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A novel fluoride-sensing scaffold by a peculiar acid-promoted trimerization of 5,6-dihydroxyindole

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ABSTRACT

An unusual rearranged trimer, 2-(2-amino-4,5-dihydroxybenzyl)-6,7-dihydroxy-3-(5,6-dihydroxyindol-3-yl)quinoline (**1a**), was obtained as the acetyl derivative (**1b**) by mild acid-promoted polymerization of 5,6-dihydroxyindole at pH 2. Compound **1b** proved to be a selective fluoride-sensing compound, transducing F^- binding into a distinct absorption at 414 nm and a marked fluorescence enhancement at 489 nm.

1. Introduction

5,6-Dihydroxyindoles are a unique group of naturally occurring, catechol-containing heterocyclic compounds, which arise biogenetically by the oxidative cyclization of catecholamines and related tyrosine-derived metabolites. A marked facility to oxidation, leading to black insoluble polymeric materials, is the distinctive chemical feature underlying the biological importance of 5,6-dihydroxyindoles.[1] This is well illustrated by their role as primary building blocks of eumelanins,[2] the key components of the human pigmentary system.[3] 5,6-Dihydroxyindoles have also been exploited in cosmetics and medicinal chemistry, e.g., as active moieties in antiviral agents and antibiotics.[4]

Recently, while pursuing a program aimed at designing novel 5,6-dihydroxyindole-based functional materials,[5] we came across an unexpected behavior of this indole when left to polymerize under mildly acidic conditions. The noticeable outcome of this reaction was the formation of a rearranged trimer featuring an unusual 2-benzyl-3-indolylquinoline skeleton. Interestingly, the acetylated derivative of the trimeric product was found to exhibit selective binding properties toward fluoride anions, as revealed by the marked F^--induced changes in the absorption and fluorescence spectra. This observation suggested a potential of the trimer as a novel prototype of fluoride-sensing scaffolds. Despite the vast literature that accumulated during the past few years, the quest for easily accessible and efficient fluoride-sensing molecular systems is still an active area of research[6] because of the significant biological, medical, industrial, and environmental relevance of fluoride chemistry.

In this paper we report details of the acid-promoted trimerization of 5,6-dihydroxyindole and describe the selective effects of the fluoride anion on the chromophoric and fluorescence properties of the acetylated trimer.

2. Results and discussion

When 5,6-dihydroxyindole was dissolved in phosphate buffer at pH 2 and left at room temperature a smooth reaction occurred, leading after ca. 24 h to a main trimeric species (LC/MS analysis). This was obtained as the heptaacetyl derivative ($[M+H]^+$ m/z 740) in 10% yield by a simple work-up procedure involving acetylation of the crude mixture followed by a chromatographic step, and was identified as 2-(2-acetamido-4,5-diacetoxybenzyl)-6,7-diacetoxy-3-(5,6-diacetoxyindol-3-yl)quinoline (**1b**) following complete spectral characterization (see Supplementary data). Inspection of the reaction mixture in the early stages showed the formation of dimer **2** and trimer **3a** as minor isolable products.[7]

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A comparative study showed that none of the other indoles examined, i.e., indole, 5-hydroxyindole, 6-hydroxyindole, 5,6-dimethoxyindole, and 5,6-dihydroxy-*N*-methylindole, give the corresponding 3-(indol-3-yl)quinoline trimer under the same conditions. Use of stronger acids, e.g., HCl, or organic acids, e.g., acetic acid, was not productive, furnishing invariably ill-defined mixtures. The facile formation of **1a** from 5,6-dihydroxyindole is therefore attributed to the specific reactivity of this indole via the 2-position,[8] steering in part the acid-promoted polymerization pathway through the usually less favorable 2-(2-amino-4,5-dihydroxyphenyl)-1-(5,6-dihydroxyindol-2-yl)-1-(5,6-dihydroxyindol-3-yl)ethane (**4**) (Scheme 1). Formation of the quinoline system of **1a** from **4** may proceed through a rearrangement step akin to that described for indole trimers in acidic media.[9]

