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Short communication

Some observations relating to the stability of the BODIPY fluorophore under acidic and basic conditions

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ABSTRACT

4,4-Difluoro-4-bora-1,3,5,7-tetramethyl-2,6-diethyl-8-methyl-3a,4a-diaza-*s*-indacene (BODIPY) fluorophore was transformed into its corresponding 4,4-dimethyl, 4,4-dimethoxy and 4,4-diphenyl analogues. The stabilities of these BODIPY fluorophores in acidic (di- and trichloroacetic acid) and basic conditions (aqueous ammonium hydroxide) were investigated using ^{11}B NMR spectroscopy.

1. Introduction

4,4-Difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY **1**, Fig. 1) fluorophores were first synthesized in 1968 by Treibs and Kreuzer [1], and have attracted much attention in recent years in various applications. Many BODIPY fluorophores possess high molar extinction coefficients and are highly fluorescent [2–4]. In addition, many BODIPY fluorophores are relatively insensitive to changes in pH and polarity, and are more photochemically stable than many other commercially available fluorophores.

While some reactivities of BODIPY have been documented in the literature [2,3], detailed studies on the stability of BODIPY and analogues under acidic and basic conditions, however, have been very rare [5]. One recent publication relates to the replacement of the fluorine atoms in BODIPY by hydroxyl in the presence of a strong Lewis acid such as aluminium chloride [6]. In another report, it was shown that BODIPY analogues can be converted to their corresponding dipyrrins when treated with potassium butoxide under microwave conditions [7]. It is noted that BODIPY has been given credit in numerous articles to be chemically stable. This contrasts the observations made in our laboratory during our attempts to incorporate BODIPY analogues into oligonucleotides by the phosphoramidite chemistry-based solid-phase synthesis, particularly during acid and base treatments [8]. The work presented herein aims at comparing the stability of a series of 4,4-disubstituted BODIPY analogues under acidic and basic conditions that are commonly used in solid-phase synthesis of oligonucleotides. In this respect, building blocks are exposed to repeated acid treatment, *e.g.* di- or trichloroacetic acid. The BODIPY analogues are also tested for their stability under the deprotection conditions that are used in solid-phase oligonucleotide synthesis, *e.g.* treatment with aqueous ammonium hydroxide at 55 °C for 15 h.

2. Materials and methods

2.1. Instrumentation

^{1}H NMR spectra were measured at 300 and 600 MHz with Bruker Avance spectrometers; spectra were calibrated to residual undeuterated NMR solvents; *J* values are given in Hz. ^{13}C and ^{19}F NMR spectra were measured at 75.5 and 282.4 MHz, respectively, and ^{11}B NMR spectra were measured at 96.3 and 192.6 MHz with the same spectrometers. Chemical shifts are given in ppm. Low and high resolution mass spectra were obtained with Kratos Concept 1S high resolution mass spectrometer using electron impact or fast

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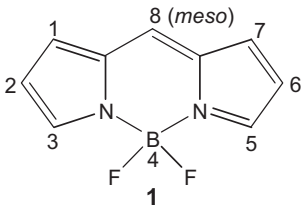


1

Fig. 1. BODIPY core structure.

atom bombardment sources interfaced with DART 32 bit acquisition system through a Sun Sparcstation 10 and Mach 3 software.

2.2. Synthesis of 4,4-difluoro, 4,4-dimethyl, 4,4,-dimethoxy, 4,4,-diphenyl, and 4,4-bis(dichloroacetyl) BODIPY analogues

Detailed synthetic procedures, NMR and mass spectroscopic data of these compounds are provided in the Supplementary materials.

2.3. Procedures for the stability of BODIPY analogues in the presence of dichloroacetic acid

2.3.1. Example: stability of 4,4-diphenyl-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-s-indacene ***4d*** in dichloroacetic acid–dichloromethane

4,4-Diphenyl-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene **4d** (10 mg, 0.023 mmol) was dissolved in dry dichloromethane (0.4 ml) in an NMR tube, followed by addition of dichloroacetic acid (95 μl, 1.15 mmol, 50 mol equiv). A sealed capillary tube containing a solution of sodium tetraphenylborate in D_2O was inserted. ^{11}B NMR spectra were acquired at appropriate interval.

