SUPPORTING INFORMATION

Understanding Complex Supramolecular Landscapes: Noncovalent Macrocyclization Equilibria Examined 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 (^{1}H) and 77.0 ppm (^{13}C), DMSO-d$_6$ calibrated at 2.50 ppm (^{1}H) and 39.5 ppm (^{13}C) and DMF-d$_7$ calibrated at 8.03 ppm (^{1}H) 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. **CD** spectra were recorded with a JASCO V-815 equipment (measurement Information: data interval = 1 nm, data pitch = 1 nm, sensitivity = standard, D.I.T. = 1 sec, slit width = 1000 um). Quartz cuvettes (1.0 cm path length) were used for the measurements. In all these three instruments the temperature was controlled using a JASCO Peltier thermostatted cell holder with a range of 263–383 K, adjustable temperature slope, and accuracy of ± 0.1 K.

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**, **A1** and **U1** (Scheme S1) have been previously reported by us.[1] 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.[2]
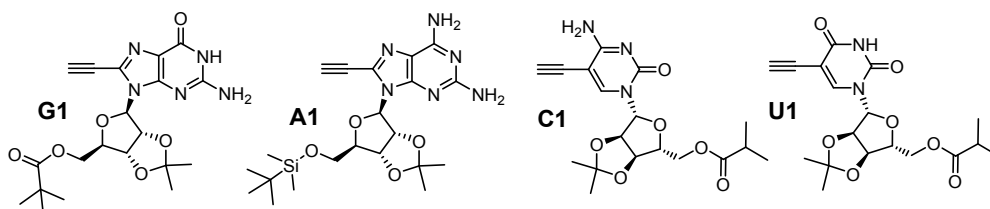
**Scheme S1.** Ethynyl-nucleobases **G1**, **C1**, **A1** and **U1** previously reported.[1]

[1] J. Camacho-García, C. Montoro-García, A. M. López-Pérez, N. Bilbao, S. Romero-Pérez and D. González-Rodríguez, *Org. Biomol. Chem.*, 2015, **13**, 4506-4513.

[2] (a) M. Mao, J.-B. Wang, Z.-F. Xiao, S.-Y. Dai and Q.-H. Song, *Dyes Pigments*, 2012, **94**, 224-232; (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**, 9219-9222.

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.
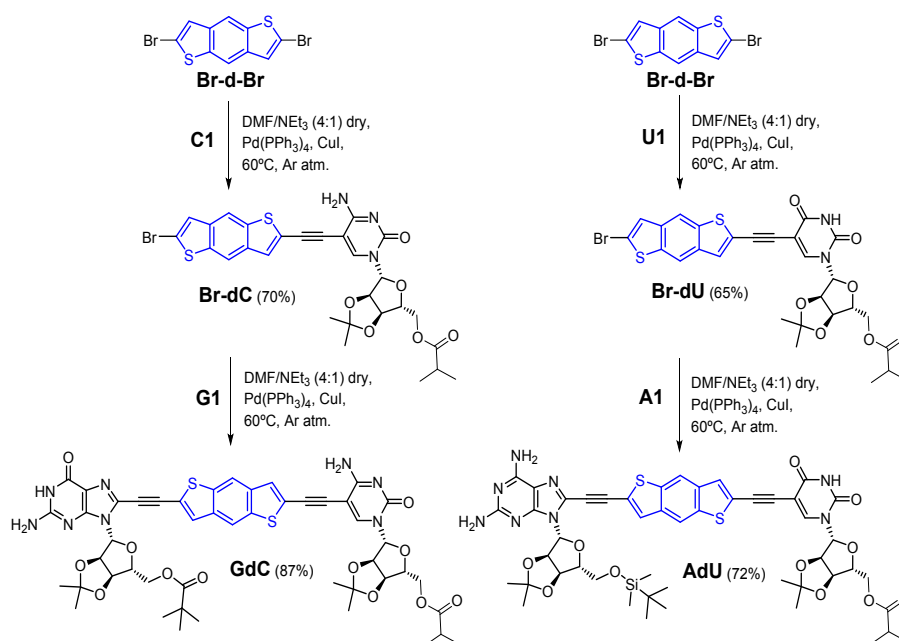
Scheme S2. Synthesis of dinucleosides **GdC** and **AdU** from dibromobithiophene **Br-d-Br** *via* two consecutive Sonogashira coupling reactions.
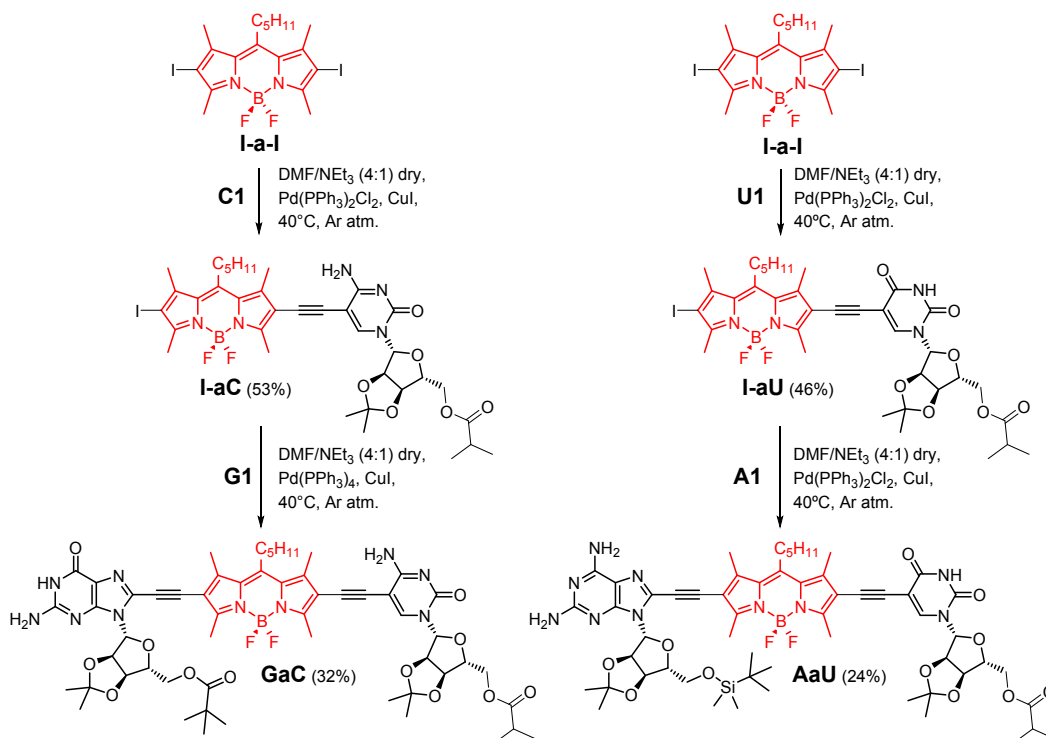
Br-dC. This compound was prepared from 2,6-dibromobenzo[1,2-b:4,5-b']dithiophene (3 eq., 0.114 g), **C1** (1 eq., 0.031 g), and a dry DMF/NEt$_3$ (4:1) mixture as solvent. The reaction mixture was stirred at 60ºC overnight. The crude was purified using chloroform/methanol (40:1) as eluent. **Br-dC** was obtained as a yellow solid in 70% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 8.47 (s, 1H), 8.40 (s, 1H), 8.18 (s, 1H), 8.00 (s, 1H), 7.81 (s, 1H), 7.69 (s, 1H), 7.36 (s, 1H), 5.84 (s, 1H), 5.02 (d, J = 6.9 Hz, 1H), 4.81 (m, 1H), 4.32 – 4.19 (m, 3H), 2.59 (m, 1H), 1.49 (s, 3H), 1.30 (s, 3H), 1.10 (dd, J = 7.0, 2.0 Hz, 6H). ^{13}C NMR (76 MHz, DMSO-d_6) δ (ppm) 175.8, 147.6, 147.5, 137.9, 137.7, 137.0, 136.3, 128.9, 126.3, 122.7, 117.0, 116.1, 115.9, 113.0, 84.6, 84.4, 80.6, 63.8, 33.1, 26.9, 25.1, 18.8, 18.7. HRMS (ESI+): Calculated for C$_{28}$H$_{26}$BrN$_3$O$_6$S$_2$: 643.0446 [M]$^+$. Found: 644.0500 [M+H]$^+$.