The 2-(2-aminobenzyl)-3-(indol-3-yl)quinoline system featured by **1a** has previously been obtained only by harsh treatment of indole under Friedel–Crafts acylation conditions[10] or in the presence of *p*-toluenesulfonic acid followed by complex work-up, extraction, and chromatographic separation steps.[9]

The absorption properties of **1b** are shown in Figure 1. The compound exhibited a distinct maximum at 330 nm in CH_3CN. Upon the addition of increasing concentrations of F^-, a yellow coloration became apparent, due to the development of an absorption at 414 nm. Examples of colorless-to-yellow color changes associated with F^- binding to an organic compound have already been reported in the literature.[11] No clear isosbestic point is apparent from data in Figure 1, which suggests that color development involves more complex equilibria than a simple 1:1 substrate–anion binding process.

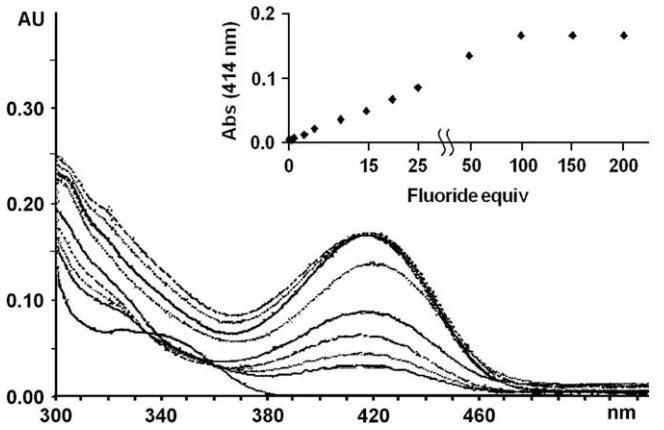


Figure 1. Changes in the UV/vis spectra of **1b** (1×10^{-5} M) in CH_3CN after addition of 0, 10, 15, 20, 25, 50, 100, 150, and 200 equiv of tetrabutylammonium fluoride (TBAF). Inset: Absorbance at 414 nm versus equiv of F^-.

The acetylated derivative **1b** exhibited a remarkable fluorescence enhancement upon the addition of F^- (Fig. 2). The fluorescence response of **1b** (5×10^{-7} M) upon addition of up to 300 equiv F^- is shown in Figure 3. In the absence of F^-, fluorescence of the free compound was weak and barely detectable. Addition of the anion to the solution caused the emergence of a distinct emission band at 489 nm following excitation at 414 nm. This effect is worthy of note since anion binding causes fluorescence quenching for most of the reported sensors,[12] with only a few exhibiting fluorescence enhancement.[11c,13]

The recognition process was selective for F^- since in the presence of other anions, including Cl^-, Br^-, I^-, AcO^-, NO_2^-, HSO_4^-, no significant changes in the fluorescence spectra were observed. Complete fluorescence quenching was noted however in the presence of water (>20%). The stoichiometry of the fluoride–**1b** interaction was determined to be 2:1 from the Job's plot (Fig. 3).

In subsequent experiments, the effects of F^- binding on the parent 5,6-diacetoxyindole and the acetylated trimer **3b** were investigated. Trimer **3b** was a suitable model to identify the chromogenic and fluorogenic systems in **1b**, since it exhibited the

