Fluorescence sensing due to allosteric switching of pyrene functionalized *cis*-cyclohexane-1,3-dicarboxylate

Carol Monahan, Jeffrey T. Bien and Bradley D. Smith*†

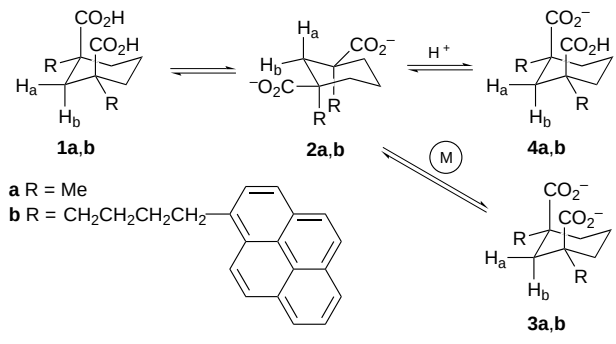
Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556, USA

The excimer/monomer fluorescence ratio for a pyrene functionalized *cis*-cyclohexane-1,3-dicarboxylate decreases upon titration with divalent and monovalent metal cations, as well as strong and weak acids.

Molecules that can be chemically switched between different conformations have been used as allosteric receptors, catalysts, transport carriers, liquid crystalline materials and sensors.[1] The main approach with allosteric sensors has been to change the proximity between two interacting fluorophores,[2] or to alter the solvent polarity surrounding a fluorophore.[3] Here we describe the allosteric properties of *cis*-1,3-disubstituted cyclohexane-1,3-dicarboxylic acids **1** and in particular how the **cis**-1,3-bispyrenyl derivative **1b** acts as a fluorescent cations sensor.[4]

Initially, the *cis*-1,3-dimethyl analogue **1a** was prepared,† and shown by ^{1}H NMR spectroscopy to adopt a chair conformation with both carboxyl groups in axial positions (Scheme 1).§ This conformational preference is in agreement with the higher steric *A* value for a methyl group compared to a carboxy group. Conversion to the dianion results in a switch to the alternate chair structure **2a** with diequatorial carboxylate groups.§ The driving force for this conformational change is the electrostatic repulsion of the proximal anionic carboxylates. In agreement with an earlier report by Menger *et al*., titration of a 1 : 1 MeOH–H_2O solution of **2a** with Mg^{2+} induces a conformational flip to **3a**.[6] Addition of Na^+, however, has a negligible effect on conformation in such a polar solvent. Two factors drive the conformational change induced by Mg^{2+}: (i) partial neutralization of the anionic carboxylates by the Mg^{2+} ion, and (ii) release of steric strain as the methyl groups transfer from diaxial to more accommodating diequatorial positions.

Based on this knowledge it was hypothesized that compound **1b** would show substantial changes in fluorescence upon conformational switching. The two pyrenyl groups in structure **2b** are in close proximity to each other and are able to exhibit intramolecular excimer fluorescence. With structures **1b**, **3b** and **4b**, the pyrenyl groups are separated and the favorability of excimer formation is decreased.[2] This was found to be the case in MeCN solution where titration of the bis(tetrabutylammonium) salt of **2b** with strong acid resulted in a stoichiometric decrease in the excimer/monomer fluorescence ratio. The titration curve (Fig. 1) indicates that only 1 equiv. of nitric acid is needed to completely switch the excimer/monomer ratio. Thus, protonation of one of the carboxylates relieves the strain due to dianionic repulsion and causes a flip to the alternate chair conformation **4b**. In contrast, alkali metal cations induce much gentler decreases in excimer/monomer ratio suggesting a gradual formation of **3b**, where M is one or two M^+ ions.§ Titration with ammonium chloride or ammonium nitrate generated plots that were identical to the nitric acid curve (Figs. 1 and 2). It appears that the ammonium also transfers a proton to **2b** and forms **4b**. Although carboxylic acids are more acidic than ammonium salts in aqueous solution, the reverse order is observed in aprotic solvents such as MeCN. For example, ammonium (pK_a 16.5) is more acidic than glutaric acid (pK_{a_1} 19.2, pK_{a_2} 29.9), a dicarboxylic acid that is structurally related to **1**.[7–9]