GdC. This compound was prepared from **Br-dC** (1 eq., 0.071 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 (10:1) as eluent. **GdC** was obtained as a yellow solid in 87% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 11.09 (s, 1H), 8.41 (s, 2H), 8.19 (s, 1H), 8.13 (s, 1H), 7.93 (s, 2H), 7.89 (s, 1H), 7.77 (s, 1H), 6.91 (s, 1H) 6.07 (s, 1H), 5.79 (s, 1H), 5.37 (d, J = 6.0 Hz, 1H), 5.26 (m, 1H), 4.96 (d, J = 6.0 Hz, 1H), 4.79 (m, 1H), 4.40 – 4.00 (m, 6H), 2.53 (m, 1H), 1.40-0.60

(m, 27H) ^{13}C NMR (76 MHz, DMSO-d_6) δ 175.4, 174.0, 162.2, 154.4, 152.8, 151.4, 148.7, 145.7, 136.3, 135.8, 135.7, 135.4, 128.8, 127.0, 126.5, 122.0, 118.9, 116.2, 115.9, 115.4, 111.7, 111.4, 97.8, 92.0, 87.9, 87.1, 86.0, 85.9, 85.2, 83.8, 83.1, 82.8, 81.9, 79.9, 79.1, 68.1, 62.5, 62.1, 36.5, 31.5, 29.6, 27.4, 27.0, 25.3, 25.2, 25.1, 23.6, 23.4, 20.4, 17.1, 17.0, 12.2. HRMS (MALDI): Calculated for $C_{48}H_{50}N_8O_{12}S_2$: 994.2990 [M]$^+$. Found: 1017.2871 [M+Na]$^+$.

Br-dU. This compound was prepared from 2,6-dibromobenzo[1,2-b:4,5-b']dithiophene (3 eq., 0.114 g), **U1** (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. **Br-dU** was obtained as a yellow solid in 65% yield. ^{1}H NMR (300 MHz, CDCl$_3$) δ (ppm) 8.55 (s, 1H), 8.06 (d, *J* = 8.3 Hz, 2H), 7.74 (s, 1H), 7.50 (s, 1H), 7.33 (s, 1H), 5.85 (d, *J* = 2.2 Hz, 1H), 4.91 (dd, *J* = 6.4, 2.2 Hz, 1H), 4.79 (dd, *J* = 6.4, 3.8 Hz, 1H), 4.43 (m, 1H), 4.36 (d, *J* = 4.1 Hz, 2H), 2.64 (hept, *J* = 7.1 Hz, 1H), 1.60 (s, 3H), 1.37 (s, 3H), 1.20 (dd, *J* = 7.0, 3.5 Hz, 6H). ^{13}C NMR (76 MHz, CDCl$_3$) δ (ppm) 176.6, 160.5, 148.8, 143.8, 138.7, 138.3, 138.0, 136.6, 129.0, 125.8, 122.9, 116.9, 116.5, 115.6, 115.1, 100.3, 93.8, 87.8, 85.8, 85.1, 85.0, 80.7, 63.8, 34.1, 27.3, 25.5, 19.3, 19.2. HRMS (ESI+): Calculated for $C_{28}H_{25}BrN_2O_7S_2$: 644.0287 [M]$^+$. Found: 667.0176 [M+Na]$^+$.

AdU. This compound was prepared from **Br-dU** (1 eq., 0.071 g), **A1** (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 (10:1) as eluent. **AdU** was obtained as a yellow solid in 72% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 11.93 (s, 1H), 8.54 (s, 2H), 8.23 (s, 1H), 8.00 (s, 1H), 7.76 (s, 1H), 7.11 (s, 2H), 6.23 (s, 2H), 6.11 (s, 1H), 5.87 (s, 1H), 5.57 (d, *J* = 7.2 Hz, 1H), 5.20 – 5.01 (m, 2H), 4.82 (d, *J* = 4.1 Hz, 1H), 4.26 (m, 3H), 4.11 (m, 1H), 3.71 (m, 2H), 2.60 (hept, *J* = 6.9 Hz, 1H), 1.55 (s, 3H), 1.50 (s, 3H), 1.36 (s, 3H), 1.31 (s, 3H), 1.10 (dd, *J* = 6.8, 3.2 Hz, 6H), 0.75 (s, 9H), -0.15 (d, *J* = 5.7 Hz, 6H). ^{13}C NMR (76 MHz, DMSO-d_6) δ (ppm) 175.8, 161.2, 156.3, 150.8, 149.2, 146.1, 138.0, 137.4, 137.3, 137.3, 130.4, 128.4, 127.9, 123.4, 120.8, 117.8, 117.5, 114.0, 113.4, 113.1, 97.7, 92.3, 88.8, 88.8, 88.0, 86.7, 85.6, 85.5, 84.3, 83.8, 82.9, 80.2, 63.7, 63.5, 33.1, 30.6, 27.0, 26.9, 25.7, 25.3, 25.1, 18.8, 18.7, 17.9, -5.5, -5.6. HRMS (MALDI): Calculated for $C_{49}H_{56}N_8O_{11}S_2Si$: 1024.3279 [M]$^+$. Found: 1047.3142 [M+Na]$^+$.