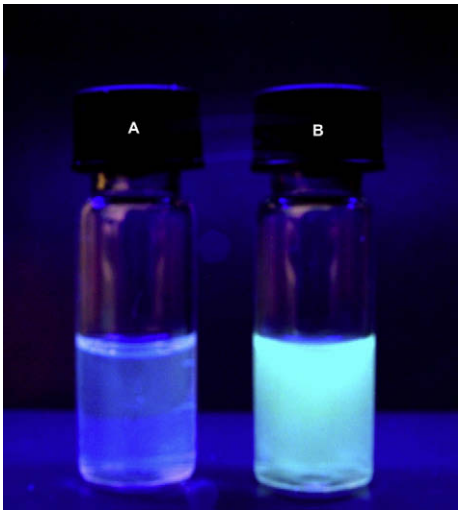
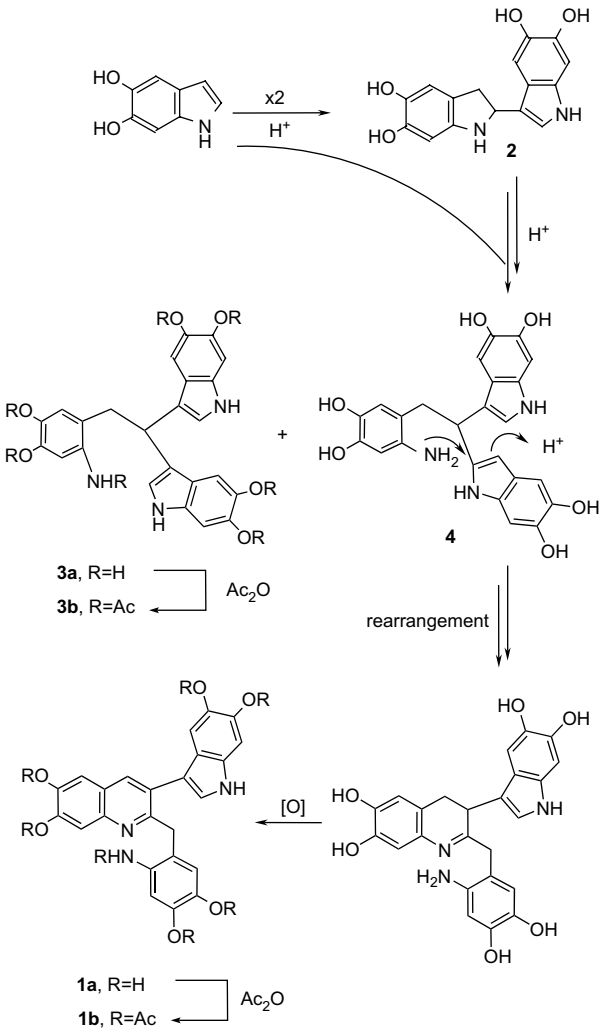


Figure 2. Fluorescence changes of **1b** (5×10^{-5} M) in CH_3CN upon addition of 25 equiv of TBAF. (A) no additive; (B) +TBAF (under a UV lamp at 366 nm).



Scheme 1.

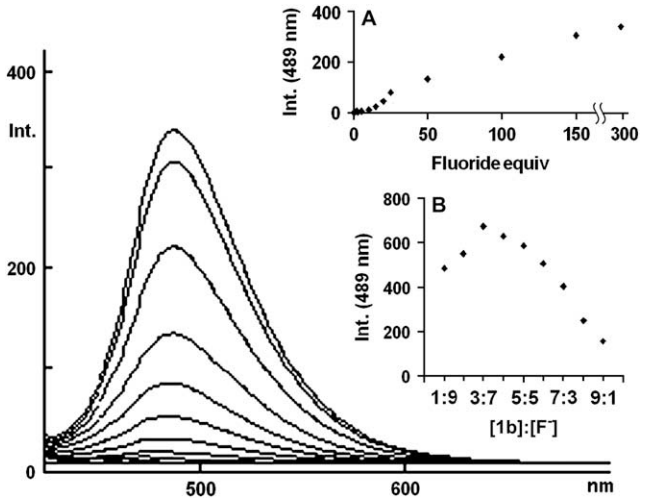


Figure 3. Changes in the emission spectra of **1b** (5×10^{-7} M) in CH_3CN after addition of TBAF from 0 to 300 equiv (excitation wavelength 414 nm). Inset A: Fluorescence intensity at 489 nm versus equiv of F^-. Inset B: Job's plot for **1b**–fluoride interaction in CH_3CN (total [**1b**]+[F^-]=5×10^{-5} M).

diacetoxyindole and acetamido moieties placed at a comparable distance by a non-conjugating spacer chain replacing the rigid quinoline ring. Upon the addition of F^- neither diacetoxyindole nor **3b** developed significant fluorescence. This observation suggests that the quinoline ring is an essential constituent of the fluoride-sensing fluorophore.

