Synthesis and Spectroscopic Characterization of BODIPY-Modified Uridines as Potential Fluorescent Probes for Nucleic Acids

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Abstract: BODIPY-modified nucleosides that contain the fluorophore attached to the 5-position of uridine via a short phenylene bridge have been prepared and characterized by optical spectroscopy and by electrochemistry. The target compounds were synthesized using a Pd-catalyzed cross-coupling of 4-formylphenylboronic acid to 5-ido-2'-desoxyuridine, followed by acid-catalyzed formation of the BODIPY chromophore. The weakly electron-donating ethyl substituents in positions 2 and 6 of the BODIPY dye, shift both the absorption and emission properties of the corresponding modified uridines bathochromically and alter their redox properties. In contrast, exchange of the fluoro ligands at the boron center of the chromophores by methoxy groups does not change the optical properties but increases the electron-rich character of the BODIPY-modified uridines significantly.

Key words: absorption, BODIPY, cyclic voltammetry, fluorescence, nucleoside

4,4-Difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) and its derivatives represent a class of dyes that are widely used for fluorescent labeling strategies in chemical biology.1 The BODIPY dyes are strongly UV-absorbing chromophores that exhibit nanosecond lifetimes and emit fluorescence with sharp peaks, narrow profiles and with high quantum yields.2,3 BODIPY is reasonably stable under physiological conditions. Without special substituents, BODIPY dyes are relatively insensitive to the polarity and pH of their environment. On the other hand, modifications to the chromophore structure help tune their fluorescence properties and extend their use to a large range of different applications.4–8

The majority of the labeling methods with BODIPY chromophores involve the use of a reactive anchor at the end of a linker in order to attach the dye to distinct positions of proteins or nucleic acids.9,10 Especially for DNA, terminal labeling using an alkyl chain linker was preferred for applications in fluorescent DNA analytics and chemical biology with DNA.9,11 However, there are no reports on the covalent modification of any of the DNA bases by BODIPY via short linkers. In principal, the attachment of chromophores to DNA bases via short bridges (e.g. acetylene or phenylene) or even via a single C–C bond, should yield unique optical properties, such as solvatochromism and exciplex-type emission that are suitable for DNA probing.12–20 Over the last years, we have synthetically attached organic chromophores, e.g. phenothiazine,18 pyrene,19 or ethynylpyrene,20 to DNA bases for electron-transfer studies and as fluorescent probes for DNA.

In general, fluorophore labeling of DNA bases using long alkyl chain linkers is rationalized as a requirement for the replication of the corresponding modified oligonucleotides or modified nucleoside triphosphates by DNA polymerases.21 Hence, a critical issue for the direct linkage between the chromophore and the DNA base is the question of whether canonical base recognition complementarity persists in DNA polymerase catalyzed primer extension experiments. Recently we could show that the DNA polymerase catalyzed nucleotide incorporation opposite to attached pyrene- and BODIPY-modified nucleosides follows Watson–Crick selectivity.22 Moreover, the Klenow fragment (exo-) is able to bypass the BODIPY modification site for further elongation. These observations make this kind of fluorescent labels promising tools for in vivo experiments in cell biology. However, in the case of BODIPY-modified oligonucleotides, a significant fluorescence quenching (92–99%) was observed as a result of the incorporation of the chromophore into duplex DNA. Hence, we are interested in the further development of BODIPY-modified uridines in order to avoid fluorescence quenching in DNA, which is presumably caused by charge-transfer processes. Herein, we present the synthesis of BODIPY-modified uridines that vary in the ligands on the boron atom and the substituents at positions 2 and 6. These target compounds were studied by optical spectroscopy and electrochemical measurements.

