[†] J. Benet-Buchholz,[†] and Anton Vidal-Ferran*[,†,‡]

ICIQ, Institut Català d'Investigació Química, Avda. Països Catalans, 16, 43007 Tarragona, Spain, and ICREA, Institució Catalana de Recerca i Estudis Avançats, Passeig Lluís Companys, 23, 08010 Barcelona, Spain

avidal@iciq.es

Received May 12, 2011

ABSTRACT



The synthesis of new P=O-disubstituted receptors with appended crown ethers and their properties as receptors for dicarboxylic acids have been studied. High affinities have been observed (oxalic and malonic acids with 4-, 5-, 6-, or 8-crown ethers). Binding of a cationic effector within the crown ether unit resulted in a positive "*allosteric*" effect, which has been determined to be $K_{rel} = 7$ in the best case (binding of malonic acid with Li^+ @ *rac*-3b).

Allosteric modulation is a quite common feature in living systems and has allowed for structural and functional regulation.[1] The term allosterism involves the modification of the properties of the biologically active site by the interaction of an external unit (effector) with a specific regulating site in the biological system. This effector can either enhance or decrease the functional properties of the biological system. The design of artificial allosteric receptors is of great significance for controlling molecular function by external stimuli. Since Rebek's pioneering design of an artificial allosteric receptor,[2a] other systems have been designed and studied to control catalysis and binding by combining allosteric sites and effectors of a diverse nature.[2b−d,3,4]

A wide variety of receptors for neutral molecules (aromatic derivatives, nucleic bases, sugars, ureas, etc.) and charged species (alkali metal ions, halide anions, carboxylates, etc.) incorporating an appended crown ether unit as the allosteric site have been reported.[3] Binding of suitable effectors not identical to the substrates (heterotropic allostery) within the crown ether unit has led to increased receptor affinity toward the aforementioned substrates (positive allosteric effects). Crown ethers are certainly not the only allosteric units that have been studied (examples on the use of bipyridine, Schiff base, cathecol, β-diketone, calixarene, resorcinarene, boronic acid groups, and other motifs as allosteric sites have been reported[3,4]). However, with the possibility to easily vary the crown ether size, shape, and topology together with

[†] ICIQ.
[‡] ICREA.

(1) For example, see: (a) Perutz, M. F.; Fermi, G.; Luisi, B.; Shaanan, B.; Liddington, R. C. *Acc. Chem. Res.* **1987**, *20*, 309. (b) Grandori, R.; Lavoie, T. A.; Pflumm, M.; Tian, G.; Niersbach, H.; Maas, W. K.; Fairman, R.; Carey, J. *J. Mol. Biol.* **1995**, *254*, 150. (c) Burke, J. R.; Witmer, M. R.; Tredup, J.; Micanovic, R.; Gregor, K. R.; Lahiri, J.; Tramposch, K. M.; Villafranca, J. J. *Biochemistry* **1995**, *34*, 15165.