Scheme S3. Synthesis of dinucleosides **GaC** and **AaU** from diiodoBODIPY **I-a-I** *via* two consecutive Sonogashira coupling reactions.
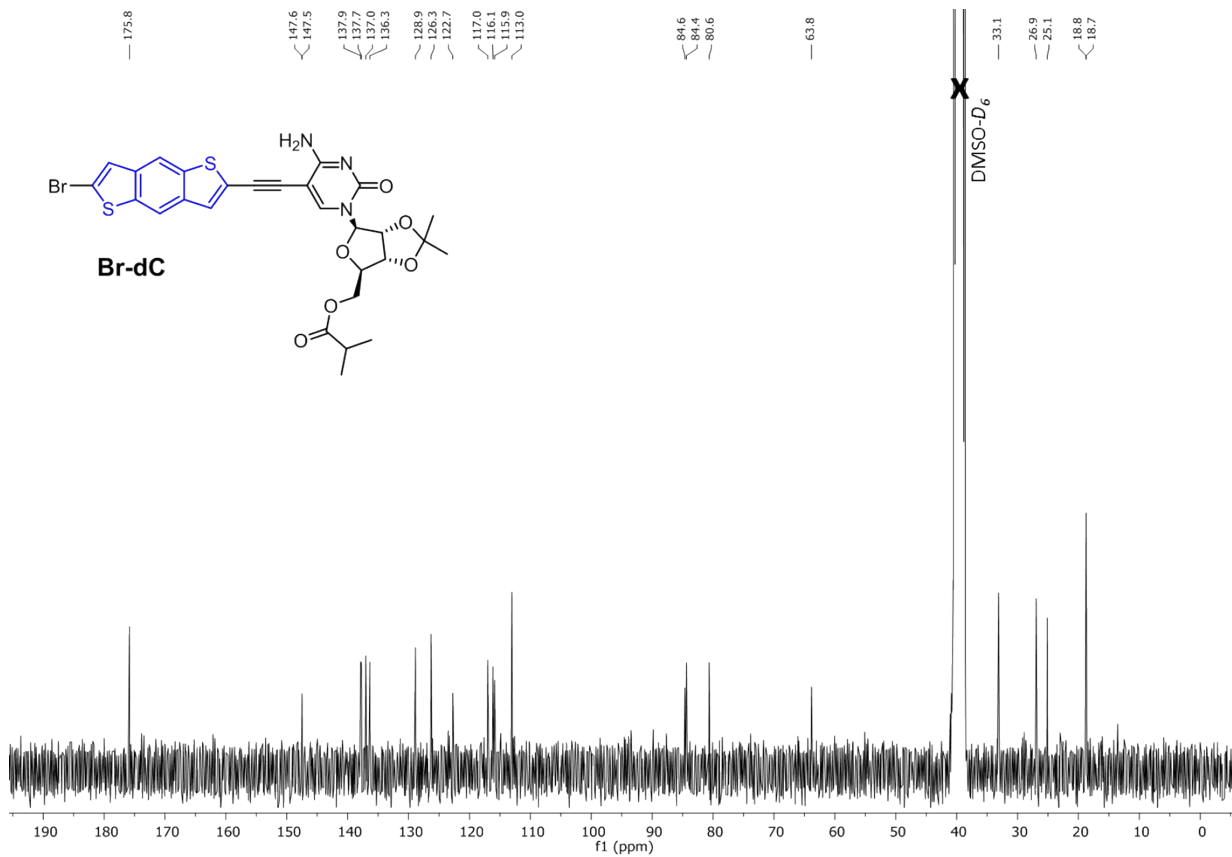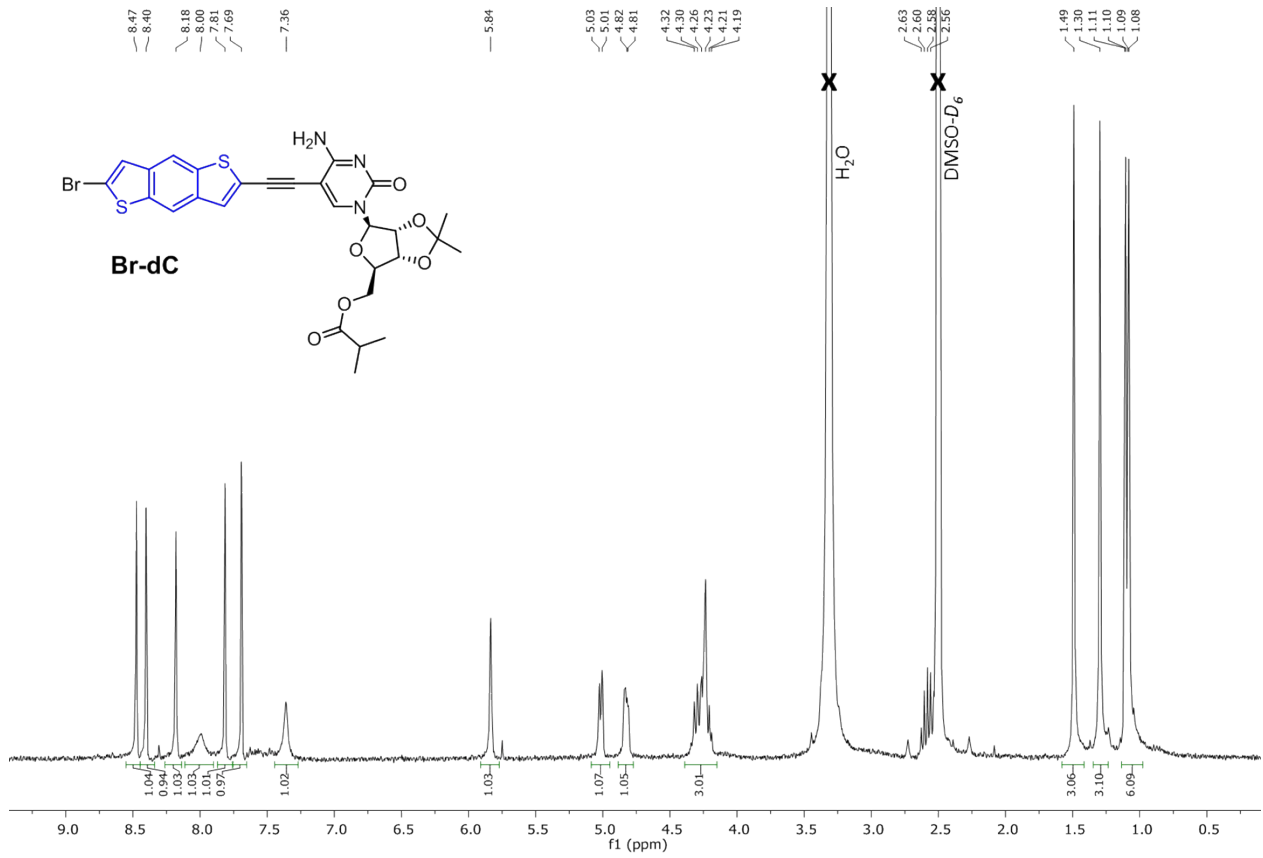
I-aC. This compound was prepared from 4,4'-difluoro-1,3,5,7-dimethyl-8-pentyl-4-bora-3a,4a-diaza-*s*-indacene (3 eq., 0.270 g), **C1** (1 eq., 0.074 g), and a dry DMF/NEt$_3$ (4:1) mixture as solvent. The reaction mixture was stirred at 40ºC overnight. The crude was purified using chloroform/methanol (40:1) as eluent. **I-aC** was obtained as a pink solid in 53% yield. ^{1}H NMR (300 MHz, CDCl$_3$) δ (ppm) 8.34 (s, 1H), 7.71 (s, 1H), 5.85 (s, 1H), 5.70 (s, 1H), 5.00 (d, *J* = 6.1 Hz, 1H), 4.85-4.77 (m, 1H), 4.49-4.26 (m, 3H), 3.00 (m, 1H), 2.70-2.40 (m, 12H), 1.76-0.94 (m, 23H). ^{13}C NMR (76 MHz, CDCl$_3$) δ (ppm) 176.4, 164.8, 157.0, 156.0, 153.9, 