Supporting Information

Efficient Synthesis of Fluorescent Alkynyl *C*-Nucleosides via Sonogashira Coupling for the Preparation of DNA-based Polyfluorophores

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General Information

All chemicals were used as received. All reactions were carried out under stirring. Reactions under inert gas were carried out in flasks equipped with septa under argon (supplied by using a standard manifold with vacuum and argon lines). Analytical TLC was performed on Merck ready-to-use plates with silica gel 60 (F254). Fisher Scientific silica gel (grade 60, 230–400 mesh) was used for column chromatography. ODFs were synthesized by using an Applied Biosystems 394 DNA/RNA synthesizer. NMR spectra were recorded at 25 °C by using Varian Mercury 400 (400 MHz (^{1}H), 376 MHz (^{19}F), 162 MHz (^{31}P), and 100 MHz (^{13}C)) and Varian Inova 500 (500 MHz (^{1}H), and 125 MHz (^{13}C)) spectrometer. All spectra are referenced to tetramethylsilane as standard (δ = 0 ppm) by using the signals of the solvents.

$CDCl_3$: 7.26 ppm ($CHCl_3$) or 77.16 ppm ($^{13}CDCl_3$)

$(CD_3)_2SO$: 2.50 ppm (CHD_2SOCD_3) or 39.52 ppm ($^{13}CD_3SOCD_3$)

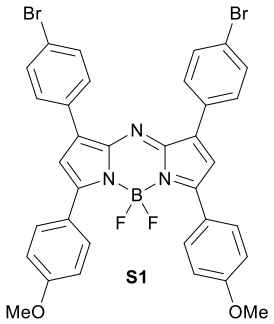
The spectra were analyzed according to first order. Multiplicities of the signals are described as follows: s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublet, ddd= doublet of doublet of doublet, m = multiplet, m_c = centered multiplet. Coupling constants (*J*) are given in Hz. Mass spectra (ESI) were obtained by using a Waters 2795 HPLC-MS (Micromass ZQ single quadrupole) spectrometer. High-resolution mass spectra (HRMS) were obtained by using a Bruker micrOTOF-O II (hybrid quadrupole-time of flight). MALDI-TOF mass spectra of ODFs were obtained by using an Applied Biosystems Voyager-DE RP spectrometer. Gas chromatography was performed on a Shimadzu GC 2014 instrument equipped with an electron capture detector. Reversed phase semi-preparative HPLC was performed using a LC-6AD Shimadzu liquid chromatography system equipped with a SPD-M10A diode array detector and a SCL-10A system controller with a Jupiter 5 µm C5 300 Å column (Phenomenex, 250 × 10.00mm). Flow rate: 3 mL/min; solvent A: 0.1 M NEt_3/AcOH buffer (pH 7) in water; solvent B: acetonitrile. Imaging of the combinatorial ODF library was performed on a Nikon Eclipse 80i epifluorescence microscope with a

Nikon Plan Fluor 4×/0.13 objective, a QIClick^{TM} digital CCD camera, and QCapture Pro 7 imaging software.

UV/Vis absorption spectra were recorded by using a Varian Cary 100 Bio UV Visible spectrophotometer and fluorescence spectra were recorded by using a Horiba Jobin Yvon Fluorolog-3 spectrofluorometer. Closed quartz cuvettes with a 1 cm path length were used in all experiments. Fluorescence quantum yield measurements were performed on the previously mentioned fluorometer and UV/Vis instrument. Relative quantum yield efficiencies were obtained by comparing the absorption values and the areas under the emission spectrum for the unkown substance and a standard. The following equation was used to calculate quantum yields:

$$\Phi_x = \Phi_s \times (F_x/F_s) \times (n_x/n_s)^2 \times (A_s/A_x)$$

Here, Φ_s is the reported quantum yield of the standard, F is the integrated emission spectrum, A is the absorbance at the excitation wavelength, and n is the refractive index of the used solvents. The subscript x denotes unkown and s denotes standard. 9,10-Diphenylanthracene in cyclohexane (Φ_F = 0.97; for nucleoside **9a**),[1] perylene in EtOH (Φ_F = 0.92; for nucleoside **9b**),[1] fluorescein in 0.1 M aqueous NaOH (Φ_F = 0.925; for nucleoside **9c**),[1] cresyl violet perchlorate in MeOH (Φ_F = 0.54; for nucleoside **9d**),[1] and aza-BODIPY dye **S1** in 1% pyridine/toluene (Φ_F = 0.42; for nucleosides **9e** and **9f**)[2] were used as standards. The slit width for both excitation and emission was 2 nm for 9,10-diphenylanthracene, perylene, fluorescein, and Nile red, and 4 nm for aza-BODIPY dye **S1**.



S1

Fig. S1 Molecular structure of aza-BODIPY dye **S1**, which was used as a quantum yield reference for the nucleosides **9e** and **9f**.[2]

Experimental Procedures and Characterization

(2*R*,3*S*)-2-(Hydroxymethyl)-5-methoxytetrahydrofuran-3-ol (**2**)

Acetyl chloride (345 µL) was added to a solution of 2-deoxy-D-ribose (**1**; 10 g, 74.6 mmol) in MeOH (120 mL). The reaction mixture was stirred for 1 h at room temperature. Subsequently, solid $NaHCO_3$ (4 g) was added and the suspension was stirred for further 5 min. The solution was filtered and the solvent was removed under reduced pressure. The product was purified via column chromatography

(CH_2Cl_2 + 5% MeOH). The title compound was obtained as a colorless liquid, yield: 7.29 g (66%). The analytical data were identical with the previously published data.[3]

(2*R*,3*S*)-3-(Benzyloxy)-2-((benzyloxy)methyl)-5-methoxytetrahydrofuran (**3**)

Sodium hydride (60% in mineral oil, 4.48 g, 112 mmol) was added to a stirred solution of sugar **2** (5.53 g, 37.7 mmol) in dry THF (100 mL) at 0 °C. The suspension was subsequently stirred for 1 h at room temperature. Afterwards, the mixture was cooled to 0 °C and benzyl bromide (13.3 mL, 112 mmol) and tetra-*n*-butylammonium iodide (400 mg) were added. The mixture was stirred overnight at room temperature. The reaction was quenched by carefully adding MeOH (10 mL) at 0 °C. Water (200 mL) was added and the product was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified via column chromatography (hexanes/EtOAc 6:1). The title compound was obtained as a colorless liquid, yield: 11.2 g (92%). The analytical data were identical with the previously published data.[4]

(4*S*,5*R*)-4-(Benzyloxy)-5-((benzyloxy)methyl)tetrahydrofuran-2-ol (**4**)

Acetyl chloride (14.5 mL, 203 mmol) was slowly added to ice-cold water (110 mL) and acetonitrile (1.1 mL) and the mixture is stirred for 30 min at room temperature. A solution of sugar **3** (11.1 g, 33.8 mmol) in dioxane (190 mL) was added and the mixture was stirred overnight at room temperature. Subsequently, the reaction mixture was neutralized by adding concentrated aqueous $NaHCO_3$ solution (230 mL). The product was extracted with CH_2Cl_2 (3 × 150 ml) and the solvent was removed under reduced pressure. The crude product was purified via column chromatography (hexanes/EtOAc 10:3 → 2:1). The title compound was obtained as a colorless liquid, yield: 8.11 g (77%). The analytical data were identical with the previously published data.[4]

(2*R*,3*S*,5*R*)-1,3-Bis(benzyloxy)hept-6-yne-2,5-diol (**5a**)

Ethynylmagnesium bromide (0.5 M in THF, 179 mL, 89.4 mmol) was added to a solution of sugar **4** (13.7 g, 43.6 mmol) in dry THF (150 mL). The reaction mixture was allowed to reach room temperature and is subsequently stirred for 2 d. The reaction was quenched with saturated aqueous NH_4Cl solution (60 mL). The solution was diluted with CH_2Cl_2 (300 mL) and washed with H_2O (3 × 100 mL) and brine (3 × 100 mL). The organic phase was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The two diastereomers **5a** and **5b** were separated via column chromatography (hexanes/EtOAc 2:1; R_f (**5a**) = 0.16; R_f (**5b**) = 0.24). The compounds **5a** and **5b** were obtained as a colorless liquids, yield: 7.37 g (**5a**, 50%); 4.39 g (**5b**, 30%). The analytical data were identical with the previously published data.[5]

(2*R*,3*S*,5*S*)-3-(Benzyloxy)-2-((benzyloxy)methyl)-5-ethynyltetrahydrofuran (**6**)

p-Toluenesulfonyl chloride (4.56 g, 23.9 mmol) and KOH (5 M in H_2O; 11.3 mL, 56.4 mmol) were sequentially added to a solution of alkyne **5a** (7.37 g, 21.7 mmol) in aceton (70 mL). The reaction mixture was stirred for 13 h at room temperature. Subsequently, the mixture was diluted with H_2O and the product was extracted with CH_2Cl_2 (3 × 100 mL). The organic phases were dried over Mg_5O4 and the solvent was removed under reduced pressure. The crude product was purified via column chromatography (hexanes/EtOAc 9:1). The title compound was obtained as a colorless liquid, yield: 5.63 g (81%). The analytical data were identical with the previously published data.[5]

(2*R*,3*S*,5*S*)-5-Ethynyl-2-(hydroxymethyl)tetrahydrofuran-3-ol (**7**)

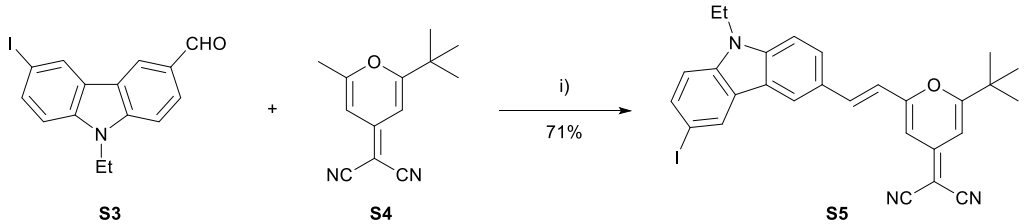
Boron trichloride (1 M in CH_2Cl_2; 70 mL, 70 mmol) was added over 20 min to a solution of ethynyl-sugar **6** (5.63 g, 17.5 mmol) in dry CH_2Cl_2 (500 mL) at –78 °C. The mixture was stirred for 1.5 h at this temperature. Subsequently, MeOH (30 mL) was added and the mixture was stirred for 14 h at room temperature. The product was extracted with water (3 × 90 mL) and the solvent was removed under reduced pressure. The crude product was purified via column chromatography ($CHCl_3$ + 5% MeOH → $CHCl_3$ + 5% MeOH). The title compound was obtained as a colorless liquid, yield: 2.02 g (81%). The analytical data were identical with the previously published data.[6]

(2*R*,3*S*,5*S*)-2-(4,4'-Dimethoxytrityloxymethyl)-5-ethynyltetrahydrofuran-3-ol (**8**)

4,4'-Dimethoxytrityl chloride (5.29 g, 15.6 mmol) was added to a solution of ethynyl-sugar **7** (2.02 g, 14.2 mmol) and 4-dimethylaminopyridine (174 mg, 1.42 mmol) in dry pyridine (135 mL). The mixture was stirred for 2 h at room temperature. Subsequently, the reaction was quenched by adding MeOH (10 mL). The solvent was removed under reduced pressure and the product was purified via column chromatography (hexanes/EtOAc 3:1 → 2:1). The title compound was obtained as a white foam, yield: 5.38 g (85%). The analytical data were identical with the previously published data.[7]

3-Bromoperylene (**S2**)

The preparation and properties of compound **S2** have been reported in reference [8].



S3 **S4** **S5**

Scheme S1 Synthesis of pyran dye **S5**. Reagents and conditions: (i) piperidine, EtOH, reflux, 2 d.

9-Ethyl-6-iodo-9*H*-carbazole-3-carbaldehyde (**S3**)

9-Ethyl-9*H*-carbazole-3-carbaldehyde (**S8**, 2.00 g, 8.96 mmol) was dissolved in acetic acid (45 mL). Potassium iodide (2.97 g, 17.9 mmol) and iodic acid (4.73 g, 26.9 mmol) were added and the mixture was heated to 70 °C for 3 h. Water (100 mL) and CH_2Cl_2 (100 mL) were added and the organic phase was separated. The organic phase was washed with saturated $Na_2SO_{3(aq)}$ (50 mL) and saturated $NaHCO_{3(aq)}$ (50 mL). The organic phase was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (hexanes/EtOAc 5:1 → 4:1). Subsequently, the product was recrystallized from EtOH. The title compound was obtained as an off-white solid, yield: 1.36 g (44%).

R_f = 0.20 (hexanes/EtOAc 5:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.42 (t, ^3J = 7.1 Hz, 3H, CH_3), 4.32 (q, ^3J = 7.1 Hz, 2H, CH_2), 7.20 (d, ^3J = 8.4 Hz, 1H, CH_{ar}), 7.43 (d, ^3J =8.4 Hz, 1H, CH_{ar}), 7.74 (d, ^3J =8.4 Hz, 1H, CH_{ar}), 8.00 (d, ^3J =8.4 Hz, 1H, CH_{ar}), 8.39 (s, 1H, CH_{ar}), 8.46 (s, 1H, CH_{ar}), 10.05 (s, 1H, CHO); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.9, 38.1, 83.0, 109.0, 111.2, 121.8, 124.4, 125.5, 127.5, 129.0, 129.7, 135.0, 139.8, 143.4, 191.6; ESI MS: *m*/*z* = 372 $[M+Na]^+$, 350 $[M+H]^+$; HRMS: *m*/*z* calcd for $C_{15}H_{12}INNaO$: 371.9856; found: 371.9850 $[M+Na]^+$.

2-(2-(*tert*-Butyl)-6-methyl-4*H*-pyran-4-ylidene)malononitrile (**S4**)

The preparation and properties of compound **S4** have been reported in reference [9].

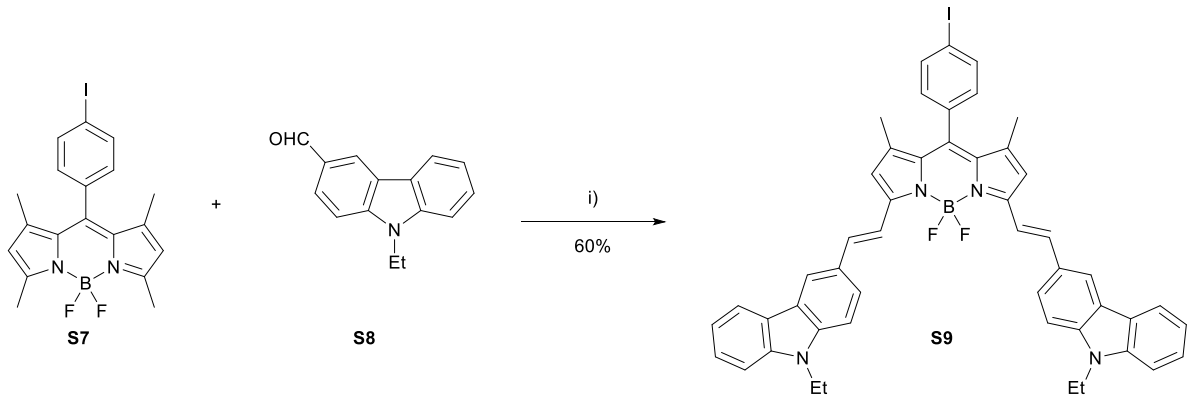
(*E*)-2-(2-(*tert*-Butyl)-6-(2-(9-ethyl-6-iodo-9*H*-carbazol-3-yl)vinyl)-4*H*-pyran-4-ylidene)malononitrile (**S5**)

Piperidine (1.30 mL) was added to a solution of pyran **S4** (866 mg, 4.04 mmol) and aldehyde **S3** (1.28 g, 3.67 mmol) in EtOH (150 mL). The mixture was heated under reflux for 2 d. The reaction mixture was cooled to room temperature and the precipitated product is filtered off and washed with EtOH (30 mL). After drying in high vacuum, the title compound was obtained as an orange solid, yield: 1.41 g (71%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.42 (s, 9H, $C(CH_3)_3$), 1.45 (t, ^3J = 7.2 Hz, 3H, CH_3), 4.37 (q, ^3J = 7.2 Hz, 2H, CH_2), 6.56 (d, ^4J = 1.9 Hz, 1H, CH_{ar}), 6.68 (d, ^4J = 1.9 Hz, 1H, CH_{ar}), 6.76 (d, ^3J = 15.9 Hz, 1H, CH), 7.23 (d, ^3J = 8.5 Hz, 1H, CH_{ar}), 7.44 (d, ^3J = 8.5 Hz, 1H, CH_{ar}), 7.60 (d, ^3J = 15.9 Hz, 1H, CH), 7.72 (dd, ^3J = 8.5 Hz, ^4J = 1.2 Hz, 1H, CH_{ar}), 7.75 (dd, ^3J = 8.5 Hz, ^4J = 1.5 Hz, 1H, CH_{ar}), 8.20 (d, ^4J = 1.2 Hz, 1H, CH), 8.45 (d, ^4J = 1.5 Hz, 1H, CH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.0, 28.3, 36.8, 38.1, 58.9, 82.5, 102.7, 106.5, 109.6, 111.2, 115.6, 115.6, 115.8, 121.6, 122.4, 125.3, 125.8, 126.2, 129.6, 134.8, 138.9, 139.7, 141.2, 156.9, 159.6, 172.2; ESI MS: *m*/*z* = 546 $[M+H]^+$; HRMS: *m*/*z* calcd for $C_{28}H_{24}IN_3NaO$: 568.0856; found: 568.0844 $[M+Na]^+$.

9-(Diethylamino)-5-oxo-5*H*-benzo[*a*]phenoxazin-2-yl trifluoromethanesulfonate (**S6**)

The preparation and properties of compound **S6** have been reported in reference [10].



Scheme S2 Synthesis of BODIPY dye **S9**. Reagents and conditions: (i) TsOH, piperidine, toluene, reflux, 1 d.

4,4-Difluoro-8-(4-iodophenyl)-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (**S7**)

The preparation and properties of compound **S7** have been reported in reference [11].

3,5-Bis((*E*)-2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)-4,4-difluoro-8-(4-iodophenyl)-1,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene (**S9**)

Piperidine (674 µL, 6.80 mmol) was added to a solution of BODIPY dye **S7** (614 mg, 1.36 mmol), aldehyde **S8** (1.21 g, 5.44 mmol) and *p*-toluenesulfonic acid monohydrate (12.9 mg, 68.0 µmol) in toluene (20 mL). The reaction mixture was heated for 1 d under reflux. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (hexanes/CH_2Cl_2 3:2 → CH_2Cl_2). Remaining impurities (mono-Knoevenagel by-product) were removed via repeated precipitation by adding hexanes to a CH_2Cl_2 solution. The title compound was obtained as a black solid, yield: 707 mg (60%).

R_f = 0.29 (hexanes/CH_2Cl_2 3:2); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.48 (t, ^3J = 7.2 Hz, 6H, 2 × CH_3), 1.52 (s, 6H, 2 × CH_3), 4.41 (q, ^3J = 7.2 Hz, 4H, 2 × CH_2), 6.72 (s, 2H, 2 × CH_{ar}), 7.14 (d, ^3J = 8.1 Hz, 2H, 2 × CH_{ar}), 7.30 (t, ^3J = 7.4 Hz, 2H, 2 × CH_{ar}), 7.43–7.46 (m, 4H, 4 × CH_{ar}), 7.49–7.53 (m, 4H, 4 × CH_{ar}), 7.83 (d, ^3J = 14.2 Hz, 2H, 2 × CH), 7.85 (d, ^3J = 8.2 Hz, 2H, 2 × CH_{ar}), 7.87 (d, ^3J = 8.2 Hz, 2H, 2 × CH_{ar}), 8.22 (d, ^3J = 7.6 Hz, 2H, 2 × CH_{ar}), 8.36 (s, 2H, 2 × CH_{ar}); ^{13}C NMR was not obtained due to poor signal-to-noise ratio; ^{19}F NMR (376 MHz, $CDCl_3$): δ = –139.0 (q, 2F, ^1J = 32.6 Hz, BF_2); ESI MS: *m*/*z* = 899 $[M+K]^+$, 883 $[M+Na]^+$, 860 $[M+H]^+$, 841 $[M–F]^+$; HRMS: *m*/*z* calcd for $C_{49}H_{40}BF_2IN_4$: 860.2361; found: 860.2366 $[M]^+$.

3,5-Bis((*E*)-4-(dimethylamino)styryl)-4,4-difluoro-8-(4-iodophenyl)-1,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene (**S10**)

The preparation and properties of compound **S10** have been reported in reference [12].

General method 1 for the preparation of the *C*-nucleosides 9a–f

Ethynyl-sugar **8** (1.00 g, 2.25 mmol, 1.50 equiv.), the respective dye (1.50 mmol, 1.00 equiv.), $Pd(PPh_3)_4$ (173 mg, 150 µmol, 0.10 equiv.), and CuI (57.1 mg, 300 µmol, 0.20 equiv.) were added to a flask. The flask was capped with a rubber septum and then purged with argon. Afterwards, dry NEt_3 (2.08 mL) and dry DMF (17.0 mL) were added via syringe and the mixture was stirred for 2.5 h at 80 °C. Subsequently, the solvent was removed under reduced pressure and the crude product was purified by column chromatography.

(2*R*,3*S*,5*S*)-2-(4,4'-Dimethoxytrityloxymethyl)-5-(pyren-1-ylethynyl)tetrahydrofuran-3-ol (**9a**)

After purification (chromatography with eluent hexanes/EtOAc 3:1 → 2:1) the title compound was obtained as a white foam from 1-bromopyrene (422 mg, 1.50 mmol) according to general method 1, yield: 711 mg (74%).

R_f = 0.35 (hexanes/EtOAc 2:1); ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 2.18 (ddd, ^2J = 12.6 Hz, ^3J = 6.9 Hz, ^3J = 5.3 Hz, 1H, CH), 2.67 (ddd, ^2J = 12.6 Hz, ^3J = 7.2 Hz, ^3J = 5.4 Hz, 1H, CH), 3.08 (dd, ^2J = 10.0 Hz, ^3J = 5.6 Hz, 1H, CH), 3.19 (dd, ^2J = 10.0 Hz, ^3J = 3.1 Hz, 1H, CH), 3.74 (s, 6H, 2 × CH_3), 4.15 (m_c, 1H, CH), 4.22 (m_c, 1H, CH), 5.24 (dd, ^3J = 7.1 Hz, ^3J = 6.0 Hz, 1H, CH), 5.31 (d, ^3J = 4.4 Hz, 1H, OH), 6.91 (d, ^3J = 8.8 Hz, 4H, 4 × CH_{ar}), 7.22–7.25 (m, 1H, CH_{ar}), 7.31 (d, ^3J = 8.8 Hz, 4H, 4 × CH_{ar}), 7.34–7.36 (m, 2H, 2 × CH_{ar}), 7.44–7.46 (m, 2H, 2 × CH_{ar}), 8.12 (d, ^3J = 7.6 Hz, 1H, CH_{ar}), 8.16–8.37 (m, 7H, 7 × CH_{ar}), 8.57 (d, ^3J = 9.2 Hz, 1H, CH_{ar}); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ = 42.2, 55.0, 64.0, 67.5, 71.8, 83.0, 84.5, 85.4, 96.3, 113.2, 116.7, 123.4, 123.6, 124.9, 125.9, 126.0, 126.7, 126.7, 127.2, 127.8, 127.9, 128.3, 128.7, 129.6, 129.8, 130.5, 130.8, 130.9, 131.3, 135.7, 145.0, 158.1; ESI MS: *m/z* = 684 $[M+K]^+$, 668 $[M+Na]^+$, 529 $[M+Na–C_{11}H_7]^+$, 507 $[M–C_{11}H_6]^+$, 303 $[M–C_{23}H_{17}O_3]^+$; HRMS: *m/z* calcd for $C_{44}H_{36}NaO_5$: 667.2455; found: 667.2459 $[M+Na]^+$.

