Locked Energy of Axial to Equatorial Transformation Monitored by Exciplex and Excimer Fluorescence

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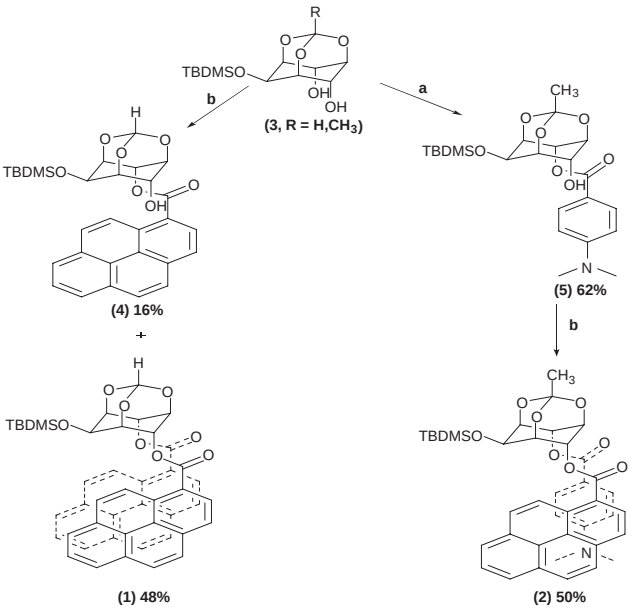
2-*O*-*tert*-Butyldimethylsilyl-4,6-bispyrenoyl-*myo*-inositol-1,3,5-orthoformate (**1**) and 2-*O*-*tert*-butyldimethylsilyl-4-[(4-dimethylamino)benzoyl]-6-pyrenoyl-*myo*-inositol-1,3,5-orthoacetate (**2**) adopt unstable chair conformations with five substituents axial, in which the aromatic esters participate in π-stacking, and give excimer and exciplex fluorescence, respectively. Upon addition of acid, the orthoformate/orthoacetate lock is cleaved, which allows the inositol ring to switch to the more stable penta-equatorial chair conformation, with loss of exciplex/excimer fluorescence.

Intramolecular excimer and exciplexes are of considerable interest as fluorescent probes with a wide range of applications.[1] The fluorescence emission by the formation of excimers and exciplexes, have been effected by various factors including solvent, alkali metals, pH, and light,[2–6] and some of the reports have attributed relative disappearance/decrease in intensity to a change in conformation of the molecules.[7] *myo*-Inositol has five equatorial hydroxy groups in its most stable chair conformation. The 1,3,5-orthoformate or 1,3,5-orthoacetate of *myo*-inositol in **1** and **2**, respectively, locks the chair in the unstable 4,6-diaxial conformation by simultaneously protecting the *cis*-1,3,5-trishydroxy groups.[9] This was done with the purpose of releasing the conformational trigger energy on treatment with acid.[8,10] This study provides proof of principle for the development of fluorescent probes for acidic compartments (e.g. vacuole in cells and hypoxic tumour cells) and other potential applications, for example artificial ribonucleases, biosensors, and signaling devices. To monitor the conformational change, the reagents **1** and **2** have been constructed, which would give fluorescent colour changes when excimer and exciplex signals are destroyed.