147.5, 145.2, 144.9, 142.8, 141.5, 139.4, 132.1, 131.9, 130.3, 128.4, 123.1, 114.0, 95.9, 85.8, 85.5, 81.1, 64.2, 33.8, 32.4, 31.5, 29.1, 27.1, 25.3, 22.5, 19.0, 18.9, 16.6, 15.3, 14.0. HRMS (FAB+): Calculated for C$_{35}$H$_{45}$BF$_2$IN$_5$O$_6$: 819.2476 [M]$^+$. Found: 820.2557 [M+H]$^+$.
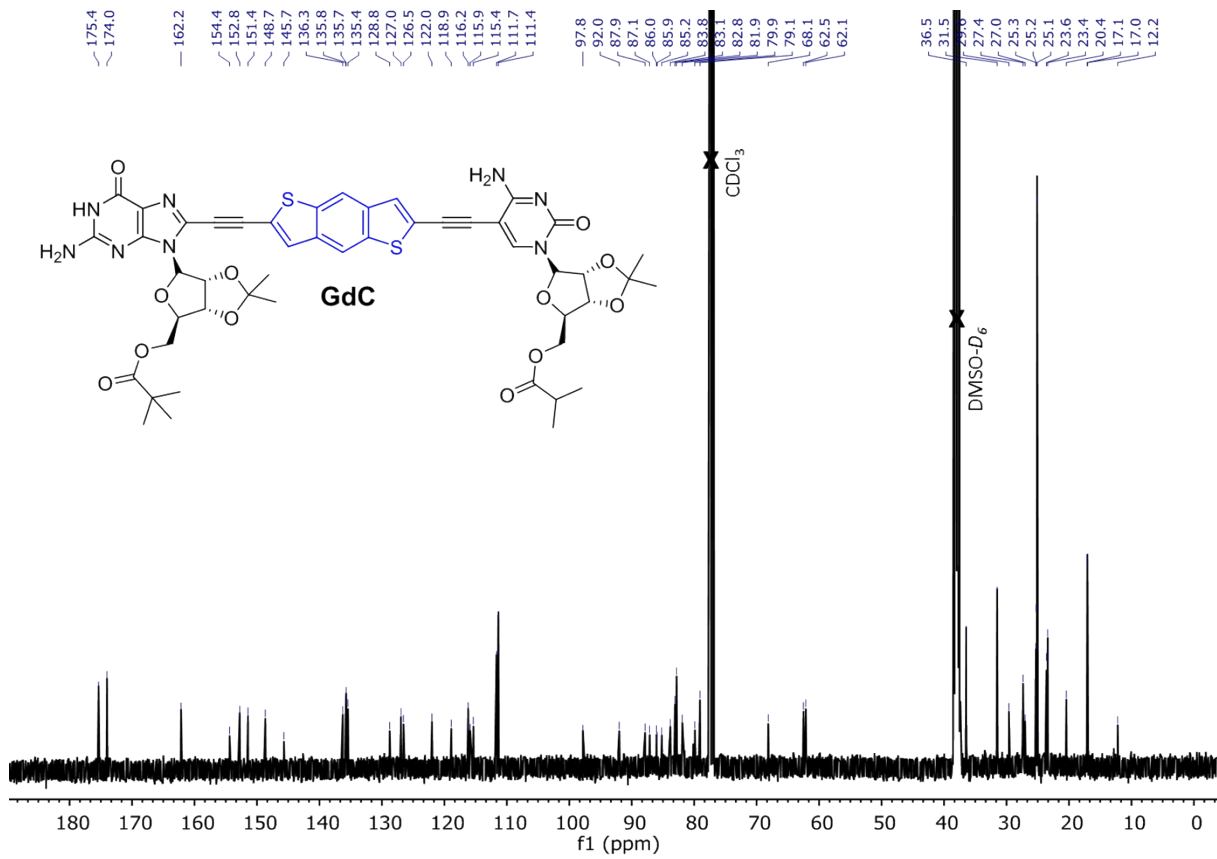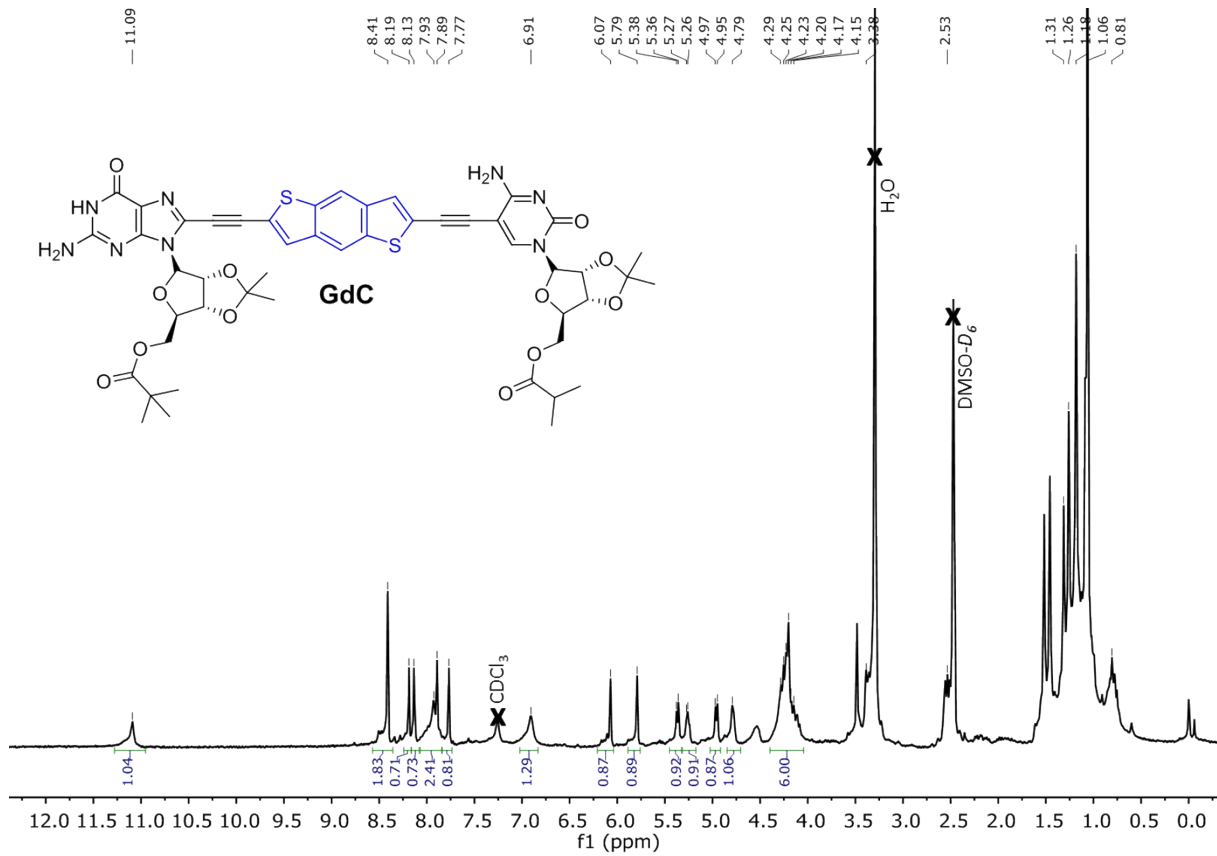
GaC. This compound was prepared from **I-aC** (1 eq., 0.115 g), **G1** (1.1 eq., 0.066 g), and a dry DMF/NEt$_3$ (4:1) mixture as solvent. The reaction mixture was stirred at 40ºC overnight. The crude was purified using chloroform/methanol (10:1) as eluent. **GaC** was obtained as a purple solid in 32% yield. ^{1}H NMR (300 MHz, DMF-*d$_7$*) δ (ppm) 11.11 (s, 1H), 8.34 (s, 1H), 7.85-7.55 (m, 2H), 7.10 (s, 1H), 6.32 (s, 1H), 5.95 (s, 1H), 5.67 (d, *J* = 6.5 Hz, 1H), 5.34 (m, 1H), 5.17 (d, *J* = 4.8 Hz, 