When processing the ^{11}B spectra, the following processing parameters were used:

Lb = 10 Hz
ME_mod = LPbr
NCOER = 32
LPBIN = 1024
Tdoff = 32
FCOR = 1
PKNL = TRUE
FT_mod = FSC

2.4. The stability of BODIPY analogues in base condition

2.4.1. Example: 1,3,5,7,8-pentamethyl-2,6-diethyl-4, 4-dimethoxy-4-bora-3a,4a-diaza-s-indacene ***4c***

1,3,5,7,8-Pentamethyl-2,6-diethyl-4,4-dimethoxy-4-bora-3a,4a-diaza-*s*-indacene **4c** (42 mg, 0.123 mmol) was dissolved in THF (4 ml). This solution was divided equally into 16 reacti-vials (*i.e.* 250 μl each). To each of the vial was added aqueous ammonium hydroxide solution (600 μl, 28%), sealed and heated at 55 °C. After 2, 4, 6, 8, 11, 13, 24 h, two vials were removed from heating, cooled, and combined. After solvents were removed under reduced pressure, the residue was dissolved in dichloromethane (600 μl) and transferred to an NMR tube. ^{11}B NMR spectra were recorded with a sealed capillary tube insert containing a solution of sodium tetraphenylborate in D_2O.

2.5. X-ray crystallography

Single crystals of compound **5** were obtained by slow evaporation of solvents from hexane–dichloromethane. CCDC 812198 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

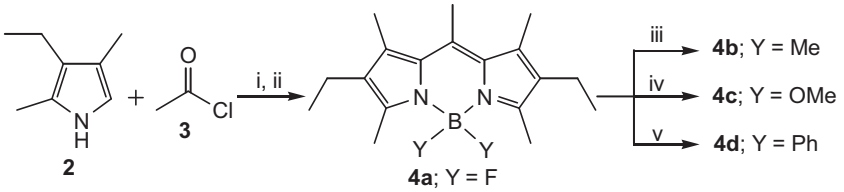
Data were collected on a Bruker-Nonius Kappa-CCD diffractometer using monochromated Mo-Kα radiation and were measured using a combination of φ scans and ω scans with κ offsets, to fill the Ewald sphere. The data were processed using the Denzo-SMN package [9]. Absorption corrections were carried out using SORTAV [10]. The structure was solved and refined using SHELXTL V6.1 [11] for full-matrix least-squares refinement that was based on F^2. All H atoms were included in calculated positions and allowed to refine in riding-motion approximation with U ~ iso ~ tied to the carrier atom.

3. Results and discussion

As a model for the stability experiments, 4,4-difluoro-4-bora-1,3,5,7-tetramethyl-2,6-diethyl-8-methyl-3a,4a-diaza-*s*-indacene **4a** was chosen since the dipyrromethene ring is fully substituted to avoid potential reactions at these sites. This compound was readily prepared using literature procedures by the treatment of 2,4-dimethyl-3-ethylpyrrole **2** with acetyl chloride, followed by complexation with boron trifluoride diethyl etherate in the presence of triethylamine [7,12]. In addition, three other analogues, *i.e.* 4,4-dimethyl **4b** [13], 4,4-dimethoxy **4c** [14] and 4,4-diphenyl **4d** [15] BODIPY were prepared according to methods documented in the literature (Scheme 1).

The stability of the BODIPY fluorophores **4a**–**d** in acidic solutions at room temperature was followed by recording ^{11}B NMR spectra at appropriate intervals. A sealed capillary insert of a solution of sodium tetraphenylborate in D_2O was used as an internal standard. In the case of an unstable substrate, the disappearance of substrate was monitored. In order to eliminate the broad ^{11}B resonance signal from the NMR tube, which overlaps with both substrate and internal standard signals, a linear prediction method [16] was used to process the ^{11}B NMR data. As shown in Fig. 2, this linear prediction method allows for the suppression of background signals from the NMR tube.

