SUPPORTING INFORMATION

Dye-conjugated Complementary Lipophilic Nucleosides as Useful Probes to Study Association Processes by Fluorescence Resonance Energy Transfer

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SUPPORTING INFORMATION

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S0A. Experimental Section

General Methods

Column chromatography was carried out on silica gel Merck-60 (230-400 mesh, 60 Å), and TLC on aluminium sheets precoated with silica gel 60 F254 (Merck). **NMR** spectra were recorded with a BRUKER AVANCE-II (300 MHz) instrument and BRUKER DRX 500 MHz. The temperature was actively controlled at 298 K. Chemical shifts are measured in ppm using the signals of the deuterated solvent as the internal standard [$CDCl_3$ calibrated at 7.26 ppm (^1H) and 77.0 ppm (^{13}C), DMSO-d_6 calibrated at 2.50 ppm (^1H) and 39.5 ppm (^{13}C) and DMF-d_7 calibrated at 8.03 ppm (^1H) and 163.2 ppm (^{13}C). **FAB MS** spectra were recorded on a VG AutoSpec instrument, **MALDI-TOF MS/HRMS** on a Bruker Reflex III spectrometer and **ESI-MS** spectra were obtained from an Applied Biosystems QSTAR equipment. **UV-Visible** experiments were conducted using a JASCO V-660 apparatus at room temperature. **Emission** spectra were recorded in a JASCO FP-8600 equipment. **Fluorescence quantum yields** were obtained following the comparative method of Williams *et al*.[1] at room temperature. A solution of anthracene in deoxygenated cyclohexane was used as standard[2] in the case of donor benzodithiophene species, as their absorption and emission range are similar to each other. Donor species were dissolved in deoxygenated toluene. The emission spectra of the corresponding compounds were measured using an excitation wavelength of 350 nm. On the other hand, for acceptor BODIPY species a solution of cresyl violet perchlorate in deoxygenated ethanol was used as standard.[2] Acceptor species were dissolved in deoxygenated toluene. The emission spectra of the corresponding compounds were measured using an excitation wavelength of 540 nm. **Electrochemical measurements** were carried out with an AUTOLAB electrochemistry system in a three-electrode cell under N_2 atmosphere in anhydrous deoxygenated CH_2Cl_2 containing 0.1 M tetrabutylammonium hexafluorophosphate ($TBAPF_6$) as the supporting electrolyte at room temperature. Cyclic and square wave voltammetry (CV and SWV, respectively) studies were carried out using a scan rate of 100 mVs^{-1}. Polycrystalline Pt was used as working electrode, the counter electrode was a Pt gauze, and the reference electrode was a silver wire quasi-reference electrode. Ferrocene (Fc) was used as internal standard, and all potentials in this work are referenced to the ferrocene couple (Fc^+/Fc).

Starting materials. Chemicals were purchased from commercial suppliers and used without further purification. Hygroscopic reagents were dried in a vacuum oven before use. Reaction solvents were thoroughly dried before use using standard methods. The synthesis and characterization of 5- (pyrimidines) or 8- (purines) ethynyl-substituted nucleosides equipped with lipophilic riboses (**G1**, **C1**, **AA1** and **U1**), and the mononucleosidic compounds **G**, **C**, **AA** and **U**, have been previously reported by us.[3] 2,6-dibromobenzo[1,2-b:4,5-b']dithiophene (CAS number: 909280-97-3) was purchased from TCI Europe N.V. The synthesis of the diiodinated BODIPY **I-a-I** was carried out as described in literature procedures.[4] The synthesis of the rest of the compounds reported in this manuscript is detailed below.

[1] A. T. R. Williams, S. A. Winfield and J. N. Miller, *Analyst*, 1983, **108**, 1067.

[2] http://omlc.org/spectra/PhotochemCAD/index.html

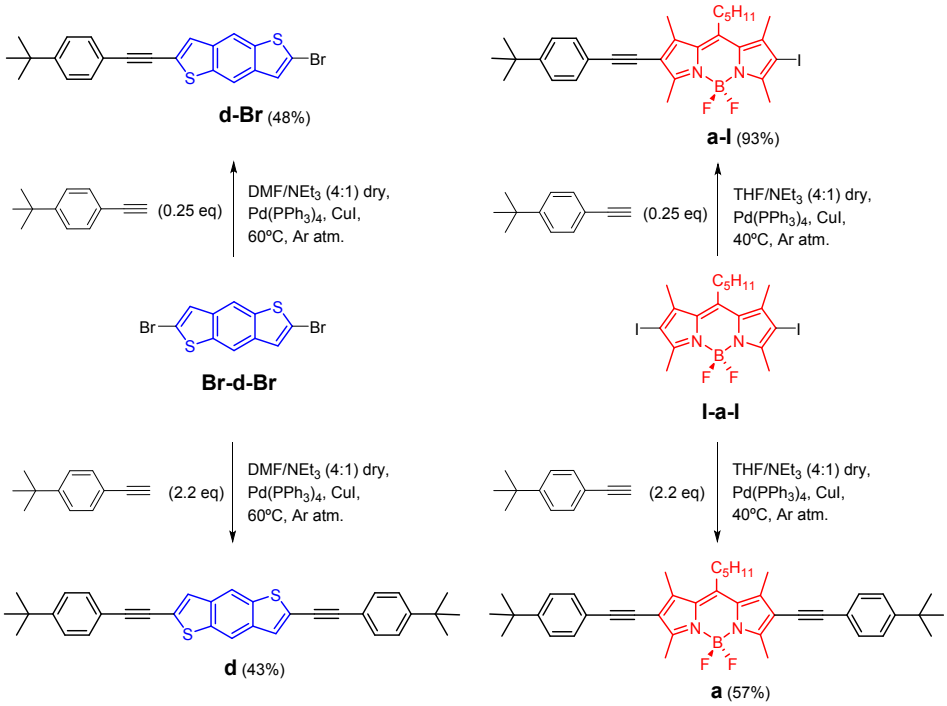
[3] J. Camacho-García, C. Montoro-García, A. M. López-Pérez, N. Bilbao, S. Romero-Pérez and D. González-Rodríguez, *Org. Biomol. Chem.*, 2015, **13**, 4506.

[4] (a) M. Mao, J.-B. Wang, Z.-F. Xiao, S.-Y. Dai and Q.-H. Song, *Dyes Pigments*, 2012, **94**, 224; (b) L. Wang, J.-W. Wang, A.-j. Cui, X.-X. Cai, Y. Wan, Q. Chen, M.-Y.He and W. Zhang, *RSC Adv.*, 2013, **3**, 9219.

Synthesis

General procedure for the Sonogashira coupling reaction

A dry THF/NEt_3 or DMF/NEt_3 (4:1) mixture (5 mL) was subjected to deoxygenation by three freeze-pump-thaw cycles with argon. It was then poured over a round-bottom flask containing the corresponding amount of the compound bearing the ethynyl group, the right proportion of halogenated species, $Pd(PPh_3)_4$ (0.02 eq., 0.0025 g) and CuI (0.01 eq., 0.0002 g). The resulting mixture was stirred for 12 hours under argon atmosphere at the corresponding temperature for each case. Once completed, the mixture was filtrated over a celite plug and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography using the respective eluent to give the desired products. The slight modifications of this procedure are remarked in each case.



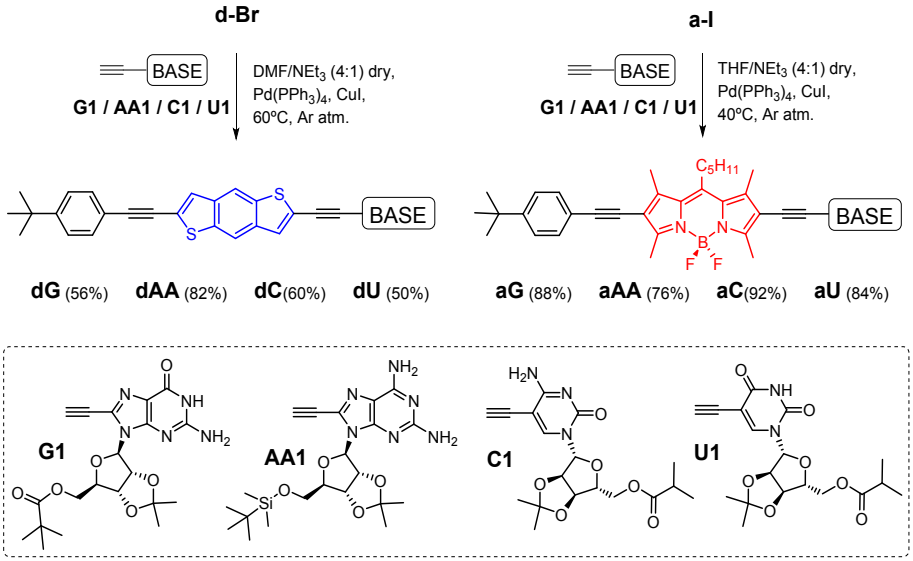
Scheme S1. Synthesis of compounds **d**, **a**, **d-Br** and **d-I** from dibromobithiophene **Br-d-Br** and diiodoBOPIPY **I-a-I**.

d-Br. This compound was prepared from 2,6-dibromobenzo[1,2-b:4,5-b']dithiophene (3 eq., 0.114 g), 1-(*tert*-butyl)-4-ethynylbenzene (1 eq., 0.015 mL), and a dry DMF/NEt_3 (4:1) mixture as solvent. The reaction mixture was stirred at 60ºC overnight. The crude was purified using cyclohexane/toluene (10:1) as eluent. **d-Br** was obtained as a white solid in 48% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.08 (s, 1H), 8.07 (s, 1H), 7.49 (d, *J* = 8.7 Hz, 3H), 7.39 (d, *J* = 8.2 Hz, 2H), 7.34 (s, 1H), 1.34 (s, 9H). HRMS (MALDI): Calculated for $C_{22}H_{17}BrS_2$: 423.9955 [M]. Found: 423.9950 [M].

d. This compound was prepared from 2,6-dibromobenzo[1,2-b:4,5-b']dithiophene (1 eq., 0.114 g), 1-(*tert*-butyl)-4-ethynylbenzene (2.2 eq., 0.13 mL), and a dry DMF/NEt_3 (4:1) mixture as solvent. The reaction mixture was stirred at 60ºC overnight. The crude was purified using cyclohexane/toluene (10:1) as eluent. **d** was obtained as a yellow solid in 43% yield. ^{1}H NMR (300 MHz, $C_2D_2Cl_4$) δ (ppm) 8.18 (s, 2H), 7.53 (d, *J* = 9.0 Hz, 4H), 7.42 (d, *J* = 8.4 Hz, 4H), 7.29 (s, 2H), 1.34 (s, 18H). HRMS (MALDI): Calculated for $C_{34}H_{30}S_2$: 502.1789 $[M]^+$. Found: 502.1783 $[M]^+$.

a-I. This compound was prepared from 4,4'-difluoro-1,3,5,7-tetrametil-8-pentil-4-bora-3a,4a-diaza-*s*-indacene (3 eq., 0.187 g), 1-(*tert*-butyl)-4-ethynylbenzene (1 eq., 0.015 mL), and a dry THF/NEt_3 (4:1) mixture as solvent. The reaction mixture was stirred at 40ºC overnight. The crude was purified using chloroform/methanol (100:1) as eluent. **a-I** was obtained as a purple solid in 93% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.46 (d, *J* = 8.2 Hz, 2H), 7.38 (d, *J* = 8.3 Hz, 2H), 3.09 – 2.95 (m, 2H), 2.67 (s, 3H), 2.63 (s, 3H), 2.57 (s, 3H), 2.49 (s, 3H), 1.73 – 1.58 (m, 2H), 1.58 – 1.37 (m, 5H), 1.34 (s, 9H), 0.95 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (76 MHz, $CDCl_3$) δ (ppm) 157.3, 154.9, 151.7, 147.0, 141.7, 141.6, 132.0, 131.2, 131.0, 125.5, 120.4, 116.9, 96.8, 86.1, 81.0, 34.9, 32.6, 31.6, 31.3, 29.2, 22.6, 18.9, 16.2, 15.4, 14.1, 13.8. HRMS (MALDI): Calculated for $C_{30}H_{36}BF_2IN_2$: 600.1984 $[M]^+$. Found: 600.1992 $[M]^+$.

a. This compound was prepared from 4,4'-difluoro-1,3,5,7-tetrametil-8-pentil-4-bora-3a,4a-diaza-*s*-indacene (1 eq., 0.187 g), 1-(*tert*-butyl)-4-ethynylbenzene (2.2 eq., 0.13 mL), and a dry THF/NEt_3 (4:1) mixture as solvent. The reaction mixture was stirred at 40ºC overnight. The crude was purified using chloroform/methanol (20:1) as eluent. **a** was obtained as a purple solid in 57% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.46 (d, *J* = 8.5 Hz, 4H), 7.38 (d, *J* = 8.6 Hz, 4H), 3.09 – 2.97 (m, 2H), 2.68 (s, 6H), 2.58 (s, 6H), 1.42-1.25 (m, 18H), 0.95 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (76 MHz, $CDCl_3$) δ (ppm) 156.8, 151.6, 141.0, 132.3, 131.4, 131.2, 125.6, 125.5, 120.5, 96.6, 81.2, 77.5, 34.9, 32.6, 31.7, 31.3, 31.2, 22.6, 15.2, 14.2, 13.7. HRMS (FAB+): Calculated for $C_{42}H_{49}BF_2N_2$: 630.3957 $[M]^+$. Found: 630.3994 $[M]^+$.