(2*R*,3*S*,5*S*)-2-(4,4'-Dimethoxytrityloxymethyl)-5-(perylen-3-ylethynyl)tetrahydrofuran-3-ol (**9b**)

After purification (chromatography with eluent hexanes/EtOAc 3:1 → 2:1) the title compound was obtained as a yellow/orange foam from 3-bromoperylene (**S2**, 497 mg, 1.50 mmol) according to general method 1, yield: 517 mg (50%).

R_f = 0.30 (hexanes/EtOAc 2:1); ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 2.09 (ddd, ^2J = 12.5 Hz, ^3J = 6.3 Hz, ^3J = 5.2 Hz, 1H, CH), 2.61 (ddd, ^2J = 12.5 Hz, ^3J = 7.3 Hz, ^3J = 5.8 Hz, 1H, CH), 3.04 (dd, ^2J = 9.8 Hz, ^3J = 5.6 Hz, 1H, CH), 3.15 (dd, ^2J = 9.8 Hz, ^3J = 2.7 Hz, 1H, CH), 3.74 (s, 6H, 2 × CH_3), 4.07 (m_c, 1H, CH), 4.16 (m_c, 1H, CH), 5.17 (dd, ^3J = 7.0 Hz, ^3J = 6.1 Hz, 1H, CH), 5.27 (bs, 1H, OH), 6.91 (d, ^3J = 8.6 Hz, 4H, 4 × CH_{ar}), 7.21–7.25 (m, 1H, CH_{ar}), 7.29 (d, ^3J = 8.6 Hz, 4H, 4 × CH_{ar}), 7.31–7.35 (m, 2H, 2 × CH_{ar}), 7.42–7.44 (m, 2H, 2 × CH_{ar}), 7.58 (d, ^3J = 7.7 Hz, 2H, 2 × CH_{ar}), 7.67 (t, ^3J = 7.9 Hz, 1H, CH_{ar}), 7.71 (d, ^3J = 7.8 Hz, 1H, CH_{ar}), 7.84 (d, ^3J = 7.9 Hz, 1H, CH_{ar}), 7.85 (d, ^3J = 8.1 Hz, 1H, CH_{ar}), 8.20 (d, ^3J = 8.2 Hz, 1H, CH_{ar}), 8.36 (d, ^3J = 7.9 Hz, 1H, CH_{ar}), 8.41 (d, ^3J = 6.9 Hz, 1H, CH_{ar}), 8.42 (d, ^3J = 6.7 Hz, 1H, CH_{ar}), 8.47 (d, ^3J = 7.6 Hz, 1H, CH_{ar}); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ = 42.2, 55.1, 64.0, 67.4, 71.7, 82.4, 84.5, 85.4, 96.6, 113.2, 119.2, 120.3, 121.4, 121.4, 121.7, 125.7, 126.7, 127.0, 127.1, 127.6, 127.7, 127.8, 127.9, 128.4, 128.7, 129.8, 129.8, 130.1, 131.0, 131.1, 131.3, 134.2, 134.2, 135.7, 135.7, 145.0,

158.1; ESI MS: *m*/*z* = 717 $[M+Na]^+$; HRMS: *m*/*z* calcd for $C_{48}H_{38}NaO_5$: 717.2611; found: 717.2607 $[M+Na]^+$.

2-(2-((*E*)-2-(6-(((2*S*,4*S*,5*R*)-5-(4,4'-Dimethoxytrityloxymethyl)-4-hydroxytetrahydrofuran-2-yl)ethynyl)-9-ethyl-9*H*-carbazol-3-yl)vinyl)-6-(*tert*-butyl)-4*H*-pyran-4-ylidene)malononitrile (**9c**)

After purification (chromatography with eluent hexanes/EtOAc 2:1 → 1:1) the title compound was obtained as an orange/red foam from pyran dye **S5** (818 mg, 1.50 mmol) according to general method 1, yield: 858 mg (67%).

R_f = 0.50 (hexanes/EtOAc 1:1); ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ = 1.33 (t, ^3J = 7.0 Hz, 3H, CH_3), 1.39 (s, 9H, $C(CH_3)_3$), 1.97–2.01 (m, 1H, CH), 2.58 (ddd, ^2J = 12.7 Hz, ^3J = 7.1 Hz, ^3J = 5.4 Hz, 1H, CH), 3.03 (dd, ^2J = 9.8 Hz, ^3J = 6.0 Hz, 1H, CH), 3.14 (dd, ^2J = 9.8 Hz, ^3J = 2.4 Hz, 1H, CH), 3.74 (s, 6H, 2 × CH_3), 3.97–4.00 (m, 1H, CH), 4.11 (m_c, 1H, CH), 4.48 (q, ^3J = 7.0 Hz, 2H, CH_2), 5.01 (dd, ^3J = 7.1 Hz, ^3J = 6.8 Hz, 1H, CH), 5.22 (d, ^3J = 4.9 Hz, 1H, OH), 6.43 (s, 1H, CH_{ar}), 6.79 (s, 1H, CH_{ar}), 6.91 (d, ^3J = 8.5 Hz, 4H, 4 × CH_{ar}), 7.21–7.24 (m, 1H, CH_{ar}), 7.28 (d, ^3J = 8.5 Hz, 4H, 4 × CH_{ar}), 7.31–7.36 (m, 3H, 3 × CH_{ar}), 7.42–7.43 (m, 2H, 2 × CH_{ar}), 7.56 (d, ^3J = 8.5 Hz, 1H, CH_{ar}), 7.66 (d, ^3J = 8.3 Hz, 1H, CH_{ar}), 7.69–7.72 (m, 2H, 2 × CH_{ar}), 7.91 (d, ^3J = 8.3 Hz, 1H, CH_{ar}), 8.31 (s, 1H, CH_{ar}), 8.64 (s, 1H, CH_{ar}); ^{13}C NMR (125 MHz, $(CD_3)_2SO$): δ = 13.9, 27.5, 36.4, 37.5, 42.2, 55.0, 56.0, 64.1, 67.1, 71.6, 73.7, 83.9, 85.3, 88.4, 101.8, 106.0, 110.1, 110.1, 113.0, 113.2, 115.5, 116.1, 122.1, 122.2, 122.4, 124.1, 126.2, 126.4, 126.7, 127.8, 127.9, 129.5, 129.8, 135.7, 138.9, 138.9, 139.8, 141.2, 145.0, 156.6, 158.1, 160.2, 172.5; ESI MS: *m*/*z* = 885 $[M+Na]^+$; HRMS: *m*/*z* calcd for $C_{56}H_{51}N_3NaO_6$: 884.3670; found: 884.3682 $[M+Na]^+$.

2-(((2*S*,4*S*,5*R*)-5-(4,4'-Dimethoxytrityloxymethyl)-4-hydroxytetrahydrofuran-2-yl)ethynyl)-9-(diethylamino)-5*H*-benzo[*a*]phenoxazin-5-one (**9d**)

After purification (chromatography with eluent hexanes/EtOAc 2:1 → 3:4) the title compound was obtained as a deep violet foam from Nile red dye **S6** (700 mg, 1.50 mmol) according to general method 1, yield: 980 mg (86%).

R_f = 0.17 (hexanes/EtOAc 1:1); ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 1.16 (t, ^3J = 6.9 Hz, 6H, 2 × CH_3), 2.02 (ddd, ^2J = 12.7 Hz, ^3J = 6.3 Hz, ^3J = 6.3 Hz, 1H, CH), 2.58 (ddd, ^2J = 12.7 Hz, ^3J = 7.3 Hz, ^3J = 7.3 Hz, 1H, CH), 3.02 (dd, ^2J = 10.1 Hz, ^3J = 5.6 Hz, 1H, CH), 3.13 (dd, ^2J = 10.1 Hz, ^3J = 2.8 Hz, 1H, CH), 3.49 (q, ^3J = 6.9 Hz, 4H, 2 × CH_2), 3.74 (s, 6H, 2 × CH_3), 3.98–4.04 (m, 1H, CH), 4.13 (m_c, 1H, CH), 5.07 (dd, ^3J = 6.9 Hz, ^3J = 6.9 Hz, 1H, CH), 5.22 (d, ^3J = 4.7 Hz, 1H, OH), 6.26 (s, 1H, CH_{ar}), 6.63 (d, ^4J = 2.4 Hz, 1H, CH_{ar}), 6.82 (dd, ^3J = 9.2 Hz, ^4J = 2.4 Hz, 1H, CH_{ar}), 6.91 (d, ^3J = 8.7 Hz, 4H, 4 × CH_{ar}), 7.21–7.25 (m, 1H, CH_{ar}), 7.28 (d, ^3J = 8.7 Hz, 4H, 4 × CH_{ar}), 7.31–7.35 (m, 2H, 2 × CH_{ar}), 7.41–7.43 (m, 2H, 2 × CH_{ar}), 7.61 (d, ^3J = 9.2 Hz, 1H, CH_{ar}), 7.69 (dd, ^3J = 8.1 Hz, ^4J = 1.5 Hz, 1H, CH_{ar}), 8.08 (d, ^3J = 8.1 Hz, 1H, CH_{ar}), 8.51 (d, ^4J = 1.5 Hz, 1H, CH_{ar}); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ = 12.5, 41.9, 44.6, 55.1, 64.0, 67.0, 71.6, 83.6, 84.3, 85.4, 93.1, 96.0, 104.6, 110.6, 113.2, 124.4, 125.1, 125.6, 126.1, 126.7, 127.7, 127.9, 129.7, 130.3, 131.2, 131.7, 132.0, 135.7, 137.1, 145.0, 146.6, 151.1, 152.0, 158.0, 181.1; ESI MS: *m*/*z* = 761 $[M+H]^+$, 303 $[M–C_{27}H_{25}N_2O_5]^+$; HRMS: *m*/*z* calcd for $C_{48}H_{45}N_2O_7$: 761.3221; found: 761.3225 $[M+H]^+$.

(2*R*,3*S*,5*S*)-5-((4-(3,5-Bis((*E*)-2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)-4,4-difluoro-1,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacen-8-yl)phenyl)ethynyl)-2-(4,4'-dimethoxytrityloxymethyl)tetrahydrofuran-3-ol (**9e**)

After purification (chromatography with eluent hexanes/EtOAc 2:1 → 1:1 → CH_2Cl_2 and subsequent precipitation from hexanes/EtOAc) the title compound was obtained as a green solid from BODIPY dye **S9** (1.29 g, 1.50 mmol) according to general method 1, yield: 1.47 g (83%).

R_f = 0.27 (hexanes/EtOAc 3:2); ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 1.36 (t, ^3J = 7.0 Hz, 6H, 2 × CH_3), 1.48 (s, 6H, 2 × CH_3), 2.01 (ddd, ^2J = 12.3 Hz, ^3J = 6.3 Hz, ^3J = 5.9 Hz, 1H, CH), 2.56 (ddd, ^2J = 12.3 Hz, ^3J = 7.2 Hz, ^3J = 5.4 Hz, 1H, CH), 3.02 (dd, ^2J = 9.9 Hz, ^3J = 5.6 Hz, 1H, CH), 3.13 (dd, ^2J = 9.9 Hz, ^3J = 2.6 Hz, 1H, CH), 3.75 (s, 6H, 2 × CH_3), 3.96–4.02 (m, 1H, CH), 4.12 (m_c, 1H, CH), 4.50 (q, ^3J = 7.0 Hz, 4H, 2 × CH_2), 5.03 (dd, ^3J = 6.9 Hz, ^3J = 6.9 Hz, 1H, CH), 5.21 (bs, 1H, OH), 6.92 (d, ^3J = 8.5 Hz, 4H, 4 × CH_{ar}), 7.03 (s, 2H, CH_{ar}), 7.22–7.36 (m, 5H, 5 × CH_{ar}), 7.28 (d, ^3J = 8.5 Hz, 4H, 4 × CH_{ar}), 7.41–7.43 (m, 2H, 2 × CH_{ar}), 7.49–7.55 (m, 4H, 4 × CH_{ar}), 7.63–7.69 (m, 16H, 6 × CH_{ar}), 7.72–7.82 (m, 6H, 6 × CH_{ar}), 8.36 (d, ^3J = 7.6 Hz, 2H, 2 × CH_{ar}), 8.49 (s, 2H, 2 × CH_{ar}); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ = 13.8, 14.5, 37.2, 41.9, 55.0, 64.0, 67.0, 71.6, 83.8, 84.2, 85.4, 94.0, 109.6, 109.9, 112.8, 113.2, 115.4, 118.2, 119.4, 120.0, 120.9, 122.2, 122.9, 125.5, 126.4, 126.7, 127.4, 127.7, 127.7, 127.9, 128.9, 129.1, 129.7, 132.2, 134.7, 135.7, 138.5, 140.1, 140.4, 141.0, 145.0, 152.5, 158.1; ^{19}F NMR (376 MHz, $(CD_3)_2SO$): δ = –135.9 to –136.3 (m, 2F, BF_2); ESI MS: *m*/*z* = 1201 $[M+Na]^+$, 1178 $[M+H]^+$; HRMS: *m*/*z* calcd for $C_{77}H_{67}BF_2N_4NaO_5$: 1199.5077; found: 1199.5059 $[M+Na]^+$.

(2*R*,3*S*,5*S*)-5-((4-(3,5-Bis((*E*)-4-(dimethylamino)styryl)-4,4-difluoro-1,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacen-8-yl)phenyl)ethynyl)-2-(4,4'-dimethoxytrityloxymethyl)tetrahydrofuran-3-ol (**9f**)

After purification (chromatography with eluent hexanes/EtOAc 2:1 → 1:1 and subsequent precipitation from hexanes/EtOAc) the title compound was obtained as a dark green solid from BODIPY dye **S10** (1.07 g, 1.50 mmol) according to general method 1, yield: 1.05 g (68%).

R_f = 0.41 (hexanes/EtOAc 1:1); ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 1.42 (s, 6H, 2 × CH_3), 1.99 (m_c, 1H, CH), 2.54–2.58 (m, 1H, CH), 2.96–3.04 (m, 1H, CH), 3.00 (s, 12H, 4 × CH_3), 3.09–3.15 (m, 1H, CH), 3.74 (s, 6H, 2 × CH_3), 3.98 (m_c, 1H, CH), 4.11 (m_c, 1H, CH), 5.02 (dd, ^3J = 6.7 Hz, ^3J = 6.7 Hz, 1H, CH), 5.19 (bs, 1H, OH), 6.79 (d, ^3J = 8.1 Hz, 4H, 4 × CH_{ar}), 6.90–6.92 (m, 6H, 6 × CH_{ar}), 7.23–7.33 (m, 9H, 9 × CH_{ar}), 7.41–7.48 (m, 10H, 10 × CH_{ar}), 7.63 (d, ^3J = 7.6 Hz, 2H, 2 × CH_{ar}); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ = 14.4, 39.8, 41.9, 55.0, 64.0, 67.0, 71.6, 83.8, 84.2, 85.3, 91.5, 112.2, 113.2, 117.8, 122.8, 123.9, 126.7, 127.7, 127.9, 128.8, 129.2, 129.7, 132.0, 132.0, 134.9, 135.7, 137.3, 140.3, 145.0, 151.0, 152.3, 158.0; ^{19}F NMR (376 MHz, $(CD_3)_2SO$): δ = –136.9 to –136.8 (m, 2F, BF_2); ESI MS: *m*/*z* = 1030 $[M+H]^+$, 1010 $[M–F]^+$, 728 $[M–C_{21}H_{17}O_2]^+$, 708 $[M–C_{21}H_{18}FO_2]^+$; HRMS: *m*/*z* calcd for $C_{49}H_{40}BF_2N_4NaO_5$: 1051.4762; found: 1051.4744 $[M+Na]^+$.

General method 2 for the preparation of the phosphoramidites 10a–f

The *C*-nucleoside **9a**–**f** (1.00 equiv.) was added to a flask, which was capped with a rubber septum and then purged with argon. Afterwards, dry CH_2Cl_2 (15 mL), dry DIPEA (5.00 equiv.) and 2-

cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (3.00 equiv.) were added via syringe and the mixture was stirred for 1.5 h at room temperature. Subsequently, the solvent was removed under reduced pressure and the crude product was purified by column chromatography.

(2*R*,3*S*,5*S*)-2-(4,4'-Dimethoxytrityloxymethyl)-5-(pyren-1-ylethynyl)tetrahydrofuran-3-yl (2-cyanoethyl) diisopropylphosphoramidite (**10a**)

After purification (chromatography with eluent hexanes/EtOAc 3:1 + 1% NEt_3) the title compound was obtained as a white foam from *C*-nucleoside **9a** (550 mg, 853 µmol) according to general method 2, yield: 647 mg (90%). The product was obtained as a mixture of two diastereoisomers (ca. 1:1 ratio).

R_f = 0.45 (hexanes/EtOAc 3:1 + 1% NEt_3); ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 0.88 (d, ^3J = 6.6 Hz, 3H, CH_3), 1.00–1.03 (m, 6H, 2 × CH_3), 1.06 (d, ^3J = 6.9 Hz, 3H, CH_3), 2.29 (ddd, ^2J = 13.1 Hz, ^3J = 3.2 Hz, ^3J = 3.2 Hz, 0.5H, CH_a), 2.40 (ddd, ^2J = 13.1 Hz, ^3J = 3.2 Hz, ^3J = 3.2 Hz, 0.5H, CH_b), 2.57 (t, ^3J = 5.9 Hz, 1H, CH_2), 2.68 (t, ^3J = 5.8 Hz, 1H, CH_2), 2.70–2.75 (m, 1H, CH), 3.07–3.13 (m, 1H, CH), 3.20–3.28 (m, 1H, CH), 3.47–3.62 (m, 3H), 3.67–3.81 (m, 1H), 3.74 (s, 6H, 2 × CH_3), 4.28 (m_c, 0.5H, CH_a), 4.34 (m_c, 0.5H, CH_b), 4.45–4.54 (m, 1H, CH), 5.36–5.39 (m, 1H, CH), 6.90 (d, ^3J = 8.5 Hz, 2H, 2 × CH_{ar}), 6.92 (d, ^3J = 8.7 Hz, 2H, 2 × CH_{ar}), 7.22–7.36 (m, 7H, 7 × CH_{ar}), 7.44–7.46 (m, 2H, 2 × CH_{ar}), 8.11–8.38 (m, 8H, 8 × CH_{ar}), 8.51 (dd, ^3J = 9.2 Hz, ^{4}J = 2.0 Hz, 1H, CH_{ar}), (1:1 mixture of diastereoisomers); ^{31}P NMR (162 MHz, $(CD_3)_2SO$): δ = 148.6, 148.7, (1:1 mixture of diastereoisomers); ESI MS: *m*/*z* = 784 $[M+NaO–C_6H_{13}N]^+$; HRMS: *m*/*z* calcd for $C_{53}H_{53}N_2NaO_6P$: 867.3533; found: 867.3536 $[M+Na]^+$.

(2*R*,3*S*,5*S*)-2-(4,4'-Dimethoxytrityloxymethyl)-5-(perylen-3-ylethynyl)tetrahydrofuran-3-yl (2-cyanoethyl) diisopropylphosphoramidite (**10b**)

After purification (chromatography with eluent hexanes/EtOAc 3:1 + 1% NEt_3) the title compound was obtained as a yellow/orange foam from *C*-nucleoside **9b** (530 mg, 763 µmol) according to general method 2, yield: 629 mg (98%). The product was obtained as a mixture of two diastereoisomers (ca. 1:1 ratio).

R_f = 0.43 (hexanes/EtOAc 3:1 + 1% NEt_3); ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ = 0.88 (d, ^3J = 6.4 Hz, 3H, CH_3), 1.01 (d, ^3J = 6.4 Hz, 6H, 2 × CH_3), 1.06 (d, ^3J = 6.4 Hz, 3H, CH_3), 2.20–2.22 (m, 0.5H, CH_a), 2.31–2.34 (m, 0.5H, CH_b), 2.54–2.58 (m, 1H, CH), 2.65–2.69 (m, 2H, 2 × CH), 3.05–3.10 (m, 1H, CH), 3.18–3.24 (m, 1H, CH), 3.43–3.59 (m, 3H, 3 × CH), 3.65–3.70 (m, 1H, CH), 3.73 (s, 6H, 2 × CH_3), 4.22–4.23 (m, 0.5H, CH_a), 4.28–4.29 (m, 0.5H, CH_b), 4.41–4.50 (m, 1H, CH), 5.29–5.32 (m, 1H, CH), 6.88–6.91 (m, 4H, 4 × CH_{ar}), 7.21–7.34 (m, 7H, 7 × CH_{ar}), 7.41–7.43 (m, 2H, 2 × CH_{ar}), 7.53–7.56 (m, 2H, 2 × CH_{ar}), 7.62–7.68 (m, 2H, 2 × CH_{ar}), 7.81–7.83 (m, 2H, 2 × CH_{ar}), 8.14–8.16 (d, ^3J = 8.2 Hz, 1H, CH_{ar}), 8.31–8.33 (m, 1H, CH_{ar}), 8.36–8.39 (m, 2H, 2 × CH_{ar}), 8.42–8.43 (m, 1H, CH_{ar}), (1:1 mixture of diastereoisomers); ^{31}P NMR (162 MHz, $(CD_3)_2SO$): δ = 148.4, 148.6, (1:1 mixture of diastereoisomers); ESI MS: *m*/*z* = 1812 $[2M+Na]^+$, 934 $[M+NaO]^+$, 918 $[M+Na]^+$, 896 $[M+H]^+$; HRMS: *m*/*z* calcd for $C_{57}H_{55}N_2NaO_6P$: 917.3690; found: 917.3705 $[M+Na]^+$.

(2*R*,3*S*,5*S*)-2-(4,4'-Dimethoxytrityloxymethyl)-5-((6-((*E*)-2-(6-(*tert*-butyl)-4-(dicyanomethylene)-4*H*-pyran-2-yl)vinyl)-9-ethyl-9*H*-carbazol-3-yl)ethynyl)tetrahydrofuran-3-yl (2-cyanoethyl) diisopropylphosphoramidite (**10c**)

After purification (chromatography with eluent hexanes/EtOAc 2:1 + 1% NEt_3) the title compound was obtained as an orange foam from *C*-nucleoside **9c** (700 mg, 812 µmol) according to general method 2, yield: 789 mg (91%). The product was obtained as a mixture of two diastereoisomers (ca. 1:1 ratio).