It was found that 4,4-diphenyl BODIPY **4d** is stable in a solution of dichloroacetic acid (50 mol equiv) in dichloromethane for 3 days (No new signals appeared after 3 days when the experiment was terminated). 4,4-Difluorine **4a** is less stable under the same conditions, with 8% decomposition after 24 h. Treatment of 4,4-dimethyl



Scheme 1. Reagents and conditions: i). CH_2Cl_2, reflux, 3 h; ii). CH_2Cl_2, NEt_3, $BF_3 \cdot Et_2O$, reflux; iii). MeMgBr, CH_2Cl_2, 0 °C; iv). PhMgBr, CH_2Cl_2, 0 °C; v). NaOMe, MeOH, CH_2Cl_2, reflux.

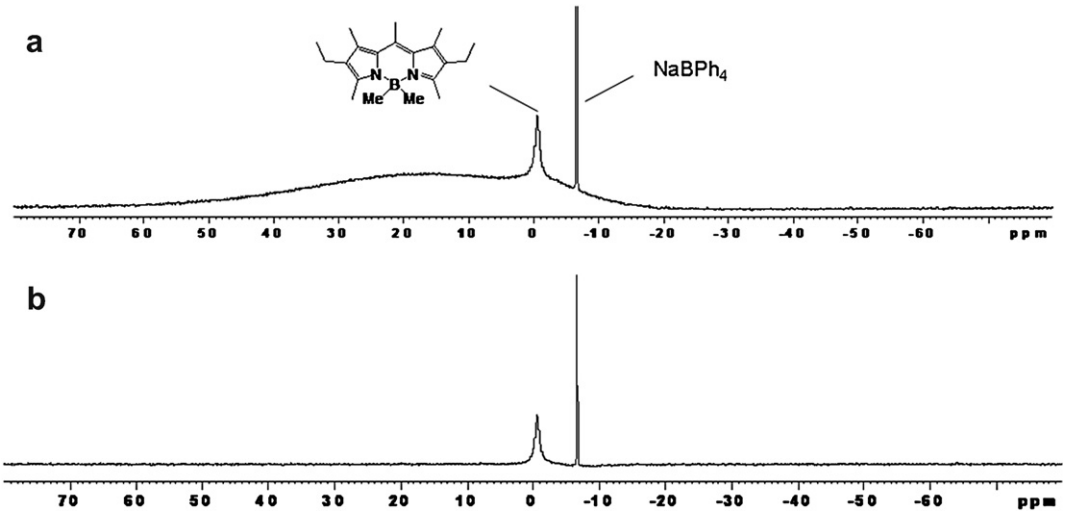
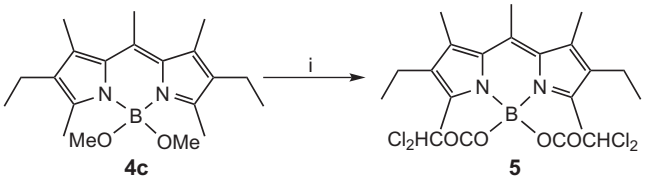


Fig. 2. ^{11}B NMR spectra of **4b** recorded in the presence of an internal stand ($NaBPh_4$). a). Before applying the linear backward prediction algorithm, and b). after applying the linear backward prediction algorithm.

BODIPY **4b** under the same conditions led to complete decomposition over 6 h to give a single boron-containing product, which was not identified (see Supplementary material for ^{11}B NMR spectra). When 4,4-dimethoxy BODIPY **4c** was treated with 10 mol equiv of dichloroacetic acid in dichloromethane, full conversion to a single product was observed in 12 h (see Supplementary material for ^{11}B NMR spectra). When this reaction was carried out on a preparative scale (Scheme 2), the product was identified as the corresponding anhydride **5** (see Supplementary material for details). The identity of this product **5** was confirmed by X-ray crystallography (Fig. 3).