The HOMO and LUMO of the unsubstituted BODIPY chromophore were calculated with the semiempirical AM1 method (Figure 1) and were obtained after full geometry optimization. The results show that excitation enhances the electron-density in position 8, whereas the electron-density in positions 2 and 6 are reduced. Hence, acceptor substituents in position 8 as well as donor substituents in positions 2 and 6 should stabilize the LUMO energetically and should cause a bathochromic shift of the absorption and the emission. Accordingly, we synthesized two sets of BODIPY-modified uridines as fluorescent nucleosides. The pyrrol moieties as part of the uridines 1–3 are substituted by methyl groups in positions 1, 3, 5 and 7. The uridines 4–6 bear two additional ethyl groups in positions 2 and 6 as weak electron-donating substituents. According to a recent publication by Gabe et al., the redox
properties of BODIPY derivatives can be easily tuned by exchange of the fluoro ligands of the boron atom by methoxy groups.\textsuperscript{13} This represents the second structural variation among the synthesized uridines 1–6. Uridines 1 and 4 contain two fluoro substituents, 2 and 5 bear one fluoro and one methoxy ligand, and in 3 and 6 both ligands are replaced by methoxy groups.

![Figure 1](image.png)

**Figure 1** Calculated HOMO and LUMO of the unsubstituted BODIPY chromophore

In all six target compounds, the BODIPY chromophores were attached to the 5-position of uridine in order to retain the preferred anti-conformation of the corresponding uridines that is a prerequisite for the Watson–Crick type base pairing in DNA. A phenylene spacer was placed between the BODIPY dye and the uridine in order to reduce steric hindrance between the two moieties. The synthesis of all BODIPY-modified uridines 1–6 started with the Pd-catalyzed reaction of 5-iodo-2′-deoxyuridine (7) with 4-formylphenylboronic acid (8) to obtain 5-(4-formylphenyl)-2′-deoxyuridine (9) in 76% yield. Both starting compounds are commercially available. This Suzuki–Miyaura type cross-coupling\textsuperscript{23} follows a methodology that we initially established for the preparation of pyrene-modified nucleosides.\textsuperscript{19} It proceeds in aqueous solution and tolerates unprotected hydroxy groups of the 2′-deoxyriboside. The dipyrromethane structures were formed from the aldehyde function of 9 under acidic conditions using either 2,4-dimethylpyrrole (10) or 3-ethyl-2,4-dimethylpyrrole (11). Subsequently, the intermediate compounds were oxidized to the dipyrromethenes by treatment with p-chloranil. Both products, 5-[4-(1′,3′,3′-tetramethylidipyrrylmethenyl)phenyl]-2′-deoxyuridine (12) and 5-[4-(2′,2′-diethyl-1′,3′,3′-tetramethylidipyrrylmethenyl)phenyl]-2′-deoxyuridine (13) were obtained in good yields (65 and 83%, respectively). Finally, the BF\textsubscript{2} group was incorporated by standard procedures yielding the BODIPY-modified uridines 1 and 4. According to the published procedure, treatment of the uridines 1 and 4 with methanolic sodium methoxide replaces the fluoro ligands of the boron atom\textsuperscript{7} and yields a mixture of the uridines 2/3 and 5/6, respectively, which could be separated by column chromatography.

The optical properties of the BODIPY-modified uridines 1–6 were determined in methanol and are summarized in Table 1. The UV/Vis absorption spectra (Figure 2) and the steady-state fluorescence spectra (Figure 3) both indicated two sets of modified uridines. The first set, 1–3, showed absorption maxima between 496 and 498 nm and extinction coefficients between 69,000 and 71,000 M\textsuperscript{-1}cm\textsuperscript{-1}; the Stokes shift of all three compounds was 12 nm. Accordingly, the fluorescence spectra exhibited maxima around 508 nm with quantum yields between 42 and 47%. As expected from our calculations, the additional weak electron-donating ethyl substituents in positions 2

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**Scheme 1** Synthesis of the BODIPY-modified uridines 1–6. Reagents and conditions: (a) Pd(dppe)\textsubscript{2}Cl\textsubscript{2}, Na\textsubscript{2}CO\textsubscript{3}, H\textsubscript{2}O–MeCN (2:1), 80 °C, 5 h, 76%; (b) (i) TFA, DMF–CH\textsubscript{2}Cl\textsubscript{2} (1:6), r.t., 20 h; (ii) p-chloranil, r.t., 2 h, 65% (12), 83% (13); (c) BF\textsubscript{3}·OEt\textsubscript{2}, Et\textsubscript{3}N, DMF, r.t., 5 h, 45% (1), 54% (4); (d) NaOMe, MeOH, r.t., 40 h (2,3), 24 h (5) or 48 h (6), 33% (2), 40% (5), 40% (3), 34% (6).