1H), 4.96 (m, 1H), 4.37 (m, 6H), 3.18 (m, 1H), 2.70 (m, 12H), 1.80-0.70 (m, 38H). ^{13}C NMR (76 MHz, DMF-*d$_7$*) δ (ppm) 178.6, 177.1, 175.6, 155.9, 133.1, 132.8, 130.0, 114.6, 114.5, 96.0, 91.2, 87.3, 86.7, 86.2, 85.0, 83.5, 82.6, 65.4, 65.3, 39.5, 32.8, 26.0, 25.8, 23.5, 23.3, 19.6, 15.9. HRMS (MALDI): Calculated for C$_{56}$H$_{69}$BF$_2$N$_{10}$O$_{12}$: 1122.5158 [M]$^+$. Found: 1145.5072 [M+Na]$^+$.
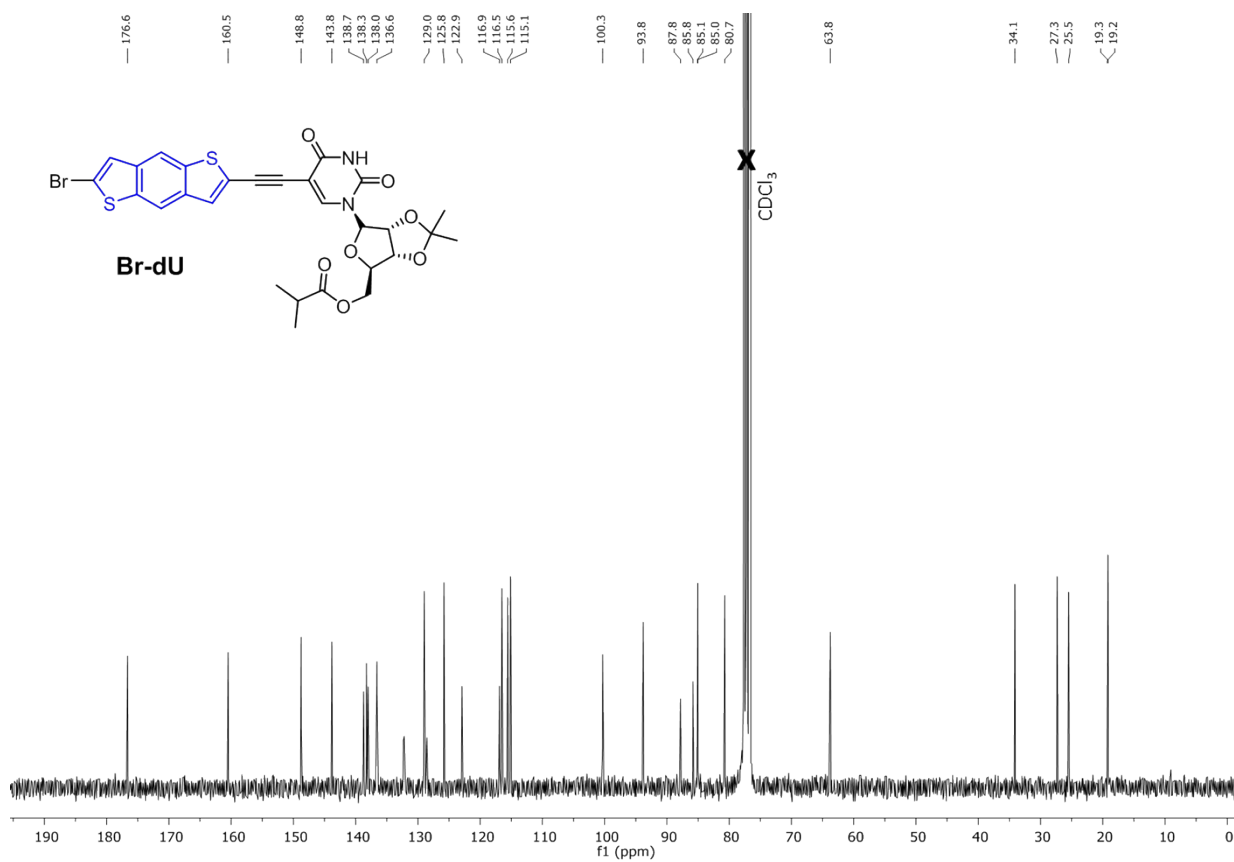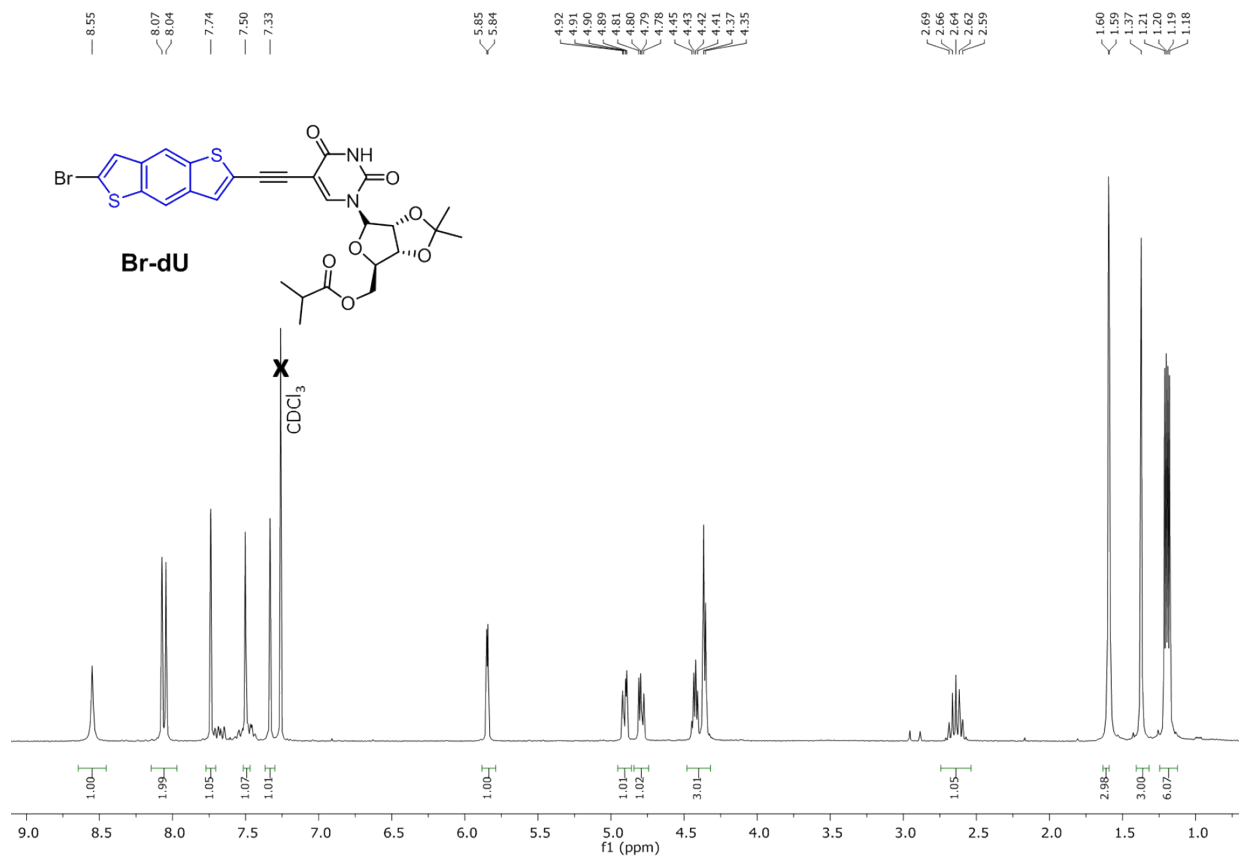
I-aU. This compound was prepared from 4,4'-difluoro-1,3,5,7-dimethyl-8-pentyl-4-bora-3a,4a-diaza-*s*-indacene (3 eq., 0.270 g), **U1** (1 eq., 0.060 g), and a dry DMF/NEt$_3$ (4:1) mixture as solvent. The reaction mixture was stirred at 40ºC overnight. The crude was purified using chloroform/methanol (20:1) as eluent. **I-aU** was obtained as a brown solid in 46% yield. ^{1}H NMR (300 