R_f = 0.18 (hexanes/EtOAc 2:1 + 1% NEt_3); ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ = 0.94 (d, ^3J = 6.6 Hz, 3H, CH_3), 1.07 (d, ^3J = 6.6 Hz, 6H, 2 × CH_3), 1.10 (d, ^3J = 6.6 Hz, 3H, CH_3), 1.33 (t, ^3J = 7.1 Hz, 3H, CH_3), 1.37 (s, 9H, $C(CH_3)_3$), 2.10–2.14 (m, 0.5H, CH_a), 2.19–2.23 (m, 0.5H, CH_b), 2.58–2.60 (m, 1H, CH), 2.62–2.67 (m, 1H, CH), 2.71–2.73 (m, 1H, CH), 3.03–3.80 (m, 1H, CH), 3.16–3.19 (m, 0.5H, CH_a), 3.20–3.23 (m, 0.5H, CH_b), 3.46–3.59 (m, 3H, 3 × CH), 3.66–3.71 (m, 1H, CH), 3.73 (s, 3H, 2 × $CH_{3,a}$), 3.74 (s, 3H, 2 × $CH_{3,b}$), 4.14–4.17 (m, 0.5H, CH_a), 4.18–4.22 (m, 0.5H, CH_b), 4.36–4.50 (m, 3H, CH, CH_2), 5.14–5.16 (m, 1H, CH), 6.41 (s, 1H, CH_{ar}), 6.76 (s, 1H, CH_{ar}), 6.88–6.91 (m, 4H, 4 × CH_{ar}), 7.21–7.34 (m, 8H, 8 × CH_{ar}), 7.40–7.43 (m, 2H, 2 × CH_{ar}), 7.52–7.54 (m, 1H, CH_{ar}), 7.64–7.71 (m, 3H, 3 × CH_{ar}), 7.89–7.91 (m, 1H, CH_{ar}), 8.26–8.28 (m, 1H, CH_{ar}), 8.55–8.56 (m, 1H, CH_{ar}), (1:1 mixture of diastereoisomers); ^{31}P NMR (162 MHz, $(CD_3)_2SO$): δ = 148.5, 148.6, (1:1 mixture of diastereoisomers); ESI MS: *m*/*z* = 1101 $[M+NaO]^+$, 1085 $[M+Na]^+$, 1063 $[M+H]^+$; HRMS: *m*/*z* calcd for $C_{65}H_{68}N_5NaO_7P$: 1084.4749; found: 1084.4761 $[M+Na]^+$.

(2*R*,3*S*,5*S*)-2-(4,4'-Dimethoxytrityloxymethyl)-5-((9-(diethylamino)-5-oxo-5*H*-benzo[*a*]phenoxazin-2-yl)ethynyl)tetrahydrofuran-3-yl (2-cyanoethyl) diisopropylphosphoramidite (**10d**)

After purification (chromatography with eluent hexanes/EtOAc 2:1 + 1% NEt_3 → hexanes/EtOAc 4:3 + 1% NEt_3) the title compound was obtained as a deep violet solid from *C*-nucleoside **9d** (205 mg, 269 µmol) according to general method 2, yield: 215 mg (83%). The product was obtained as a mixture of two diastereoisomers (ca. 1:1 ratio).

R_f = 0.34 (hexanes/EtOAc 4:3 + 1% NEt_3); ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 0.98 (d, ^3J = 6.7 Hz, 3H, CH_3), 1.08 (d, ^3J = 6.7 Hz, 3H, CH_3), 1.09 (d, ^3J = 6.7 Hz, 3H, CH_3), 1.10 (d, ^3J = 7.0 Hz, 3H, CH_3), 1.16 (t, ^3J = 7.0 Hz, 6H, 2 × CH_3), 2.17 (m_c, 0.5H, CH_a), 2.27 (m_c, 0.5H, CH_b), 2.59 (t, ^3J = 6.0 Hz, 1H, CH_2), 2.60–2.67 (m, 1H, CH), 2.72 (t, ^3J = 5.8 Hz, 1H, CH_2), 3.02–3.08 (m, 1H, CH), 3.14–3.23 (m, 1H, CH), 3.47–3.62 (m, 7H), 3.70–3.79 (m, 1H), 3.74 (s, 6H, 2 × CH_3), 4.18 (m_c, 0.5H, CH_a), 4.23 (m_c, 0.5H, CH_b), 4.40–4.47 (m, 1H, CH), 5.21–5.22 (m, CH), 6.28 (s, 1H, CH_{ar}), 6.66 (s, 1H, CH_{ar}), 6.86 (d, ^3J = 9.3 Hz, 1H, CH_{ar}), 6.90 (d, ^3J = 8.5 Hz, 2H, 2 × CH_{ar}), 6.92 (d, ^3J = 8.5 Hz, 2H, 2 × CH_{ar}), 7.23–7.35 (m, 7H, 7 × CH_{ar}), 7.40–7.42 (m, 2H, 2 ×CH_{ar}), 7.61 (dd, ^3J = 9.0 Hz, ^4J = 2.0 Hz, 1H, CH_{ar}), 7.69 (dd, ^3J = 8.1 Hz, 1H, CH_{ar}), 8.09 (d, ^3J = 8.1 Hz, 1H, CH_{ar}), 8.53 (s, 1H, CH_{ar}), (1:1 mixture of diastereoisomers); ^{31}P NMR (162 MHz, $(CD_3)_2SO$): δ = 148.4, 148.5, (1:1 mixture of diastereoisomers); ESI MS: *m*/*z* = 900 $[M+NaO–C_6H_{13}N]^+$, 878 $[M+O–C_6H_{12}N]^+$; HRMS: *m*/*z* calcd for $C_{57}H_{61}N_4NaO_8P$: 983.4119; found: 983.4122 $[M+Na]^+$.

(2*R*,3*S*,5*S*)-5-((4-(3,5-Bis((*E*)-2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)-4,4-difluoro-1,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacen-8-yl)phenyl)ethynyl)-2-(4,4'-dimethoxytrityloxymethyl)tetrahydrofuran-3-yl (2-cyanoethyl) diisopropylphosphoramidite (**10e**)

After purification (chromatography with eluent hexanes/EtOAc 2:1 + 1% NEt_3 → CH_2Cl_2 + 1% NEt_3) the title compound was obtained as a green solid from *C*-nucleoside **9e** (1.31 g, 1.11 mmol) according to general method 2, yield: 1.28 g (84%). The product was obtained as a mixture of two diastereoisomers (ca. 1:1 ratio).

R_f = 0.34 (hexanes/EtOAc 2:1 + 1% NEt_3); ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ = 0.97 (d, ^3J = 6.1 Hz, 3H, CH_3), 1.05–1.17 (m, 9H, 3 × CH_3), 1.32–1.38 (m, 6H, 2 × CH_3), 1.47 (s, 6H, 2 × CH_3), 2.15 (m_c, 0.5H, CH_a), 2.25 (m_c, 0.5H, CH_b), 2.54–2.62 (m, 1H, CH), 2.61 (t, ^3J = 5.4 Hz, 1H, CH_2), 2.74 (t, ^3J = 5.4 Hz, 1H, CH_2), 3.01–3.08 (m, 1H, CH), 3.13–3.22 (m, 1H, CH), 3.49–3.62 (m, 3H), 3.69–3.79 (m, 1H), 3.74 (s, 6H, 2 × CH_3), 4.17 (m_c, 0.5H, CH_a), 4.21 (m_c, 0.5H, CH_b), 4.38–4.54 (m, 5H, CH, 2 × CH_2), 5.16–5.21 (m, 1H, CH), 6.88–6.93 (m, 4H, 4 × CH_{ar}), 7.03 (s, 2H, 2 × CH_{ar}), 7.22–7.35 (m, 9H, 9 × CH_{ar}), 7.39–7.44 (m, 2H, 2 × CH_{ar}), 7.45–7.55 (m, 4H, 4 × CH_{ar}), 7.60–7.69 (m, 6H, 6 × CH_{ar}), 7.69–7.75 (m, 2H, 2 × CH_{ar}), 7.75–7.83 (m, 4H, 4 × CH_{ar}), 8.32–8.38 (m, 2H, 2 × CH_{ar}), 8.49 (s, 2H, 2 × CH_{ar}), (1:1 mixture of diastereoisomers); ^{19}F NMR (377 MHz, $(CD_3)_2SO$): δ = –136.4 to –136.0 (m, 2F, BF_2), (1:1 mixture of diastereoisomers); ^{31}P NMR (162 MHz, $(CD_3)_2SO$): δ = 148.4, 148.5, (1:1 mixture of diastereoisomers); ESI MS: *m*/*z* = 1317 $[M+NaO–C_6H_{13}N]^+$, 1295 $[M+O–C_6H_{12}N]^+$; HRMS: *m*/*z* calcd for $C_{86}H_{84}BF_2N_6NaO_6P$: 1399.6156; found: 1399.6159 $[M+Na]^+$.

(2*R*,3*S*,5*S*)-5-((4-(3,5-Bis((*E*)-4-(dimethylamino)styryl)-4,4-difluoro-1,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacen-8-yl)phenyl)ethynyl)-2-(4,4'-dimethoxytrityloxymethyl)tetrahydrofuran-3-yl (2-cyanoethyl) diisopropylphosphoramidite (**10f**)

After purification (chromatography with eluent hexanes/EtOAc 2:1 + 1% NEt_3 → hexanes/EtOAc 4:3 + 1% NEt_3) the title compound was obtained as a black solid from *C*-nucleoside **9f** (728 mg, 707 µmol) according to general method 2, yield: 794 mg (91%). The product was obtained as a mixture of two diastereoisomers (ca. 1:1 ratio).

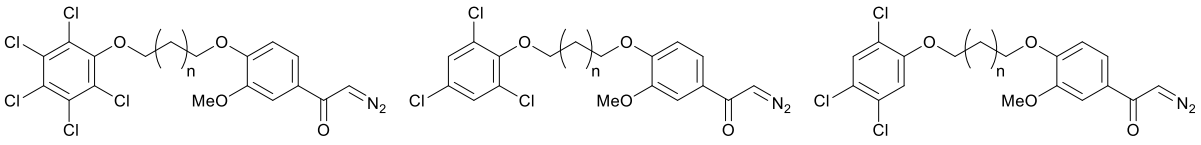
R_f = 0.49 (hexanes/EtOAc 4:3 + 1% NEt_3); ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 0.97 (d, ^3J = 6.7 Hz, 3H, CH_3), 1.06–1.11 (m, 9H, 3 × CH_3), 1.42 (s, 6H, 2 × CH_3), 2.15 (m_c, 0.5H, CH_a), 2.24 (m_c, 0.5H, CH_b), 2.55–2.63 (m, 1H, CH), 2.60 (t, ^3J = 5.8 Hz, 1H, CH_2), 2.73 (t, ^3J = 5.8 Hz, 1H, CH_2), 3.00 (s, 12H, 4 × CH_3), 3.00–3.07 (m, 1H, CH), 3.14–3.21 (m, 1H, CH), 3.48–3.62 (m, 3H), 3.67–3.74 (m, 1H), 3.74 (s, 6H, 2 × CH_3), 4.17 (m_c, 0.5H, CH_a), 4.21 (m_c, 0.5H, CH_b), 4.37–4.47 (m, 1H, CH), 5.16–5.19 (m, 1H, CH), 6.79 (d, ^3J = 8.4 Hz, 4H, 4 × CH_{ar}), 6.88–6.93 (m, 6H, 6 × CH_{ar}), 7.22–7.35 (m, 9H, 9 × CH_{ar}), 7.39–7.50 (m, 10H, 10 × CH_{ar}), 7.60 (d, ^3J = 7.3 Hz, 2H, 2 × CH_{ar}), (1:1 mixture of diastereoisomers); ^{19}F NMR (377 MHz, $(CD_3)_2SO$): δ = –137.1 to –136.7 (m, 2F, BF_2), (1:1 mixture of diastereoisomers); ^{31}P NMR (162 MHz, $(CD_3)_2SO$): δ = 148.4, 148.5, (1:1 mixture of diastereoisomers); ESI MS: *m*/*z* = 1169 $[M+NaO–C_6H_{13}N]^+$, 1147 $[M+O–C_6H_{12}N]^+$, 844 $[M–C_{27}H_{30}NO]^+$; HRMS: *m*/*z* calcd for $C_{74}H_{80}BF_2N_6NaO_6P$: 1251.5842; found: 1251.5828 $[M+Na]^+$.

Synthesis of a combinatorial library

The synthesis of the combinatorial library was carried out on a PEG-grafted polystyrene-based resin (130 µm, NovaSyn TG amino resin, Novabiochem) which was functionalized with an aliphatic spacer (Scheme S3). Upon functionalization, the loading of the resin was 280 µmol/g, which was determined by deprotecting a small part of the resin and measuring the absorbance of the released trityl cation.

Scheme S3 Functionalization of the solid support for the synthesis of the combinatorial library.

The combinatorial library was synthesized according to the previously reported split-and-pool procedure.[13] Multiple washing steps with CH_2Cl_2 were performed between each step as described below. In total, 37.8 mg of the solid support was used, which was divided equally into 7 portions (5.4 mg each). Prior to each coupling, the beads of each reaction vessel were labelled with a unique combination of electrophoric α-diazonketone tags (Fig. S2, originally developed by Still *et al.*)[14] to allow for subsequent decoding of the one-bead-one-compound library via analysis by gas chromatography with electron capture detection. The tagging was performed as previously described.[15] In essence, the respective tag (4 mg) was dissolved in dry CH_2Cl_2 (100 µL) and added to the dry resin. After swelling the resin for 45 min at room temperature in the tag solution, $Rh_2(CF_3CO_2)_4$ (100 µL; prepared by dissolving 4 mg of the rhodium catalyst in 10 mL dry CH_2Cl_2) was added and the mixture was shaken for 4 h at room temperature. If several tags were coding for the same monomer, the tagging procedure was repeated accordingly.



7A: n = 7
8A: n = 8
9A: n = 9

4B: n = 4
5B: n = 5
6B: n = 6
7B: n = 7
8B: n = 8
9B: n = 9
10B: n = 10

9D: n = 9
10D: n = 10

Fig. S2 Molecular structures of the used GC tags.

The DMT protection group was cleaved by washing the resin repeatedly with trichloroactic acid solution (3% in CH_2Cl_2) until the deprotection solution remained colorless. The deprotection solutions were collected and combined to quantify the concentration of the released trityl cation via measuring its absorbance. Thereby, the average coupling yield of each coupling cycle could be calculated. The monomer dSpacer CE Phosphoramidite (Glen Research) were used as 0.1 M solutions in acetonitrile. The monomers **10a**–**f** were used as 0.1 M solutions in CH_2Cl_2/THF 9:1. For each coupling, 200 µL of the respective monomer solution and 200 µL of the activator 5-ethylthio-1*H*-tetrazole (0.25 M in acetonitrile) were used and the reaction mixture was shaken for 1 h at room

temperature. Subsequently, the reaction solution was filtered and the resin was treated a second time with freshly prepared reaction solution under the same conditions as described above (double coupling). The capping with acetic anhydride and the oxidation with iodine was done by a DNA synthesizer using standard procedures. The whole procedure was repeated four times in total to obtain a combinatorial library which theoretically contains 2401 different sequences. The combinatorial library was obtained with an overall average coupling yield of 94% (according to detritylation). After the synthesis, the library was deprotected by incubating with 0.05 M K_2CO_3 solution in MeOH (1 mL, ultra-mild deprotection) overnight at room temperature. Subsequently, the library was washed with MeOH, saturated disodium ethylenediaminetetraacetate dihydrate solution in DMSO, and MeOH. The library was dried under high vacuum and stored in the dark.

Library screening

Samples of the ODF library were dispersed on a double sided tape (3M Scotch removable double sided tape) adhered to a Petri dish (Tissue Culture Dish 35 × 10 mm, Falcon). The beads were submerged in PBS (Corning cellgro Cell Culture Phosphate Buffered Saline, 1×). After an incubation time of 10 min, the beads were imaged using an epifluorescence microscope with 4× objective and a long-pass filter (λ_{ex} = 340–380 nm; λ_{em} > 420 nm). The exposure time was set to 500 ms with an electronic gain of 8. Bright beads were isolated manually and placed into a melting point capillary tube (Kimax, Kimble Chase). Ceric ammonium nitrate solution (5 µL, 0.5 M, water/acetonitrile 1:1) was added to cleave the GC tags and the capillaries were centrifuged briefly. Decane (8 µL) was added and the capillaries were centrifuged again, ensuring that the liquids and the beads were at the bottom. The capillaries were sealed with a flame torch and kept at 37 °C overnight. The capillaries were sonicated for 15 min and the decane layer was mixed with *N*,*O*-bis(trimethylsilyl)acetamide (1 µL) and injected into a GC instrument (split ratio 1:14). The sequence of the ODFs was obtained by comparing the GC signals with the known retention times of respective GC tags.

Fig. S3 Representative images from the 2401-member tetramer ODF library on PEG-polystyrene beads submerged in PBS, taken with 340–380 nm excitation. Note that many beads had very dim emission and were thus barely visible under UV light excitation, presumably due to the presence of the BODIPY-containing monomers C and/or D, which leads to fluorescence quenching.

General method 3 for the synthesis of ODF sequences

ODFs were synthesized with a DNA synthesizer using standard phosphoramidite chemistry. 3'-Phosphate CPG columns (1 µmol, Glen Research) were used for the synthesis. The monomers Spacer Phosphoramidite C3 and dSpacer CE Phosphoramidite (Glen Research) were used as 0.1 M solutions in acetonitrile. The monomers **10a**–**f** were used as 0.1 M solutions in CH_2Cl_2/THF 9:1. The coupling time was 999 s for all building blocks. After the synthesis, the ODFs were deprotected and cleaved from the solid support by incubating with 0.05 M K_2CO_3 solution in MeOH (1 mL, ultra-mild deprotection) overnight at room temperature. The solid support was filtered off and 1.5 mL of aqueous 2 M NEt_3/AcOH buffer (1.5 mL, pH 7) was added. The solvent was removed under reduced pressure and the ODFs were purified by reversed phase semi-preparative HPLC.

5'-PYYSSP-3'

After HPLC purification the title compound was obtained as a slightly yellowish solid from Spacer Phosphoramidite C3, dSpacer CE Phosphoramidite, and phosphoramidites **10a** according to general method 3, yield: 1.0 mg.

MALDI-TOF MS (matrix: 3-hydroxypicolinic acid): *m*/*z* = 1461 $[M–H]^–$.

5'-PSSYEP-3'

After HPLC purification the title compound was obtained as a yellow solid from Spacer Phosphoramidite C3, dSpacer CE Phosphoramidite, phosphoramidite **10a**, and phosphoramidite **10b** according to general method 3, yield: 1.0 mg.

MALDI-TOF MS (matrix: 3-hydroxypicolinic acid): *m*/*z* = 1511 $[M–H]^–$.

5'-PKYYYP-3'

After HPLC purification the title compound was obtained as a reddish solid from Spacer Phosphoramidite C3, phosphoramidite **10a**, and phosphoramidite **10c** according to general method 3, yield: 0.5 mg.

MALDI-TOF MS (matrix: 3-hydroxypicolinic acid): *m*/*z* = 2170 $[M+2Na–3H]^–$, 2148 $[M+Na–2H]^–$, 2126 $[M–H]^–$.

5'-PSKKKP-3'

After HPLC purification the title compound was obtained as an orange solid from Spacer Phosphoramidite C3, dSpacer CE Phosphoramidite, and phosphoramidite **10c** according to general method 3, yield: 0.4 mg.

MALDI-TOF MS (matrix: 3-hydroxypicolinic acid): *m*/*z* = 2380 $[M+2Na–3H]^–$, 2358 $[M+Na–2H]^–$, 2336 $[M–H]^–$.

5’-PYYKNP-3’

After HPLC purification the title compound was obtained as a brownish/red solid from Spacer Phosphoramidite C3, phosphoramidite **10a**, phosphoramidite **10c**, and phosphoramidite **10d** according to general method 3, yield: 0.8 mg.

MALDI-TOF MS (matrix: 3-hydroxypicolinic acid): *m*/*z* = 2242 $[M–H]^–$.

5’-YSSS-3’

After HPLC purification the title compound was obtained as a yellow oil from dSpacer CE Phosphoramidite and phosphoramidite **10b** according to general method 3, yield: 0.5 mg.

MALDI-TOF MS (matrix: 3-hydroxypicolinic acid): *m*/*z* = 961 $[M–H]^–$.

5’-ESSS-3’

After HPLC purification the title compound was obtained as a colorless oil from dSpacer CE Phosphoramidite and phosphoramidite **10a** according to general method 3, yield: 0.6 mg.

MALDI-TOF MS (matrix: 3-hydroxypicolinic acid): *m*/*z* = 1011 $[M–H]^–$.

5’-KSSS-3’

After HPLC purification the title compound was obtained as an orange oil from dSpacer CE Phosphoramidite and phosphoramidite **10c** according to general method 3, yield: 0.8 mg.

MALDI-TOF MS (matrix: 3-hydroxypicolinic acid): *m*/*z* = 1216 $[M+K–2H]^–$; 1200 $[M+Na–2H]^–$; 1178 $[M–H]^–$.

5’-NSSS-3’

After HPLC purification the title compound was obtained as a blue oil from dSpacer CE Phosphoramidite and phosphoramidite **10d** according to general method 3, yield: 0.5 mg.

MALDI-TOF MS (matrix: 3-hydroxypicolinic acid): *m*/*z* = 1116 $[M+K–2H]^–$; 1077 $[M–H]^–$.

5’-CSSS-3’

After HPLC purification the title compound was obtained as a green oil from dSpacer CE Phosphoramidite and phosphoramidite **10e** according to general method 3, yield: 0.5 mg.

MALDI-TOF MS (matrix: 3-hydroxypicolinic acid): *m*/*z* = 1516 $[M+Na-2H]^-$; 1494 $[M-H]^-$; 1447 $[M+H-BF_2]^-$.

5’-KSSS-3’

After HPLC purification the title compound was obtained as a green oil from dSpacer CE Phosphoramidite and phosphoramidite **10f** according to general method 3, yield: 0.6 mg.

MALDI-TOF MS (matrix: 3-hydroxypicolinic acid): *m*/*z* = 1384 $[M+K-2H]^-$; 1368 $[M+Na-2H]^-$; 1346 $[M-H]^-$.

Spectral Data

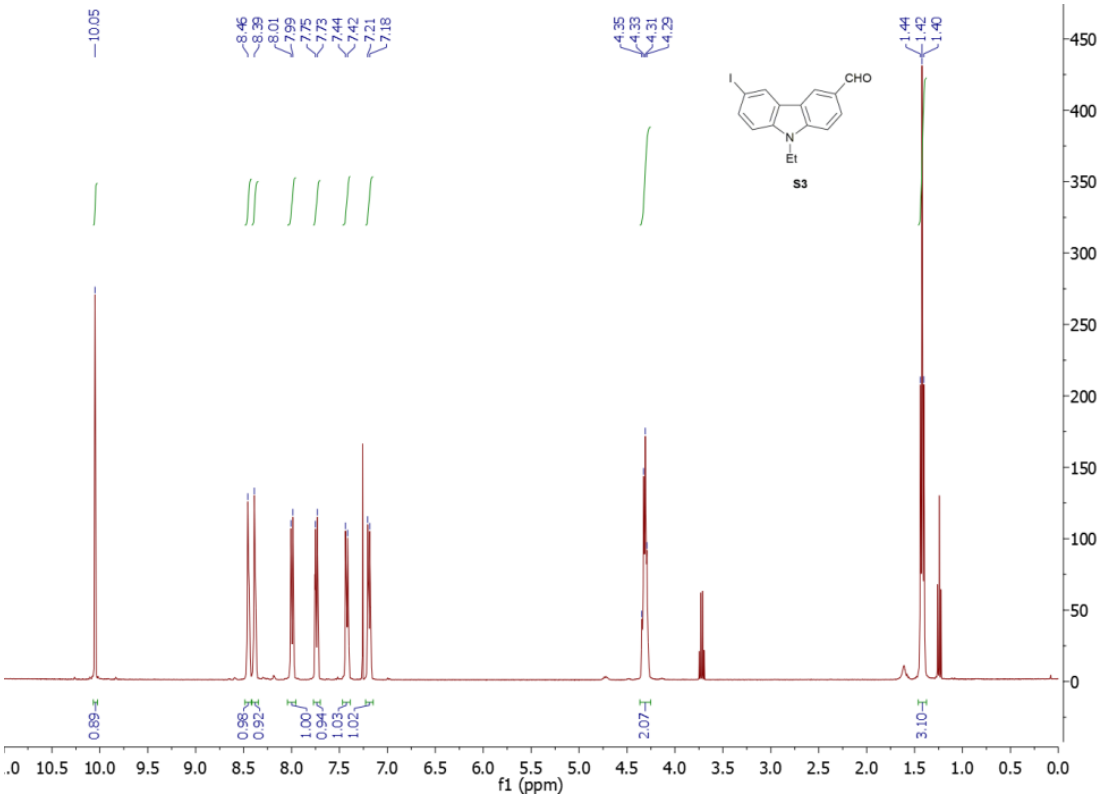


Fig. S4 ^{1}H NMR ($CDCl_3$, 400 MHz) of aldehyde **S3**.