Scheme S2. Synthesis of mononucleosides **dG**, **dAA**, **dC**, **dU**, **aG**, **aAA**, **aC** and **aU** *via* Sonogashira coupling reaction between **d-Br** or **a-I** and ethynyl-nucleobases **G1**, **AA1**, **C1** and **U1**.

dG. This compound was prepared from **d-Br** (1 eq., 0.045 g), **G1** (1.1 eq., 0.052 g), and a dry DMF/NEt_3 (4:1) mixture as solvent. The reaction mixture was stirred at 60ºC overnight. The crude was purified using chloroform/methanol (40:1) as eluent. **dG** was obtained as a yellow solid in 56% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.15 (s, 1H), 8.10 (s, 1H), 7.68 (s, 1H), 7.54 – 7.34 (m, 5H), 6.53 (s, 1H), 6.27 (s, 1H), 5.46 (s, 1H), 5.07 (m, 1H), 4.80 (m, 1H), 4.41 (m, 1H), 4.11 (m, 1H), 3.65 (s, 1H), 1.42 (s, 3H), 1.34 (s, 9H), 1.26 (s, 3H), 1.18 (s, 9H). ^{13}C NMR (76 MHz, DMSO-d_6) δ (ppm) 177.1, 154.3, 152.4, 150.4, 138.1, 137.4, 137.3, 137.2, 131.2, 130.8, 128.5, 125.7, 123.7, 121.6, 120.4, 117.9, 113.4, 96.0, 85.4, 83.4, 81.4, 64.1, 38.1, 34.7, 30.8, 27.0, 26.7, 25.3. HRMS (MALDI): Calculated for $C_{42}H_{41}N_5O_6S_2$: 775.2498 $[M]^+$. Found: 798.2396 $[M+Na]^+$.

dAA. This compound was prepared from **d-Br** (1 eq., 0.045 g), **AA1** (1.1 eq., 0.056 g), and a dry DMF/NEt_3 (4:1) mixture as solvent. The reaction mixture was stirred at 60ºC overnight. The crude was purified using chloroform/methanol (40:1) as eluent. **dAA** was obtained as a yellow solid in 82% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.21 (s, 1H), 8.17 (s, 1H), 7.70 (s, 1H), 7.52 (d, *J* = 8.2 Hz, 3H), 7.42 (d, *J* = 8.4 Hz, 2H), 6.29 (d, *J* = 2.1 Hz, 1H), 5.68 (dd, *J* = 6.4, 2.3 Hz, 1H), 5.51 (s, 2H), 5.11 (dd, *J* = 6.5, 3.4 Hz, 1H), 4.79 (s, 2H), 4.38-4.24 (m, 1H), 3.82 (m, 2H), 1.67 (s, 3H), 1.45 (s, 3H), 1.36 (s, 9H), 0.87 (s, 9H), 0.02 (s, 3H), 0.00 (s, 3H). ^{13}C NMR (76 MHz, $CDCl_3$ and a drop of DMSO-d_6) δ (ppm) 160.2, 155.8, 152.0, 150.6, 138.0, 137.6, 137.6, 136.7, 131.0, 130.1, 129.8, 129.3, 127.1, 125.1, 124.6, 120.8, 118.7, 116.8, 116.1, 114.5, 113.6, 96.0, 89.4, 87.8, 87.3, 84.1, 82.7, 82.0, 82.0, 63.2, 62.4, 34.5, 30.8, 29.3, 27.0, 25.5, 25.3, 18.0. HRMS (MALDI): Calculated for $C_{43}H_{48}N_6O_4S_2Si$: 804.2948 $[M]^+$. Found: 827.2828 $[M+Na]^+$.

dC. This compound was prepared from **d-Br** (1 eq., 0.045 g), **C1** (1.1 eq., 0.031 g), and a dry DMF/NEt_3 (4:1) mixture as solvent. The reaction mixture was stirred at 60ºC overnight. The crude was purified using chloroform/methanol (40:1) as eluent. **dC** was obtained as a yellow solid in a 60% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.16 (s, 1H), 8.15 (s, 1H), 7.83 (s, 1H), 7.50 (d, *J*= 6.6 Hz, 4H), 7.40 (d, *J* = 8.3 Hz, 2H), 5.86 (s, 1H), 5.77 (s, 1H), 4.98-4.96 (m, 1H), 4.82 – 4.79 (m, 1H), 4.44-4.36 (m, 3H), 2.61 (hept, *J*= 7.1 Hz, 1H), 1.58 (s, 3H), 1.36 (s, 3H), 1.34 (s, 9H), 1.18 (dd, *J* = 6.9, 1.2 Hz, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 175.8, 163.8, 152.9, 152.3, 147.6, 137.5, 137.4, 137.2, 137.0, 131.2, 128.8, 128.5, 125.7, 123.1, 123.0, 117.2, 117.2, 113.0, 95.8, 93.4, 89.3, 87.6, 84.6, 82.3, 80.6, 63.8, 56.0, 34.6, 33.1, 30.8, 26.9, 25.1, 18.8, 18.7, 18.5. HRMS (MALDI): Calculated for $C_{40}H_{39}N_3O_6S_2$: 721.2280 $[M]^+$. Found: 744.2172 $[M+Na]^+$.

dU. This compound was prepared from **d-Br** (1 eq., 0.045 g), **U1** (1.1 eq., 0.044 g), and a dry DMF/NEt_3 (4:1) mixture as solvent. The reaction mixture was stirred at 60ºC overnight. The crude was purified using chloroform/methanol (40:1) as eluent. **dU** was obtained as a yellow solid in a 50% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.44 (s, 1H), 8.12 (d, *J* = 4.4 Hz, 2H), 7.74 (s, 1H), 7.55 – 7.46 (m, 4H), 7.39 (d, *J* = 8.5 Hz, 2H), 5.85 (d, *J* = 2.3 Hz, 1H), 4.91 (dd, *J* = 6.4, 2.3 Hz, 1H), 4.79 (dd, *J* = 6.4, 3.7 Hz, 1H), 4.43 (m, 1H), 4.36 (d, *J* = 4.0 Hz, 2H), 2.65 (hept, *J* = 7.0 Hz, 1H), 1.60 (s, 3H), 1.38 (s, 3H), 1.34 (s, 9H), 1.20 (dd, *J* = 7.0, 3.3 Hz, 6H). ^{13}C NMR (76 MHz, $CDCl_3$) δ (ppm) 176.6, 160.4, 152.5, 148.7, 143.7, 138.2, 138.1, 138.0, 137.5, 131.5, 129.0, 127.5, 125.6, 125.0, 122.9, 119.5, 116.8, 116.5, 115.1, 100.3, 96.3, 93.7, 87.9, 85.8, 85.0, 82.5, 80.7, 63.7, 35.0, 34.1, 31.3, 27.3, 27.0, 25.4, 19.2, 19.1. HRMS (MALDI): Calculated for $C_{40}H_{38}N_2O_7S_2$: 722.2120 $[M]^+$. Found: 722.2116 $[M]^+$.

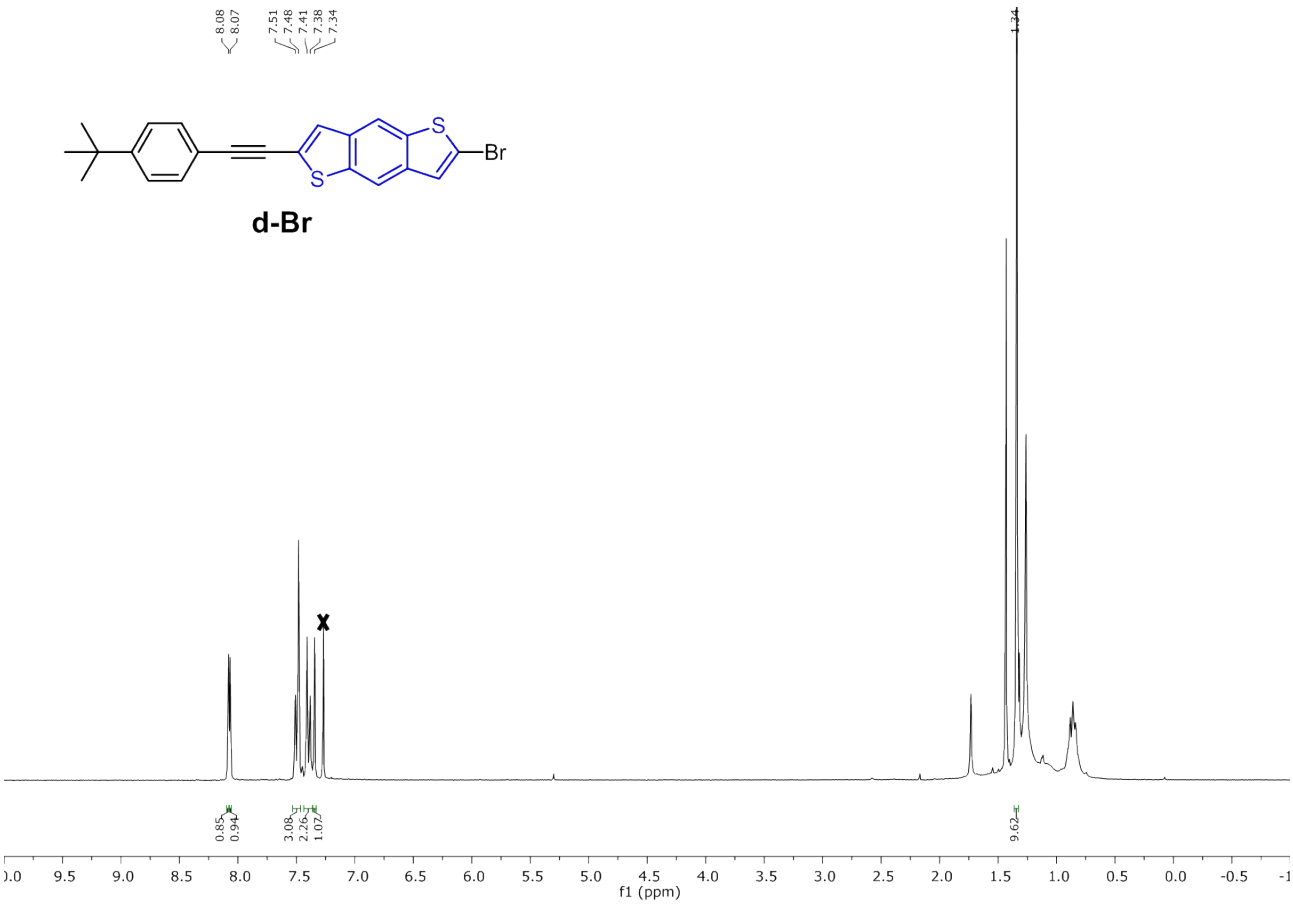
aG. This compound was prepared from **a-I** (1 eq., 0.066 g), **G1** (1.1 eq., 0.052 g), and a dry THF/NEt_3 (4:1) mixture as solvent. The reaction mixture was stirred at 40ºC overnight. The crude was purified using chloroform/methanol (40:1) as eluent. **aG** was obtained as a purple solid in 88% yield. ^{1}H NMR (300 MHz, $CDCl_3$ and a drop of DMSO-d_6) δ (ppm) 7.51 – 7.35 (m, 4H), 6.31 (s, 1H), 6.12 (s, 2H), 5.55 – 5.39 (m, 1H), 5.12 – 4.97 (m, 1H), 4.97 – 4.83 (m, 1H), 4.43-4.38 (m, 1H), 4.06 – 3.94 (m, 1H), 3.73 – 3.58 (m, 1H), 3.11 – 2.96 (m, 2H), 2.70 (s, 6H), 2.59 (s, 6H), 1.56 (s, 3H), 1.43 (s, 3H), 1.39-1.12 (m, 24H), 0.96 (t, *J* = 7.2 Hz, 3H). HRMS (MALDI): Calculated for $C_{50}H_{60}BF_2N_7O_6$: 903.4666 $[M]^+$. Found: 926.4585 $[M+Na]^+$.

aAA. This compound was prepared from **a-I** (1 eq., 0.066 g), **AA1** (1.1 eq., 0.056 g), and a dry THF/NEt_3 (4:1) mixture as solvent. The reaction mixture was stirred at 40ºC overnight. The crude was purified using chloroform/methanol (40:1) as eluent. **aAA** was obtained as a purple solid in 76% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.57 – 7.35 (m, 4H), 6.31 (s, 1H), 5.79 (d, *J* = 4.7 Hz, 1H), 5.53 – 5.33 (m, 2H), 5.10 (d, *J* = 9.0 Hz, 1H), 4.75 (s, 2H), 4.37 – 4.22 (m, 1H), 4.01 (d, *J* = 7.3 Hz, 1H), 3.88 – 3.63 (m, 2H), 3.08 (t, *J* = 10.5 Hz, 2H), 2.72 (s, 6H), 2.63 (s, 6H), 1.62 (s, 3H), 1.44 (s, 3H), 1.37– 0.84 (m, 18H), -0.01 (s, 6H). HRMS (MALDI): Calculated for $C_{51}H_{67}BF_2N_8O_4Si$: 932.5116 $[M]^+$. Found: 955.5021 $[M+Na]^+$.