Scheme 2. Reagents and conditions: i). CH_2Cl_2, $Cl_2CHCOOH$, r.t.

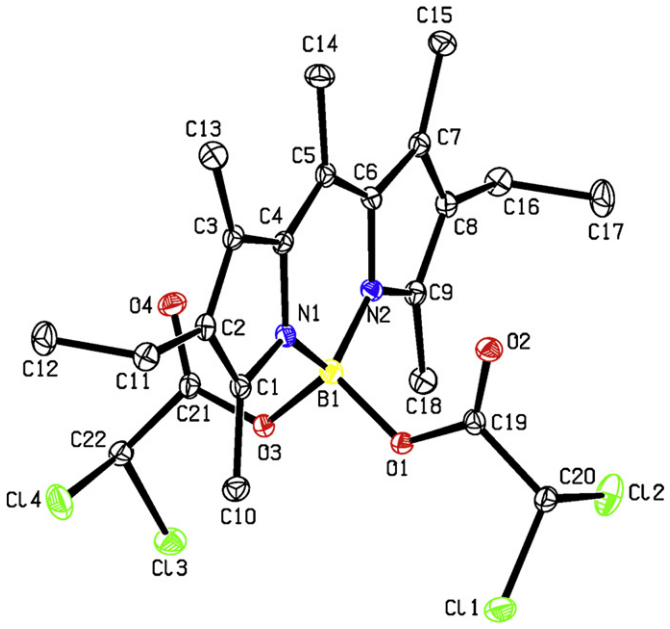


Fig. 3. The molecular structure of **5** with 30% probability displacement ellipsoids for non-H atoms (prepared with PLATON) [17].

When a stronger acid, *i.e.* trichloroacetic acid (50 mol equiv), was used, both 4,4-difluorine **4a** and 4,4-diphenyl **4d** BODIPY became unstable. Thus, 50% of 4,4-difluorine BODIPY **4a** undergoes decomposition after incubation in dichloromethane in the presence of 50 mol equiv of trichloroacetic acid for 3 h. Under the same conditions, 50% of 4,4-diphenyl BODIPY **4d** undergoes decomposition in 4 h.

Stability of the BODIPY analogues under basic conditions was assessed as follows. The substrate was dissolved in tetrahydrofuran followed by addition of aqueous ammonia and methanol (Methanol was not added in the case of 4,4-dimethoxy BODIPY **4c**). THF and methanol were added in order to generate a homogeneous solution. A series of this identical solution were prepared and heated to 55 °C in sealed vials. At appropriate time intervals, samples were removed and ^{11}B NMR spectra were recorded. The results showed that 4,4-dimethoxy BODIPY **4c** is the least stable under this condition. Within about 8 h, half of the starting materials degraded, forming a product with a ^{11}B shift of 1.2 ppm (see Supplementary material for ^{11}B NMR spectra). Under this condition 4,4-dimethyl **4b** and 4,4-difluoro BODIPYs **4a** are stable for up to 8 h, 4,4-diphenyl BODIPY **4d** is stable for 7 days.

4. Conclusions

From the results obtained in this study, 4,4-diphenyl substituted BODIPY **4d** appears to be the most stable under both acidic and basic conditions. It is also noted that substitution of the fluorine by phenyl does not compromise the fluorescent intensity of the fluorophore ($\Phi = 0.91$ in dichloromethane for **4d** v 0.83 for **4a**) [3]. The phenyl analogue **4d** shows a larger Stokes shift (35 nm) than the fluorine BODIPY **4a** (21 nm) [3]. It is possible that the 4,4-diphenyl substituted BODIPY analogues could be more suitable for oligonucleotide labelling via the phosphoramidite chemistry-based solid-phase synthesis. Work in this area is currently underway.

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Appendix. Supplementary material

Supplementary material related to this article can be found online at doi:10.1016/j.dyepig.2011.03.027.

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