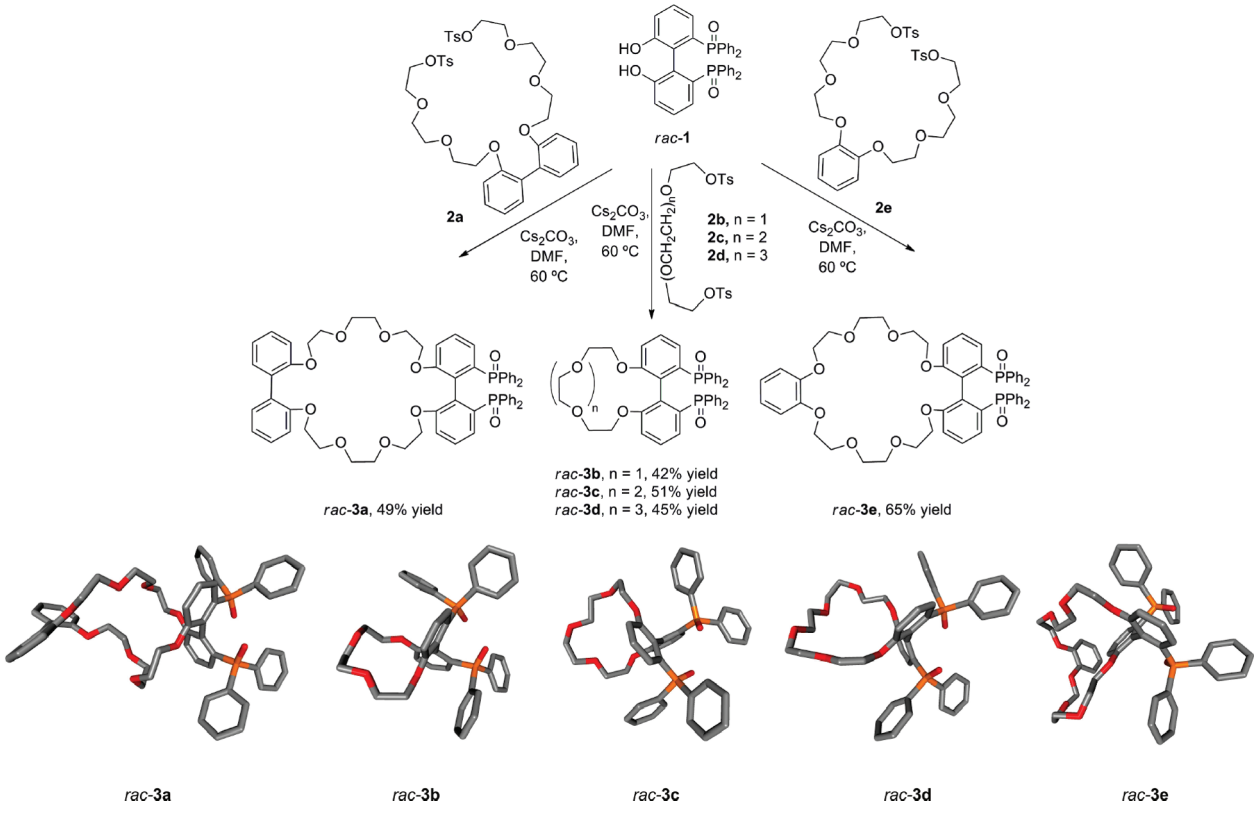
(2) (a) Rebek, J., Jr.; Trend, J. E. *J. Am. Chem. Soc.* **1978**, *100*, 4315. (b) Rebek, J., Jr.; Trend, J. E.; Wattley, R. V.; Chakravorti, S. *J. Am. Chem. Soc.* **1979**, *101*, 4333. (c) Rebek, J., Jr. *Acc. Chem. Res.* **1984**, *17*, 258. (d) Rebek, J., Jr.; Costello, T.; Marshall, L.; Wattley, R.; Gadwood, R. C.; Onan, K. *J. Am. Chem. Soc.* **1985**, *107*, 7481.

(3) (a) Nabeshima, T. *Coord. Chem. Rev.* **1996**, *148*, 151. (b) Takeuchi, M.; Ikeda, M.; Sugasaki, A.; Shinkai, S. *Acc. Chem. Res.* **2001**, *34*, 865. (c) Kovbasyuk, L.; Kraemer, R. *Chem. Rev.* **2004**, *104*, 3161. (d) Kubo, Y.; Ishii, Y. *J. Nanosci. Nanotechnol.* **2006**, *6*, 1489.

(4) As recent examples, see: (a) Hirata, O.; Takeuchi, M.; Shinkai, S. *Chem. Commun.* **2005**, 3805. (b) Meyer, M.; Fremond, L.; Espinosa, E.; Brandes, S.; Yves Vollmer, G.; Guilard, R. *New J. Chem.* **2005**, *29*, 1121. (c) Sessler, J. L.; Tomat, E.; Lynch, V. M. *J. Am. Chem. Soc.* **2006**, *128*, 4184. (d) Setsune, J.-i.; Watanabe, K. *J. Am. Chem. Soc.* **2008**, *130*, 2404. (e) Zahn, S.; Reckien, W.; Kirchner, B.; Staats, H.; Matthey, J.; Luetzen, A. *Chem.—Eur. J.* **2009**, *15*, 2572. (f) Ercolani, G.; Schiaffino, L. *Angew. Chem., Int. Ed.* **2011**, *50*, 1762.