MHz, CDCl$_3$) δ (ppm) 8.50 (s, 1H), 7.62 (s, 1H), 5.78 (d, *J* = 2.1 Hz, 1H), 4.95 (dd, *J* = 6.3, 1.7 Hz, 1H), 4.80 (dd, *J* = 6.1, 4.0 Hz, 1H), 4.44-4.32 (m, 3H), 2.95 (m, 1H), 2.61 (m, 6H), 2.51 (s, 3H), 2.45 (s, 3H), 1.80-0.80 (m, 23H). ^{13}C NMR (76 MHz, CDCl$_3$) δ (ppm) 176.5, 160.7, 156.5, 155.7, 148.7, 147.4, 142.6, 142.4, 142.0, 132.2, 130.6, 114.9, 101.0, 94.3, 87.1, 86.6, 86.2, 85.1, 84.8, 80.8, 63.7, 33.9, 32.5, 31.5, 29.1, 27.2, 25.3, 22.5, 19.1, 19.0, 18.9, 16.1, 15.3, 14.0, 13.7. HRMS (FAB+): Calculated for C$_{36}$H$_{44}$BF$_2$IN$_4$O$_7$: 820.2316 [M]$^+$. Found: 820.2316 [M]$^+$.
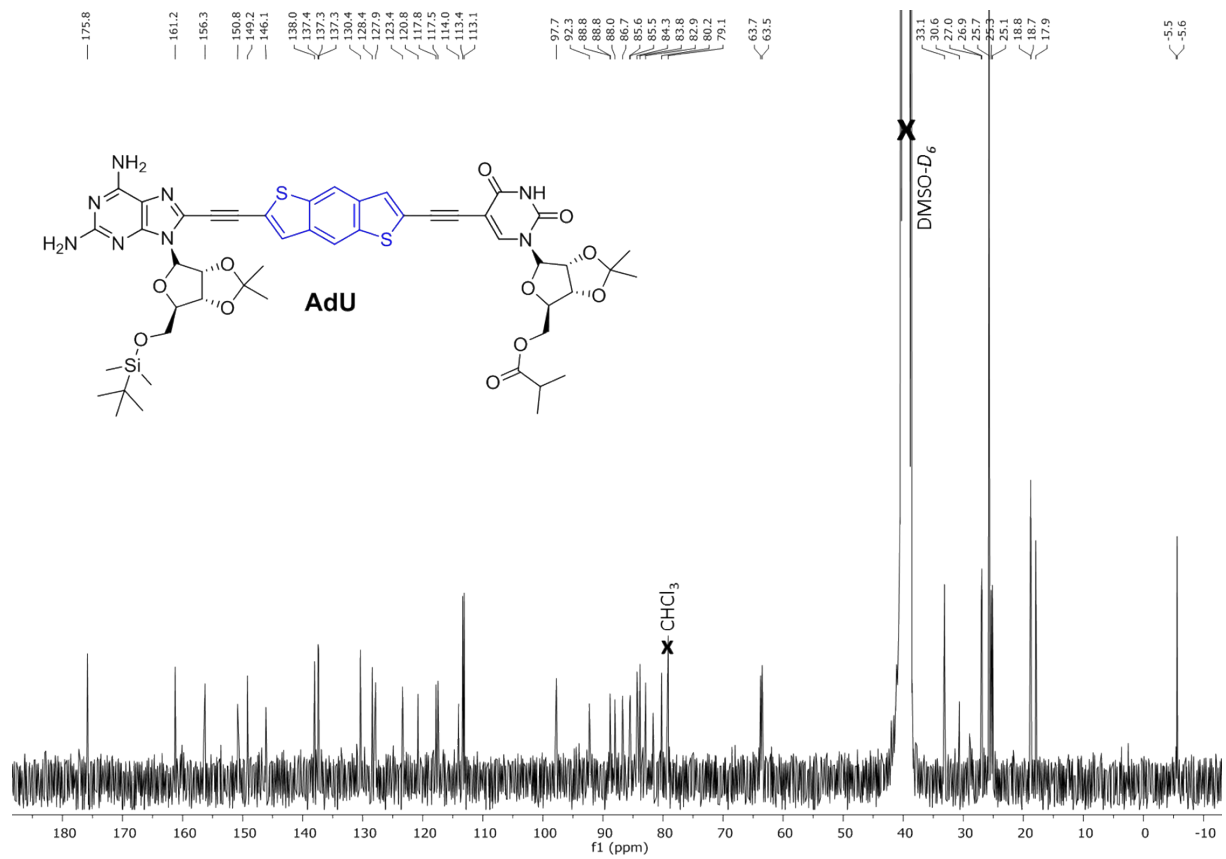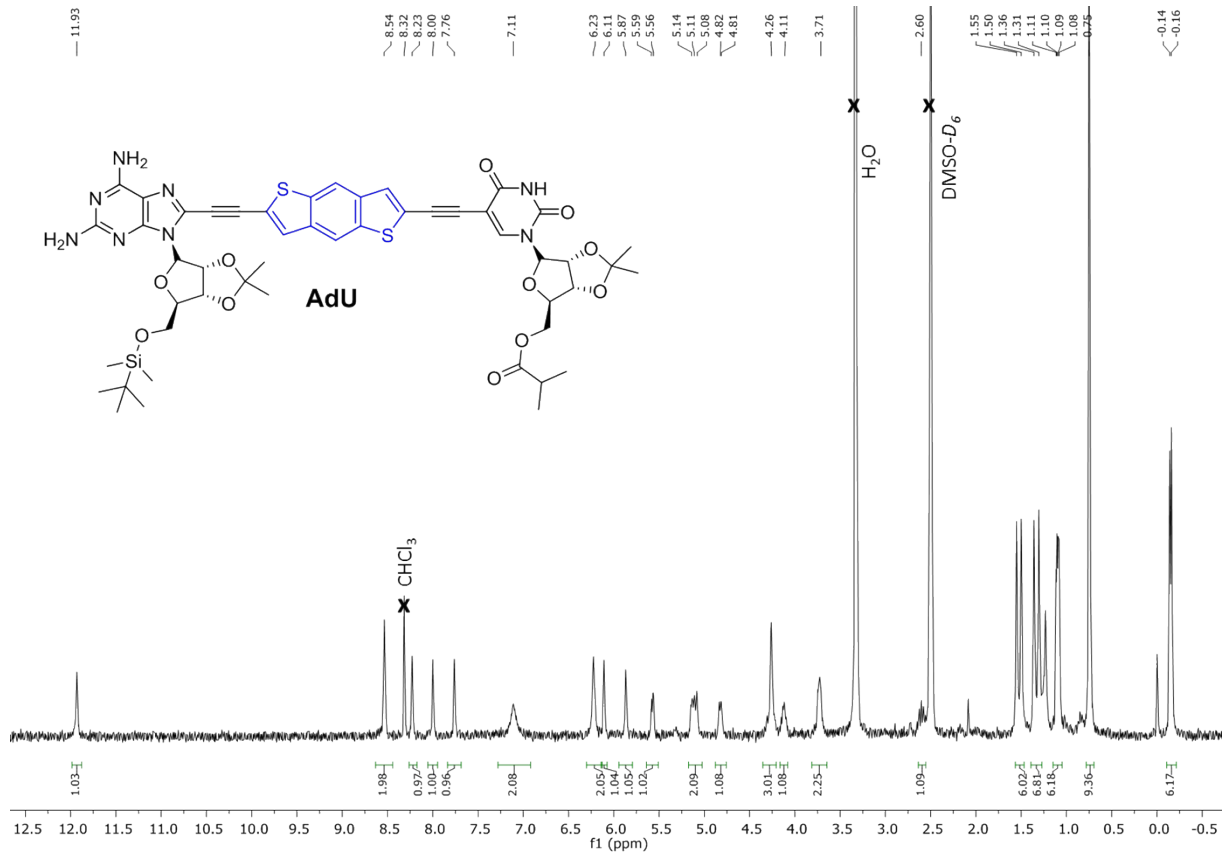
AaU. This compound was prepared from **I-aU** (1 eq., 0.060 g), **A1** (1,1 eq., 0.080 g), and a dry DMF/NEt$_3$ (4:1) mixture as solvent. The reaction mixture was stirred at 40ºC overnight. The