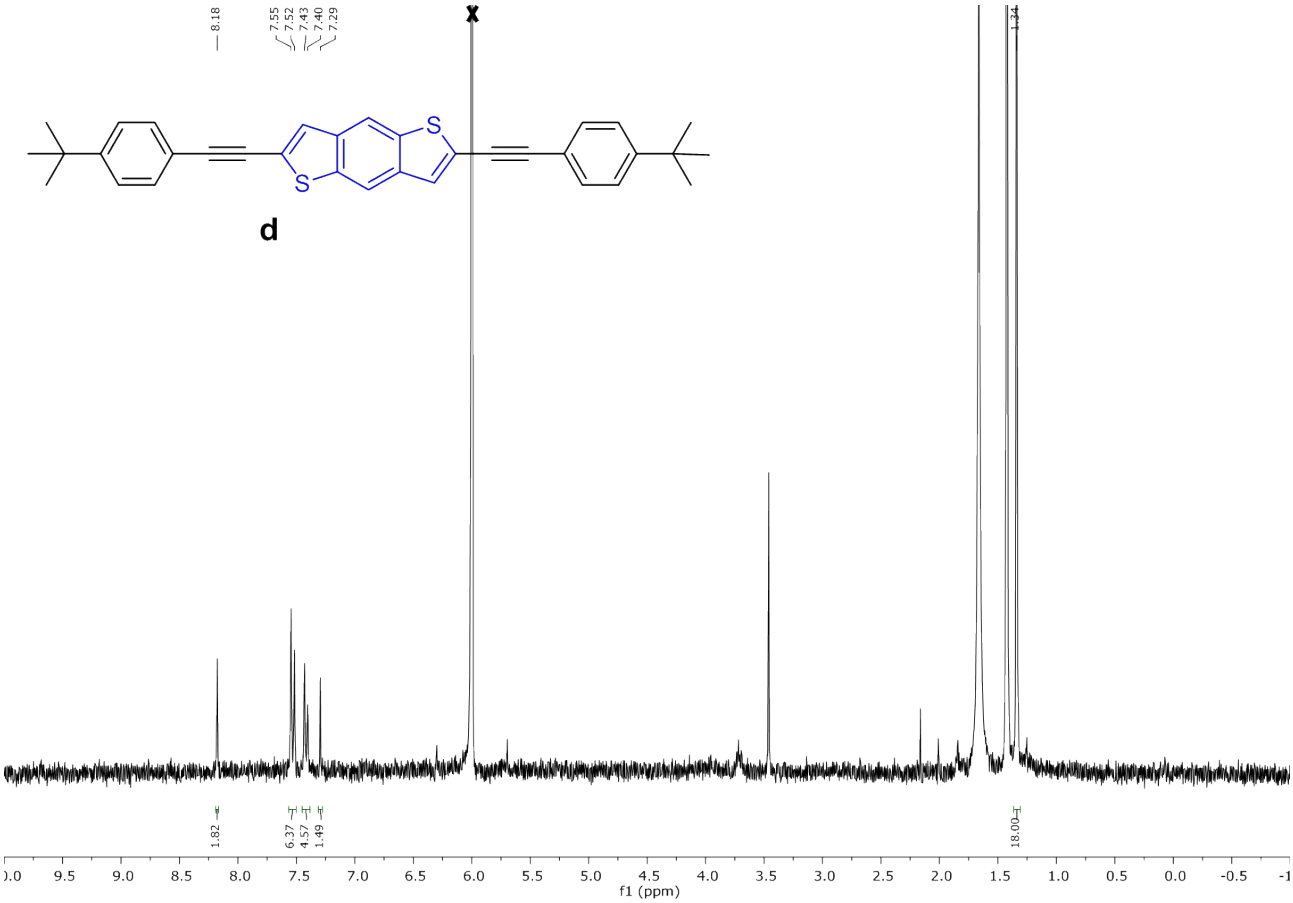
aC. This compound was prepared from **a-I** (1 eq., 0.066 g), **C1** (1.1 eq., 0.046 g), and a dry THF/NEt_3 (4:1) mixture as solvent. The reaction mixture was stirred at 40ºC overnight. The crude was purified using chloroform/methanol (40:1) as eluent. **aC** was obtained as a purple solid in 92% yield. ^{1}H NMR (300 MHz, $CDCl_3$ and a drop of DMSO-d_6) δ (ppm) δ 7.95 (s, 1H), 7.73 (s, 1H), 7.56 – 7.32 (m, 4H), 5.85 (s, 1H), 5.71 (s, 1H), 5.01 (d, *J* = 6.1 Hz, 1H), 4.89 – 4.77 (m, 1H), 4.51 – 4.27 (m, 3H), 3.16 – 2.93 (m, 2H), 2.69-2.53 (m, 13H), 1.74 – 1.22 (m, 21H), 1.15 (d, *J* = 6.9 Hz, 6H), 1.00 – 0.89 (m, 3H). ^{13}C NMR (76 MHz, $CDCl_3$ and a drop of DMSO-d_6) δ (ppm) 176.5, 164.8, 155.6, 153.9, 151.7, 148.07, 145.2, 142.1, 140.7, 131.9, 131.2, 130.9, 125.4, 120.2, 117.3, 114.4, 114.1, 97.1, 96.0, 91.6, 85.9, 85.5, 81.2, 80.7, 64.2, 34.8, 33.9, 32.5, 31.7, 31.2, 29.7, 28.9, 27.2, 25.3, 22.5, 19.1, 18.9, 15.3, 15.2, 14.0, 13.7, 13.6. HRMS (MALDI): Calculated for $C_{48}H_{58}BF_2N_5O_6$: 849.4448 $[M]^+$. Found: 872.4316 $[M+Na]^+$.

aU. This compound was prepared from **a-I** (1 eq., 0.066 g), **U1** (1.1 eq., 0.046 g), and a dry THF/NEt_3 (4:1) mixture as solvent. The reaction mixture was stirred at 40ºC overnight. The crude was purified using chloroform/methanol (40:1) as eluent. **aU** was obtained as a purple solid in 84% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.09 (s, 1H), 7.54 (s, 1H), 7.42 – 7.26 (m, 4H), 5.71 (d, *J* = 2.2 Hz, 1H), 4.87 (dd, *J* = 6.4, 2.2 Hz, 1H), 4.72 (dd, *J* = 6.4, 3.8 Hz, 1H), 4.40 – 4.22 (m, 3H), 3.01 – 2.89 (m, 2H), 2.60 (s, 3H), 2.56 (s, 3H), 2.53 (m, 1H), 2.50 (s, 3H), 2.48 (s, 3H), 1.52 (s, 3H), 1.48 – 1.32 (m, 6H), 1.30 (s, 3H), 1.26 (s, 9H), 1.10 (dd, *J* = 7.0, 3.8 Hz, 6H), 0.87 (t, *J* = 7.1 Hz, 3H).^{13}C NMR (76 MHz, $CDCl_3$) δ (ppm) 176.6, 160.6, 157.8, 156.3, 151.7, 148.7, 148.0, 142.5, 141.7, 141.3, 131.3, 125.5, 120.4, 115.0, 101.2, 97.0, 94.3, 87.5, 86.1, 85.2, 84.9, 80.9, 63.8, 34.9, 34.0, 32.6, 31.7, 31.3, 29.0, 27.3, 25.4, 22.6, 19.2, 19.1, 15.3, 15.3, 14.1, 13.8, 13.7. HRMS (MALDI): Calculated for $C_{48}H_{57}BF_2N_4O_7$: 850.4288 $[M]^+$. Found: 873.4201 $[M+Na]^+$.