10.1021/ol201277d
Published on Web 06/13/2011

Scheme 1. Synthesis and X-ray Structures of *rac*-**3a**–**e**



well established synthetic strategies for their preparation, they have become popular artificial allosteric sites. Most surprisingly, allosteric receptors for dicarboxylic acids are scarce in the literature,[5] and this work details our initial efforts in the design, preparation, and binding studies of P=O-based receptors *rac*-**3a**–**e** for the recognition of dicarboxylic acids with a crown ether mediated allosteric modulation mechanism (Scheme 1). It was envisaged that binding of cationic species (allosteric effectors) within the crown ether moiety via ion–dipole interactions would induce conformational changes to the [1,1′-biphenyl]-2,2′-diylbis(diphenylphosphine oxide) unit resulting in modified binding properties toward the dicarboxylic acids.[6] Molecular recognition of both mono- and dicarboxylic acids is of considerable interest because of their important roles in biology. Over the past few years, a variety of receptors containing different functional groups have been reported for selective binding of carboxylic acids.[6] However, to the best of our knowledge, no reports on P=O-based receptors have been made for selective dicarboxylic acid recognition.

New receptors *rac*-**3a**–**e** were straightforwardly synthesized from easily available compound *rac*-**1**[7] by reaction with the corresponding bistosylated polyethylene oxide derivative **2**[8] and an excess of Cs_2CO_3 (10 equiv) in DMF at 60 °C for 24 h. Compounds *rac*-**3a**–**e** were isolated as white solids in 42–65% yield (Scheme 1) and characterized by standard analytical methods (NMR and HRMS). X-ray analysis[9] confirmed the proposed structures.

Binding properties of P=O-based crown receptors *rac*-**3b**–**e** with different dicarboxylic acids were studied with several spectroscopic techniques. Changes in the UV spectra of *rac*-**3b**–**e** were observed after the addition of increasing amounts of the acid solutions to solutions of *rac*-**3b**–**e** in DCM/MeOH (99.5/0.5 v/v): the absorption band at 300 nm experienced a 5 nm bathochromic shift upon binding, and association constants between *rac*-**3b**–**e** with

(5) (a) Takeuchi, M.; Imada, T.; Shinkai, S. *Angew. Chem., Int. Ed.* **1998**, *37*, 2096. (b) Ikeda, T.; Hirata, O.; Takeuchi, M.; Shinkai, S. *J. Am. Chem. Soc.* **2006**, *128*, 16008.

(6) See the following reviews and references cited therein: (a) Rebek, J., Jr. *Mol. Struct. Energ.* **1988**, *10*, 219. (b) Chen, H.; Weiner, W. S. Hamilton, A. D. *Curr. Opin. Chem. Biol.* **1997**, *1*, 458. (c) Fitzmaurice, R. J.; Kyne, G. M.; Douheret, D.; Kilburn, J. D. *J. Chem. Soc., Perkin Trans. 1* **2002**, 841. (d) Korendovych, I. V.; Roesner, R. A.; Rybak-Akimova, E. V. *Adv. Inorg. Chem.* **2007**, *59*, 109.

(7) (a) Schmid, R.; Foricher, J.; Cereghetti, M.; Schoenholzer, P. *Helv. Chim. Acta* **1991**, *74*, 370. (b) Qiu, L. Q.; Qi, J. Y.; Ji, J. X.; Zhou, Z. Y.; Yeung, C. H.; Choi, M. C. K.; Chan, A. S. C. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **2003**, *C59*, o33.

(8) Compounds **2b**–**d** are commercially available, and **2e** was prepared as reported in the literature: Zhu, X.-Z.; Chen, C.-F. *J. Am. Chem. Soc.* **2005**, *127*, 13158. Compound **2a** was synthesized following the same synthetic strategy than for **2e**.