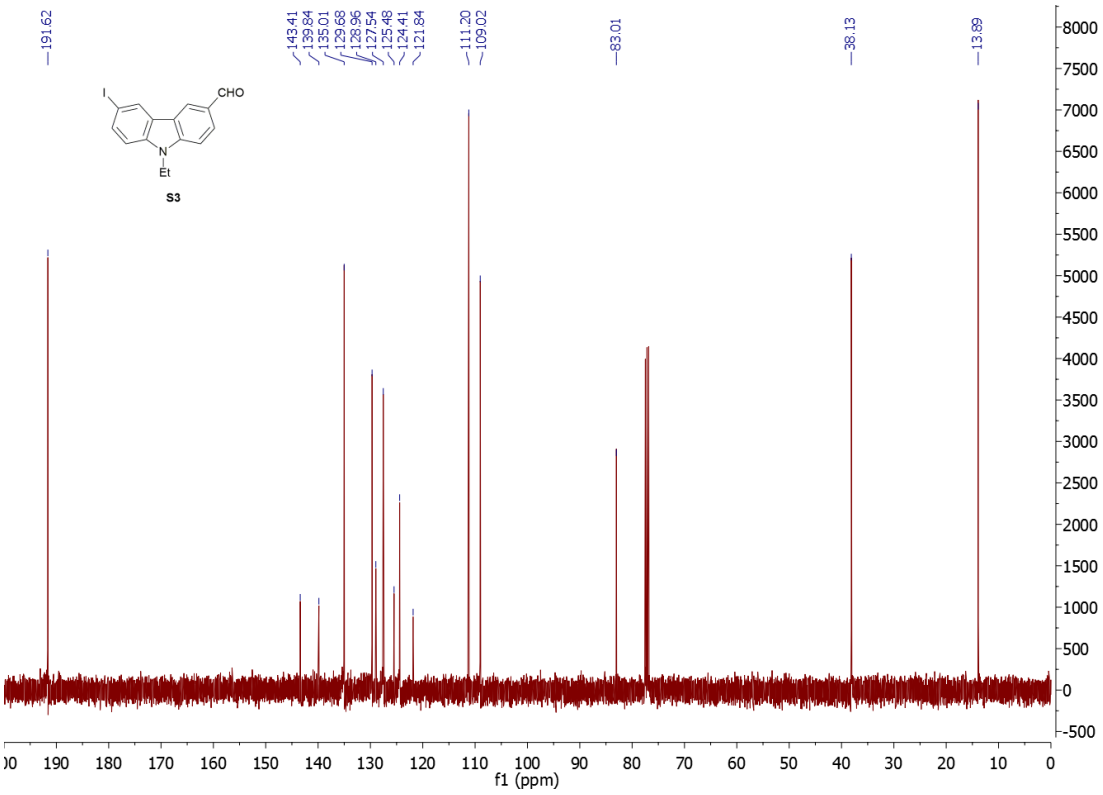


Fig. S5 ^{13}C NMR ($CDCl_3$, 100 MHz) of aldehyde **S3**.

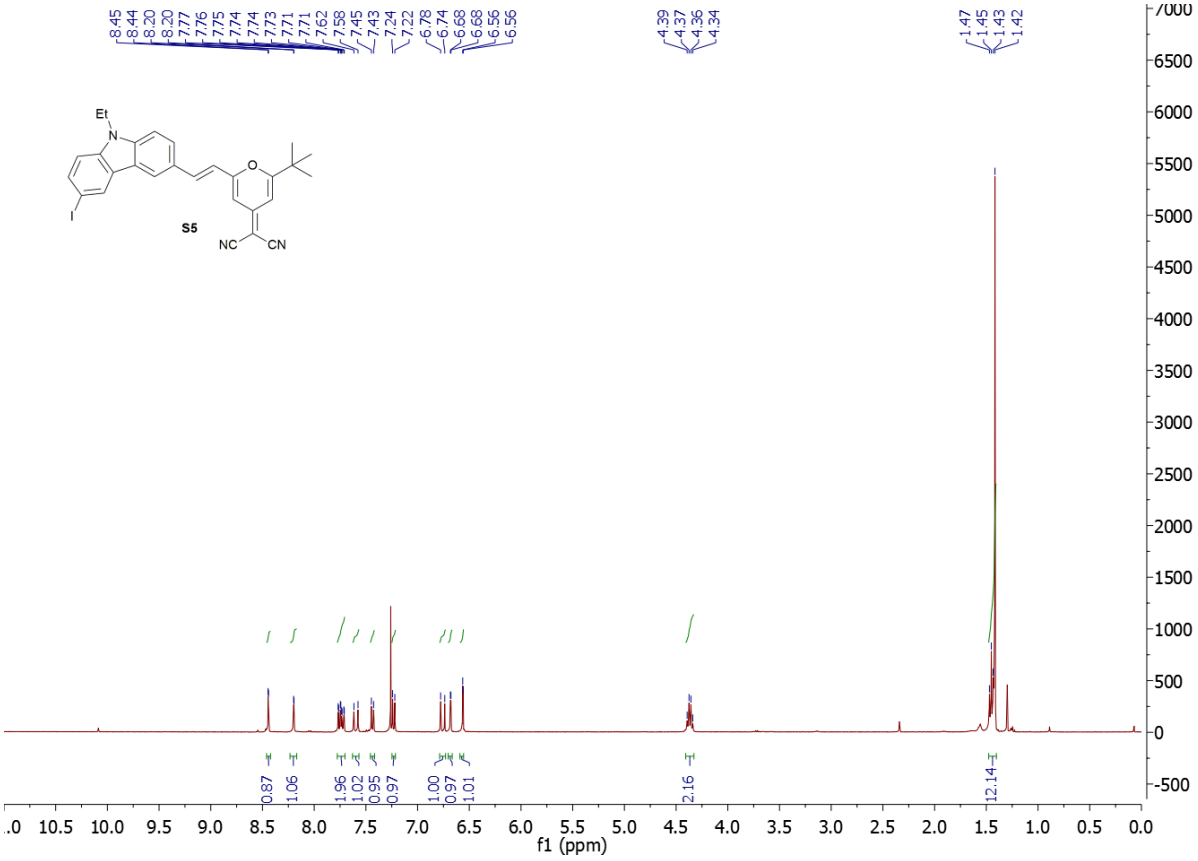


Fig. S6 ^{1}H NMR ($CDCl_3$, 400 MHz) of pyran dye **S5**.

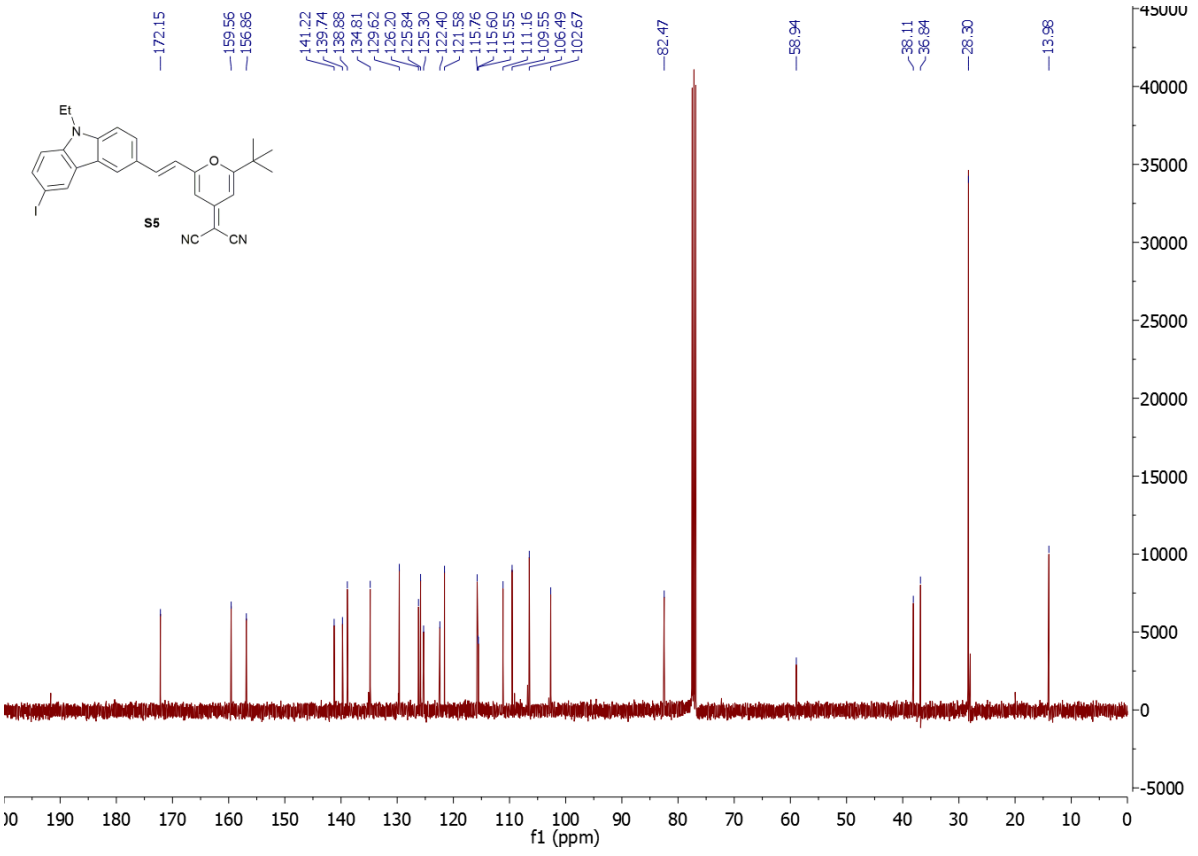


Fig. S7 ^{13}C NMR ($CDCl_3$, 100 MHz) of pyran dye **S5**.

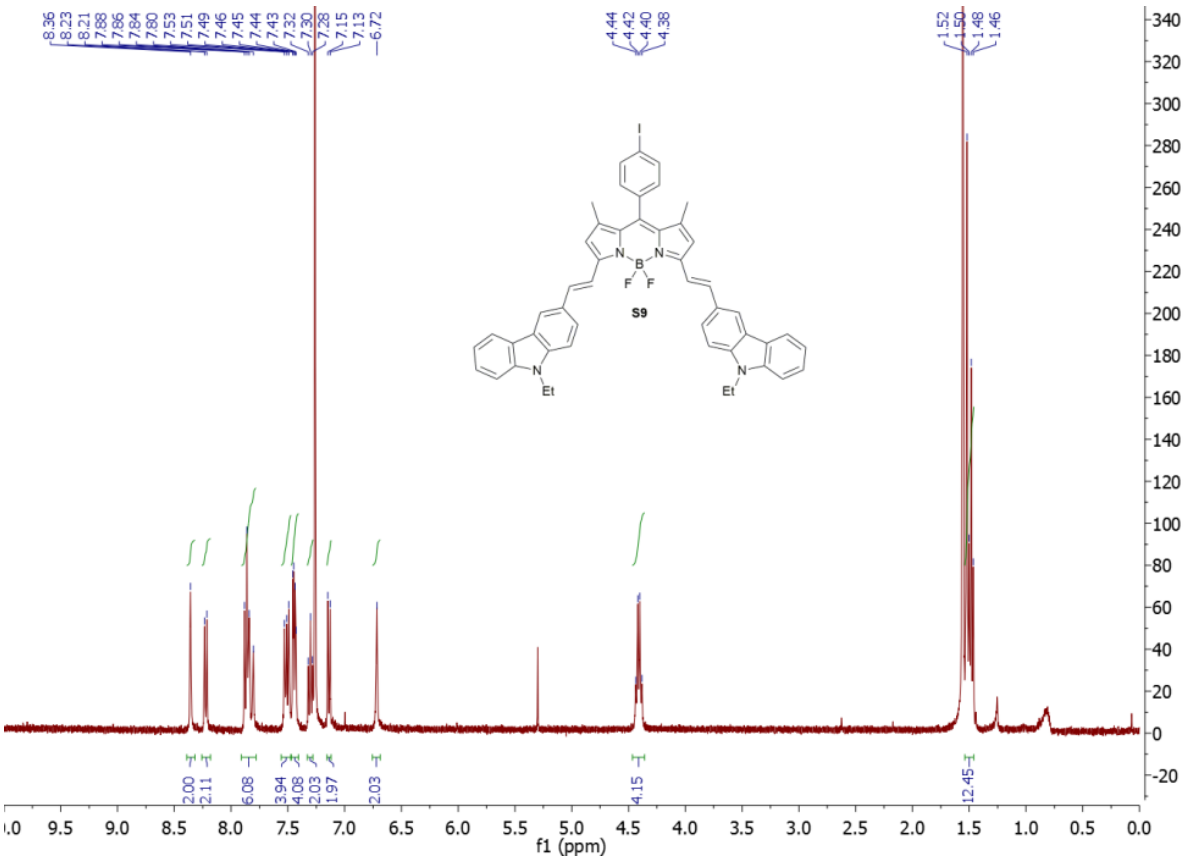


Fig. S8 ^{1}H NMR ($CDCl_3$, 400 MHz) of BODIPY dye **S9**.

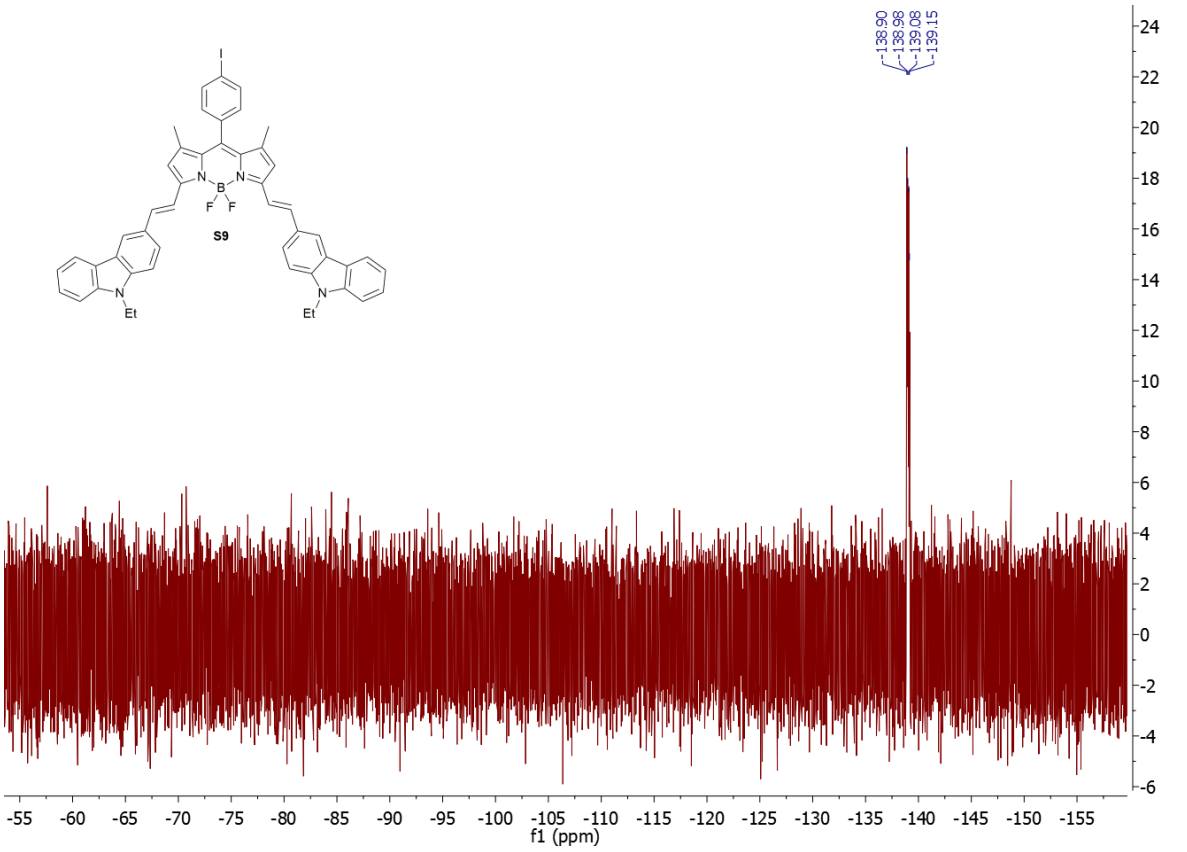


Fig. S9 ^{19}F NMR ($CDCl_3$, 377 MHz) of BODIPY dye **S9**.

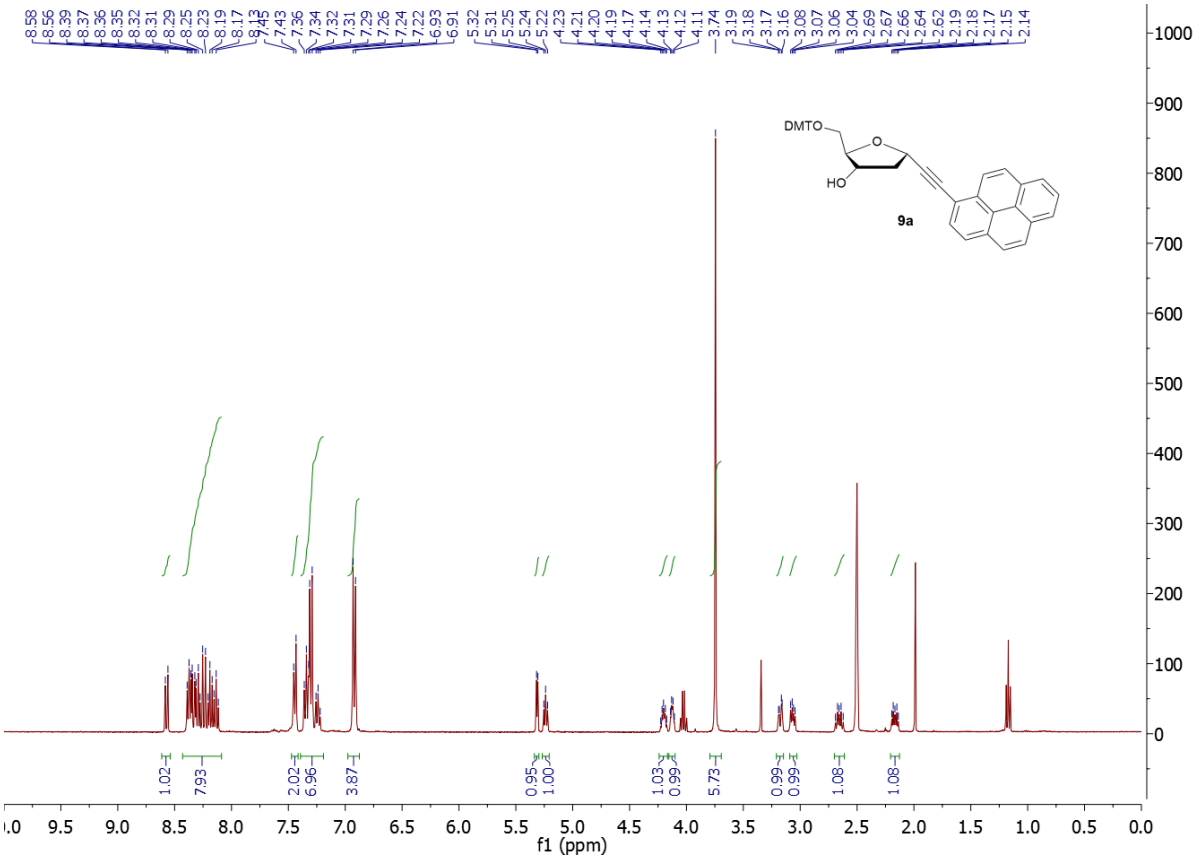


Fig. S10 ^{1}H NMR ([D_6]-DMSO, 400 MHz) of *C*-nucleoside **9a**.

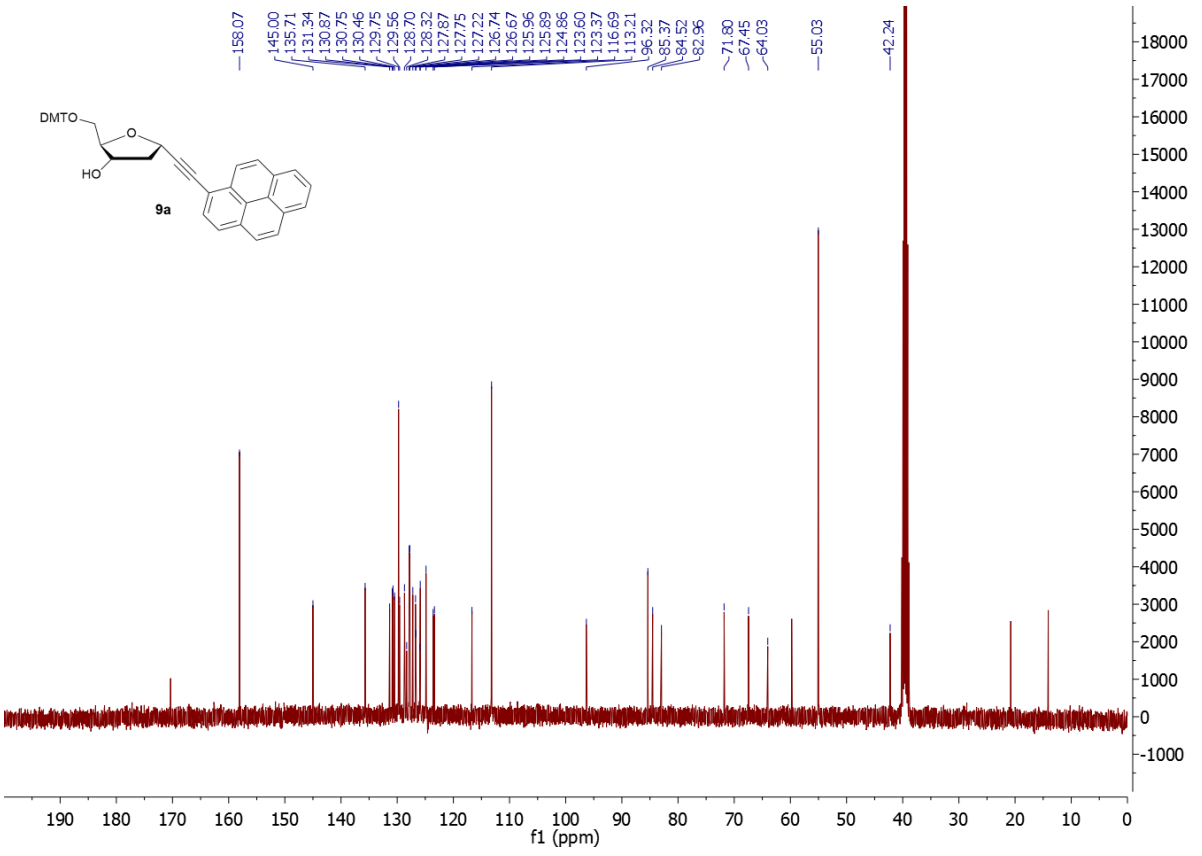


Fig. S11 ^{13}C NMR ([D_6]-DMSO, 100 MHz) of *C*-nucleoside **9a**.