To gain a deeper insight into the mechanism of F^- coordination by **1b**, ^{1}H NMR titration experiments were carried out in DMSO-d_6. With 0.5 equiv F^-, the indole NH proton signal at δ 11.6 (Fig. 4A) disappeared (Fig. 4B). A similar behavior has previously been reported for several systems containing NH protons, indicating interaction with F^- anions.[11a,c,13a,14] Addition of further amounts of the anion caused disappearance of the amide NH proton signal at δ 9.81, accompanied by a slight upfield shift of the other proton signals (Fig. 4C).[13c,14,15] A visible fluorescence with concomitant color change was well apparent at this stage.

No alteration of the signals due to the acetyl groups was observed, ruling out deacetylation during F^- coordination.

The ROESY spectrum of **1b** in the absence of F^- (see Supplementary data) showed distinct cross peaks between: (a) the quinoline H-4 and the indole H-4 resonances; (b) the quinoline H-8 and the amide NH signals; and (c) the benzylic proton singlet and the indole H-2 proton doublet. These contacts suggested a preferential conformation with the indole NH group close to the benzylic methylene and the amide group facing the quinoline nitrogen, as previously described for related systems on the basis of X-ray analysis.[10] Interestingly, in the ROESY spectrum recorded after addition of 0.5 equiv of F^-, at a stage when only the indole NH has disappeared, the cross peak between the amide NH and the quinoline H-8 signals was no longer detectable.

Based on the above NMR data and the 2:1 stoichiometry inferred from the titration experiments, it is suggested that the initial coordination of F^- to the indole NH hydrogen induces a rotation of 180° of the benzylic ring. Upon the addition of further aliquots of fluoride, significant deprotonation may occur,[16] and the deprotonated indole can be stabilized by an intramolecular hydrogen bond with the amide NH group (Scheme 2).[17] A locked coplanar disposition of the indole and quinoline rings would then ensue, with consequent fluorescence enhancement and UV bathochromic shift.[13a,17b] Attempts to detect the HF_2^- signal in the ^{1}H NMR spectrum at ca. δ 16 were however unsuccessful. This has been