(9) Crystals of compounds *rac*-**3a**–**e** and the complex between *rac*-**3c** and oxalic acid were obtained by slow evaporation of their solutions in organic solvents at rt. Supplementary crystallographic data for this paper (CCDC 791802–791806 and 823794) can also be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

oxalic and malonic acids could in this way be determined (see Table 1).[10] Other dicarboxylic acids with a longer spacer between the carboxylic groups (i.e., succinic, glutaric, and adipic acids) induced very small changes in the absorption spectra, which led us to conclude that weak binding was taking place in these cases. Binding studies consistently revealed that the binding affinity of the receptor decreased upon increasing the spacer chain length of the dicarboxylic acid, thus the selectivity order being HOOC−COOH > HOOC−CH_2−COOH > HOOC−$(CH_2)_2$−COOH ≫ HOOC−$(CH_2)_{n>2}$−COOH (Table 1). It is worth mentioning that the ring size of the crown ether in the receptors does not seem to influence the affinity of the P═O binding groups toward oxalic and malonic acids.

Table 1. Association Constants of Receptors *rac*-**3b**−**e** with Dicarboxylic Acids

		binding constants (M^{-1})[a,b]	
entry	receptor	oxalic acid	malonic acid
1	*rac*-**3b**	6.7×10^3	4.5×10^3 [c]
2	*rac*-**3c**	4.9×10^3	4.4×10^3
3	*rac*-**3d**	6.2×10^3	4.6×10^3
4	*rac*-**3e**	7.4×10^3	5.0×10^3

[a] Measured in DCM/MeOH (99.5/0.5 v/v) at 25 °C with UV−vis absorption spectroscopy. [b] Average value of at least two measurements. Standard deviations (expressed in % with respect to the binding constant value) were 17% (average value for all measurements indicated in the table). [c] This binding constant was also measured by ^{31}P NMR, and its value was found to be 2.8×10^3 M^{-1}. The binding constant determined by NMR agrees reasonably well with that measured by UV−vis spectroscopy. However, the magnitude of the binding contants (*ca.* 10^3 M^{-1}) forced us to use dilute solutions of *rac*-**3b** during the NMR titration (*ca.* 10^{-3} M), with the correspondingly long acquisition times and experiment duration. For the sake of convenience, UV−vis instead of NMR spectroscopy was used in all other cases for determining the association constants.

The interaction between the COOH and the P═O groups could be confirmed both in solution and in the solid state (see Figure 1). ^{31}P NMR analysis showed a broadening and a downfield shift of the P═O signal upon addition of oxalic or malonic acids (2.8 and 2.6 ppm, respectively),[11] thus confirming the interaction between the P═O and COOH groups in solution and also allowing the measurement of the binding constant with this technique (see footnote c in Table 1).[11] Crystals of the complex between *rac*-**3c** and oxalic acid suitable for X-ray analysis could be grown and its crystal structure investigated. In the solid state, the substrate molecules have formed hydrogen bonds between the P═O and COOH groups (see dashed lines in Figure 1; $d_{P=O-HOOC}$ = 2.580 Å and $d_{P=O-HOOC}$ = 1.70 Å), leading to a well-defined linear complex with an overall 1:1 stoichiometry).[9,11]

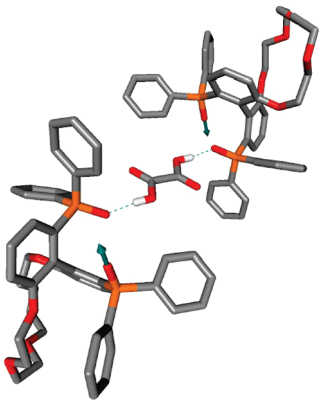


Figure 1. X-ray structure of *rac*-**3c** with oxalic acid (arrows indicate binding groups for further oxalic acid molecules).

The next step in the investigation of our allosteric receptors consisted of the study of the complexation of several potential allosteric "effectors" within the crown ether cavity (SI Table 1 in the Supporting Information). Crown ethers are well-known to form complexes with positively charged species.[12] Association of *rac*-**3a**−**e** with potential crown ether binders was studied with fluorescence emission spectroscopy: the emission band was partially quenched and red-shifted (*ca.* 5−7 nm) upon addition of a solution of crown ether binders to solutions of receptors *rac*-**3a**−**e**. Association constants (see SI Table 1) were calculated from the titration data by nonlinear curve fitting assuming a 1:1[13] binding model. Our binding studies revealed that crown ether containing receptors *rac*-**3a**−**e** did not show a pronounced selectivity for any of the different cationic species that were analyzed (Li^+, Na^+, K^+, Ca^{2+}, Ba^{2+}, and NH_4^+), *K*-values at 25 °C in DCM/MeOH (99.5/0.5 v/v) being around 10^4 M^{-1} in almost every case.[14] However, and as expected from the ring size, the receptor incorporating the smallest ring (*rac*-**3b**) showed a small preference for Li^+ over Na^+. Receptor *rac*-**3c** presented a slightly higher binding affinity for Na^+ and K^+ over Li^+; *rac*-**3d** had a small preference for Ba^{2+} whereas *rac*-**3a** and *rac*-**3e** had a preference for Ca^{2+}. All