S0B. ^{1}H NMR Spectra



d-Br



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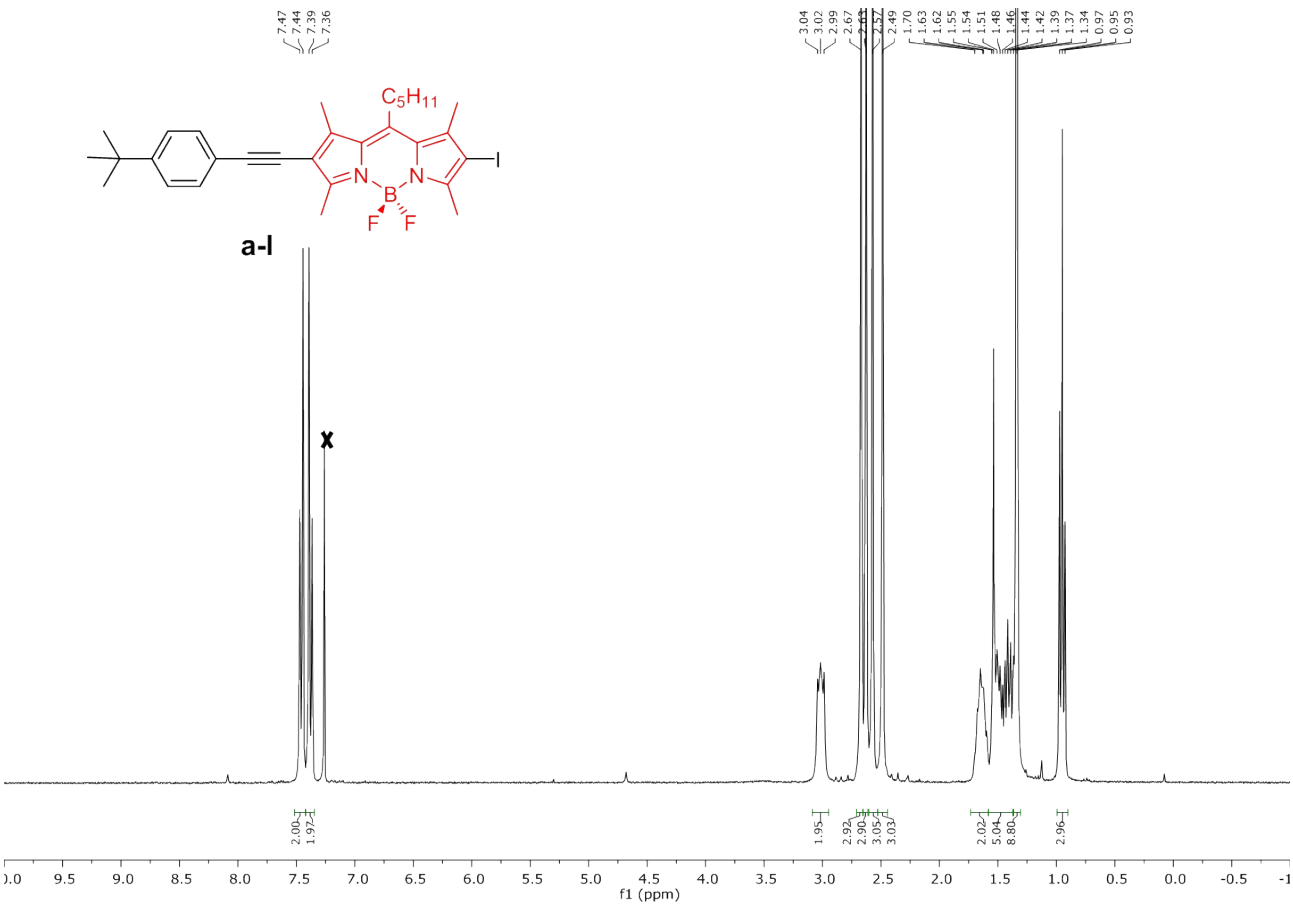
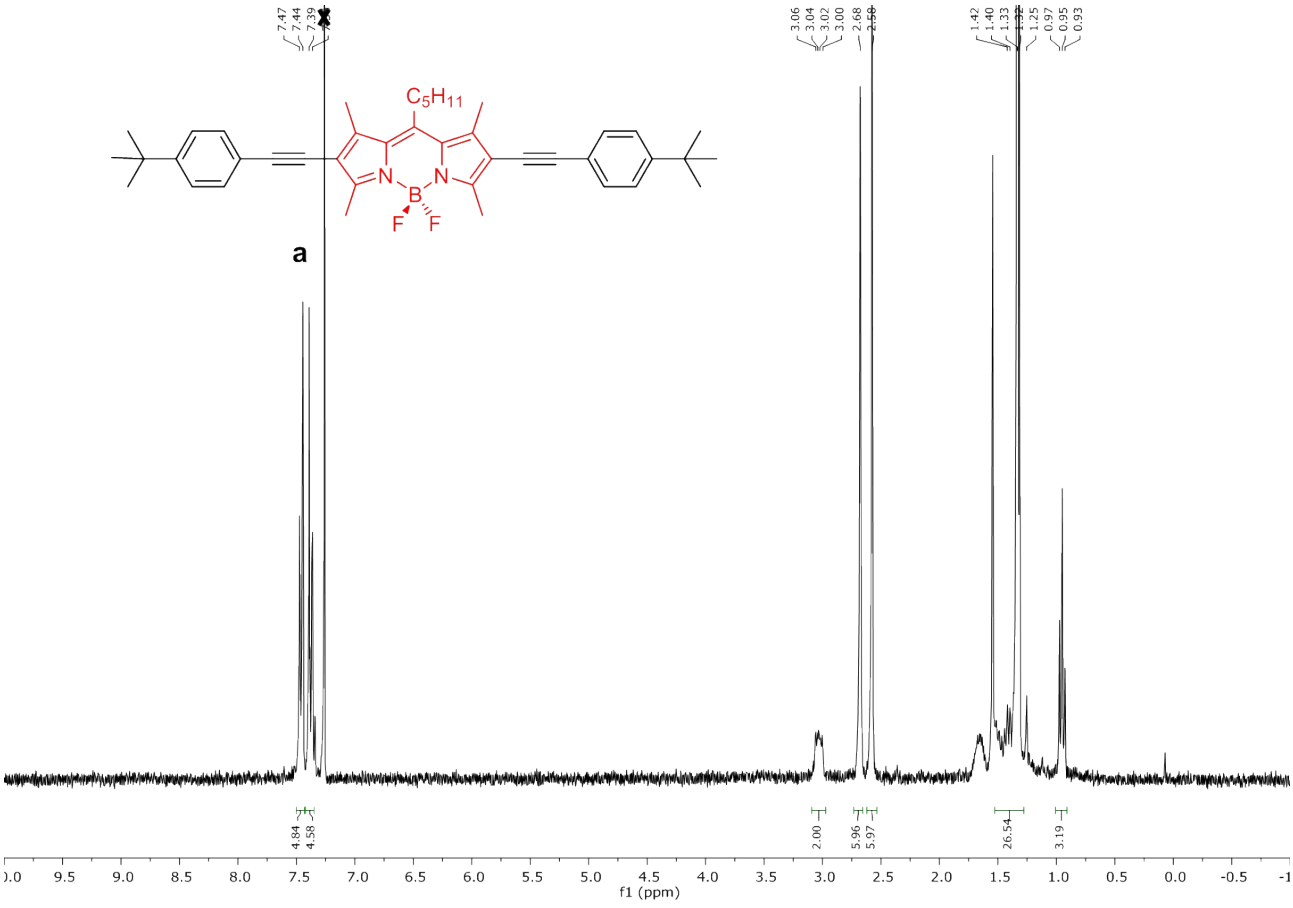
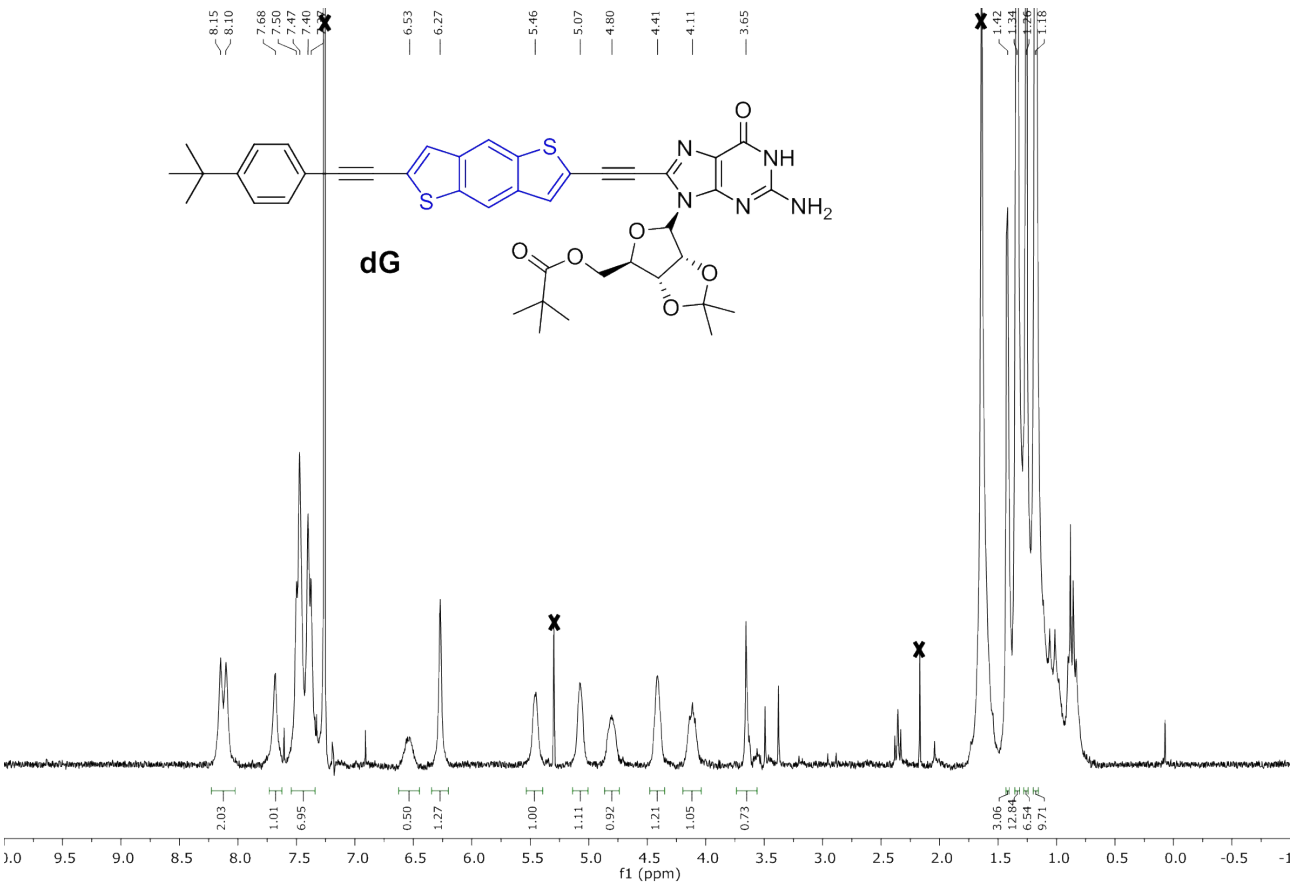
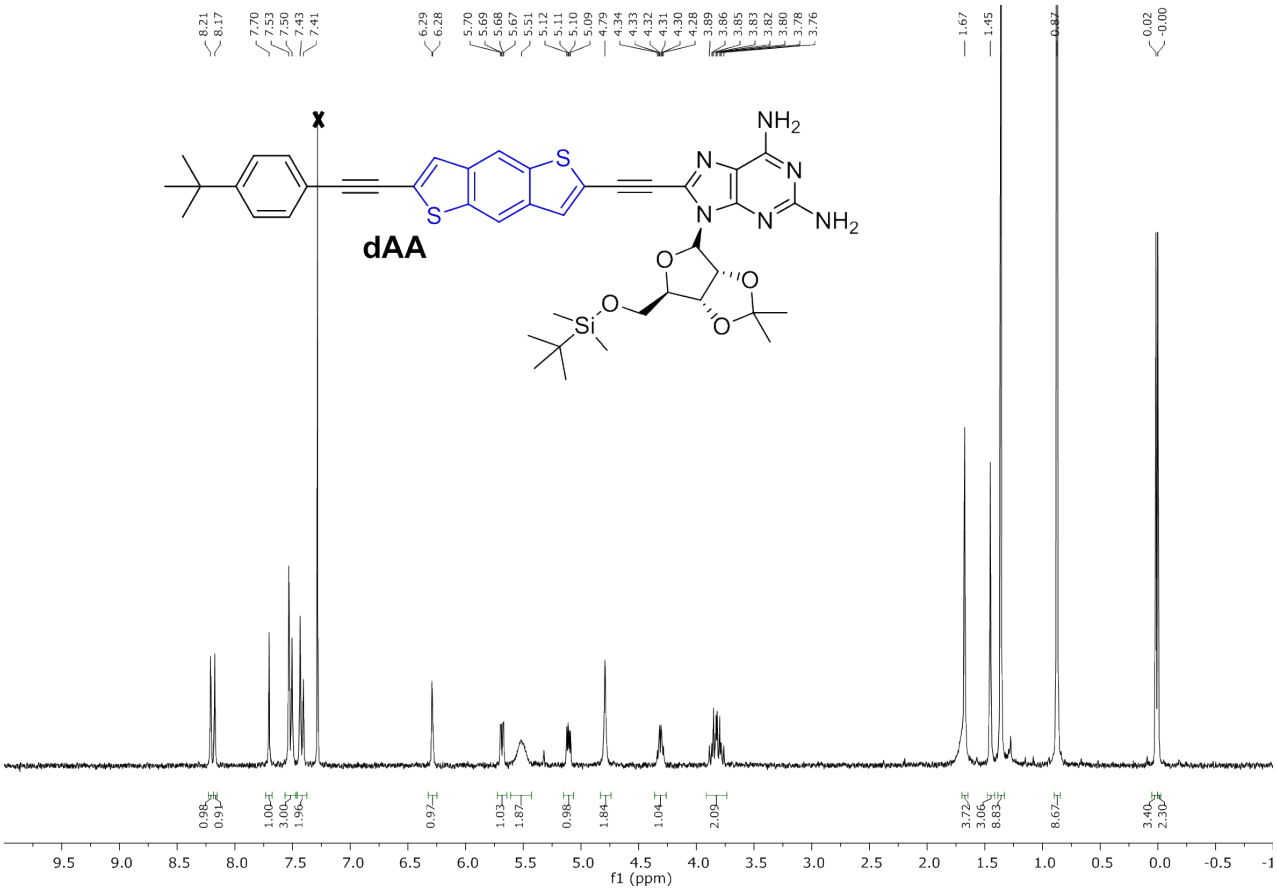

a-I
x
f1 (ppm)


a
x
f1 (ppm)


dG
f1 (ppm)