crude was purified using chloroform/methanol (20:1) as eluent. **AaU** was obtained as a purple solid in 24% yield. ^{1}H NMR (300 MHz, DMSO-*d$_6$*) δ (ppm) 11.86 (s, 1H), 8.14 (s, 1H), 6.14 (m, 3H), 5.84 (s, 1H), 5.37 (d, *J* = 6.0 Hz, 1H), 5.12 (m, 3H), 4.82 (m, 1H), 4.25 (m, 3H), 4.13 (m, 1H), 3.69 (m, 3H), 3.05 (m, 1H), 2.58 (m, 12H), 1.70-0.50 (m, 38H), -0.16 (d, *J* = 6.0 Hz, 6H). ^{13}C NMR (76 MHz, DMSO-*d$_6$*) δ (ppm) 175.8, 161.3, 160.9, 156.2, 155.3, 150.7, 149.3, 145.0, 142.7, 131.3, 129.3, 113.6, 113.2, 112.8, 98.5, 92.9, 89.0, 88.3, 86.1, 84.5, 83.8, 82.6, 80.5, 67.0, 63.7, 45.8, 33.1, 31.8, 26.9, 25.7, 25.5, 25.2, 25.1, 21.7, 18.7, 18.7, 17.9, 14.9, 14.7, 13.8, 13.3, -5.5, -5.6. HRMS (MALDI): Calculated for C$_{57}$H$_{75}$BF$_2$N$_{10}$O$_{11}$Si: 1152.5447 [M]$^+$. Found: 1152.5427 [M]$^+$.
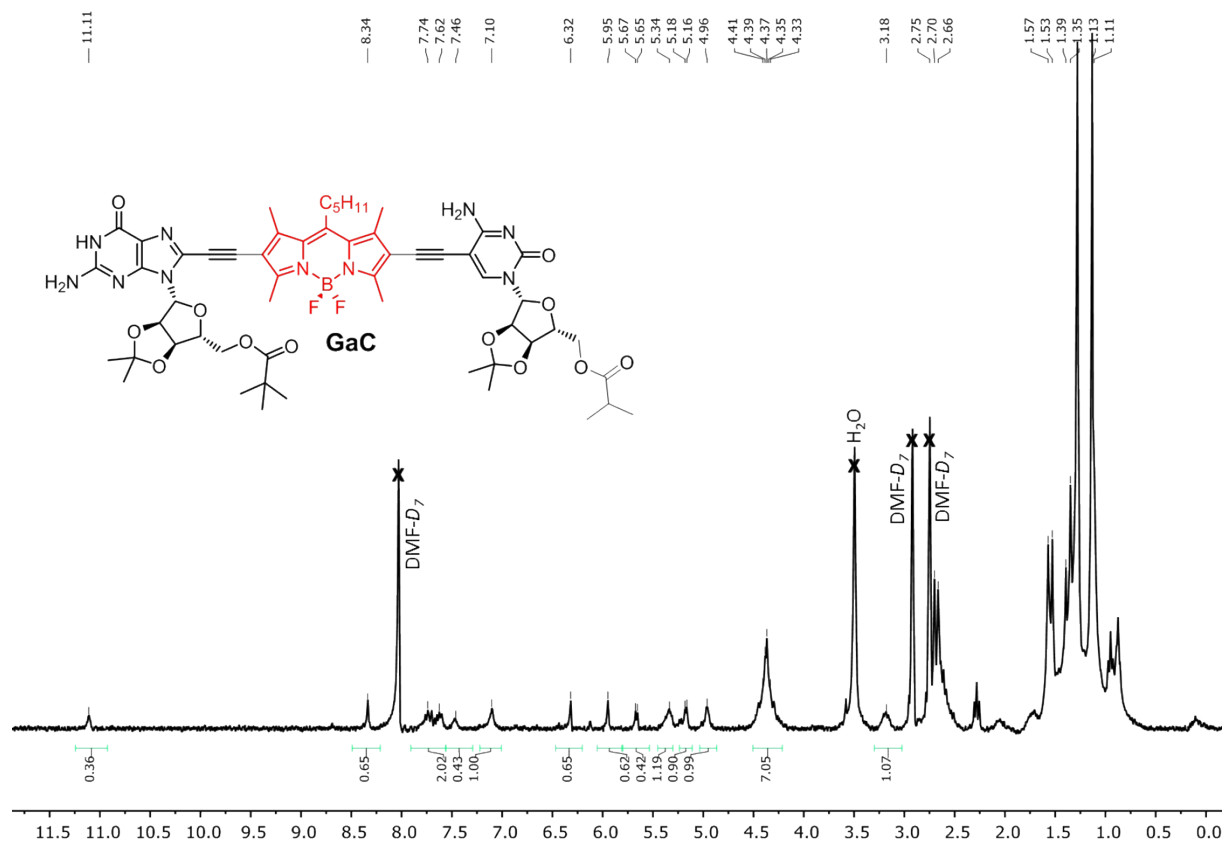
S0B. ¹H NMR Spectra