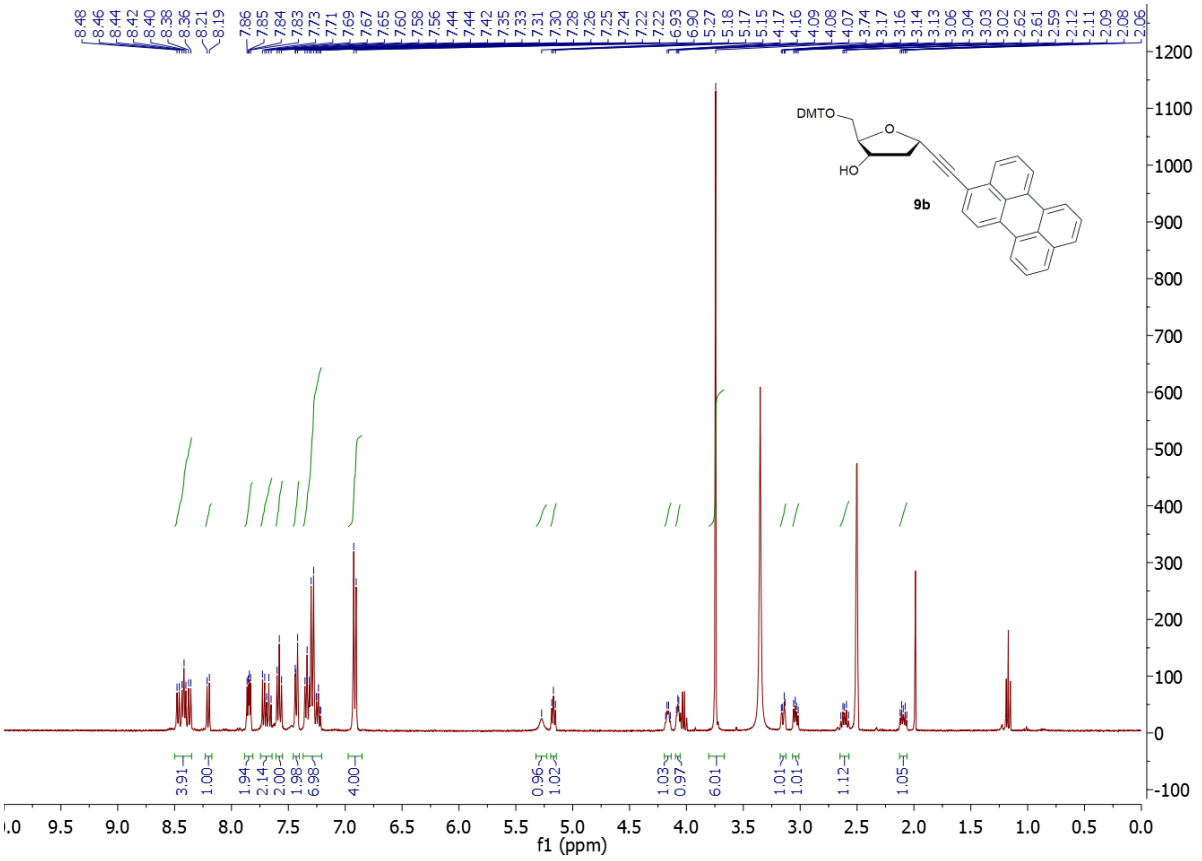


Fig. S12 ^{1}H NMR ([D_6]-DMSO, 400 MHz) of *C*-nucleoside **9b**.

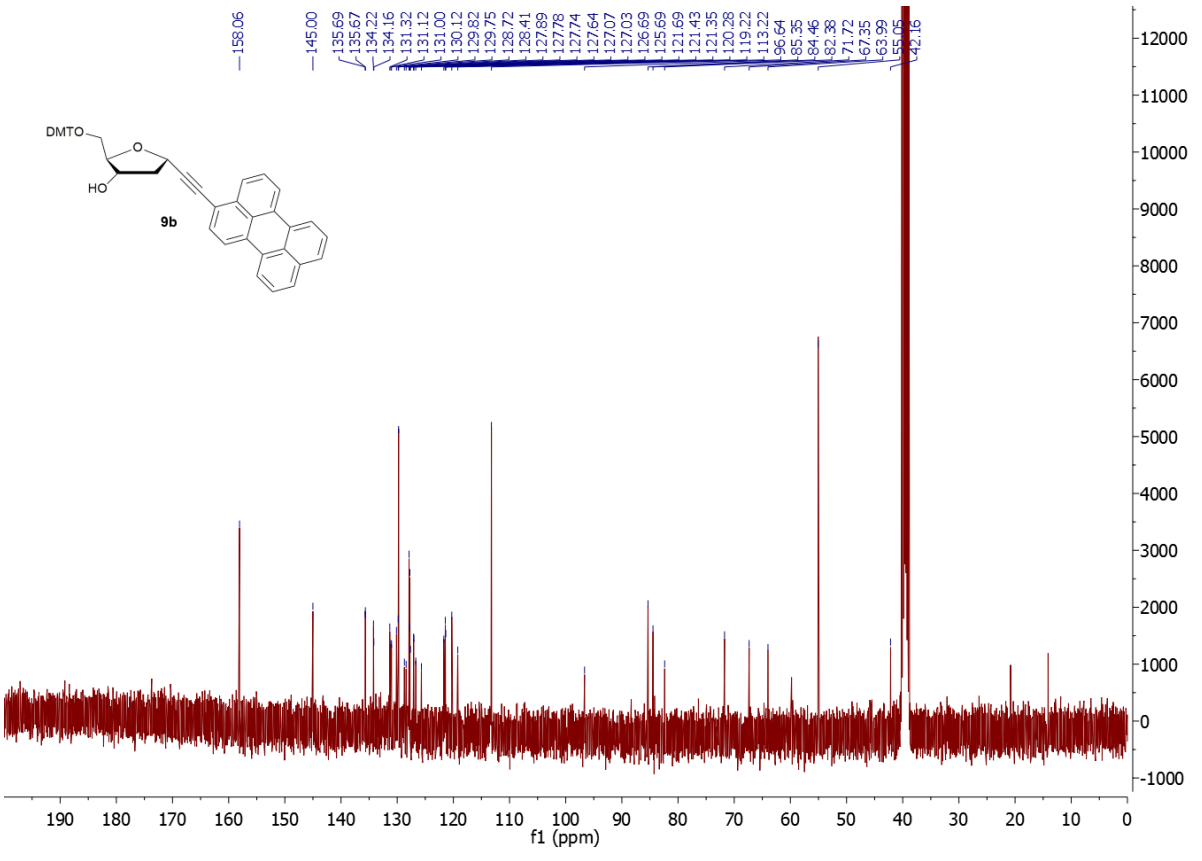


Fig. S13 ^{13}C NMR ([D_6]-DMSO, 100 MHz) of *C*-nucleoside **9b**.

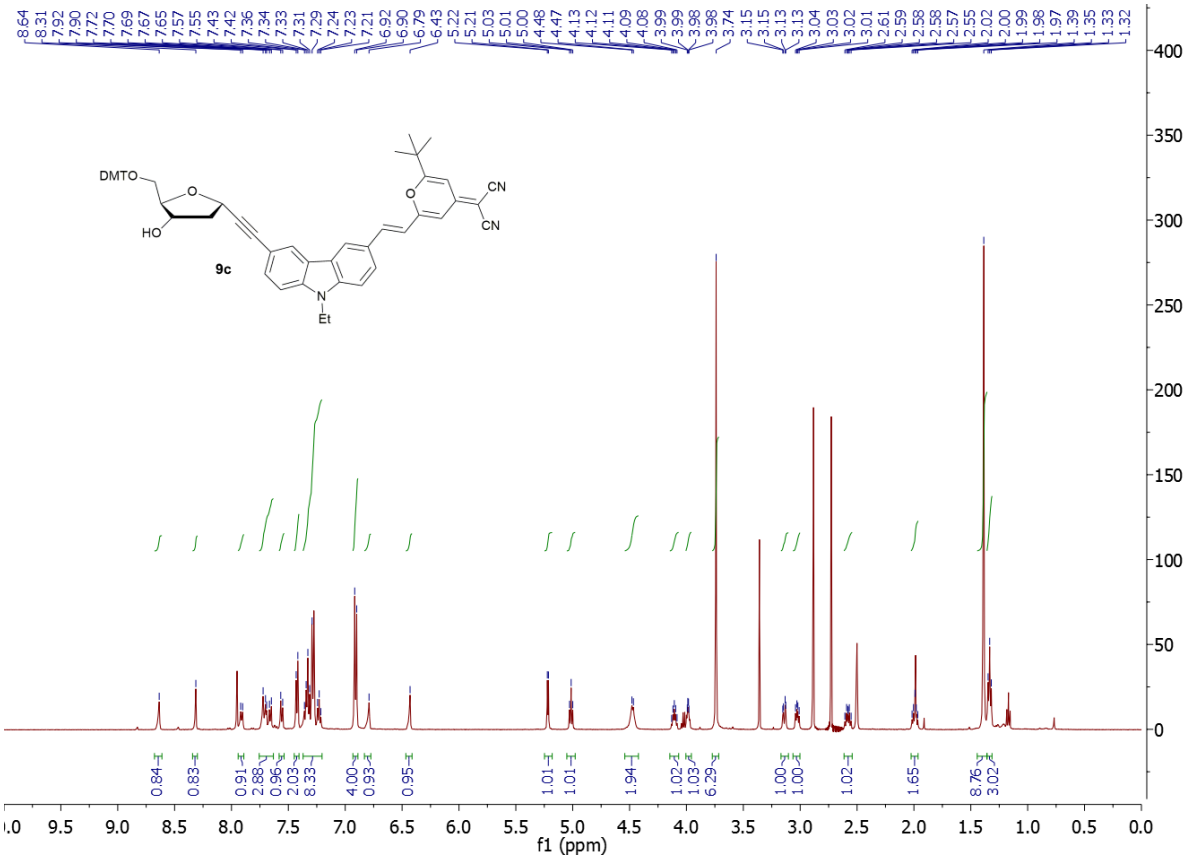


Fig. S14 ^{1}H NMR ([D_6]-DMSO, 500 MHz) of *C*-nucleoside **9c**.

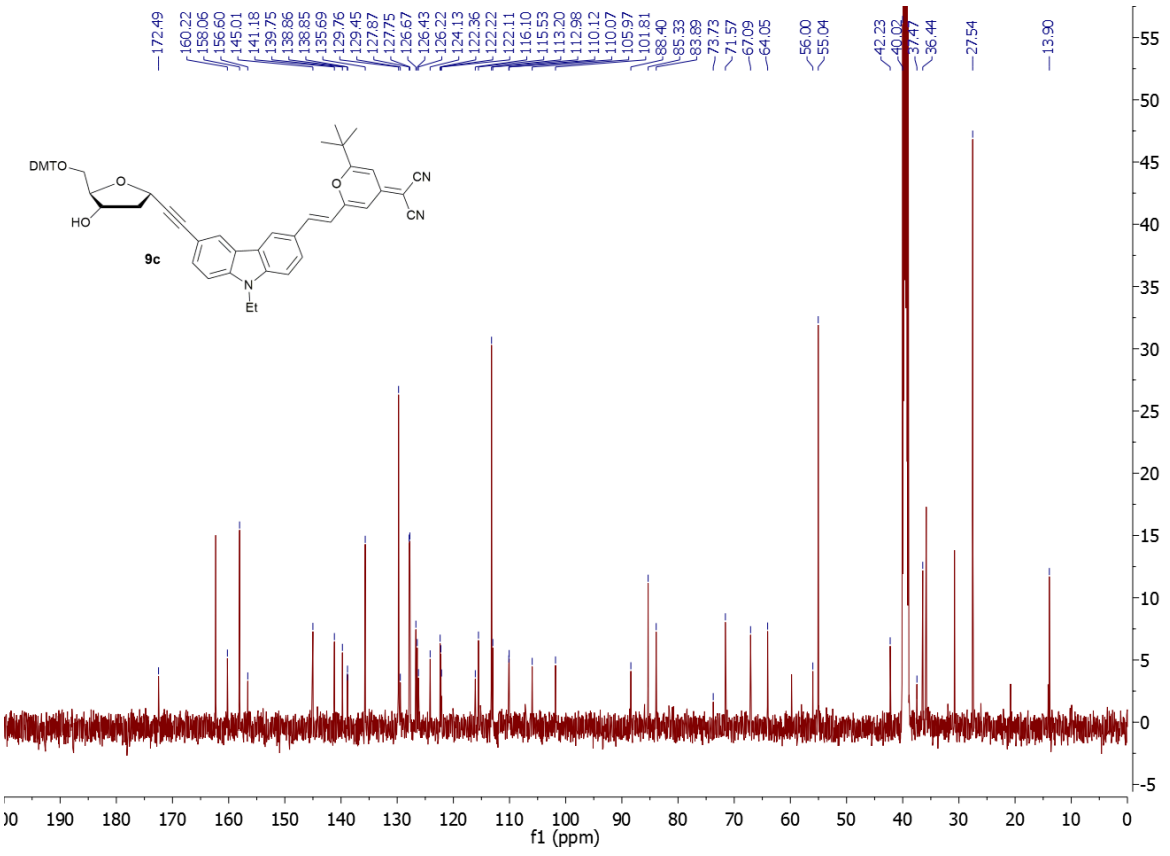


Fig. S15 ^{13}C NMR ([D_6]-DMSO, 125 MHz) of *C*-nucleoside **9c**.

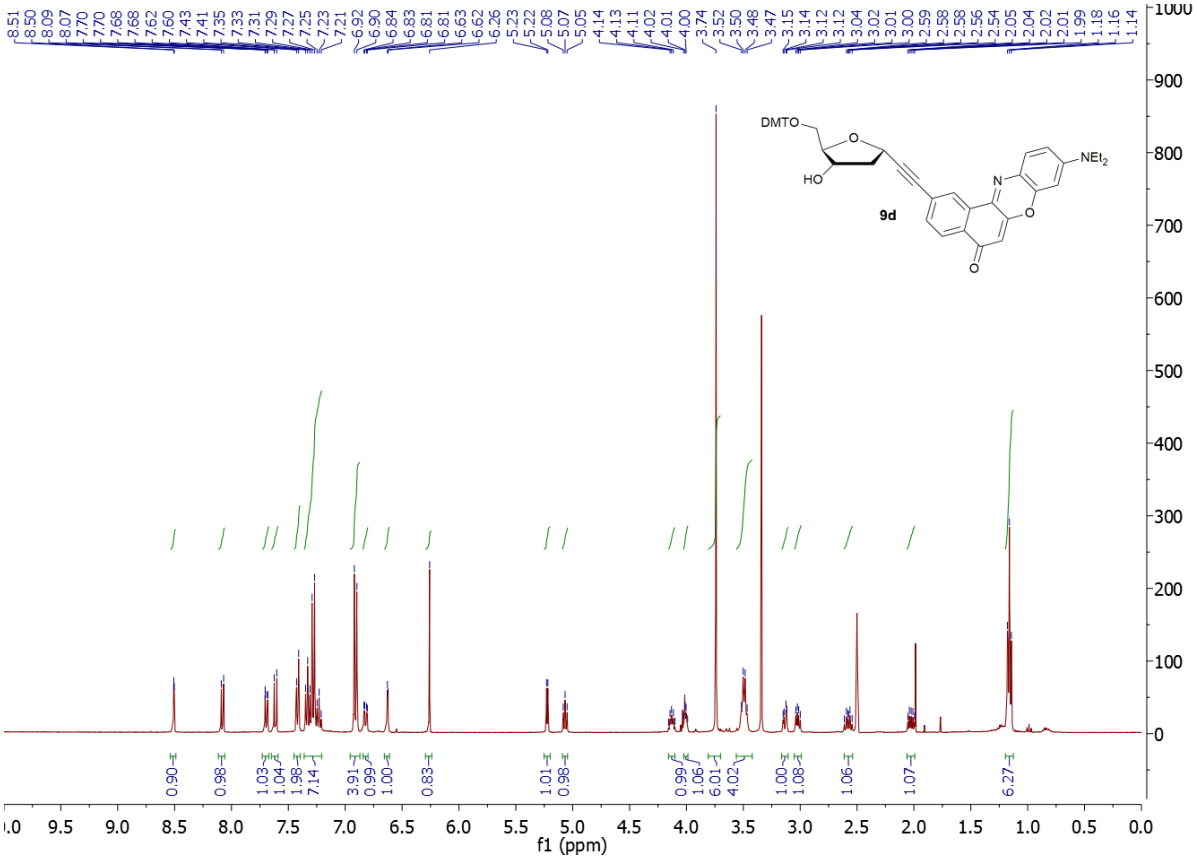


Fig. S16 ^{1}H NMR ([D_6]-DMSO, 400 MHz) of *C*-nucleoside **9d**.

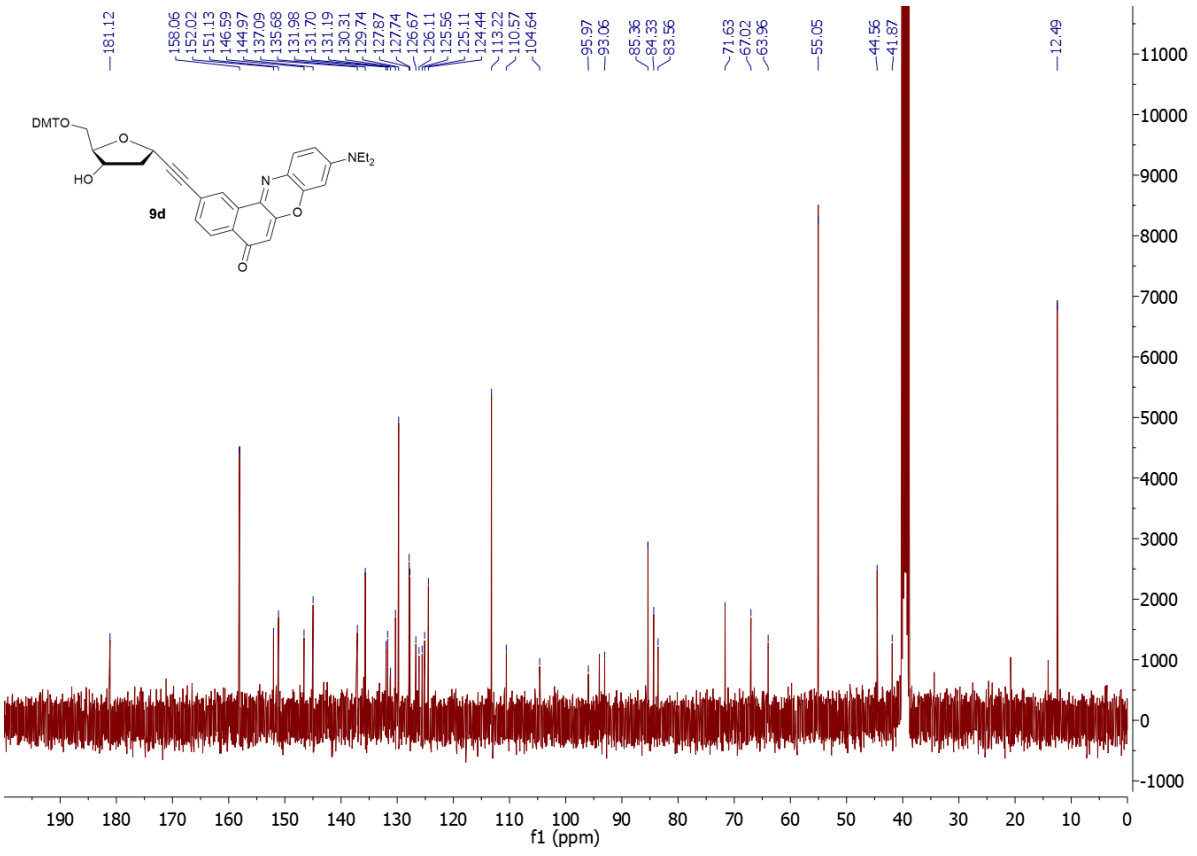


Fig. S17 ^{13}C NMR ([D_6]-DMSO, 100 MHz) of *C*-nucleoside **9d**.

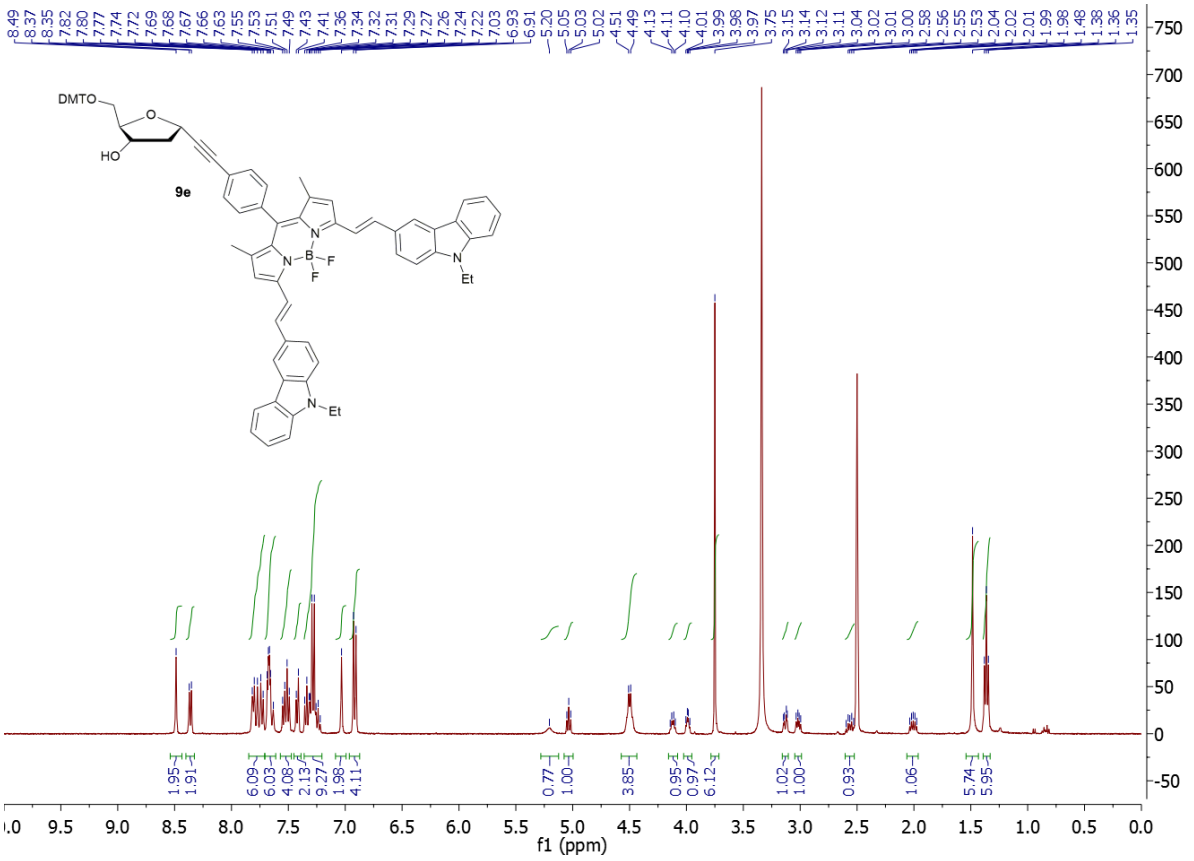


Fig. S18 ^{1}H NMR ([D_6]-DMSO, 400 MHz) of *C*-nucleoside **9e**.