In compounds **1** and **2** (Scheme 1) the pyrenoyl and 4-dimethylaminobenzoyl esters are in the conformationally locked 4,6-diaxial positions, perfectly aligned for π-stacking. In a non-fluoresence study, alignment of the aromatic rings in the crystal structure of an analogue in which two ferulic acids are in the 4,6-diaxial positions has been reported.[8] The syntheses of **1** and **2** are shown in Scheme 1. Intermediates **3** (R = H and CH_3) were prepared by reaction of *myo*-inositol with triethyl orthoformate/orthoacetate followed by regiospecific protection of the equatorial 2-hydroxy group with *tert*-butyldimethylsilyl chloride.[9,10] The remaining diaxial 4,6-bishydroxy groups of **3** (R = H) were conjugated with pyrene-1-carboxylic acid using DCC and DMAP[11] to give 4,6-bispyrenoyl-*myo*-inositol-1,3,5-orthoformate **1**. Some 4-pyrenoyl-*myo*-inositol-1,3,5-orthoformate **4** was also isolated. The mixed diester **2** was prepared by reaction of **3** (R = CH_3) with donor 4-dimethylaminobenzoic acid to give **5**, followed by reaction with pyrene-1-carboxylic acid (Scheme 1). In the ^{1}H NMR spectrum, the bispyrenoyl analogue **1** and mixed ester **2** showed upfield shifts of 0.62 ppm (Pyrene H-10) and 1.33 ppm (Aryl H-3/5),[12] respectively, compared with the monoester analogues **4** and **5**. This shift is consistent with π-stacking of the bispyrenes in **1** and pyrene/4-dimethylaminobenzoyl in **2**. Interestingly, the protons of the 4-dimethylamino group in **2** also showed an upfield shift of 0.89 ppm compared with monoester **5**.[12] The inositol proton (H-4) of **1** and **2** gave triplets with small $J_{eq/eq}$ coupling constants of 3.8 Hz, confirming the penta-axial chair conformation.



Scheme 1. Syntheses of 4,6-diaxial *myo*-inositol esters **1**, **2**, **4**, and **5**: (a) 4-dimethylaminobenzoic acid, DCC, DMAP, dry DCM; (b) pyrene-1-carboxylic acid, DCC, DMAP, dry DCM.

The NOESY on mixed ester **2** showed through-space interactions between the pyrenoyl and 4-dimethylaminobenzoyl groups.[12] In addition, fluorescence studies supported the formation of excimer and exciplex for **1** and **2**, with green fluorescence observed at 524 and 520 nm, respectively (large Stokes shift of 156 nm) (Figure 1). Release of the 1,3,5-orthoformate or 1,3,5-orthoacetate conformational lock is achieved by acid-catalysed hydrolysis.[10] To investigate the anticipated conformational change on deprotection, the 4,6-bispyrenoyl diester **1** and mixed 4-[(4-dimethylamino)benzoyl]-6-pyrenoyl diester **2** were treated with 80% trifluoroacetic acid[8,10] to give **6** and **7**, respectively (Scheme 2).

Fluorescence studies on **6** and **7** (Figure 1) showed the disappearance of the excimer and exciplex emission observed for **1** and **2**, with the observation of blue fluorescence at

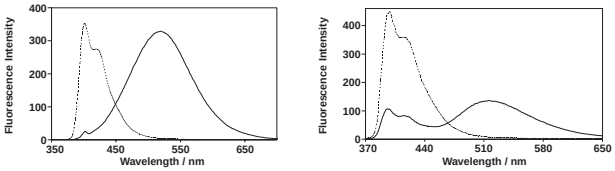
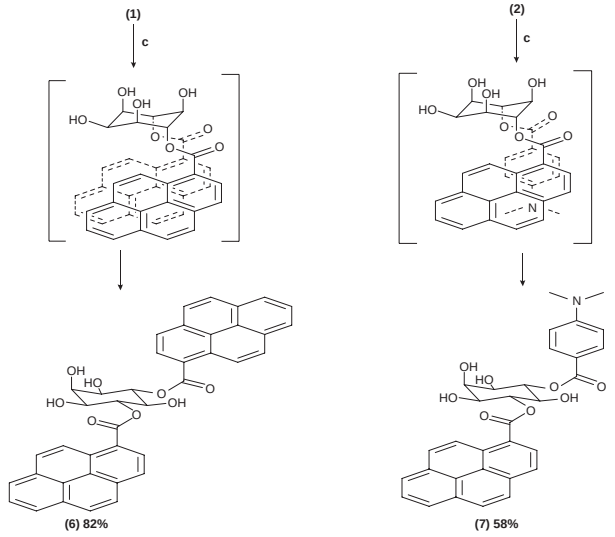