dAA
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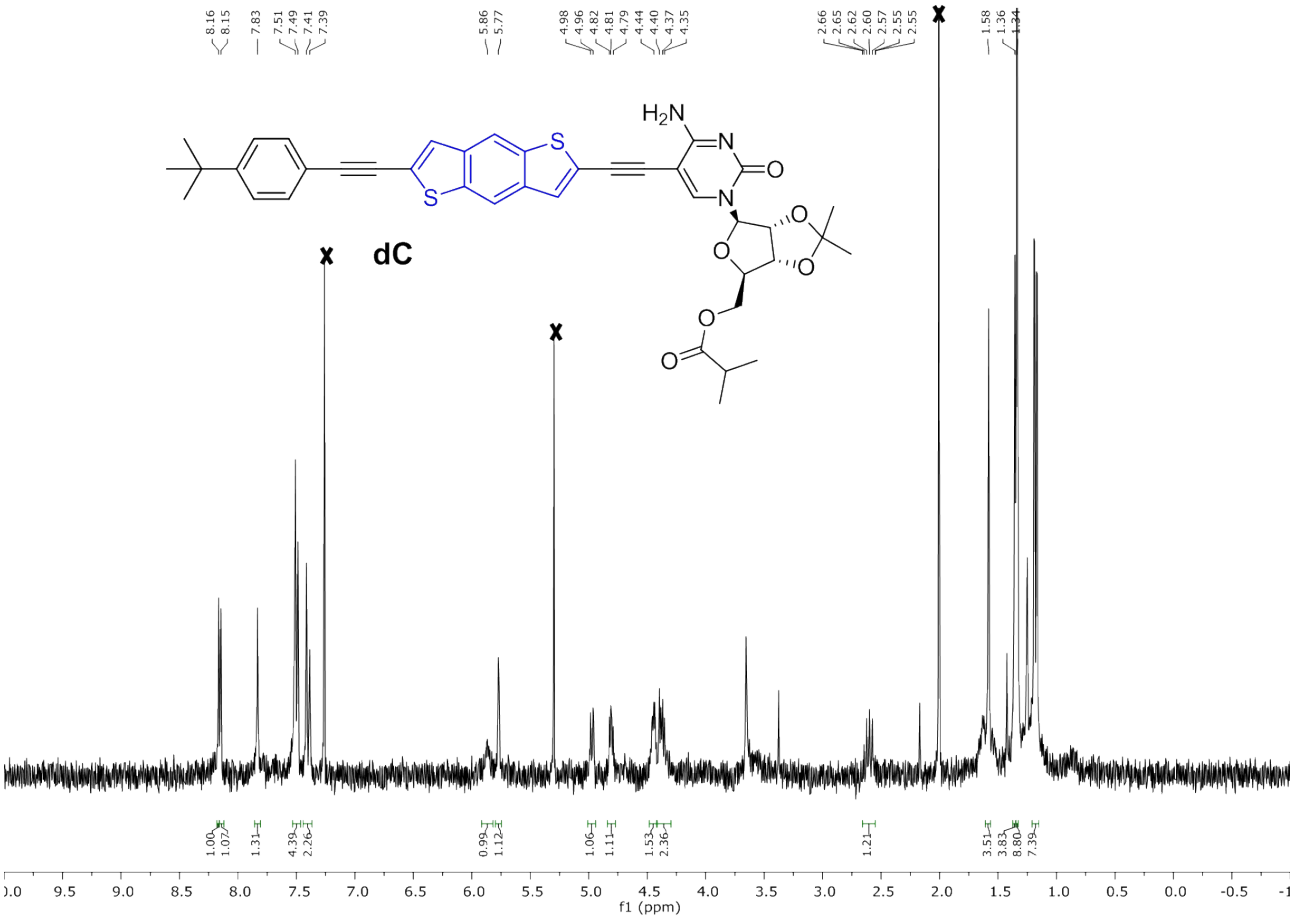
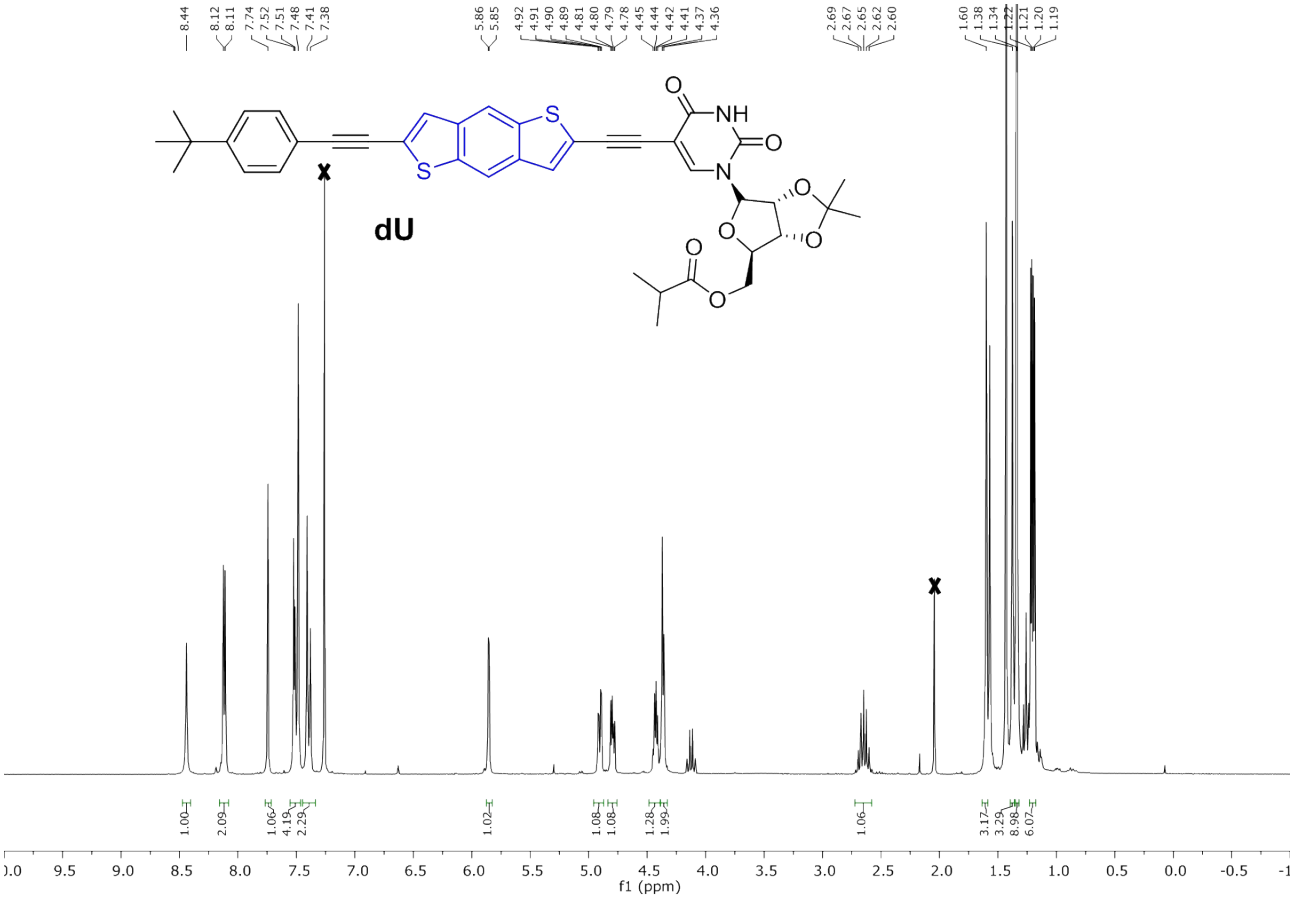
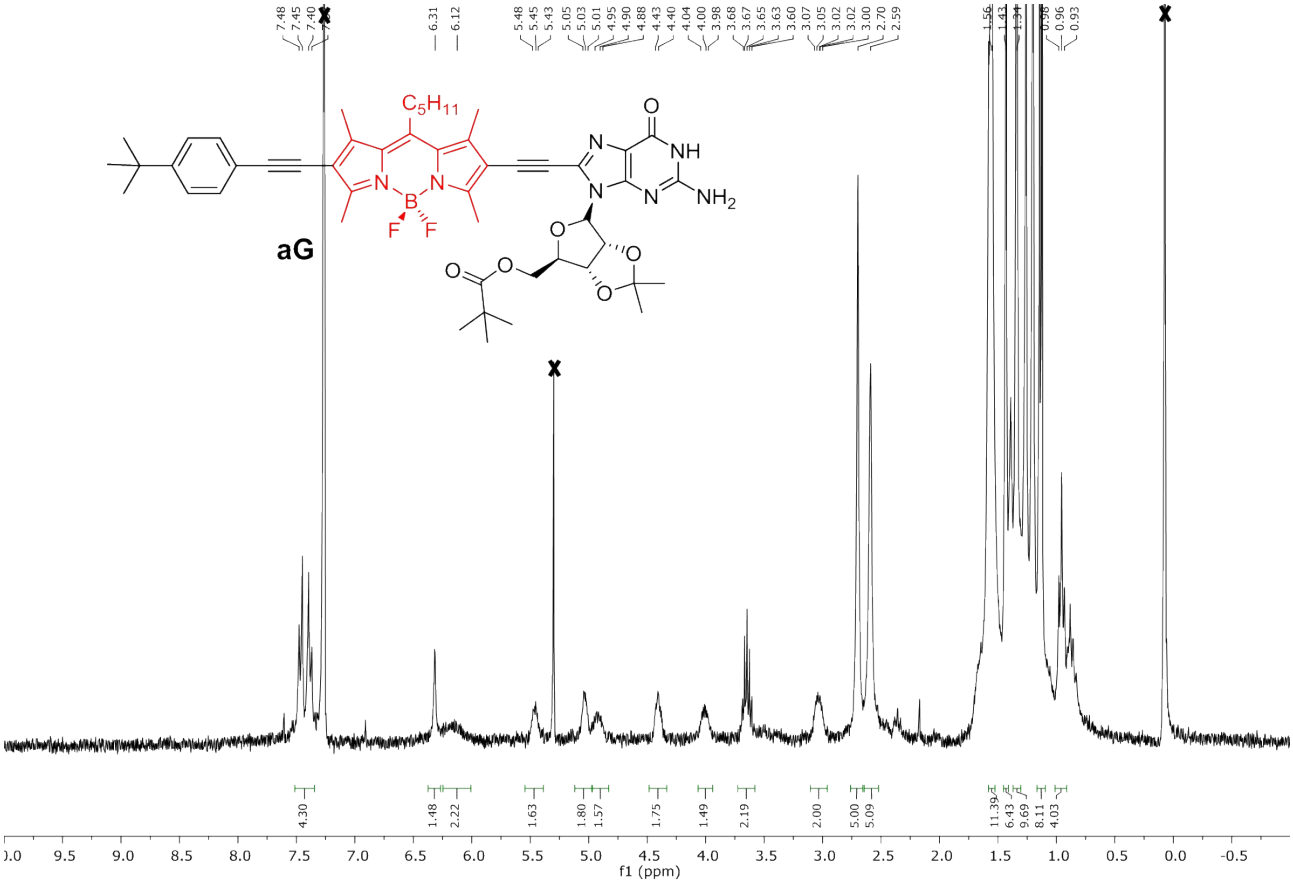
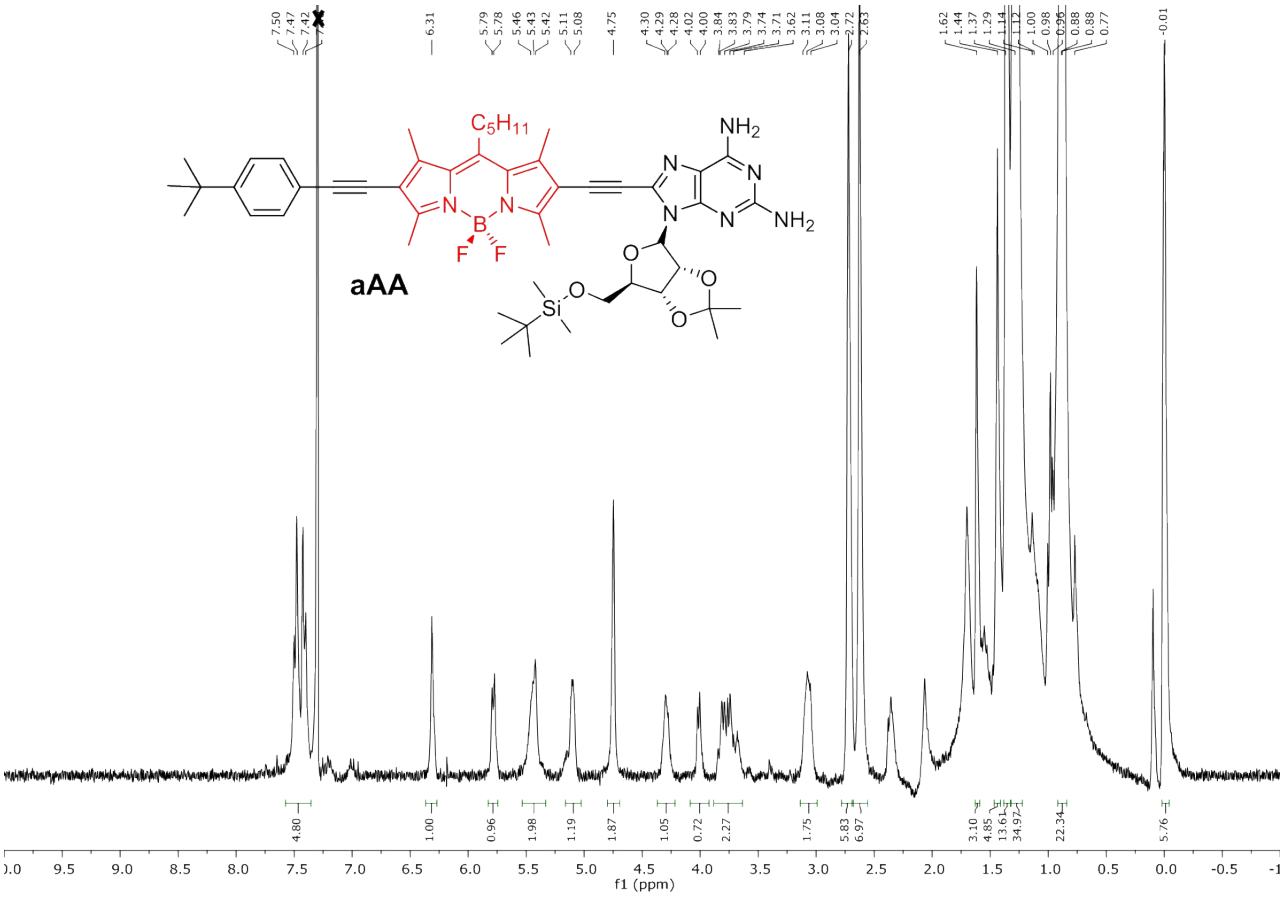
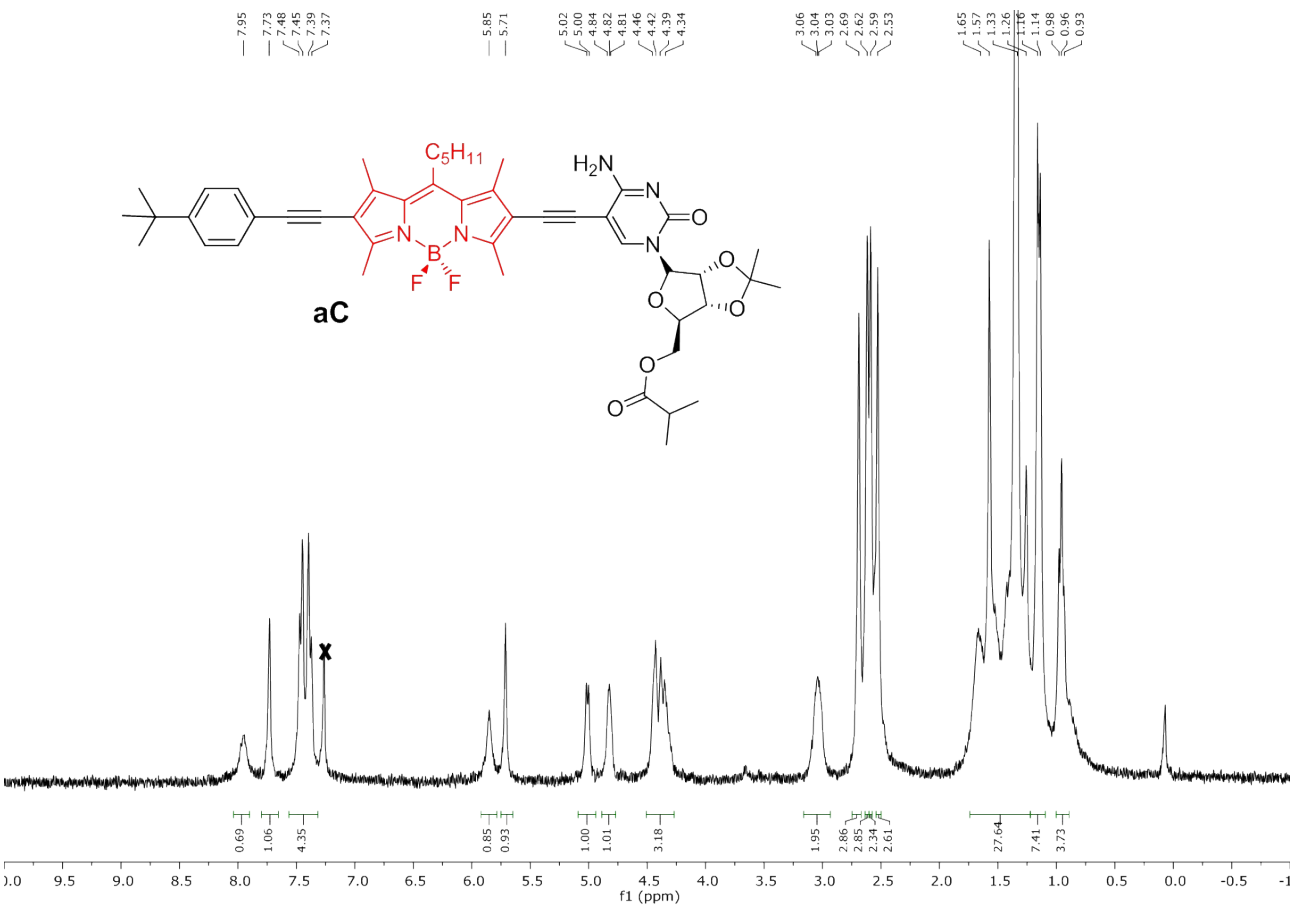
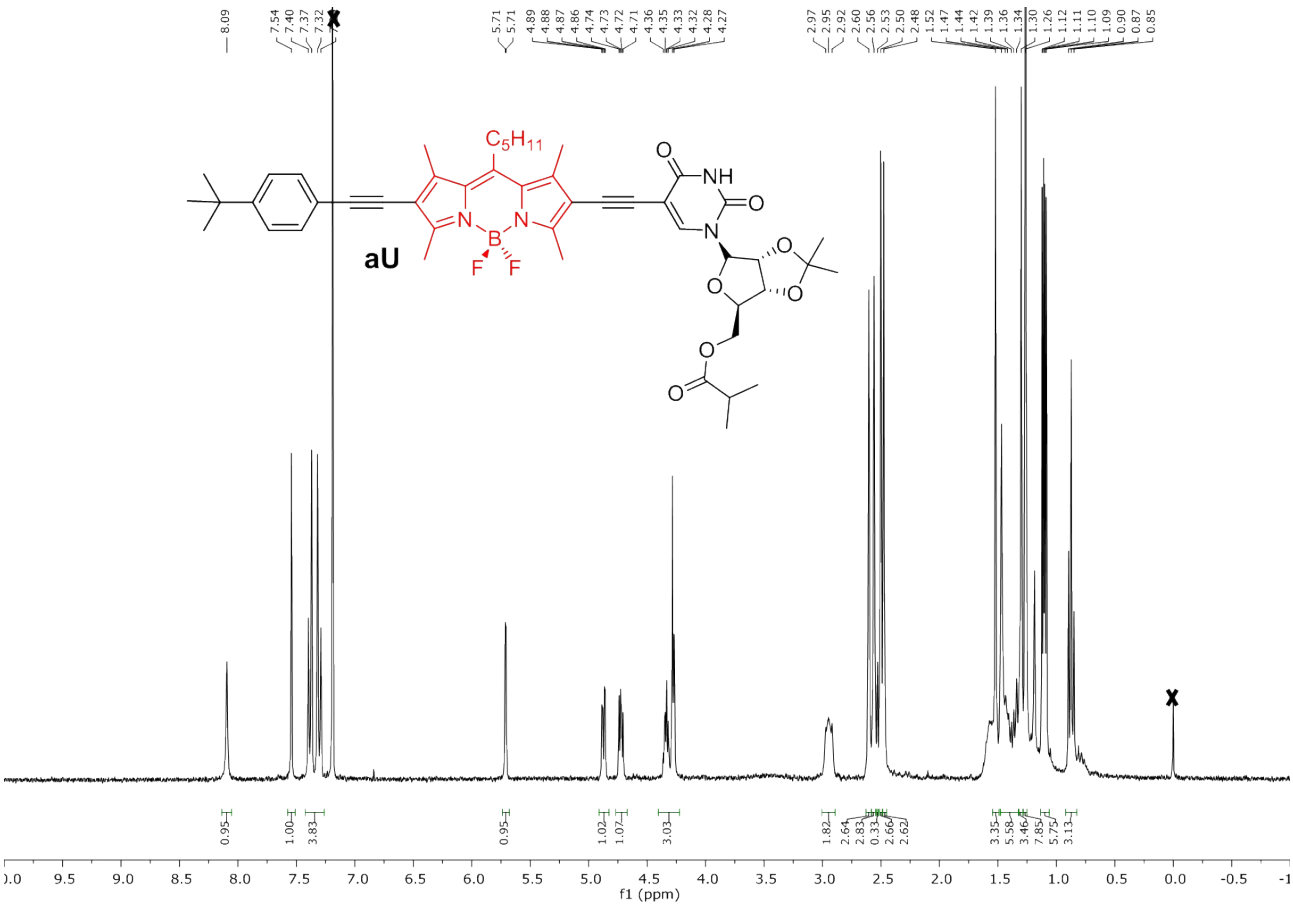