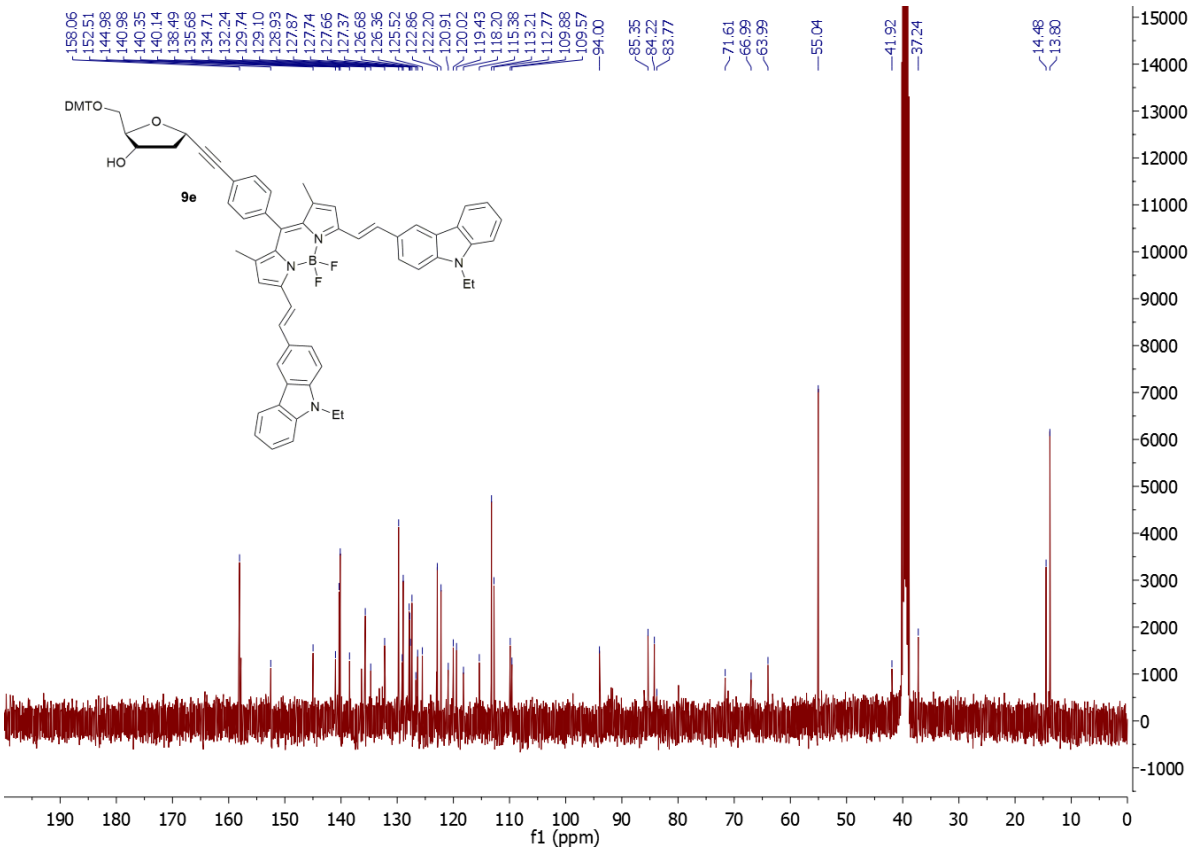


Fig. S19 ^{13}C NMR ([D_6]-DMSO, 100 MHz) of *C*-nucleoside **9e**.

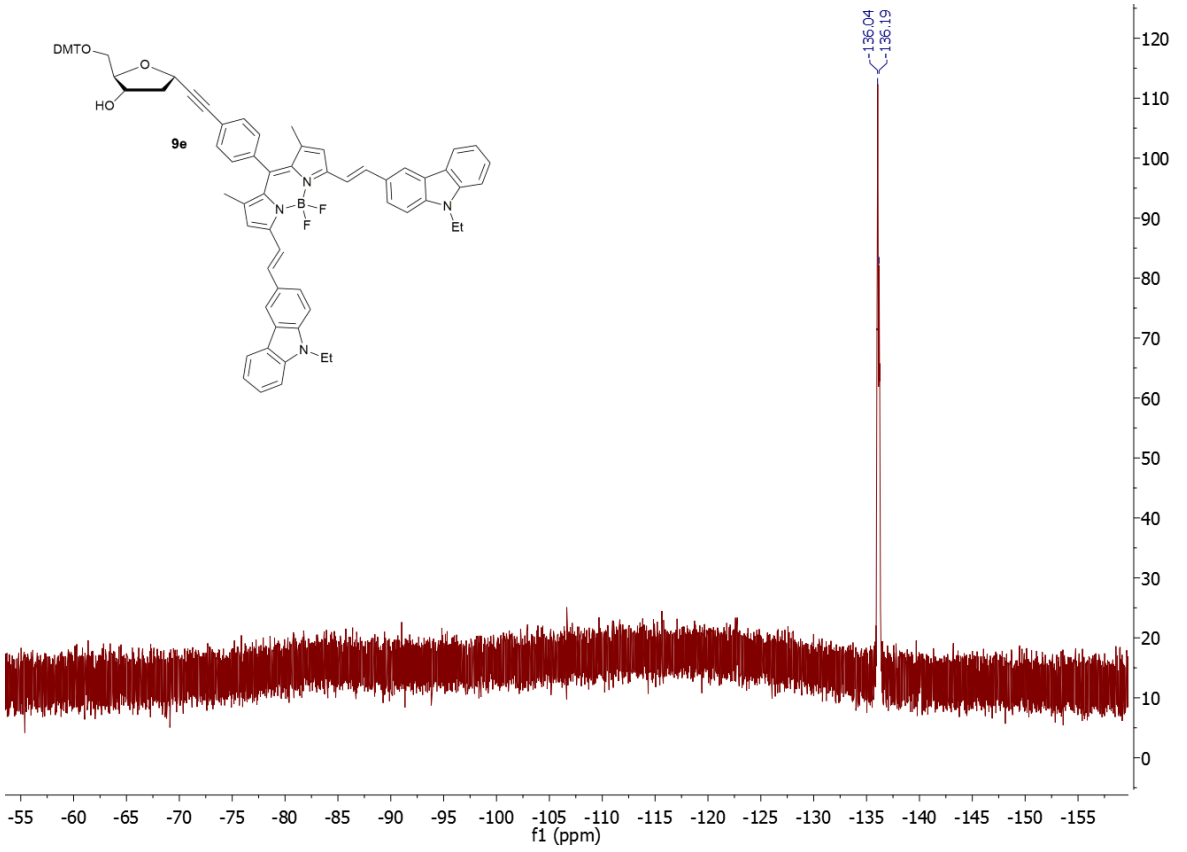


Fig. S20 ^{19}F NMR ([D$_6$]-DMSO, 377 MHz) of *C*-nucleoside **9e**.

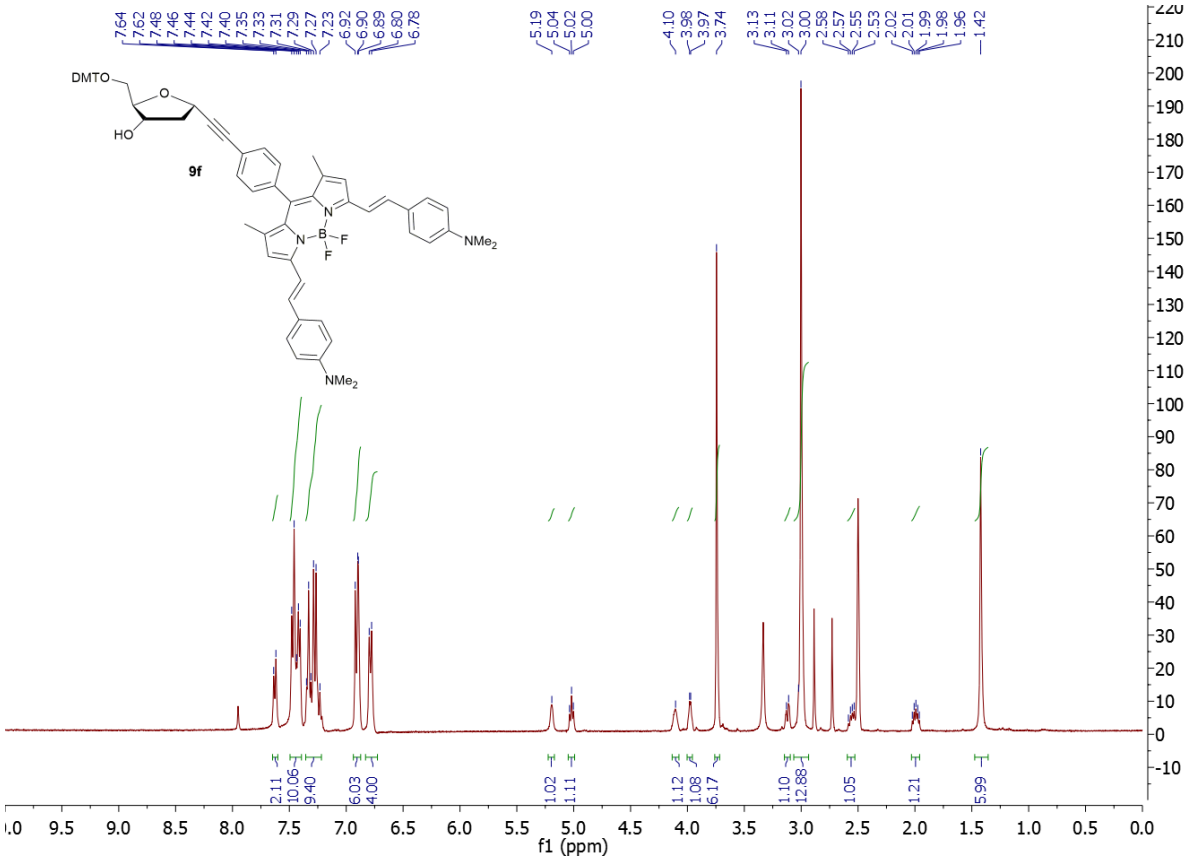


Fig. S21 ^{1}H NMR ([D$_6$]-DMSO, 400 MHz) of *C*-nucleoside **9f**.

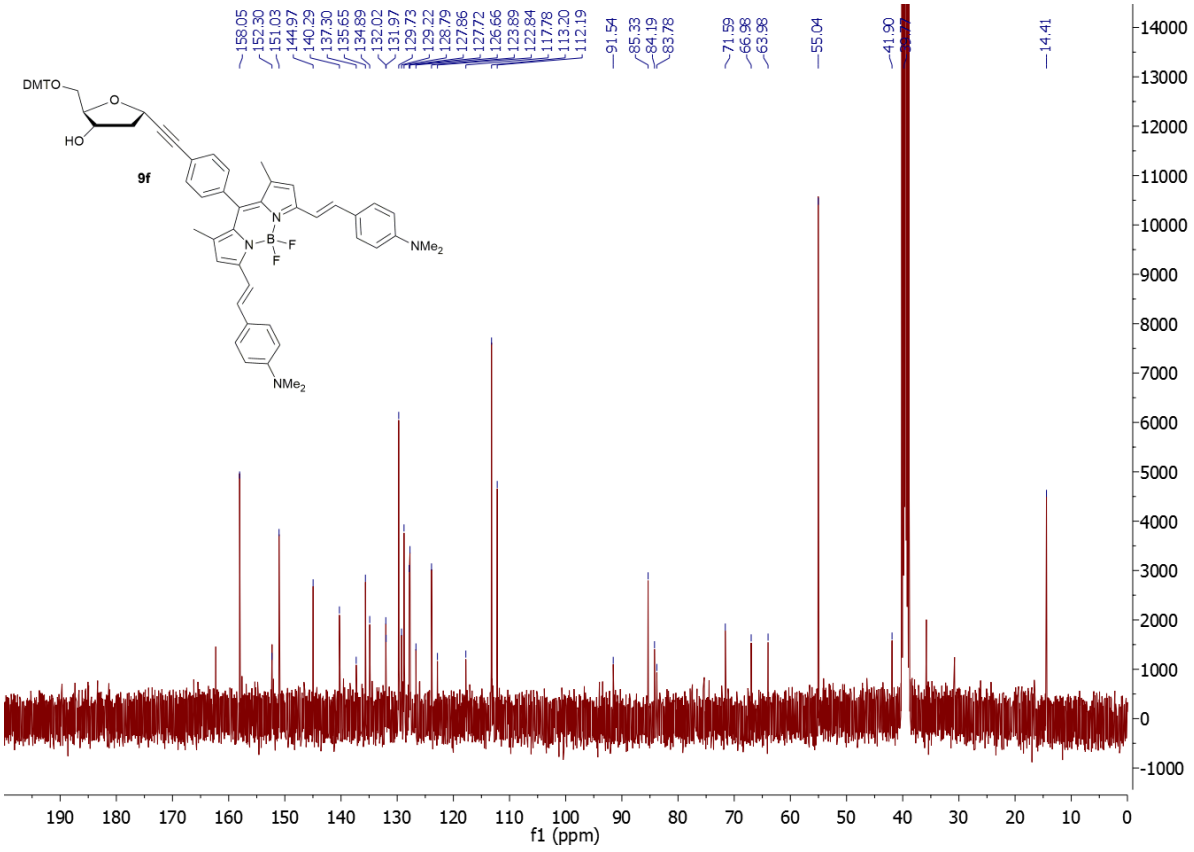


Fig. S22 ^{13}C NMR ([D$_6$]-DMSO, 100 MHz) of *C*-nucleoside **9f**.

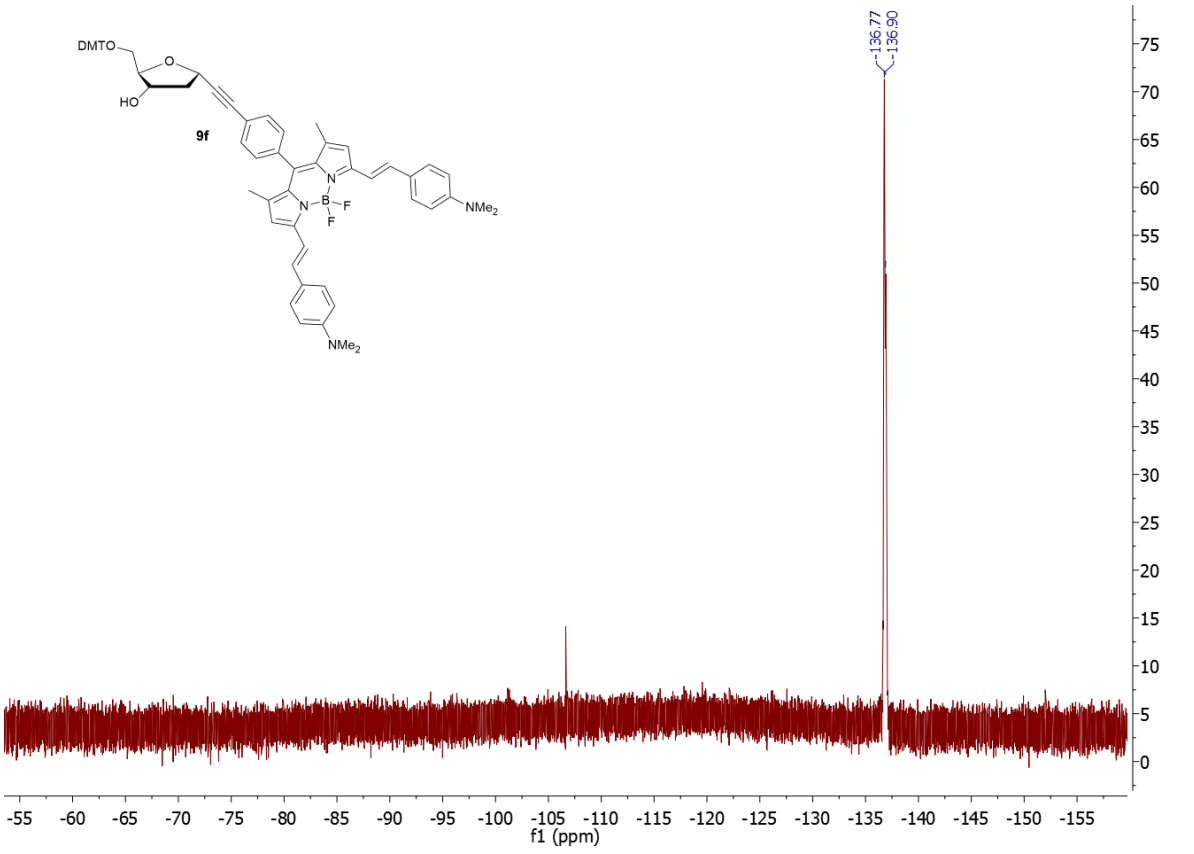


Fig. S23 ^{19}F NMR ([D$_6$]-DMSO, 377 MHz) of *C*-nucleoside **9f**.

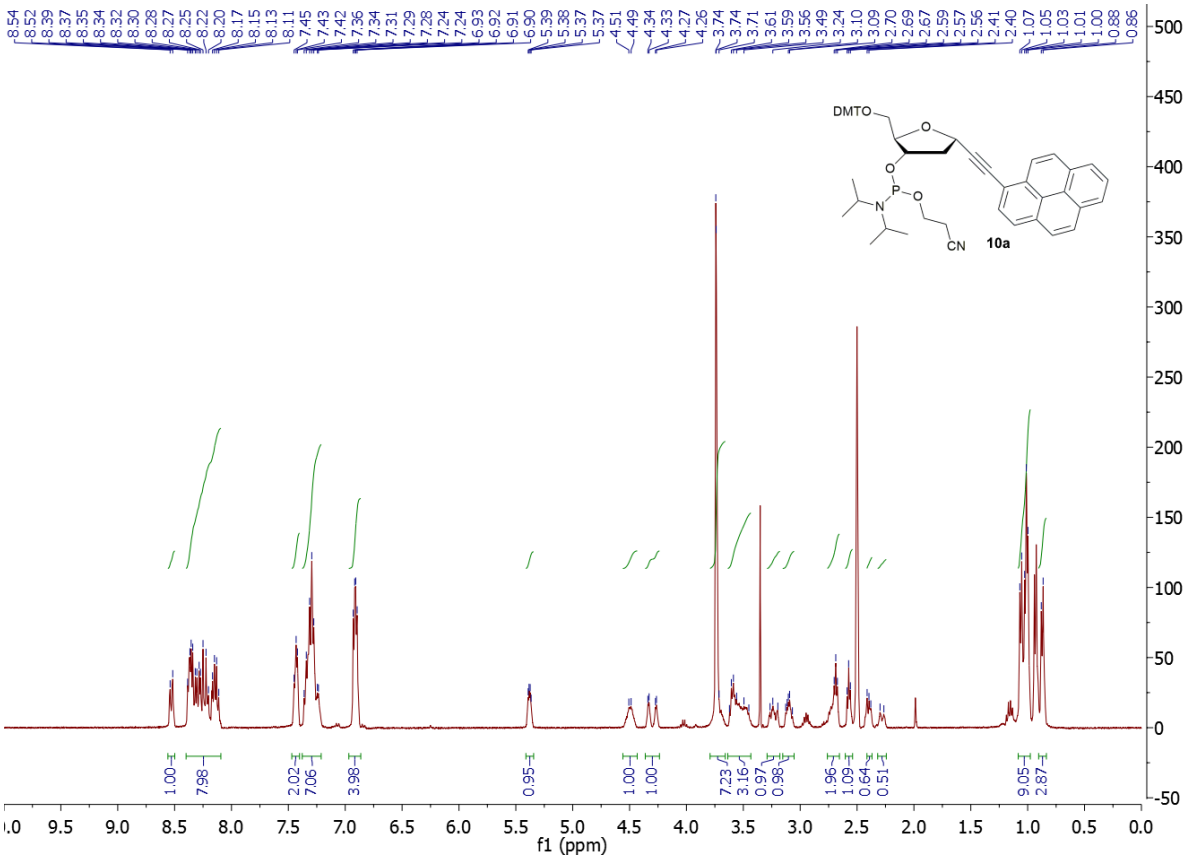


Fig. S24 ^{1}H NMR ([D_6]-DMSO, 400 MHz) of phosphoramidite **10a**.

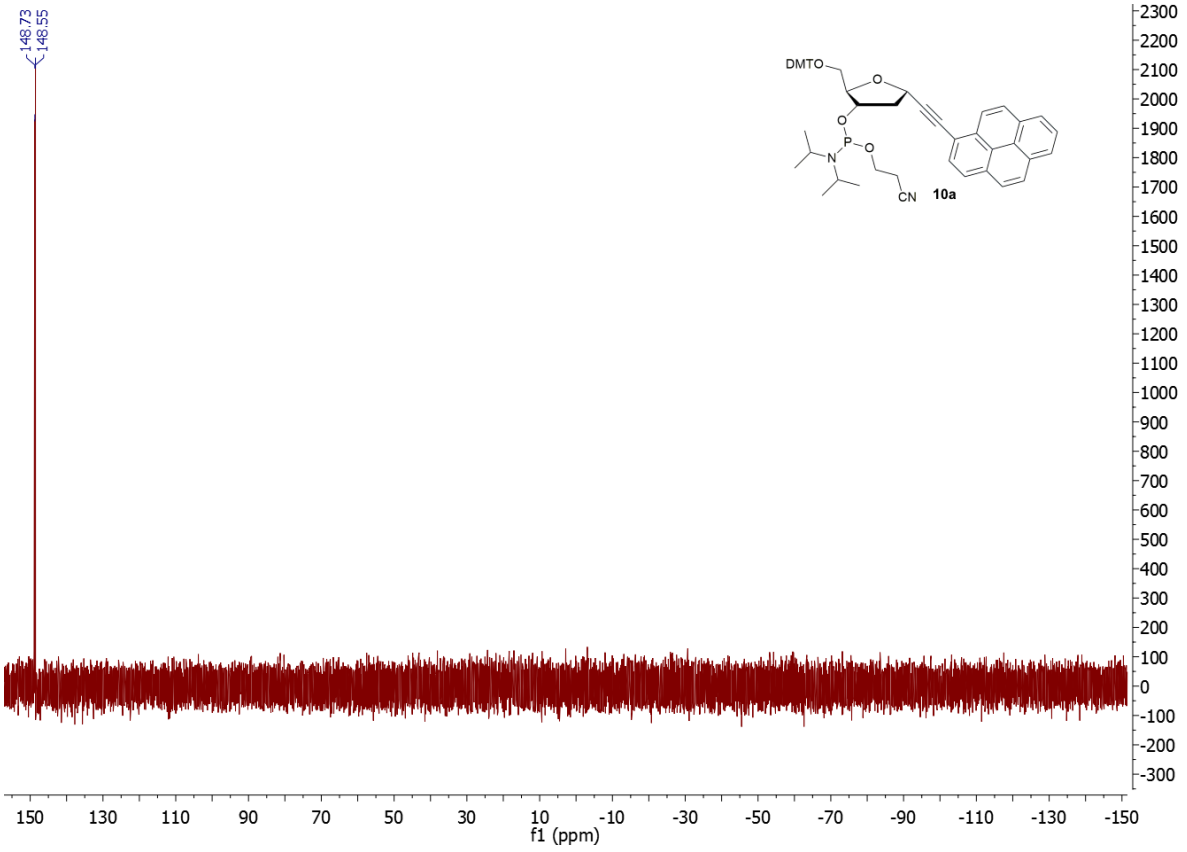


Fig. S25 ^{31}P NMR ([D_6]-DMSO, 162 MHz) of phosphoramidite **10a**.