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**Table 1** UV/Vis absorption spectra (Figure 2) and steady-state fluorescence spectra (Figure 3) both indicated two sets of modified uridines. The first set, 1–3, showed absorption maxima between 496 and 498 nm and extinction coefficients between 69,000 and 71,000 M\textsuperscript{-1}cm\textsuperscript{-1}; the Stokes shift of all three compounds was 12 nm. Accordingly, the fluorescence spectra exhibited maxima around 508 nm with quantum yields between 42 and 47%. As expected from our calculations, the additional weak electron-donating ethyl substituents in positions 2.
and 6 of the BODIPY chromophore in the second set of modified uridines (4–6) influenced their optical properties significantly: The absorption maxima were shifted bathochromically to values between 522 and 523 nm and the extinction coefficients were reduced to values between 53,000 and 58,000 M\(^{-1}\)cm\(^{-1}\). The emission spectra are also shifted bathochromically to ~535 nm. The Stokes shift of all three compounds was 11 nm. Interestingly, the quantum yields were enhanced to values between 59 and 62%. Clear differences in the optical properties of both sets of compounds could be seen by eye: The first set of uridines (1–3) formed solutions with a yellow color and a green emission, whereas solutions of the second set of uridines (4–6) were an orange color with a yellow fluorescence. With respect to the variation of the ligands of the boron atom, the optical properties in each set of compounds did not differ significantly. Obviously, the boron ligand exchange does not influence the optical properties of the corresponding BODIPY-modified uridines.

In order to elucidate the redox properties, we measured the potentials for reduction and oxidation of the BODIPY-modified uridines 1–6 by cyclic voltammetry. The corresponding data are summarized in Table 2. Consistent with the observed optical properties, the weak electron-donating character of the ethyl substituents in positions 2 and 6 of the modified uridines 4–6 changed the redox properties in comparison with the uridines 1–3. The potentials for the irreversible oxidation of the BODIPY chromophore (\(E_{\text{ox}}\)) are shifted by 0.12–0.14 V to less positive values, and the potentials for the reversible reduction of the BODIPY dye (\(E_{\text{red}}\)) are shifted by 0.05–0.10 V to more negative values. The redox potentials clearly reflect additionally the exchange of the fluoro ligands by the electron-donating methoxy ligands at the boron center. This observation stands in contrast to the observed optical properties, as discussed above. In both sets of duplexes, 1–3 and 4–6, the potentials \(E_{\text{ox}}\) are shifted by 0.13–0.15 V to less positive values and the potentials \(E_{\text{red}}\) by 0.13–0.18 V to more negative values.

The most critical issue about fluorophore labeling of nucleic acids is fluorescence quenching caused by charge-transfer and oxidation of guanines.\(^{24}\) According to the literature, the oxidation potential of guanine is ~1.3–1.4 V vs. NHE.\(^{25}\) Using a correction value of ~0.63 V, the potential is transferred to a value of ~0.6–0.7 V versus the ferrocene (Fc+/Fc) reference we used in our electrochemical experiments. Based on this electrochemical characterization, the potential applicability of the BODIPY-modified uridines 1–6 as fluorescent probes for nucleic acids can be estimated. According to the Rehm–Weller equation, the energy of the singlet–singlet transition \(E_0\) has to be added to the reduction potential \(E_{\text{red}}\) in order to estimate the reduction potential of the excited state \(E^*_{\text{red}}\) (Table 2).\(^{26}\) The uridines 1 and 2 show excited state potentials that are clearly sufficient for guanine oxidation. On the other hand, the uridines 5 and 6 exhibit excited state potentials that indicate that neither of the compounds are able to oxidize guanine photochemically. Among the modified uridines presented herein, the latter two represent the most promising candidates as fluorescent labels for nucleic acids. The remaining two uridines, 3 and 4, represent borderline cases.