Scheme 1

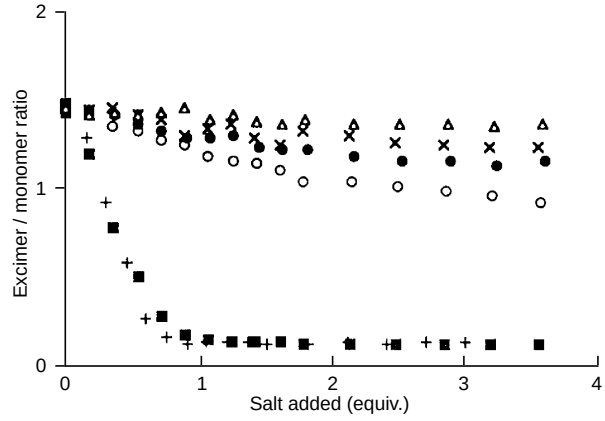


Fig. 1 Change in excimer/monomer ratio for **2b** (3 μM) in MeCN upon titration with (Δ) $CsNO_3$, (x) $RbNO_3$, (●) KNO_3, (○) $NaNO_3$, (■) NH_4NO_3 and (+) HNO_3. Excitation at 346 nm, monomer emission at 397 nm, excimer emission at 470 nm, T = 298 K.

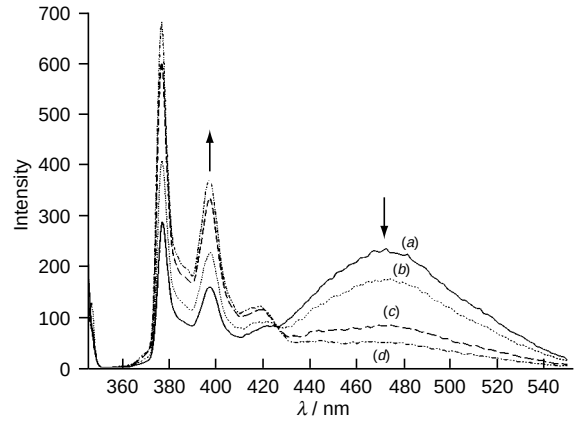


Fig. 2 Fluorescence emission for **2b** in MeCN (3 μM, excitation at 346 nm) in the presence of (*a*) 0, (*b*) 1.0, (*c*) 2.2 and (*d*) 10.6 μM of NH_4NO_3

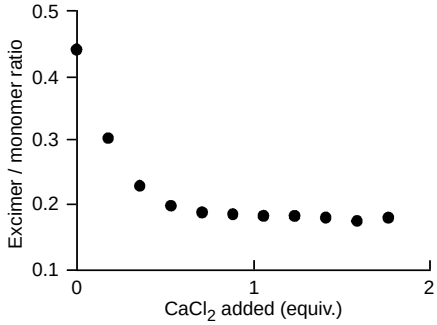


Fig. 3 Change in excimer/monomer ratio for **2b** (3 μM in MeOH upon titration with $CaCl_2$. Excitation at 346 nm, monomer emission at 397 nm, excimer emission at 470 nm, T = 298 K.

Strong evidence that the change in fluorescence is due to a conformational change was obtained from the following control experiments. (i) All of the fluorescence titrations were repeated using tetrabutylammonium pyrene-1-butyrate as a replacement fluorophore. In all cases, negligible changes in fluorescence were observed, indicating that the excimer/monomer switching is not due to due to intermolecular or environmental factors. (ii) Treatment of a CD_3CN solution of **1a** with 2 equiv. of tetramethylammonium hydroxide changes the difference in ^1H NMR chemical shifts for H_a and H_b $\Delta\delta$ = 1.36 to 0.50 ppm, which is consistent with a change from **1a** to **2a**.§ A subsequent titration of this solution with ammonium thiocyanate results in smooth migration back to $\Delta\delta$ = 1.61 ppm, suggesting that **2a** becomes protonated and converts to **4a**. The fluorescence switching effects of ammonium and alkali metal cations are essentially negligible in polar, competitive solvents such as MeOH. However, moderate descreases in excimer/monomer ratio are induced by titrating **2**b with alkaline metal dichlorides to produce **3b** (Fig. 3).

In summary, a simple but sensitive allosteric system is described that can undergo large changes in molecular shape. Depending on the experimental conditions, the conformational switching can be induced by divalent and monovalent metal cations, as well as strong and weak acids. Analogue **1b** exhibits large changes in fluorescence and is thus sensor for Lewis and Brønsted acids. Future efforts will attempt to incorporate this conformational switch into the structures of other molecular devices.