dC
f1 (ppm)


dU
f1 (ppm)


aG
f1 (ppm)


aAA
f1 (ppm)


aC
f1 (ppm)


aU
f1 (ppm)

S1. Cyclic voltammograms of reference compounds

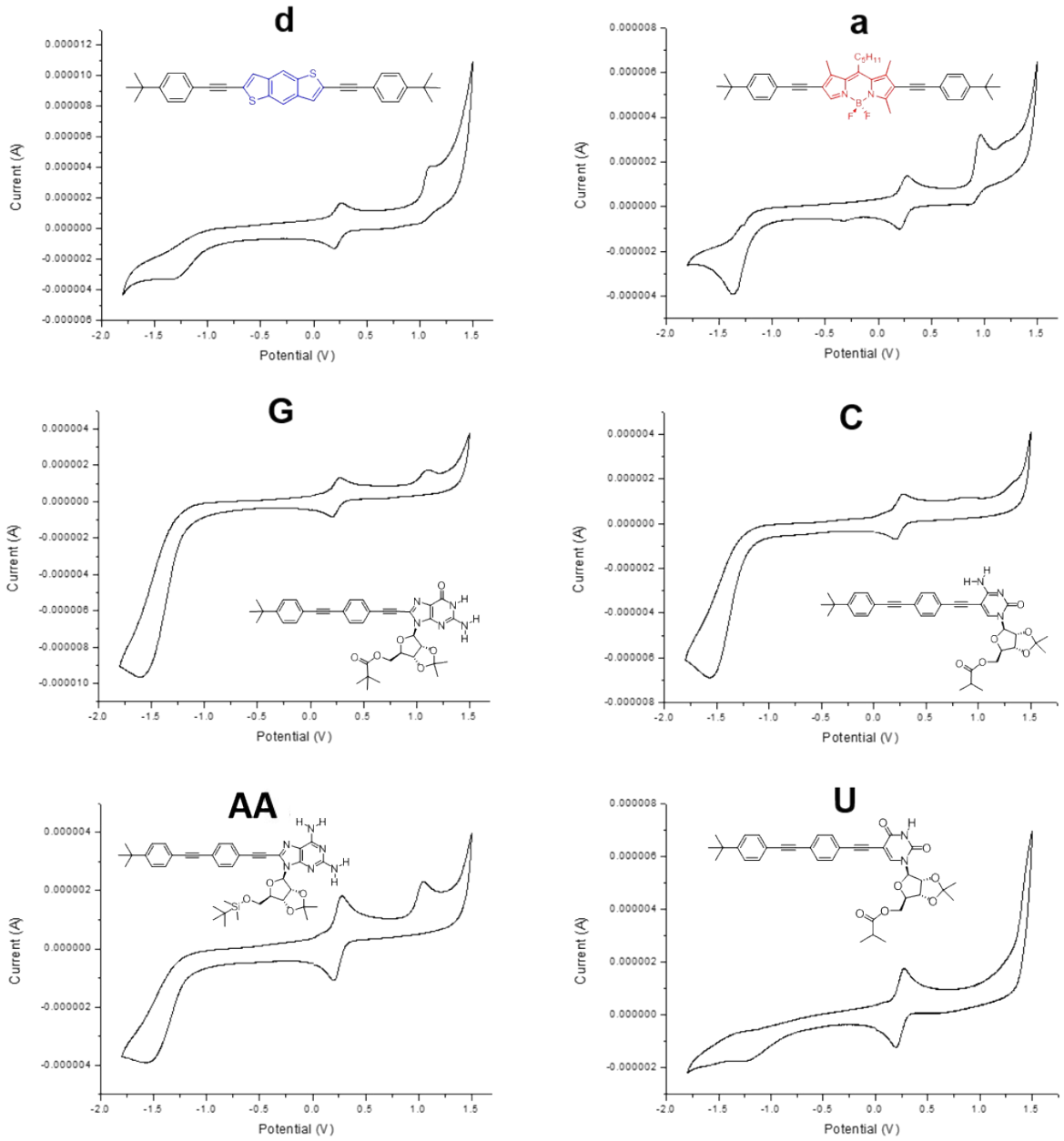


Figure S1. Cyclic voltammetry curves of reference compounds **d**, **a**, **G**, **C**, **AA** and **U** at a scan rate of 100 mVs^{-1} in anhydrous deoxygenated CH_2Cl_2 at Pt working electrode. Supporting electrolyte 0.1 M $TBAPF_6$. Ferrocene (Fc) was used as internal standard.

S2. Absorption, emission, excitation and temperature-dependent emission spectra of nucleoside donor and acceptor molecules and their binary mixtures

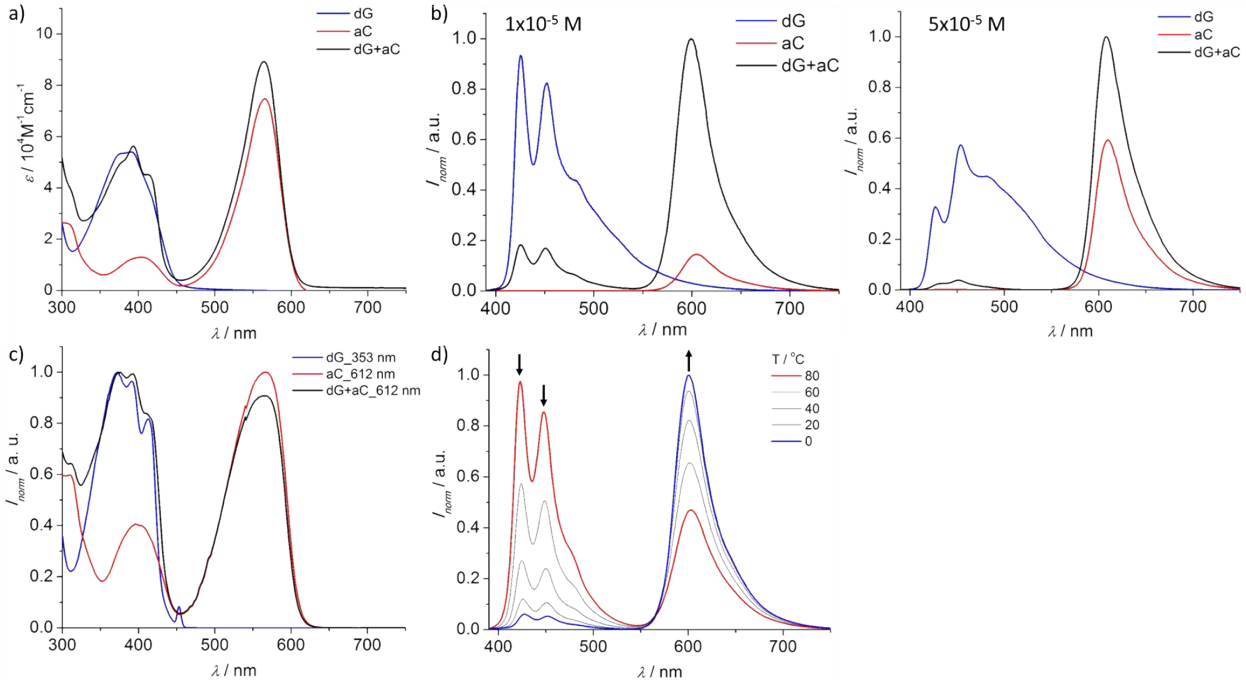


Figure S2A. a) Absorption, (**b**) emission (λ_{exc} = 353 nm) and (**c**) excitation (λ_{em} = 353, 612 nm) spectra of compounds **dG** and **aC** and their 1:1 mixtures **dG+aC**. **d**) Emission spectra (λ_{exc} = 353 nm) of the **dG+aC** 1:1 mixtures as a function of temperature in the 0-80 ºC range. Arrows indicate the evolution of donor and acceptor emission maxima when decreasing temperature. In all cases the concentration energy donor and acceptor compounds was set at *C* = 1x10^{-5} M in toluene.