| Table 1 Optical Properties of 1–6 in MeOH (5 μM) |
|-----------------|-----------------|-----------------|---------------|---------------|
| Uridine | \(\lambda_{\text{max (abs)}}\) (nm) | \(\varepsilon_{\text{max (M}^{-1}\text{cm}^{-1})}\) | \(\lambda_{\text{max (em)}}\) (nm) | \(\Phi\) |
| 1 | 496 | 69,300 | 507 | 0.42 |
| 2 | 497 | 69,100 | 508 | 0.42 |
| 3 | 498 | 70,800 | 509 | 0.47 |
| 4 | 522 | 57,500 | 534 | 0.61 |
| 5 | 523 | 55,200 | 535 | 0.62 |
| 6 | 523 | 53,600 | 535 | 0.59 |

In conclusion, we presented herein a new type of BODIPY-modified uridine that has been synthesized by a Pd-catalyzed cross-coupling of 4-formylphenylboronic acid (8) to 5-iodo-2′-deoxyuridine (7) followed by acid-catalyzed formation of the BODIPY chromophores. The weakly electron-donating ethyl substituents in positions 2 and 6 of the BODIPY dyes 4–6 shift the absorption and
emission properties bathochromically and influence the redox properties in comparison to the uridines 1–3. Exchange of the fluoro ligands at the boron center by methoxy groups in uridines 2, 3 and 5, 6 does not alter the optical properties in comparison with the uridines 1 and 3, respectively, but increases the electron-rich character of the modified BODIPYs significantly.

**Table 2**

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<th>Uridine</th>
<th>E_{on} (B/B') (V)</th>
<th>E_{red} (B/B') (V)</th>
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<td>2.34</td>
<td>0.50</td>
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*B* = uridine-modified BODIPY.

TLC was carried out with silica gel 60 on aluminum foil (Merck). 1H and 13C NMR spectra were recorded on Bruker Advance 300, Advance 400 and Advance 600 instruments; chemical shifts (δ) are referred to residual protonated solvent. LR-MS spectra were determined with a Finnigan TSQ 7000. HR-MS spectra were measured on Ionspec QFT-7 (Varian Inc.) (2, 5 and 6), microTOF-Q (Bruker) (1), or 6520 QTOF (Agilent Technologies) (3) instruments. Absorption measurements were carried out on a Cary 100 (Varian) at 20 °C. Fluorescence measurements were performed on a FluoroMax-3 (Horiba Jobin Yvon) at 20 °C.

5-[4-(4-Fluoro-4-methoxy-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacyl)-phenyl]-2'-desoxyuridine (1)

Yield: 104 mg (33%); orange solid; Rf = 0.34 (CH₃CH₂-acetone–MeOH, 4:1:0.2).

1H NMR (400 MHz, DMSO-d₆): δ = 1.39 (s, 6 H, BDP-CH₃), 2.25 (m, 2 H, 2'-H), 2.44 (s, 6 H, BDP-CH₃), 2.82 (s, 3 H, OCH₃), 3.64 (m, 2 H, 5'-H), 3.84 (m, 1 H, 3'-H), 4.32 (m, 1 H, 4'-H), 5.16 (t, J = 4.6 Hz, 1 H, 5'-OH), 5.27 (d, J = 4.4 Hz, 1 H, 3'-OH), 6.15 (s, 2 H, BDP-H), 6.23 (t, J = 6.2 Hz, 1 H, 1'-H), 7.32 (m, 2 H, Ph-H), 7.64 (m, 2 H, Ph-H), 8.44 [s, 1 H, H-6 (dU)] and 11.55 [s, 1 H, N-H (dU)].