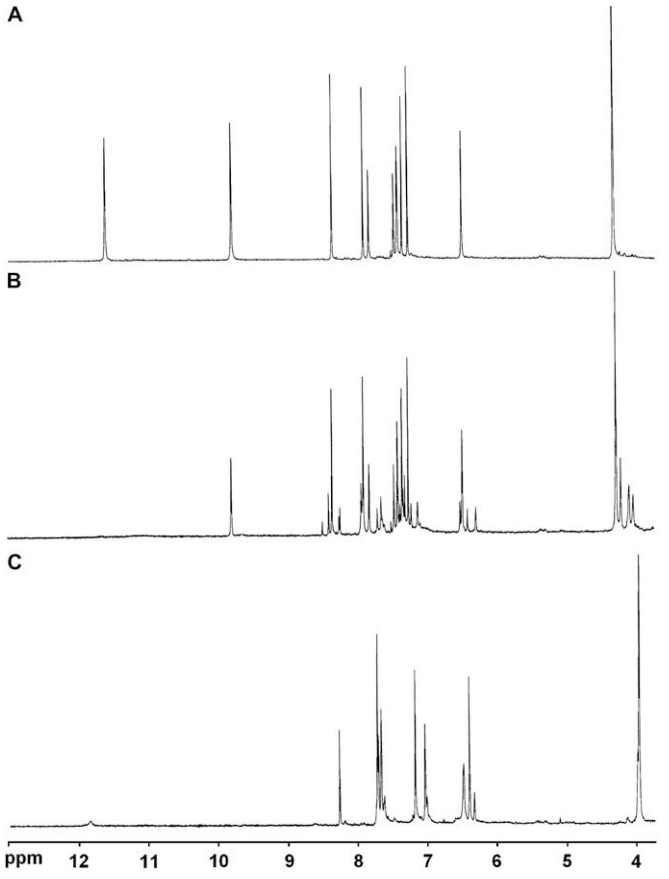
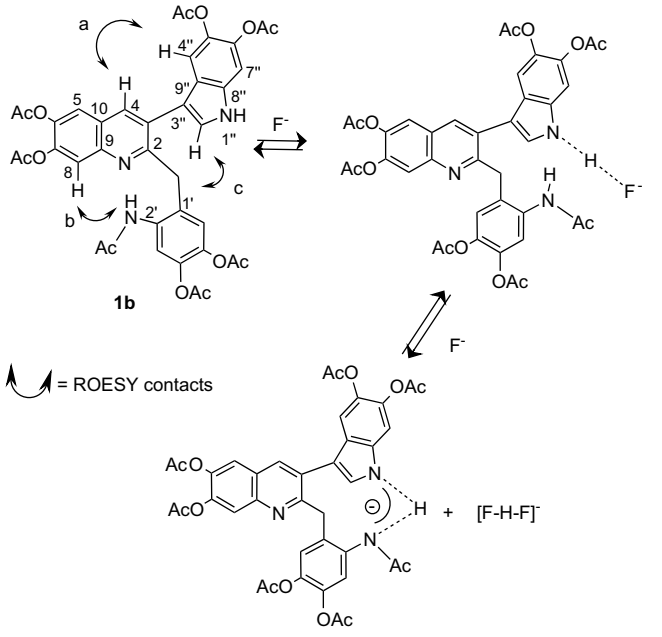


Figure 4. Partial ^{1}H NMR spectra of **1b** in DMSO-d_6 (A) in the absence or in the presence of (B) 0.5 or (C) 2.5 equiv of TBAF.

attributed to fast proton exchange with the water impurity present in the DMSO solvent.[13c]

Verification of the proposed Brønsted acid–base reaction by titration experiments with the strong base [$(Bu)_4N$]OH (TBAOH) was precluded by deacetylation of the catechol functions under such



Scheme 2.

conditions.[1b] Because of this deacetylation reaction and the lack of effect of acetate and hydrogen sulfate anions, **1b** should be regarded as a specific fluoride sensor and not a mere acid–base sensor.

The fluorescence titration profile in Figure 3 apparently supported the two-step process depicted in Scheme 2. Quantitative measurements of the F^- affinity from the titration data by previously reported procedures[18] gave values of log K=6.04 and 4.08, which are comparable with those of many known fluorescence-based sensors.[13c,14,17b,19]

3. Conclusion

We have reported herein a novel fluoride-sensing scaffold, which was obtained by a variant of the classic acid-promoted trimerization of indoles. The mild one-pot conversion of 5,6-dihydroxyindole to **1b** provides an expedient and practical access route to the 2-(2-amidobenzyl)-3-(indol-3-yl)quinoline system, and the ease of preparation would offset the relatively small product yield. Compound **1b** represents a chromogenic and fluorogenic fluoride-sensing system[20] operating in the turn-on mode, and its characterization may stimulate further studies on the potential fluoride-sensing properties of related indolylquinoline systems.