This work was supported by the National Science Foundation (USA).

Notes and References

† E-mail: smith.115@nd.edu

‡ Compound **1a** was synthesized by treating dimethyl cyclohexane-1,3-dicarboxylate with LDA (2 equiv). followed by Me_2SO_4 (2 equiv.). Saponification gave **1a** in 40% overall yield. Similarly, compound **1b** was prepared by treating the di-*tert*-butyl cyclohexane-1,3-dicarboxylate with LDA (2 equiv.) followed by 4-(pyren-1-yl)butyl trifluoromethylsulfonate (2 equiv.). Acid hydrolysis gave **1b** in 15% overall yield.

§ The conformational assignments are based on the close homology of the NMR data with Kemp *et al*. (ref. 5) and Menger *et al*. (ref. 6), who examined the conformational switching of *cis,cis*-1,3,5-trimethylcyclohexane-1,3,5-tricarboxylic acid (Kemp's triacid). In particular, the non-equivalent methylene protons between the two carboxy group in **1a** (H_a and H_b) resonate at δ 2.63 and 1.13, respectively, in 1 : 1 MeOH–H_2O ($\Delta\delta$ = 1.50 ppm). The chemical shift for H_a is strongly deshielded due to the anisotropy of the neighbouring carbonyl groups. In the case of dianion **2a**, the resonances for H_a and H_b are much closer together ($\Delta\delta$ = 0.35 ppm) indicating that they are nearly equidistant from the carboxylates, which can only occur if the carboxylates have assumed equatorial positions. Structure **3** is drawn as a classical chair with the carboxylates in diaxial positions, however, another possibility is a flattened half-chair which provides the carboxylates with slightly more spacious psuedo-axial environments. (ref. 6).

1 H.-J. Schneider and A. K. Mohammad-Ali, in *Comprehensive Supramolecular Chemistry*, ed. J. L. Atwood, J. E. D. Davies, D. D. MacNicol and F. Vögtle, Pergamon, New York, 1996, vol. 2, pp. 81–86.
2 G. L. Arnold and S. A. van Arman, *Tetrahedron Lett*., 1997, **38**, 4745; G. E. Collins and L.-S. Choi, *Chem. Commun*., 1997, 1135; H. Matsumoto and S. Shinkai, *Tetrahedron Lett*., 1996, **37**, 77; M. Takeshita and S. Shinkai, *Chem. Lett*., 1994, 125; I. Aoki, T. Harada, T. Sakaki, Y. Kawahara and S. Shinkai, *J. Chem. Soc., Chem. Commun*., 1992, 1341.
3 G. K. Walkup and B. Imperiali, *J. Am. Chem. Soc*., 1996, **118**, 3053; H. Ikeda, M. Nakamura, N. Ise, N. Oguma, A. Nakamura, T. Ikeda, F. Toda and A. Ueno, *J. Am. Chem. Soc*., 1996, **118**, 10 980.
4 Other switchable cyclohexane-based systems include the following: V. V. Samoshin, V. A. Chertkov, L. P. Vatlina, E. K. Dobretsova, N. A. Simonov, L. P. Kastorsky, D. E. Gremyachinsky and H.-J. Schneider, *Tetrahedron Lett*., 1996, **37**, 3981; M. Goodall, P. M. Kelly, D. Parker, K. Gloe and H. Stephan, *J. Chem. Soc., Perkin Trans. 2*, 1997, 59; S. M. Shirdkar and G. R. Weisman, *J. Chem. Soc., Chem. Commun*., 1989, 236.
5 D. S. Kemp and K. S. Petrakis, *J. Org. Chem*., 1981, **46**, 5140.
6 F. M. Menger, P. A. Chicklo and M. J. Sherrod, *Tetrahedron Lett*., 1989, **30**, 6943.
7 K. Izutsu, *Dissociation Constants in Dipolar Aprotic Solvents*, Blackwell Scientific Publications, Oxford, 1990.
8 I. M. Kolthoff, M. K. Chantooni and S. Bhowmik, *J. Am. Chem. Soc*., 1968, **90**, 23.
9 I. M. Kolthoff and M. K. Chantooni, *J. Am. Chem. Soc*., 1975, **97**, 1376.

Received in Columbia, MO, USA, 28th July 1997; 7/05445I