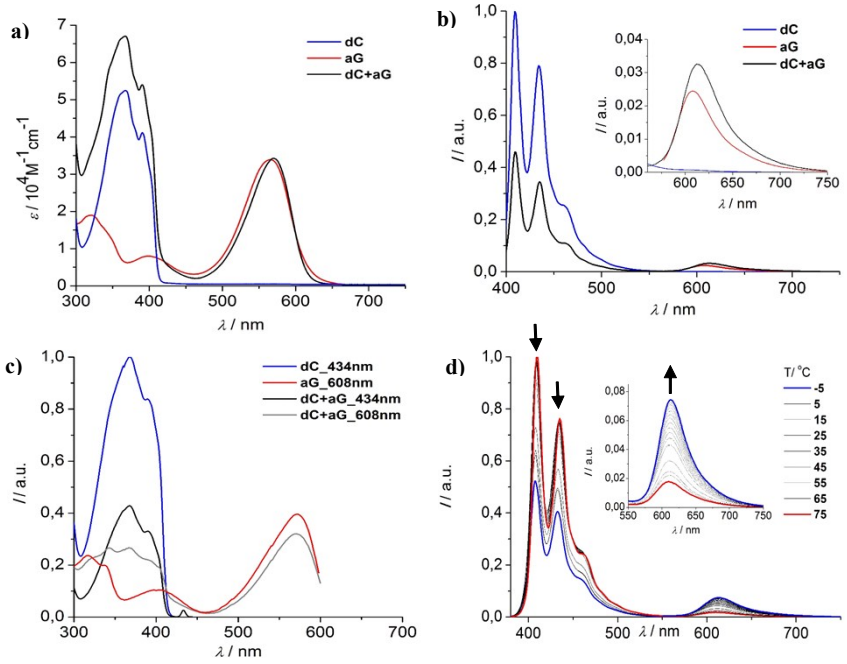


Figure S2B. a) Absorption, (**b**) emission (λ_{exc} = 368 nm) and (**c**) excitation (λ_{em} = 434, 608 nm) spectra of compounds **dC** and **aG** and their 1:1 mixtures **dC+aG**. **d**) Emission spectra (λ_{exc} = 368 nm) of the **dC+aG** 1:1 mixtures as a function of temperature in the (-5)-75ºC range. Arrows indicate the evolution of donor and acceptor emission maxima when decreasing temperature. In all cases the concentration energy donor and acceptor compounds was set at *C* = 1x10^{-5} M in toluene.

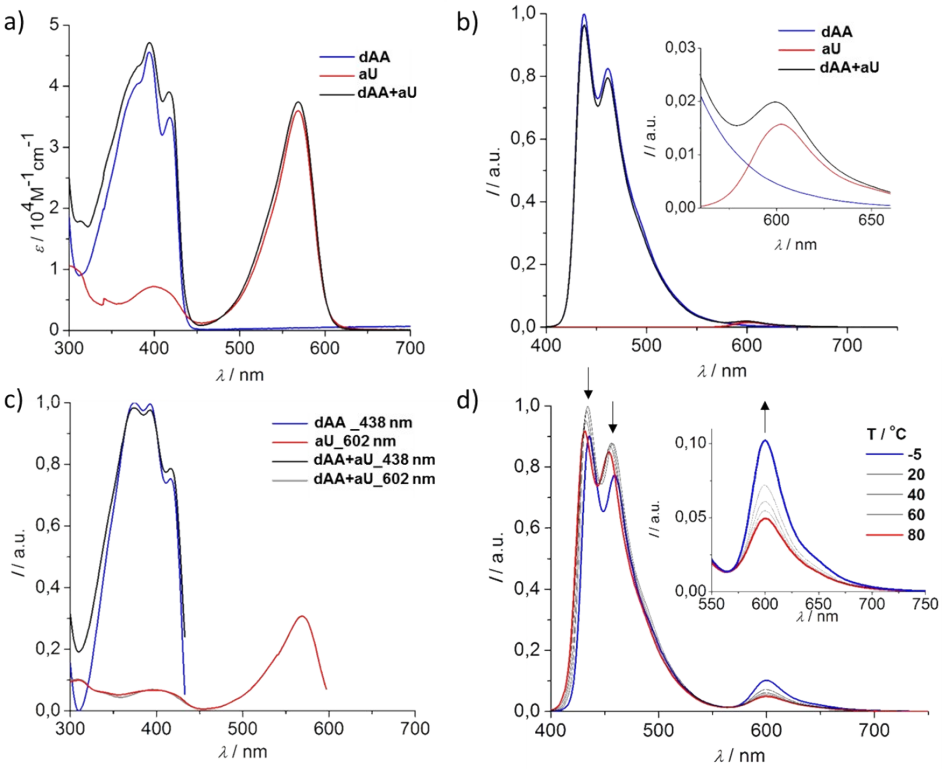


Figure S2C. a) Absorption, (**b**) emission (λ_{exc} = 360 nm) and (**c**) excitation (λ_{em} = 438, 602 nm) spectra of compounds **dAA** and **aU** and their 1:1 mixtures **dAA**+**aU**. **d**) Emission spectra (λ_{exc} = 360 nm) of the **dAA**+**aU** 1:1 mixtures as a function of temperature in the (-5)-80 ºC range. Arrows indicate the evolution of donor and acceptor emission maxima when decreasing temperature. In all cases the concentration energy donor and acceptor compounds was set at *C* = 1x10^{-5} M in toluene.

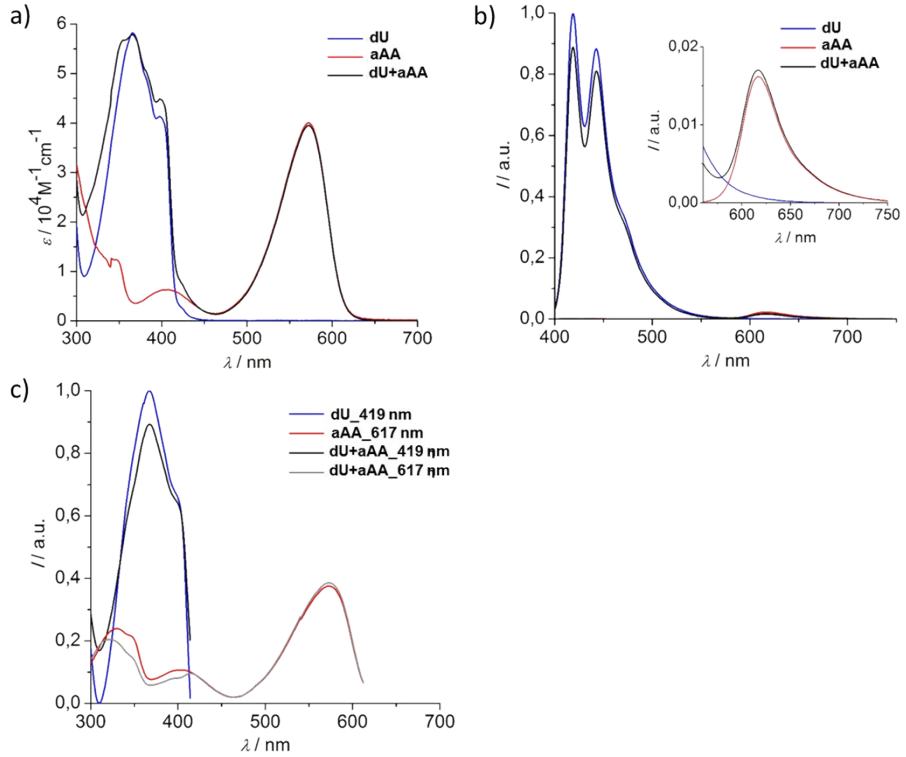


Figure S2D. a) Absorption, (**b**) emission (λ_{exc} = 366 nm) and (**c**) excitation (λ_{em} = 419, 617 nm) spectra of compounds **dU** and **aAA** and their 1:1 mixtures **dU**+**aAA**. In all cases the concentration energy donor and acceptor compounds was set at *C* = 1x10^{-5} M in toluene.

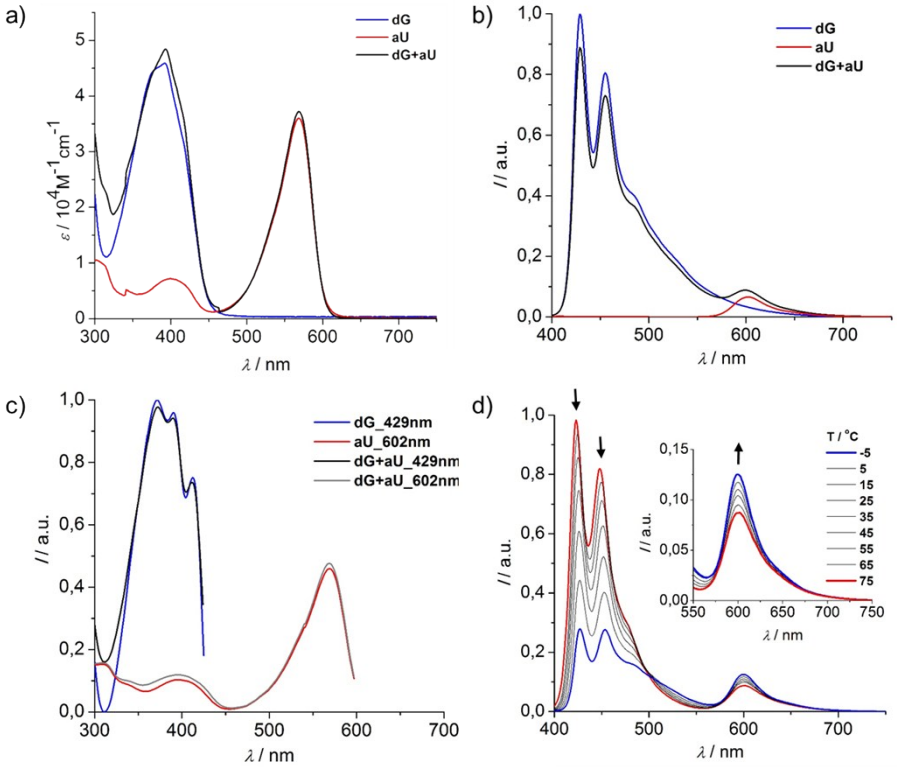


Figure S2E. a) Absorption, (**b**) emission (λ_{exc} = 373 nm) and (**c**) excitation (λ_{em} = 429, 602 nm) spectra of compounds **dG** and **aU** and their 1:1 mixtures **dG**+**aU**. **d**) Emission spectra (λ_{exc} = 373 nm) of the **dG**+**aU** 1:1 mixtures as a function of temperature in the (-5)-75 ºC range. Arrows indicate the evolution of donor and acceptor emission maxima when decreasing temperature. In all cases the concentration energy donor and acceptor compounds was set at *C* = 1x10^{-5} M in toluene.