13C NMR (100 MHz, DMSO-d₆): δ = 14.1, 40.1, 48.3, 60.6, 69.8, 84.7, 87.4, 111.9, 121.0, 127.6, 127.9, 131.4, 132.9, 133.9, 138.6, 141.5, 141.6, 149.7, 154.7, 162.0.

ESI-MS: m/z (%) = 561.4 (100) [M – H]⁻.


 Yield: 129 mg (40%); red solid; Rf = 0.16 (CH₃CH₂-acetone–MeOH, 4:1:0.2).

1H NMR (400 MHz, DMSO-d₆): δ = 1.37 (s, 6 H, BDP-CH₃), 2.23 (m, 2 H, 2'-H), 2.41 (s, 6 H, BDP-CH₃), 2.78 (s, 6 H, OCH₃), 3.64 (m, 2 H, 5'-H), 3.83 (m, 1 H, 3'-H), 4.32 (m, 1 H, 4'-H), 5.15 (t, J = 4.6 Hz, 1 H, 5'-OH), 5.26 (d, J = 4.4 Hz, 1 H, 3'-OH), 6.09 (s, 2 H, BDP-H), 6.22 (t, J = 6.2 Hz, 1 H, 1'-H), 7.27 (d, J = 8.4 Hz, 2 H, Ph-H), 8.44 [s, 1 H, H-6 (dU)] and 11.53 [s, 1 H, N-H (dU)].

13C NMR (100 MHz, DMSO-d₆): δ = 14.2, 40.3, 48.5, 60.7, 69.9, 84.8, 87.5, 112.0, 120.9, 127.7, 127.9, 132.3, 133.4, 133.8, 138.7, 140.5, 141.5, 149.7, 154.6, 162.0.

ESI-MS: m/z (%) = 573.4 (100) [M⁺].

HRMS (ESI-TOF): m/z [M + H]⁺ calcld for C₃₂H₃₇BF₃N₅O₇: 575.2677; found: 575.2674.

5-[4-(4,6-Diethyl-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacyl)-phenyl]-2'-desoxyuridine (4)

Yield: 760 mg (54%); red solid; Rf = 0.23 (CH₃CH₂-acetone–MeOH, 4:1:0.1).

1H NMR (300 MHz, DMSO-d₆): δ = 0.94 (t, J = 7.4 Hz, 6 H, BDP-CH₂CH₃), 1.31 (s, 6 H, BDP-CH₃), 2.25 (m, 2 H, 2'-H), 2.29 (q, J = 7.4 Hz, 4 H, BDP-CH₂CH₃), 2.44 (s, 6 H, BDP-CH₃), 3.64 (m, 2 H, 4'-H), 5.18 (t, J = 6.2 Hz, 1 H, 5'-OH), 5.26 (d, J = 4.4 Hz, 1 H, 3'-OH), 6.15 (s, 2 H, BDP-H), 6.23 (t, J = 6.2 Hz, 1 H, 1'-H), 7.32 (m, 2 H, Ph-H), 7.64 (m, 2 H, Ph-H), 8.44 [s, 1 H, H-6 (dU)] and 11.53 [s, 1 H, N-H (dU)].

13C NMR (100 MHz, DMSO-d₆): δ = 14.2, 40.3, 48.5, 60.7, 69.9, 84.8, 87.5, 112.0, 120.9, 127.7, 127.9, 132.3, 133.4, 133.8, 138.7, 140.5, 141.5, 149.7, 154.6, 162.0.

ESI-MS: m/z (%) = 573.4 (100) [M⁺].