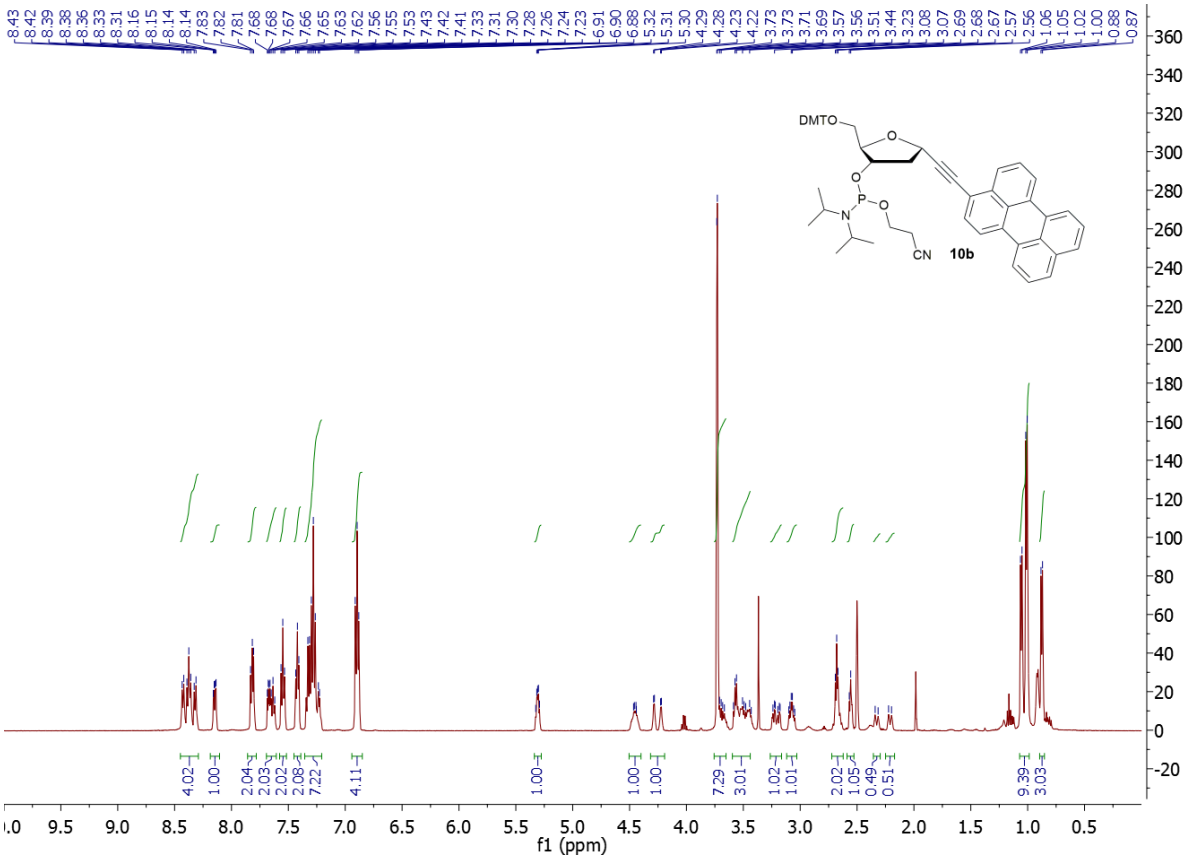


Fig. S26 ^{1}H NMR ([D_6]-DMSO, 500 MHz) of phosphoramidite **10b**.

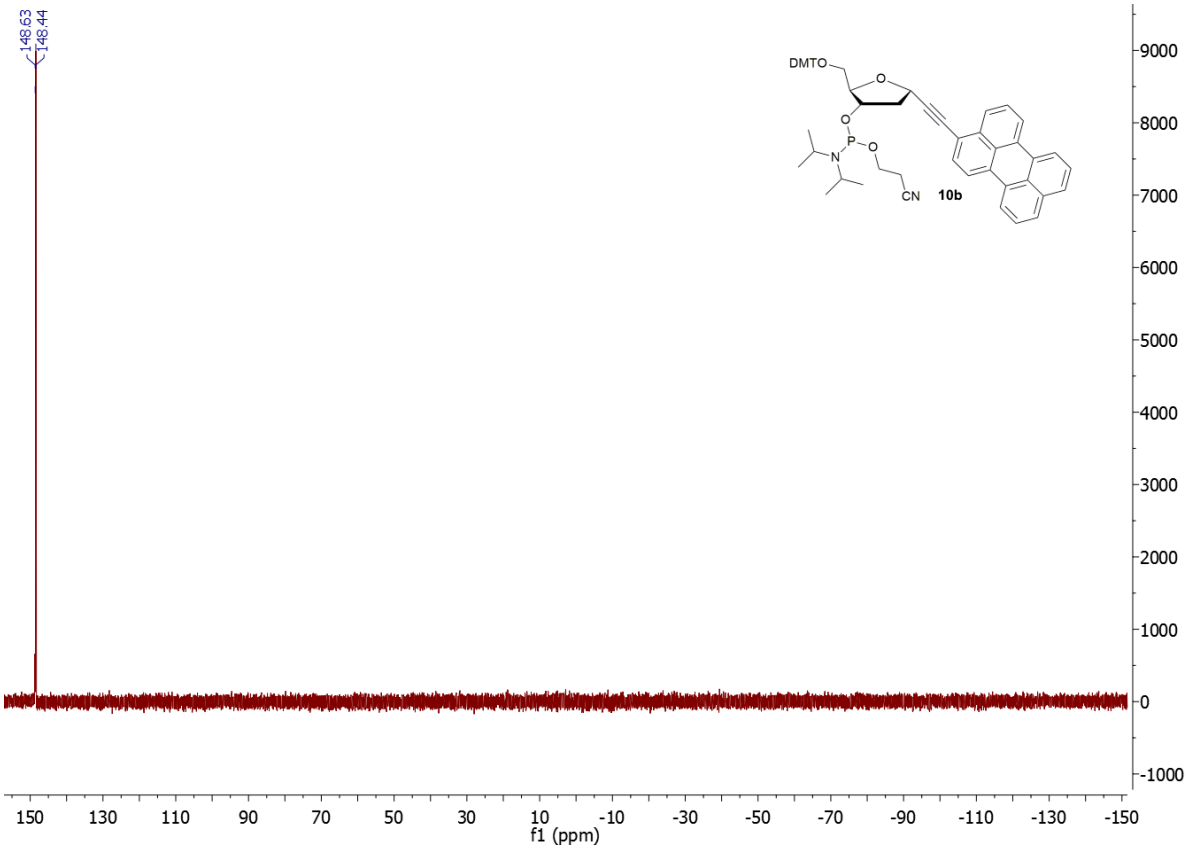


Fig. S27 ^{31}P NMR ([D_6]-DMSO, 162 MHz) of phosphoramidite **10b**.

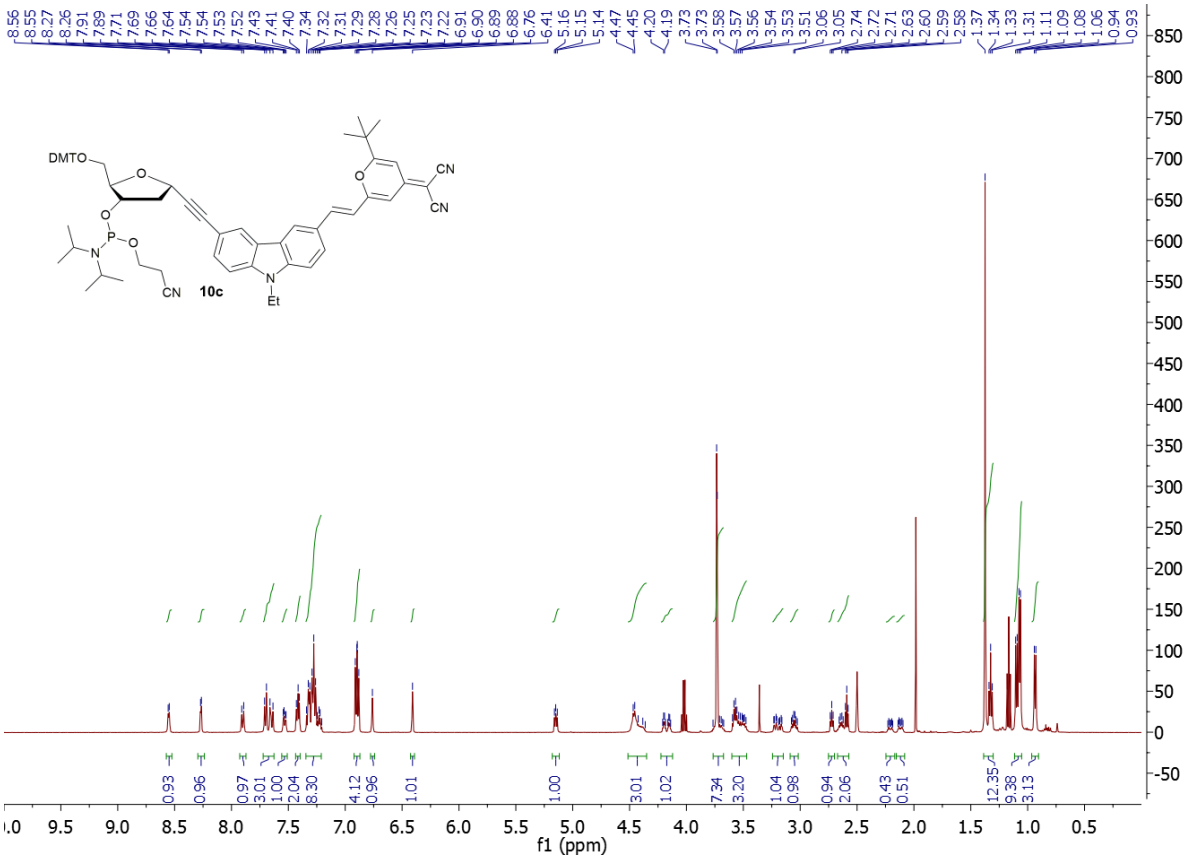


Fig. S28 ^{1}H NMR ([D_6]-DMSO, 500 MHz) of phosphoramidite **10c**.

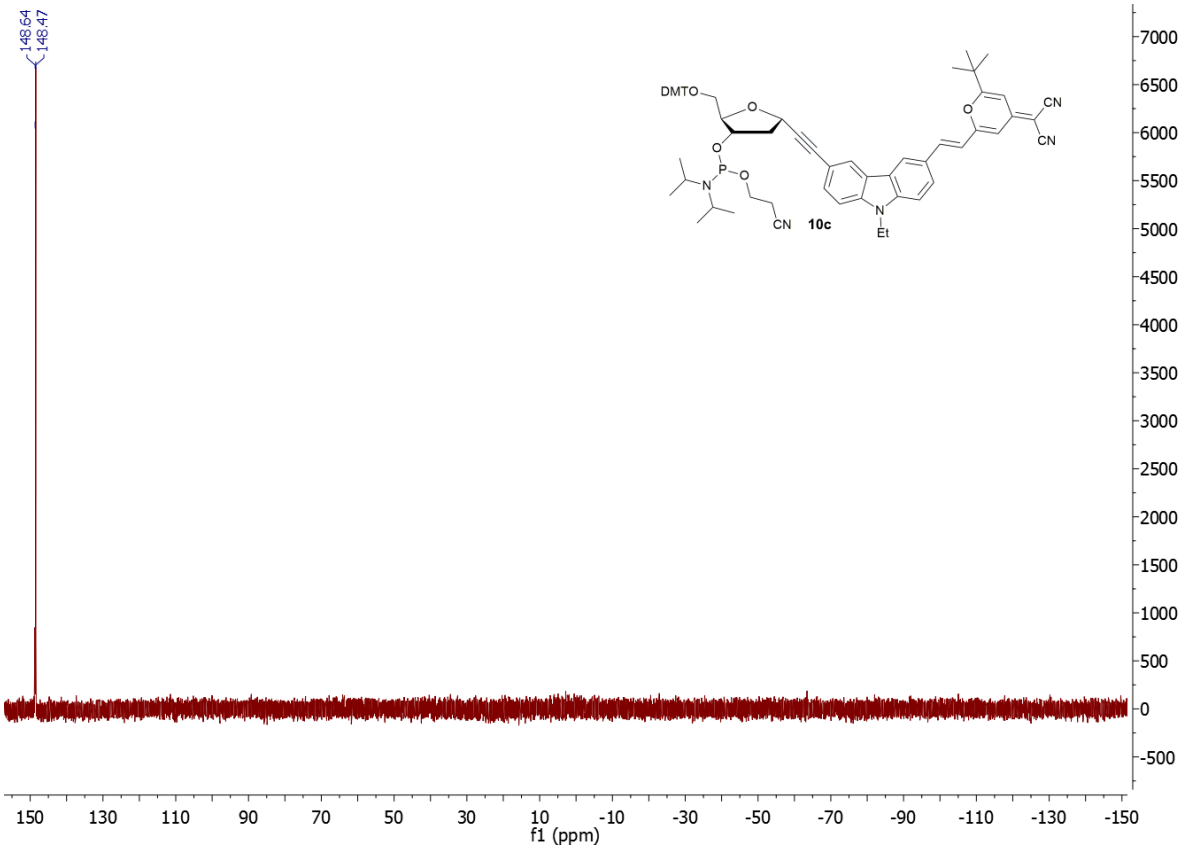


Fig. S29 ^{31}P NMR ([D_6]-DMSO, 162 MHz) of phosphoramidite **10c**.

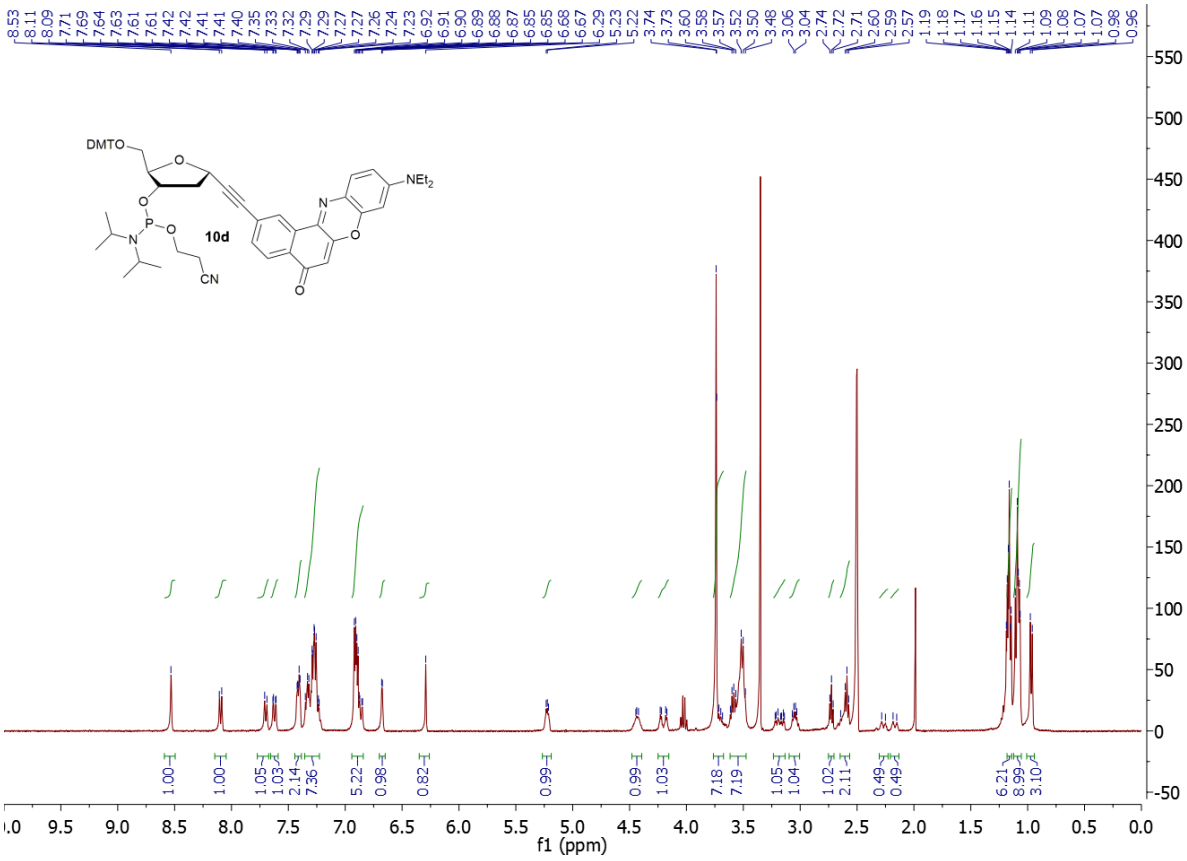


Fig. S30 ^{1}H NMR ([D_6]-DMSO, 400 MHz) of phosphoramidite **10d**.

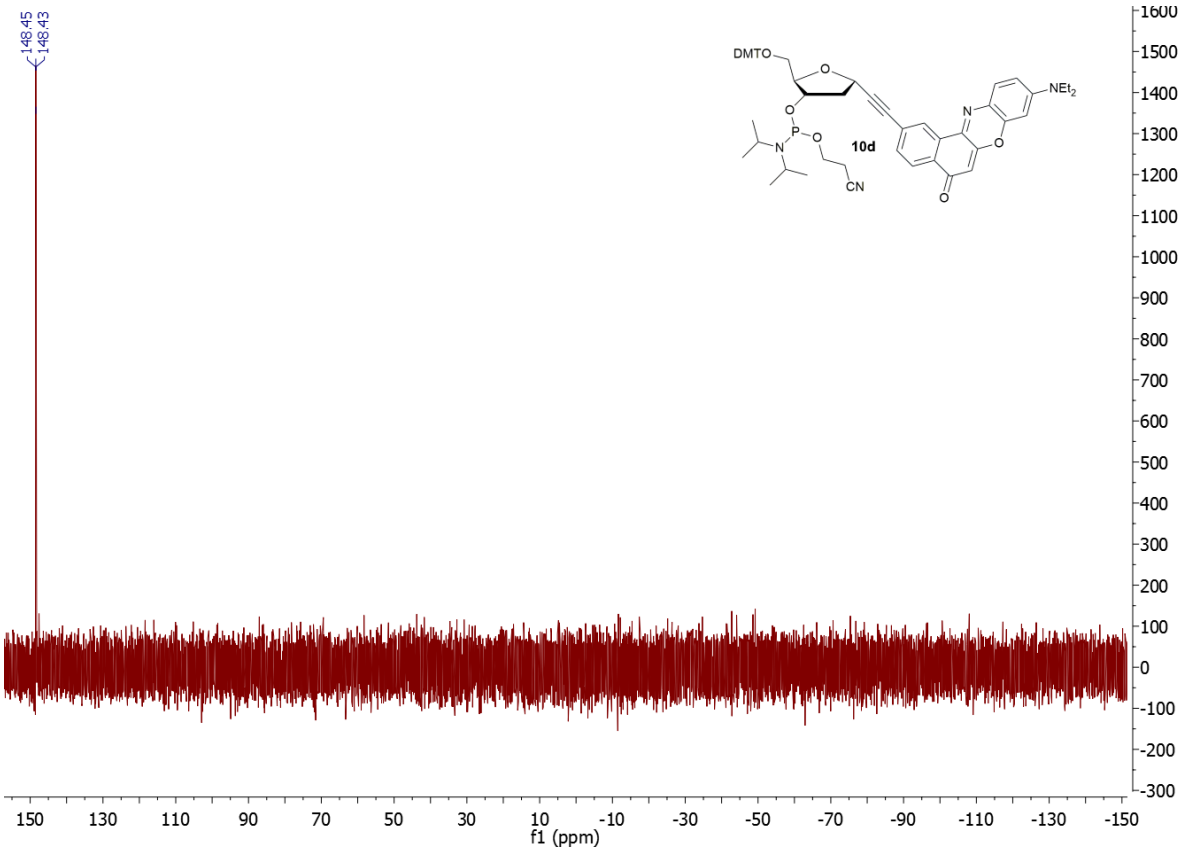


Fig. S31 ^{31}P NMR ([D_6]-DMSO, 162 MHz) of phosphoramidite **10d**.

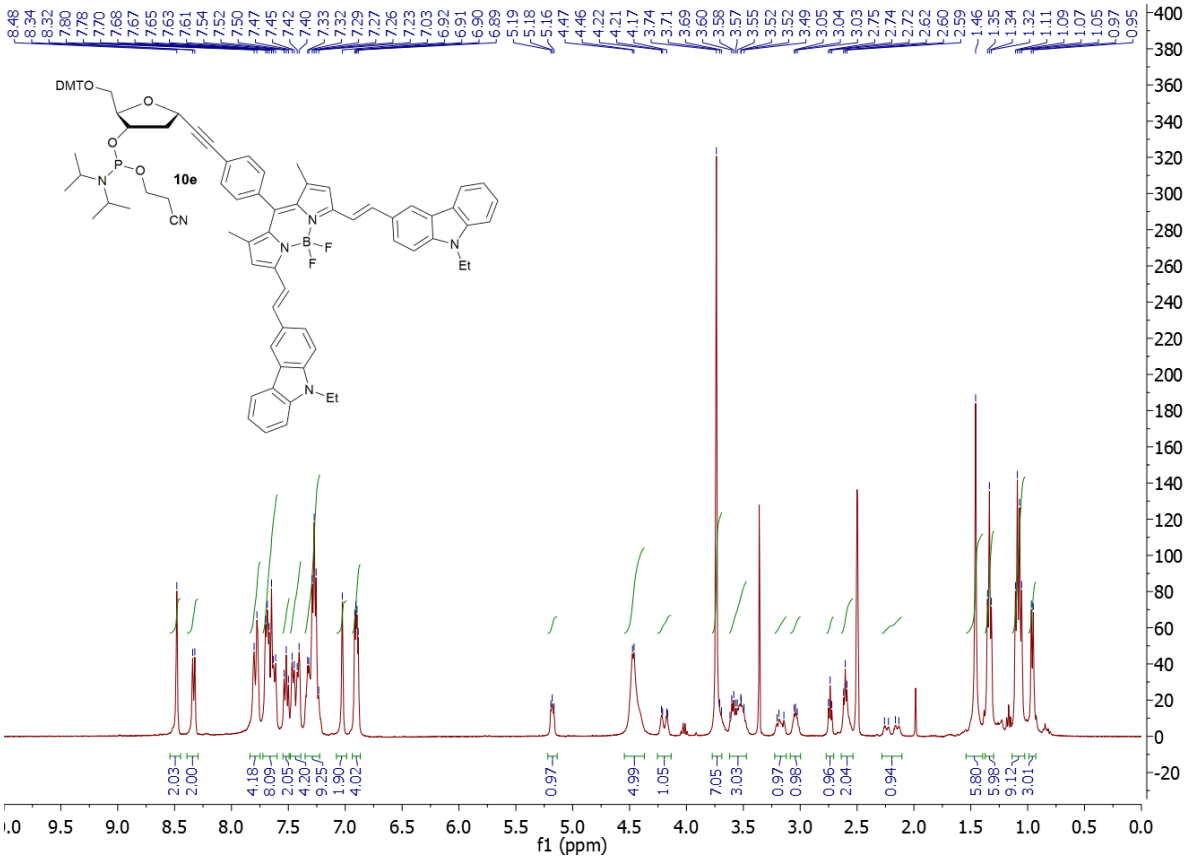


Fig. S32 ^{1}H NMR ([D_6]-DMSO, 400 MHz) of phosphoramidite **10e**.