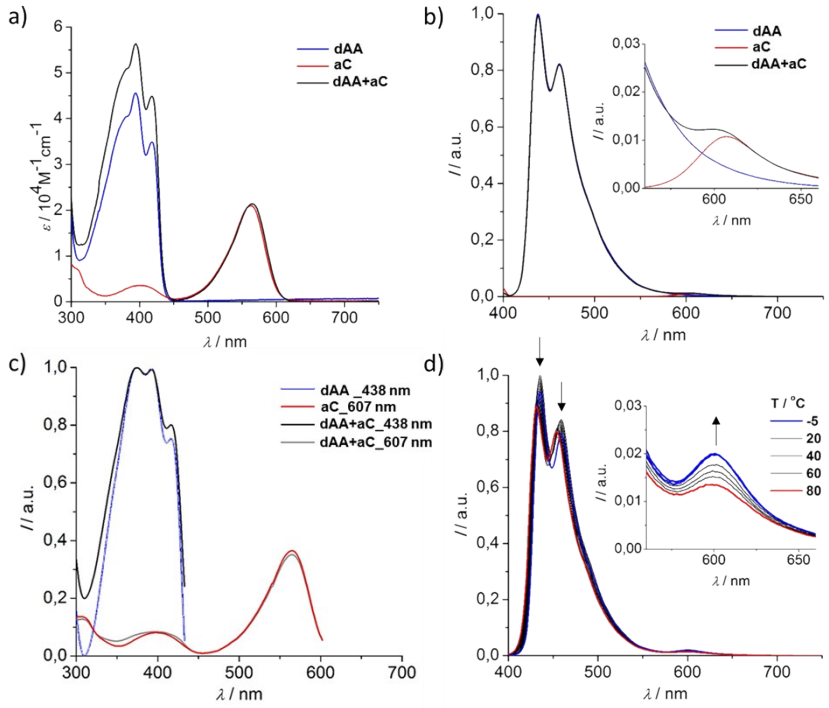


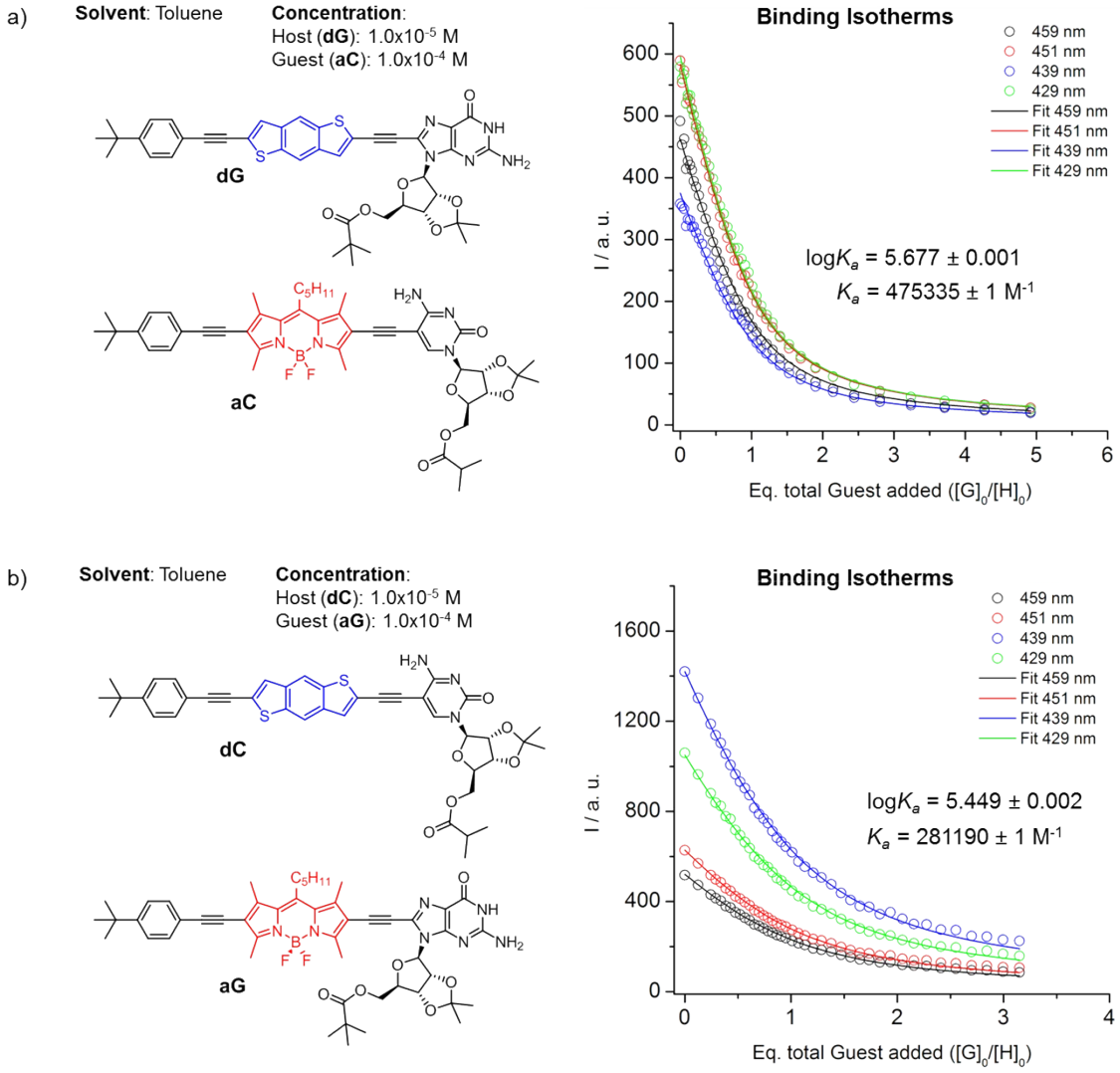
Figure S2F. a) Absorption, (**b**) emission (λ_{exc} = 394 nm) and (**c**) excitation (λ_{em} = 438, 607 nm) spectra of compounds **dAA** and **aC** and their 1:1 mixtures **dAA**+**aC**. **d**) Emission spectra (λ_{exc} = 394 nm) of the **dAA**+**aC** 1:1 mixtures as a function of temperature in the (-5)-80 ºC range. Arrows indicate the evolution of donor and

acceptor emission maxima when decreasing temperature. In all cases the concentration energy donor and acceptor compounds was set at C = $1x10^{-5}$ M in toluene.

S3A. Analysis of the Titration Emission Data to obtain K_a

ReactLab EQUILIBRIA is a program developed and commercialized by Jplus Consulting Pty Ltd (http://jplusconsulting.com/; 8 Windsor Road, East Fremantle, WA 6158, Australia). It allows for the global fitting of multiwavelength spectroscopic data in equilibrium titration measurements to chemical reaction schemes, and determines all equilibrium constants in the underlying mechanism. *ReactLab*™ algorithms fit complete reaction models directly to multivariate data and delivers all the required parameters in one step. The analysis also yields the concentration distributions of all species and the individual spectra of all the participating species. The program, including all algorithms and the GUI frontend has been developed in Matlab and compiled to produce the final deployable application.

A wavelength region in the absorption spectra (from 410 to 460 nm; each wavelength representing one set of data) was fitted by this software. However, only 4 selected wavelengths are plotted (see Figure S3A and Table S1).



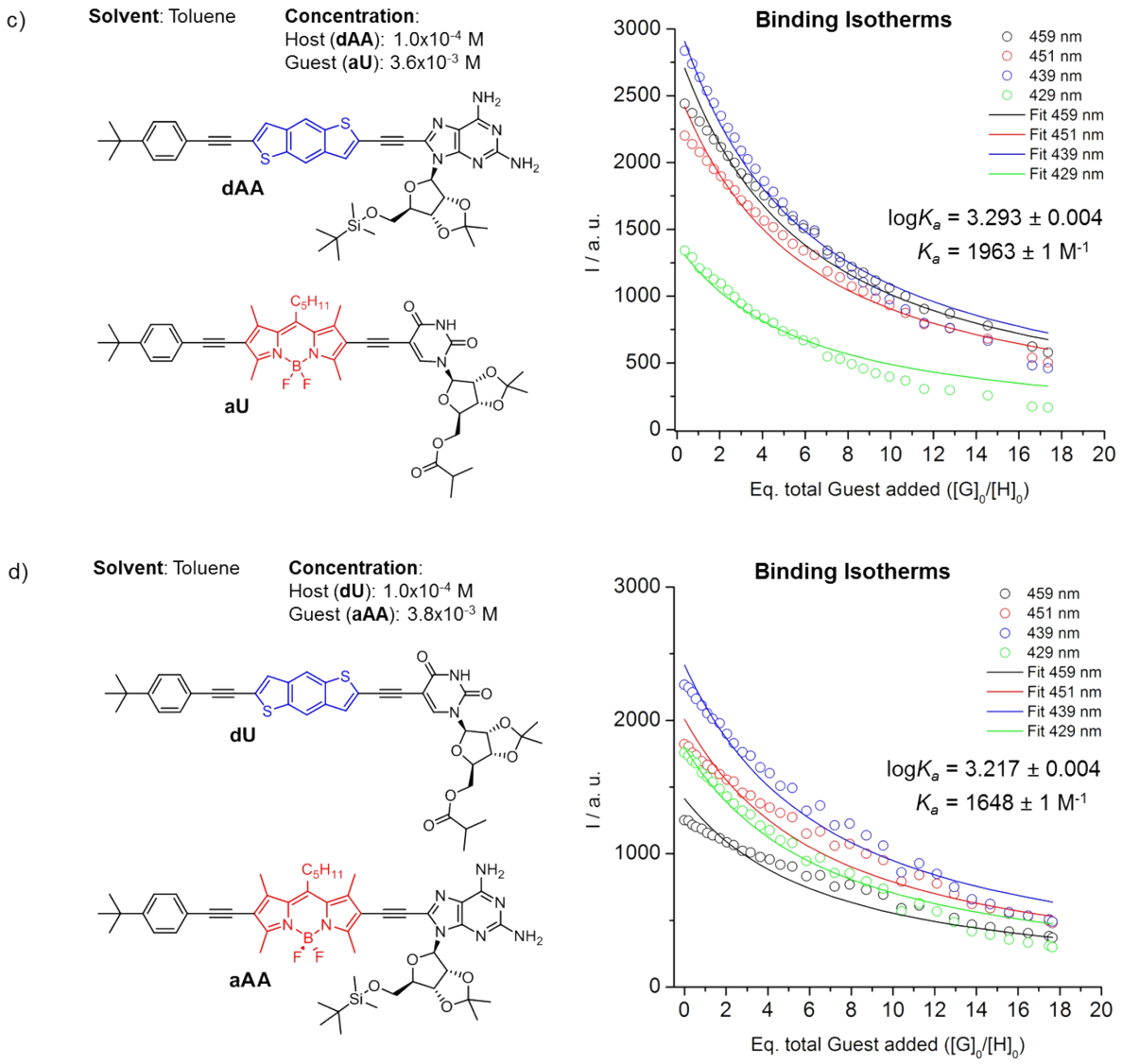


Figure S3A. Fitting of the emission data of (**a**) **dG+aC,** (**b**) **dC+aG,** (**c**) **dAA+aU** and (**d**) **dU+aAA** in toluene by the ReactLab™ EQUILIBRIA software.

The 1:1 binding constants have been also calculated using a custom written global nonlinear regression analysis program developed by P. Thordarson[5] within the Matlab R2012b package utilizing the Simplex algorithm.[6]

The standard errors (*SEy*) were calculated by:

$$SE_y = \sqrt{\frac{\sum (y_{data} - y_{calc})^2}{N - k}}$$

Where *N* is the number of data points and *k* the number of parameters to be fitted.

The calculated K_a values are shown in Table S1.

S3B. Analysis of the NMR Titration data to obtain K_a

[5] P. Thordarson, *Chem. Soc. Rev*. 2011, **40**, 1305.
[6] (a) J. A. Nelder and R. Mead, *Comp. J.* 1965, **7**, 308; (b) J. C. Lagarias, J. A. Reeds, M. H. Wright and P. E. Wright, *SIAM J. Optim.* 1998, **9**, 112.

The 1:1 binding constants of the NMR experiments have been also calculated using the Matlab® scripts developed by P. Thordarson[5] (Figure S3B and Table S1).

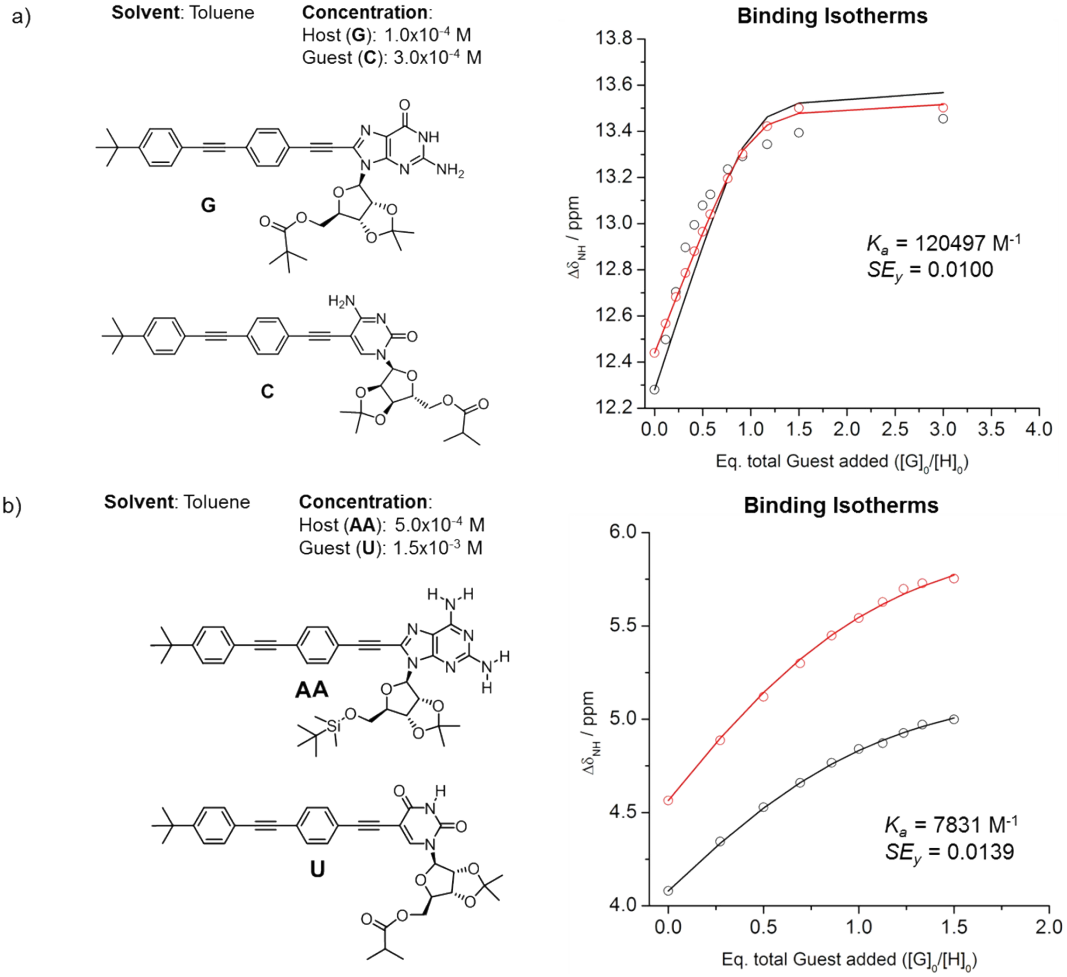


Figure S3B. NMR data of the (**a**) **G**-**C** and (**b**) **AA-U** pairs[7] in toluene fitted with the Matlab® scripts developed by P. Thordarson.[5]

Table S1. Calculated association contants (K_a, M^{-1}) between complementary nucleosides in toluene

	aG	**aC**	**aAA**	**aU**
dG		$5.0 \cdot 10^5$ [a] $4.1 \cdot 10^5$ (SE_y = 10.040)[b] $1.2 \cdot 10^5$ (SE_y = 0.010)[c]		
dC	$3.0 \cdot 10^5$ [a] $4.2 \cdot 10^5$ (SE_y = 9.395)[b]			
dAA				$2.0 \cdot 10^3$ [a] $7.4 \cdot 10^2$ (SE_y = 57.631)[b] $7.8 \cdot 10^3$ (SE_y = 0.014)[c]
dU			$1.6 \cdot 10^3$ [a] $8.1 \cdot 10^2$ (SEy = 4.065)[b]	

[a] Association constants calculated from emission data and fitted with ReactLab™ EQUILIBRIA.[b] Emission data fitted with the Matlab® scripts developed by P. Thordarson.[5] [c] NMR data fitted with the Matlab® scripts developed by P. Thordarson. In this case, nucleosides **G**, **C**, **AA**, **U** (see Figure 1) were employed instead.

[7] C. Montoro-García, J. Camacho-García, A. M. López-Pérez, M. J. Mayoral, N. Bilbao and D. González-Rodríguez, *Angew. Chem. Int. Ed.*, 2016, **55**, 223.