HRMS (ESI-TOF): m/z [M + H]⁺ calcld for C₃₂H₃₇BF₃N₅O₇: 575.2677; found: 575.2674.
2 H, 5'-H), 3.84 (m, 1 H, 5'-H), 2.42 (s, 6 H, BDP-CH3), 2.76 (s, 6 H, OCH3), 1.29 (s, 6 H, BDP-CH3), 2.28 (m, 4 H, BDP-CH2), 2.36 (m, 2 H, 2'-H), 3.72 (m, 2 H, 3'-H), 4.11 (m, 1 H, 4'-H), 5.12 (d, J = 4.4 Hz, 1 H, 5'-OH), 5.28 (d, J = 4.4 Hz, 1 H, 3'-OH), 6.23 (t, J = 6.3 Hz, 1 H, 1'-H), 7.20 (t, J = 8.2 Hz, 2 H, Ph-H), 7.82 (d, J = 8.2 Hz, 2 H, Ph-H), 8.46 [s, 1 H, H-6 (dU)], 11.56 [s, 1 H, N-H (dU)].

ESI-MS: m/z (%) = 607.3 (100) [M + H]+.

HRMS (MALDI-TOF): m/z [M + H]+ calcd for C35H43BN4O7: 618.3025; found: 618.3025.

5-(4-Formylphenyl)-2'-desoxyuridine (9)
To a solution of 5-ido-2'-desoxyuridine (7; 600 mg, 1.69 mmol) in H2O–MeCN (2:1, 22.5 mL) 4-formylphenylboronic acid (8; 305 mg, 2.05 mmol), Na2CO3 (365 mg, 3.44 mmol) and Pd(dppf)Cl2 (102 mg, 0.11 mmol) were added. The mixture was stirred for 5 h at 80 °C then cooled to r.t., H2O (20 mL) was added and the solution was neutralized with NH4Cl. After extraction with hot EtOAc (70 °C), the organic phase was evaporated and the residue was washed with cold EtOAc (0 °C) and dried under vacuum to yield 9.

Yield: 428 mg (76%); pale-brown solid; Rf = 0.55 (EtOAc–MeOH–H2O; 10:1:0.5).

1H NMR (600 MHz, DMSO-d6): δ = 2.22 (m, 2 H, 2'-H), 3.62 (m, 2 H, 5'-H), 3.82 (m, 1 H, 3'-H), 4.30 (m, 1 H, 4'-H), 5.18 (s, 1 H, 5'-OH), 5.25 (s, 1 H, 3'-OH), 6.21 (t, J = 6.3 Hz, 1 H, 1'-H), 7.81 (d, J = 8.4 Hz, 2 H, Ph-H), 7.88 (d, J = 8.4 Hz, 2 H, Ph-H), 8.43 [s, 1 H, H-6 (dU)], 9.99 [s, 1 H, N-H (dU)], 10.54 (br s, 1 H, CHO).

13C NMR (150 MHz, DMSO-d6): δ = 40.3, 60.7, 69.9, 84.8, 87.4, 112.0, 128.1, 139.6, 136.9, 149.7, 161.8, 192.6.

ESI-MS: m/z (%) = 331.0 (100) [M + H]+.

5-[4-(1,1,3,3-Tetramethylpyrromethenyl)phenyl]-2'-desoxyuridine (12)
9 (1.26 mg, 3.80 mmol) was dissolved in anhydrous CH2Cl2–DMF (6:1, 40 mL). 2,4-Dimethylpyrrole (10; 1.0 mL, 9.7 mmol) was added and the mixture was stirred at r.t. for 15 min. After addition of TFA (2 drops), the reaction was stirred at r.t. for 20 h. A solution of p-chloranil (970 mg, 3.80 mmol) in anhydrous CH2Cl2 (200 mL) was added. After 2 h at r.t. the reaction was quenched with H2O (100 mL) and the aqueous phase was extracted with CH2Cl2 (5 × 50 mL). The organic phase was dried with Na2SO4 and evaporated under vacuum to give the crude product, which was purified by flash chromatography (SiO2, CH2Cl2–acetone–MeOH; 5:2:1) to yield 12.

Yield: 1.23 g (65%); red solid; Rf = 0.26 (CH2Cl2–acetone–MeOH; 4:1:0.1).