(10) UV−visible titration data were analyzed using multivariate factor analysis and considering a binding model with two colored stoichiometric states of the crown-ethers: unbound and 1:1 complex. SPECFIT software (Version 3.0; Spectra Software Associates) was used: (a) Gampp, H.; Maeder, M.; Meyer, C. J.; Zuberbühler, A. D. *Talanta* **1985**, *32*, 95. (b) Gampp, H.; Maeder, M.; Meyer, C. J.; Zuberbühler, A. D. *Talanta* **1986**, *33*, 943. See Supporting Information for details.

(11) See Supporting Information for details.

(12) For example, see: (a) Cram, D. J. *Angew. Chem., Int. Ed. Engl.* **1988**, *100*, 1009. (b) Pedersen, C. J. *Angew. Chem., Int. Ed. Engl.* **1988**, *100*, 1021.

(13) Job plots of *rac*-**3c** with all positively charged species tested revealed a 1:1 stoichiometry of the complex (see Supporting Information for details).

(14) A preference in the coordination of monovalent (Li^+ and NH_4^+) and divalent cations (Ba^{2+}) towards the crown-ether moiety instead of the P═O group has been observed. Whilst association constants between the aforementioned cationic species and *rac*-**3a**−**e** lie around 10^4 M^{-1}, binding constants between Li^+, NH_4^+, and Ba^{2+} with [1,1′-biphenyl]-2,2′-diylbis(diphenylphosphine oxide) (compound analogue to *rac*-**3** but without the crown-ether moiety) were found to be 2 to 3 orders of magnitude lower (6.7×10^1, 1.0×10^2, and 2.6×10^2 M^{-1}, respectively) in DCM/MeOH (99.5/0.5 v/v) at 25 °C.

receptors bound NH_4^+ with similar affinities (entry 7, SI Table 1).

We have also investigated the binding behavior between *rac*-**3c** and Ba^{2+} by isothermal calorimetry (ITC).[11,15] The binding constant determined by ITC had the same order of magnitude as that measured by fluorescence ($K = 2 \times 10^4\ M^{-1}$ by ITC in DCM/MeOH (98/2 v/v) and $K = 3 \times 10^4\ M^{-1}$ by fluorescence in DCM/MeOH (99.5/0.5 v/v) at 25 °C).[11] A 1:1 ratio between Ba^{2+} and *rac*-**3c** was deduced from the ITC data. ΔH and ΔS values (7.5 kcal/mol, 44.4 cal/mol/K, 298 K in DCM/MeOH (98/2 v/v)) were also extracted from the titration data and revealed that complexation is an entropically driven process.

After studying the binding affinities of several cationic species toward the different crown ether moieties, we turned our attention to the effect of Li^+ and Na^+ as allosteric effectors in the recognition of oxalic and malonic acids by receptors *rac*-**3b**−**e**. The results obtained are summarized in Table 2. It is noteworthy to mention that binding constant values[10] of dicarboxylic acids increased as compared to the receptor with unbound "allosteric" effectors. For example, malonic acid to Li^+ @ *rac*-**3b** ($K = 3.3 \times 10^4\ M^{-1}$; entry 1 in Table 2) is approximately seven times more tightly bound than to *rac*-**3b** ($K = 4.5 \times 10^3\ M^{-1}$; entry 1 in Table 1). Similar effects were observed in the interaction between Na^+ @ *rac*-**3b** and oxalic acid (entry 2 in Table 2).