Figure 1. Left: Emission spectra of 2-*O*-*tert*-butyldimethylsilyl-4,6-bispyrenoyl-*myo*-inositol-1,3,5-orthoformate (**1**; solid) and its deprotected analogue, 4,6-bispyrenoyl-*myo*-inositol (**6**; dashed). Right: Emission spectra of 2-*O*-*tert*-butyldimethylsilyl-4-[(4-dimethylamino)benzoyl]-6-pyrenoyl-*myo*-inositol-1,3,5-orthoformate (**2**; solid) and its deprotected analogue 4-[(4-dimethylamino)benzoyl]-6-pyrenoyl-*myo*-inositol (**7**; dashed). Spectra recorded at 10^{-5} M concentration in chloroform at 20 °C. Excitation and emission slit width was 3 nm for **1** and **2** and 1.5 nm for **6** and **7**.



Scheme 2. Ring flip of penta-axial diesters **1** and **2** upon trifluoroacetic acid-catalyzed hydrolysis to give penta-equatorial diesters **6** and **7**: (c) 80% trifluoroacetic acid.

386 nm attributed to locally excited state of pyrene monomer. Excitation spectrum of **1** showed a strong red shift (ca. 43 nm) of the major excitation bands as compared with those of the deprotected analogue **6**.

This shift is attributed to π–π-stacking interactions of the pyrenoyl moieties in the ground state of **1**. In addition, there is a significant downfield shift of the aromatic peaks in the ^{1}H NMR spectrum of **6** and **7** compared with **1** and **2**. Inositol H-4 triplets with $J_{ax/ax}$ values of 9.9 Hz (**6**) and 9.7 Hz (**7**), are also consistent with the more stable penta-equatorial ring-flipped chair conformation. In contrast to **2**, NOESY interactions were not observed between the pyrenoyl and (4-dimethylamino)-benzoyl groups in **7**.[12]

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References and Notes

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12 Partial ^{1}H NMR data of key peaks include: **1** δ ($CDCl_3$) 8.74 (d, 2H, $J = 9.4$ Hz, Pyr H-2), 7.93 (d, 2H, $J = 8.4$ Hz, Pyr H-10), 5.94 (t, 2H, $J = 3.8$ Hz, inos H-4/6). **2** δ ($CDCl_3$) 8.89 (d, 1H, $J = 9.4$ Hz, Pyr H-2), 8.34 (d, 1H, $J = 8.1$ Hz, Pyr H-10), 7.15 (d, 2H, $J = 9.3$ Hz, Ar H-2/6), 5.81 (t, 1H, $J = 3.8$ Hz, inos H-6), 5.67 (t, 1H, $J = 3.9$ Hz, inos H-4), 5.30 (d, 2H, $J = 9.1$ Hz, Ar H-3/5), 2.16 (s, 6H, NMe_2). **4** δ ($CDCl_3$) 9.26 (d, 2H, $J = 9.4$ Hz, Pyr H-2), 8.55 (d, 2H, $J = 8.1$ Hz, Pyr H-10). **5** δ ($CDCl_3$) 7.79 (d, 2H, $J = 8.8$ Hz, Ar H-2/6), 6.63 (d, 2H, $J = 9.0$ Hz, Ar H-3/5), 3.05 (s, 6H, NMe_2). **6** δ (DMSO-d_6) 9.11 (d, 2H, $J = 9.6$ Hz, Pyr H-2), 8.66 (d, 2H, $J = 8.1$ Hz, Pyr H-10), 5.66 (t, 2H, $J = 9.9$ Hz, inos H-4/6). **7** δ (DMSO-d_6) 9.09 (d, 1H, $J = 9.4$ Hz, Pyr H-2), 8.66 (d, 1H, $J = 8.1$ Hz, Pyr H-10), 7.87 (d, 2H, $J = 9.2$ Hz, Ar H-2/6), 6.75 (d, 2H, $J = 9.2$ Hz, Ar H-3/5), 5.59 (t, 1H, $J = 9.8$ Hz, inos H-6), 5.40 (t, 1H, $J = 9.7$ Hz, inos H-4), 2.99 (s, 6H, NMe_2).

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