1H NMR (300 MHz, D2O): δ = 1.65 (s, 6 H, BDP-CH3), 2.32 (s, 6 H, BDP-CH3), 2.36 (m, 2 H, 2'-H), 3.72 (m, 2 H, 5'-H), 3.96 (m, 1 H, 4'-H), 4.41 (m, 1 H, 3'-H), 6.23 (t, J = 6.3 Hz, 1 H, 1'-H), 6.36 (s, 2 H, BDP-H), 7.35 (d, J = 8.5 Hz, 2 H, Ph-H), 7.57 (d, J = 8.5 Hz, 2 H, Ph-H), 8.15 [s, 1 H, H-6 (dU)].

13C NMR (100 MHz, D2O): δ = 13.1, 39.4, 60.7, 70.1, 85.9, 86.9, 114.0, 114.9, 117.8, 120.0, 128.6, 133.4, 135.4, 136.8, 140.1, 146.7, 150.9, 152.1, 164.0.

ESI-MS: m/z (%) = 503.2 (100) [M + H]+.

5-[4-(2,2-Diethyl-4,4-dimethoxy-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacyl)phenyl]-2'-desoxyuridine (13)
9 (420 mg, 1.26 mmol) was dissolved in anhydrous CH2Cl2–DMF (2:1, 15 mL). 3-Ethyl-2,4-dimethylpyrrole (11; 430 µL, 3.22 mmol) was added and the mixture was stirred at r.t. for 15 min. After addition of TFA (2 drops), the reaction was stirred at r.t. for 20 h. A solution of p-chloranil (328 mg, 1.29 mmol) in anhydrous CH2Cl2 (80 mL) was added. After 2 h at r.t. the reaction was quenched with H2O (100 mL) and the aqueous phase was extracted with CH2Cl2 (5 × 50 mL). The organic phase was dried over Na2SO4 and evaporated under vacuum. The crude product was purified by flash chromatography (SiO2, CH2Cl2–acetone–MeOH; 100:2→3:1) to yield 13.

Yield: 599 mg (83%); red solid; Rf = 0.11 (CH2Cl2–MeOH, 20:3).

1H NMR (300 MHz, CD3OD): δ = 1.13 (t, J = 7.4 Hz, 6 H, BDP-CH2), 1.72 (s, 6 H, BDP-CH2), 2.35 (m, 2 H, 2'-H), 2.43 (s, 6 H, BDP-CH3), 2.53 (q, J = 7.4 Hz, 4 H, BDP-CH2CH3), 3.82 (m, 4 H, BDP-CH2), 6.22 (d, J = 8.4 Hz, 2 H, Ph-H), 7.29 (d, J = 8.4 Hz, 2 H, Ph-H), 8.43 [s, 1 H, H-6 (dU)], 11.56 [s, 1 H, N-H (dU)].

13C NMR (75 MHz, DMSO-d6): δ = 11.5, 11.9, 14.6, 16.5, 48.5, 60.6, 69.8, 84.7, 87.4, 112.0, 127.7, 132.3, 133.5, 140.3, 152.5, 164.1.

ESI-MS: m/z (%) = 629.4 (100) [M + H]+.

2 H, 5'-H), 3.98 (m, 1 H, 3'-H), 4.47 (m, 1 H, 4’-H), 6.36 (t, J = 6.3 Hz, 1 H, 1’-H), 7.39 (d, J = 8.5 Hz, 2 H, Ph-H), 7.88 (d, J = 8.5 Hz, 2 H, Ph-H); 8.62 [s, 1 H, H-d (dU)].

13C NMR (75 MHz, CD3OD): δ = 12.2, 12.3, 14.8, 18.3, 42.0, 62.5, 72.0, 87.1, 89.3, 114.3, 129.7, 134.1, 134.5, 134.6, 136.9, 139.5, 141.3, 147.1, 151.7, 164.3.

ESI-MS: m/z (%) = 559.4 (100) [M + H]+.

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