4. Experimental section

4.1. General methods and materials

5,6-Dihydroxyindole was prepared as reported.[21] TBAF 1.0 M solution in THF was used as obtained. LC/MS analysis was carried out on an instrument equipped with an ESI ion source; an octadecylsilane-coated column, 150 mm×4.6 mm, 3.5 μm particle size, at 0.4 mL/min was used. The eluant system was 0.2% formic acid, solvent A; acetonitrile, solvent B; 5% B, 0–10 min; from 5 to 30% B, 10–25 min; from 30 to 70% B, 25–50 min. HR ESI$^+$/MS spectra were obtained in 0.2% formic acid–acetonitrile 1:1 v/v. ^{1}H and ^{13}C NMR spectra were recorded at 400 or 100 MHz, respectively. ^{1}H,^{1}H COSY, ^{1}H,^{13}C HMBC, ^{1}H,^{13}C HSQC-DEPT, and ROESY spectra were run at 400 MHz using standard pulse programs. Chemical shifts are reported in δ values (ppm) downfield from TMS.

4.2. 2-(2-Acetamido-4,5-diacetoxybenzyl)-6,7-diacetoxy-3-(5,6-diacetoxyindol-3-yl)quinoline (1b)

To a solution of 5,6-dihydroxyindole (200 mg) in methanol (4 mL) 0.1 M phosphate buffer (pH 2.0) (40 mL) was added and the reaction mixture was taken under stirring. After 24 h, when LC/MS analysis showed the formation of a product at t_R 24 min with a pseudomolecular ion peak $[M+H]^+$ at m/z 446 as a major constituent, the mixture was taken to dryness. The residue was treated with acetic anhydride (2 mL) and pyridine (80 μL) for 16 h at room temperature and fractionated by silica gel column chromatography (2 cm×36 cm) using chloroform–ethyl acetate as the eluant (9:1 to 4:6 gradient mixtures). Fractions eluted with chloroform–ethyl acetate 7:3 were collected and taken to dryness to give **1b** (33 mg, 10% yield, >97% purity estimated by ^{1}H NMR analysis) as a pale yellow oil. HR ESI$^+$/MS: found m/z 740.2089 ($[M+H]^+$), calcd for $C_{38}H_{34}N_3O_{13}$ m/z 740.2092; UV (CH_3CN): 276, 330 nm; IR ($CHCl_3$): ν_{max} 3468, 1767, 1691, 1601, 1550, 1497, 1417, 1365, 1326, 1197, 1117, 1012, 903 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.18–2.39 (21H, $COCH_3$), 4.14 (2H, s, $-CH_2$), 6.28 (1H, s, H-6′), 7.04 (1H, br s, H-2″), 7.09 (1H, s, H-4″), 7.32 (1H, s, H-7″), 7.59 (1H, s, H-5), 7.87 (1H, s, H-8), 7.96 (1H, s, H-3′), 8.02 (1H, s, H-4), 8.99 (1H, br s, NH), 10.82 (1H, br s, N*H*COCH$_3$); ^{13}C NMR ($CDCl_3$): δ 20.5–20.8 (CO*C*H$_3$), 24.9 (NHCO*C*H$_3$), 39.1 (-CH_2), 106.2 (C-7″), 112.3 (C-4″), 112.8 (C-3″), 117.3 (C-3′), 120.4 (C-5), 120.9 (C-8), 124.8 (C-6′, C-9″), 125.3 (C-10), 127.1 (C-1′, C-2″), 128.6 (C-3), 133.1 (C-8″), 135.9 (C-2′), 136.9 (C-5″), 137.5 (C-5′), 138.6 (C-6″), 139.0 (C-4), 140.5 (C-4′), 141.7 (C-6), 144.0 (C-9), 144.4 (C-7), 160.5 (C-2), 167.9–169.1 (*C*OCH$_3$).

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Supplementary data

^{1}H NMR, ^{13}C NMR, ^{1}H,^{1}H COSY, ROESY, ^{1}H,^{13}C HSQC-DEPT, and ^{1}H, ^{13}C HMBC spectra of **1b** are provided. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2009.01.003.

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