The complex formation of dicarboxylic acids with cation bound crown ethers was also confirmed by ^1H NMR experiments (see SI Figure 38 in the Supporting Information): Addition of increasing amounts of malonic acid to the solution of Li^+ @ *rac*-**3b** resulted in the appearance of a pseudotriplet at 3.35 ppm (2H) due to the malonic methylene unit. This signal multiplicity can be justified by transformation of the two magnetically equivalent protons of the unbound malonic CH_2-unit into a pair of diastereotopic nuclei due to the close proximity of the malonic acid to the stereogenic axis of *rac*-**3b**.[11] Despite many attempts, it has not been possible to grow crystals suitable for X-ray analysis of any of the complexes indicated in Table 2. We hypothesize that the changes in the torsion angle between the central phenylene rings[16] produced by the inclusion of the effectors into the crown ether strengthen binding in the aforementioned examples.

In conclusion, we have described an efficient synthesis of new P=O-disubstituted biphenyl derivatives with appended crown ethers *rac*-**3a**−**e**. Their properties as receptors for dicarboxylic acids have been evaluated, and high affinities have been observed (oxalic and malonic acids with 4-, 5-, 6-, or 8-crown ethers). Binding of cationic effectors within the crown ether unit resulted in a positive "*allosteric*" effect, which has been determined to be $K_{rel} = 7$ in the best case (binding of malonic acid with Li^+ @ *rac*-**3b**). Work is in progress to develop asymmetric catalysts based on receptors *rac*-**3a**−**e** with an allosteric regulation mechanism.

Table 2. Allosteric Effects on Binding of *rac*-**3b**−**e** with Dicarboxylic Acids

		binding constants (M^{-1})[a,b] and K_{rel}[c]	
entry	receptor	oxalic acid	malonic acid
1	**3b-Li**$^+$	1.1×10^4; 1.7	3.3×10^4; 7.4[d]
2	**3b-Na**$^+$	4.3×10^4; 6.5	1.3×10^4; 2.9
3	**3c-Li**$^+$	1.8×10^4; 3.7	1.3×10^4; 2.9
4	**3c-Na**$^+$	1.6×10^4; 3.3	1.4×10^4; 3.1
5	**3d-Li**$^+$	1.0×10^4; 1.7	1.4×10^4; 3.1
6	**3d-Na**$^+$	2.0×10^4; 3.3	8.5×10^3; 1.9
7	**3e-Li**$^+$	3.9×10^4; 5.3	8.2×10^3; 1.6
8	**3e-Na**$^+$	1.4×10^4; 1.9	7.7×10^3; 1.5

[a] Measured in DCM/MeOH (99.5/0.5 v/v) at 25 °C. [b] Average value of at least two measurements. Standard deviations (expressed in % with respect to the binding constant value) were 16% (average value for all measurements indicated in the table). [c] Binding constant quotient with and without allosteric effector calculated from UV−vis absorption titrations. [d] This binding constant was also measured by ^{31}P NMR, to confirm the K_{rel} values obtained by UV−vis. However, the magnitude of the binding contants (*ca.* $10^4\ M^{-1}$) forced us to use dilute solutions of *rac*-**3b** during the NMR titration (*ca.* 10^{-4} M). No *K*-value could be extracted with this technique, as very broad and weak ^{31}P NMR signals were observed.

Acknowledgment. We thank MICINN (Grant CTQ-2008-00950/BQU), DURSI (Grant 2009GR623), Consolider Ingenio 2010 (Grant CSD2006-0003), and ICIQ Foundation for financial support. A.J. gratefully acknowledges the *"Marie Curie Incoming Program"* and H.F.-P. the *"Programa Torres y Quevedo"* for financial support.

Supporting Information Available. Experimental details, characterization data, and NMR spectra for **1**−**3**, crystallographic data for **3** and details on binding studies. This material is available free of charge via the Internet at http://pubs.acs.org.

(15) Binding studies of other positively charged species were not possible by ITC, since heat evolved due to dilution was much higher than the heat involved in the binding process.

(16) (a) Benniston, A. C.; Li, P.; Sams, C. *Tetrahedron Lett.* **2003**, *44*, 3947. (b) Benniston, A. C.; Harriman, A.; Patel, P. V.; Sams, C. A. *Eur. J. Org. Chem.* **2005**, 4680.