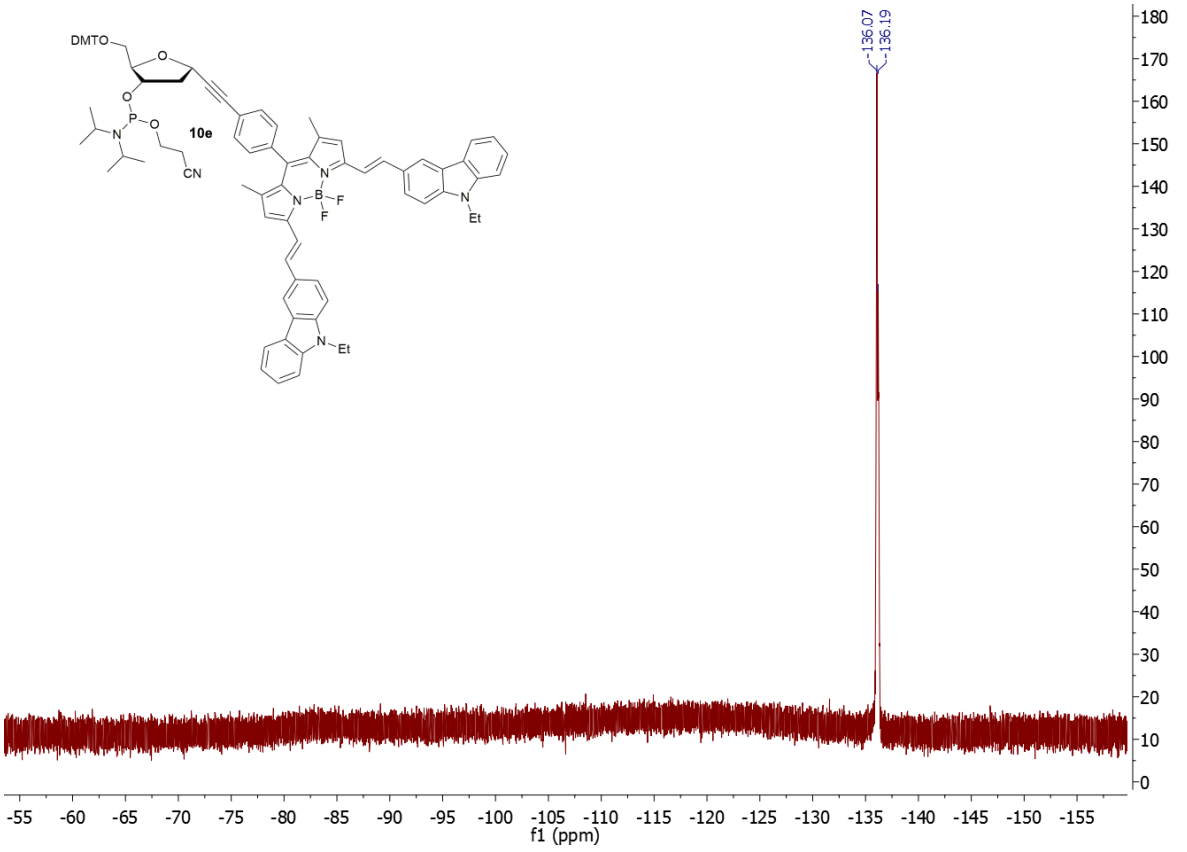


Fig. S33 ^{19}F NMR ([D_6]-DMSO, 377 MHz) of phosphoramidite **10e**.

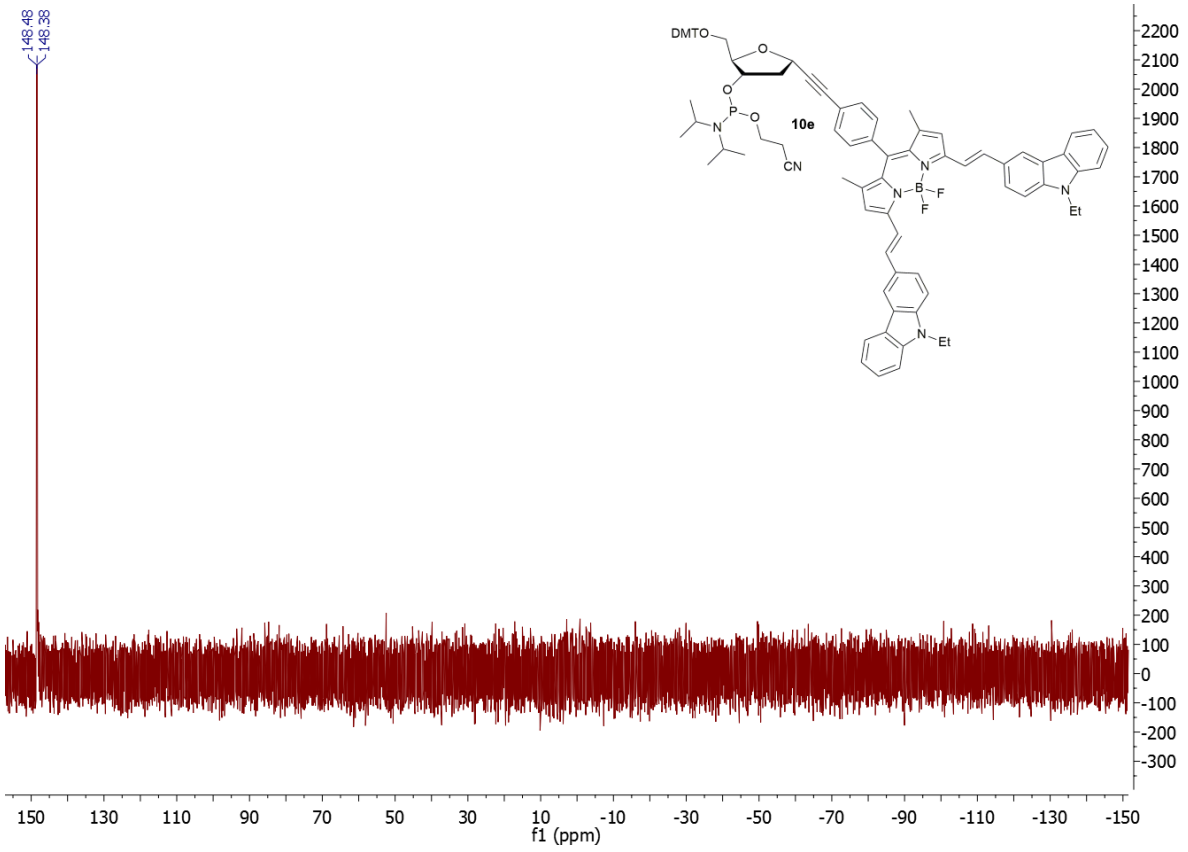


Fig. S34 ^{31}P NMR ([D$_6$]-DMSO, 162 MHz) of phosphoramidite **10e**.

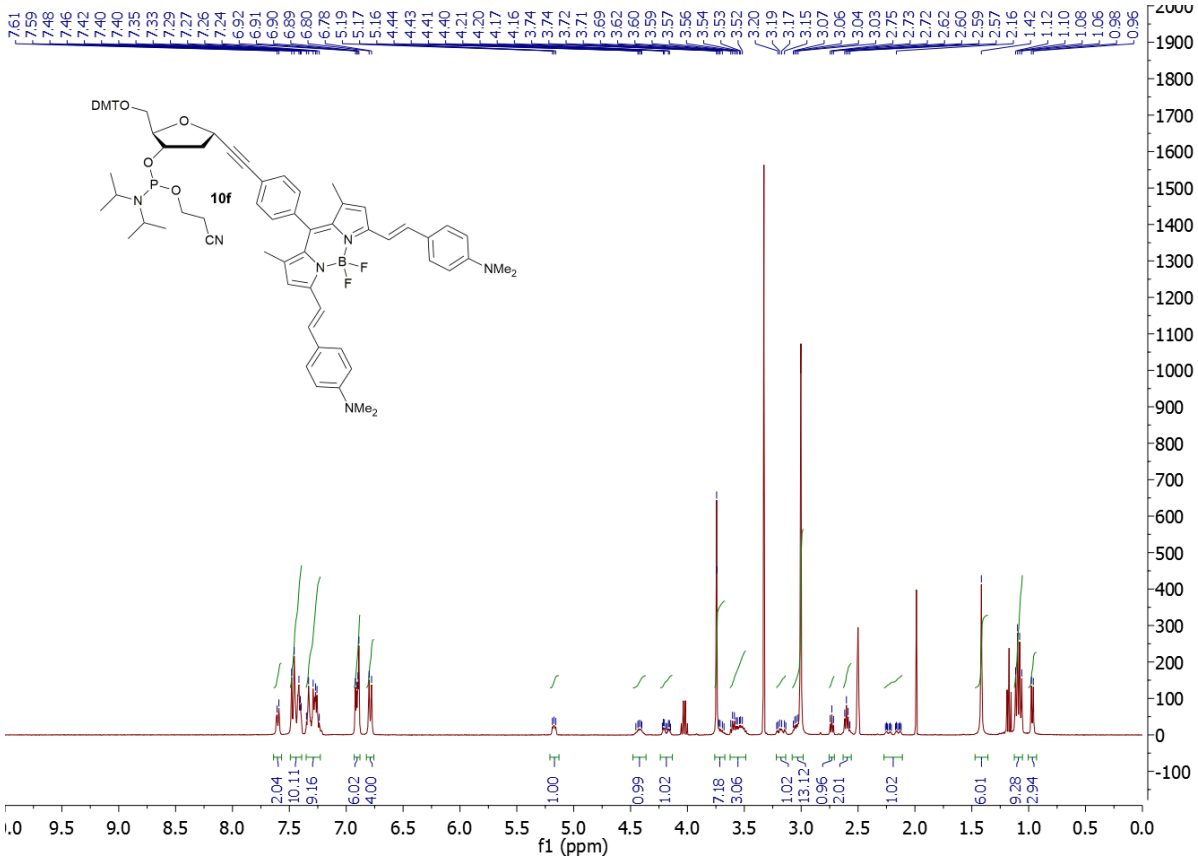


Fig. S35 ^{1}H NMR ([D$_6$]-DMSO, 400 MHz) of phosphoramidite **10f**.

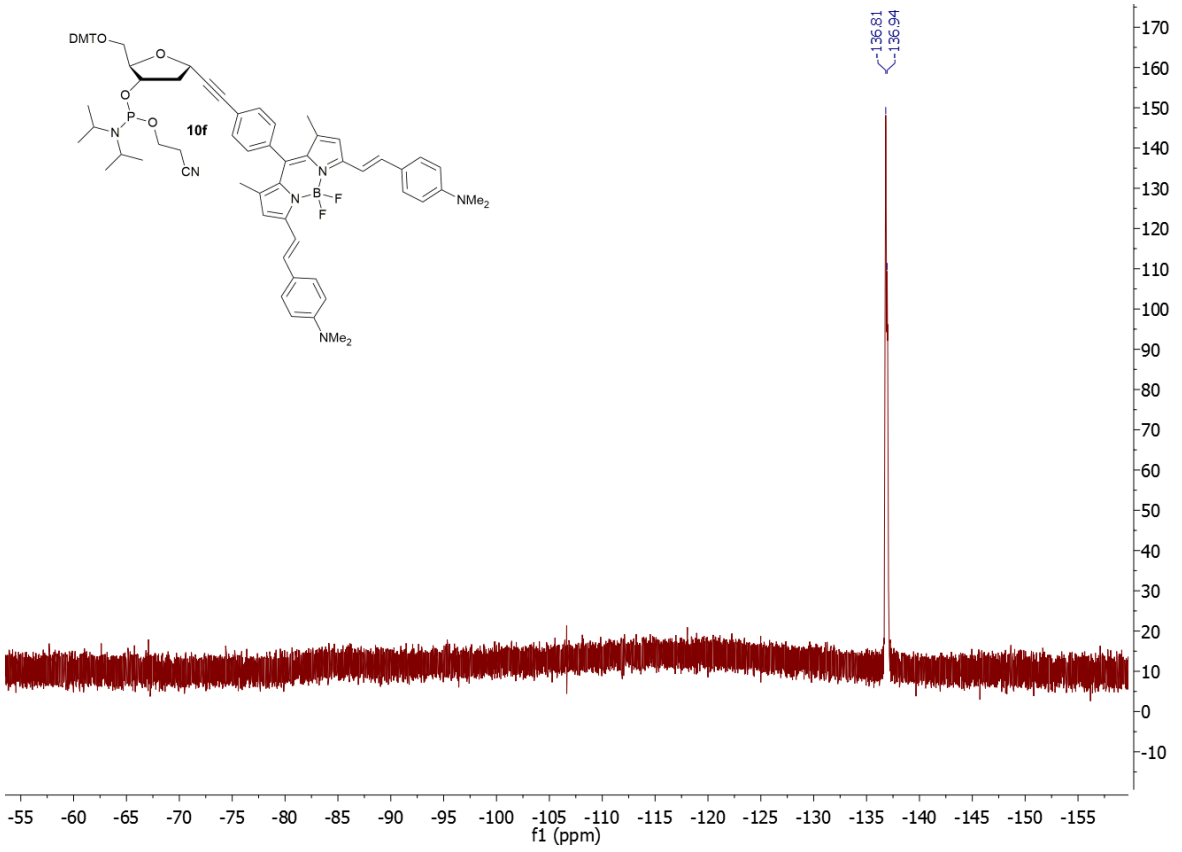


Fig. S36 ^{19}F NMR ([D_6]-DMSO, 377 MHz) of phosphoramidite **10f**.

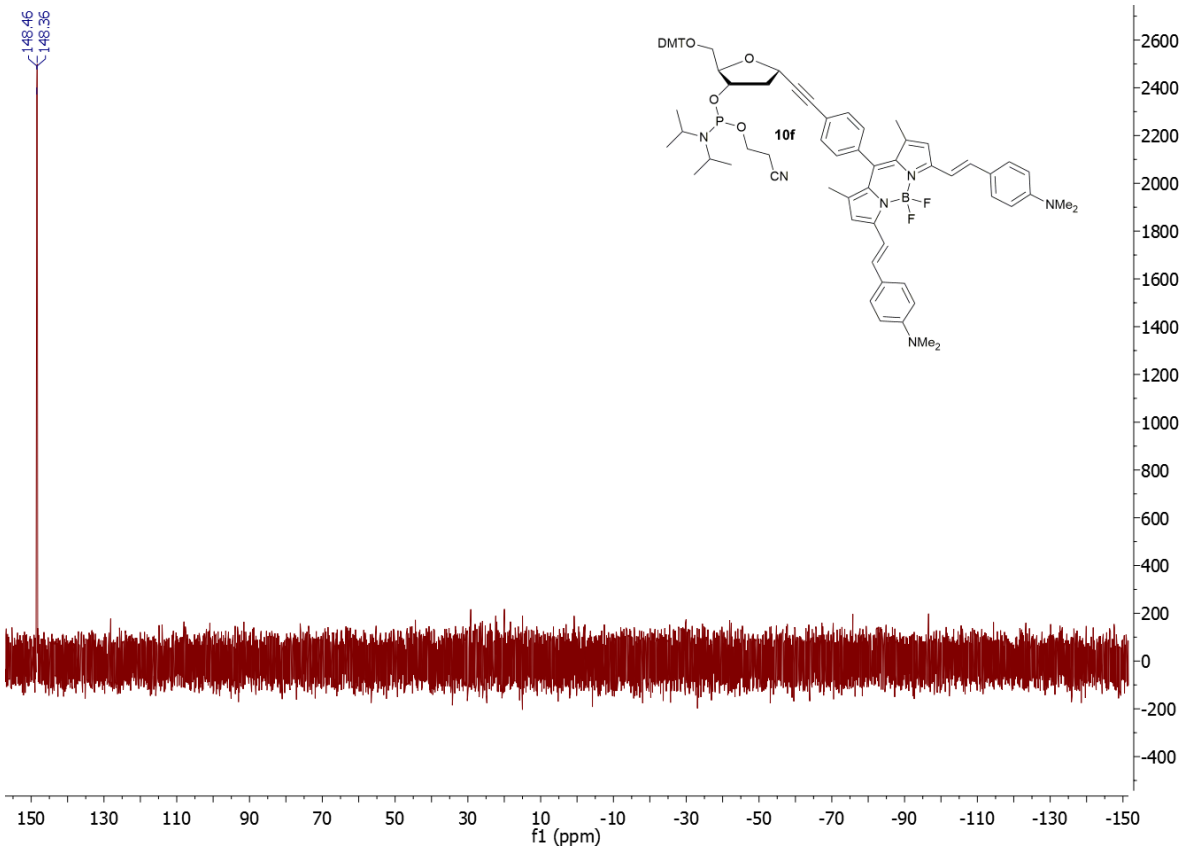


Fig. S37 ^{31}P NMR ([D_6]-DMSO, 162 MHz) of phosphoramidite **10f**.

Additional spectral data

Table S1 Spectral properties of tetrameric ODFs, which consist of three abasic spacers and one dye. All spectra were measured in PBS

Sequence	$\lambda_{max,abs}$ [a] [nm]	$\lambda_{max,em}$ [b] [nm]	Φ_F
YSSS	361	383	0.81 ± 0.03
ESSS	455	464	0.68 ± 0.04
KSSS	431	580	0.0155 ± 0.0009
NSSS	580	663	0.047 ± 0.004
CSSS	687	-	-
DSSS	678	-	-

[a] Absorption maxima have a ±1 nm imprecision. [b] Fluorescence maxima are reproducible within a ±2 nm range.

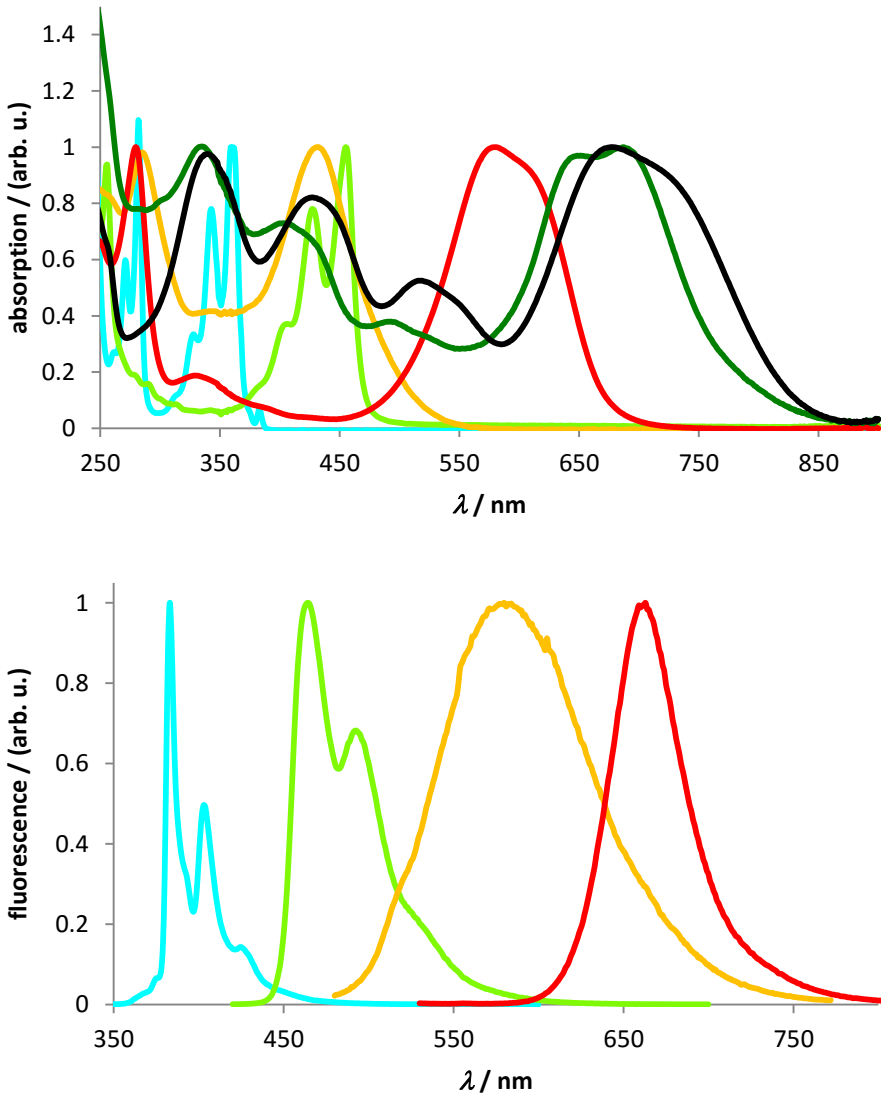


Fig. S38 Normalized absorption and fluorescence spectra of tetrameric ODFs, which consist of three abasic spacers and one dye. The spectra were recorded in PBS. Blue: YSSS; light green: ESSS; orange: KSSS; red: NSSS; dark green: CSSS; black: DSSS.

References

1. A. M. Brouwer, *Pure Appl. Chem.*, 2011, **83**, 2213-2228.
2. A. Loudet, R. Bandichhor, L. Wu and K. Burgess, *Tetrahedron*, 2008, **64**, 3642-3654.
3. M. Quintiliani, J. Balzarini and C. McGuigan, *Tetrahedron*, 2013, **69**, 9111-9119.
4. O. R. Ludek and V. E. Marquez, *Tetrahedron*, 2009, **65**, 8461-8467.
5. M. F. A. Adamo and R. Pergoli, *Org. Lett.*, 2007, **9**, 4443-4446.
6. M. F. A. Adamo, R. Pergoli and M. Moccia, *Tetrahedron*, 2010, **66**, 9242-9251.
7. M. Nakahara, T. Kuboyama, A. Izawa, Y. Hari, T. Imanishi and S. Obika, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 3316-3319.
8. A. R. Karikachery, H. B. Lee, M. Masjedi, A. Ross, M. A. Moody, X. Cai, M. Chui, C. D. Hoff and P. R. Sharp, *Inorg. Chem.*, 2013, **52**, 4113-4119.
9. Y. J. Chang and T. J. Chow, *J. Mater. Chem.*, 2011, **21**, 3091-3099.
10. J. Han, J. Jose, E. Mei and K. Burgess, *Angew. Chem. Int. Ed.*, 2007, **46**, 1684-1687.
11. S. Telitel, N. Blanchard, S. Schweizer, F. Morlet-Savary, B. Graff, J.-P. Fouassier and J. Lalevée, *Polymer*, 2013, **54**, 2071-2076.
12. R. Ziessel, G. Ulrich, A. Harriman, M. A. H. Alamiry, B. Stewart and P. Retailleau, *Chem. Eur. J.*, 2009, **15**, 1359-1369.
13. J. M. Gao, S. Watanabe and E. T. Kool, *J. Am. Chem. Soc.*, 2004, **126**, 12748-12749.
14. H. P. Nestler, P. A. Bartlett and W. C. Still, *J. Org. Chem.*, 1994, **59**, 4723-4724.
15. H. E. Blackwell, L. Perez, R. A. Stavenger, J. A. Tallarico, E. C. Eatough, M. A. Foley and S. L. Schreiber, *Chem. Biol.*, 2001, **8**, 1167-1182.