¹H NMR

8.55

8.07
8.04
7.74
7.50
7.33

5.85
5.84

4.92
4.91
4.90
4.89
4.81
4.80
4.79
4.78
4.45
4.43
4.42
4.41
4.37
4.35

2.69
2.66
2.64
2.62
2.59

1.60
1.59
1.37
1.21
1.20
1.19
1.18

Br-dU

X

CDCl₃

1.00 1.99 1.05 1.07 1.01 1.00 1.01 1.02 3.01 1.05 2.98 3.00 6.07

9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0

f1 (ppm)

¹³C NMR

176.6

160.5

148.8
143.8
138.7
138.3
138.0
136.6

129.0
125.8
122.9

116.9
116.5
115.6
115.1

100.3

93.8

87.8
85.8
85.1
85.0
80.7

63.8

34.1

27.3
25.5

19.3
19.2

Br-dU

X

CDCl₃

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

f1 (ppm)

S8

11.86

8.14

6.14
6.13
5.84
5.68
5.66
5.14
5.12
5.09
4.82
4.80
4.26
4.25
4.13
3.70
3.68

2.60
2.58
2.56
1.52
1.50
1.40
1.35
1.30
1.23
1.09
1.07
1.06
1.05
0.93
0.91
0.88
0.76
-0.15
-0.17

H₂O

DMSO-*D₆*

C₅H₁₁

AaU

0.88

0.97

3.00
0.98
1.00

2.66
1.21

3.31
1.17
2.30

12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5

175.8

161.3
160.9
156.2
155.3
150.7
149.3
145.0
142.7

131.3
129.3

113.6
113.2
112.8

98.5
92.9
89.0
88.3
86.1
84.5
83.8
82.6
80.5

67.0
63.7

45.8

33.1
31.8
26.9
25.7
25.5
25.2
25.1
21.7
18.7
18.7
17.9
14.9
14.7
13.8
13.3

-5.5
-5.6

DMSO-*D₆*

C₅H₁₁

AaU

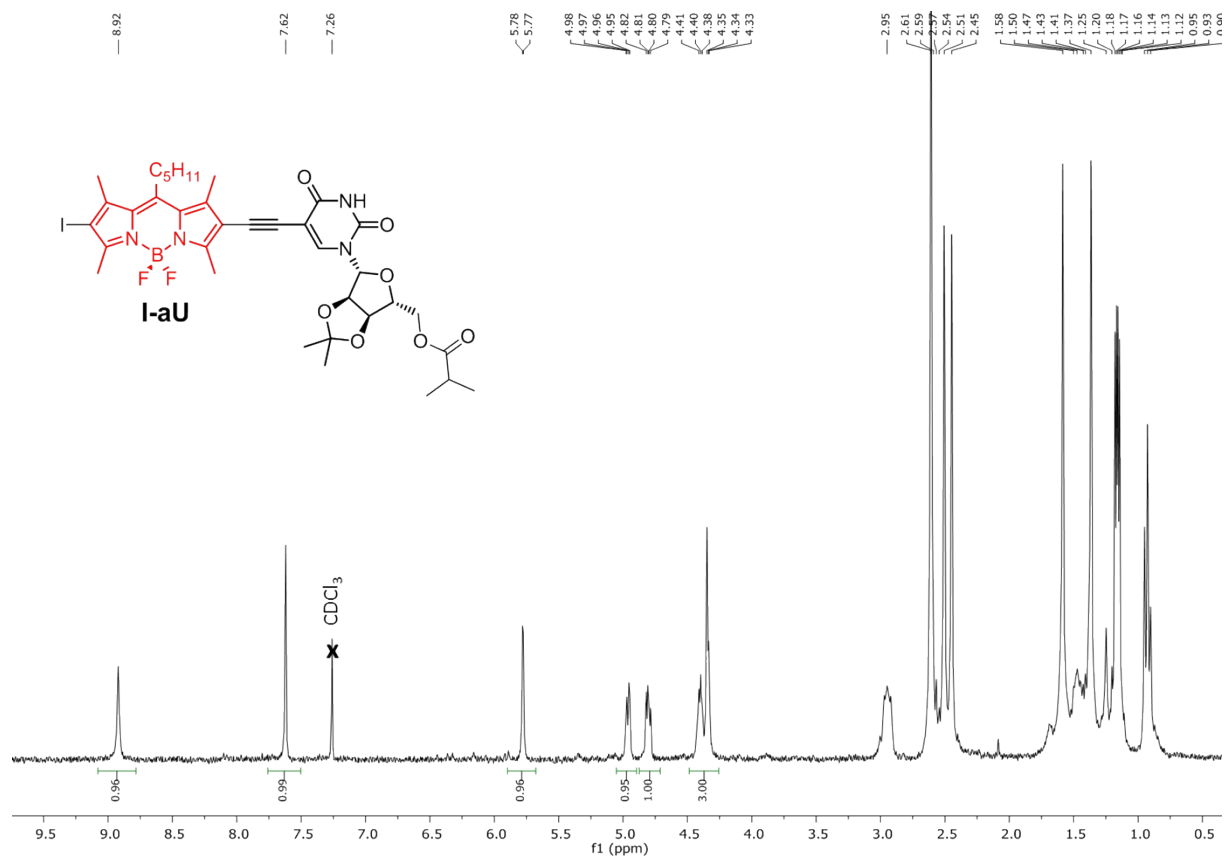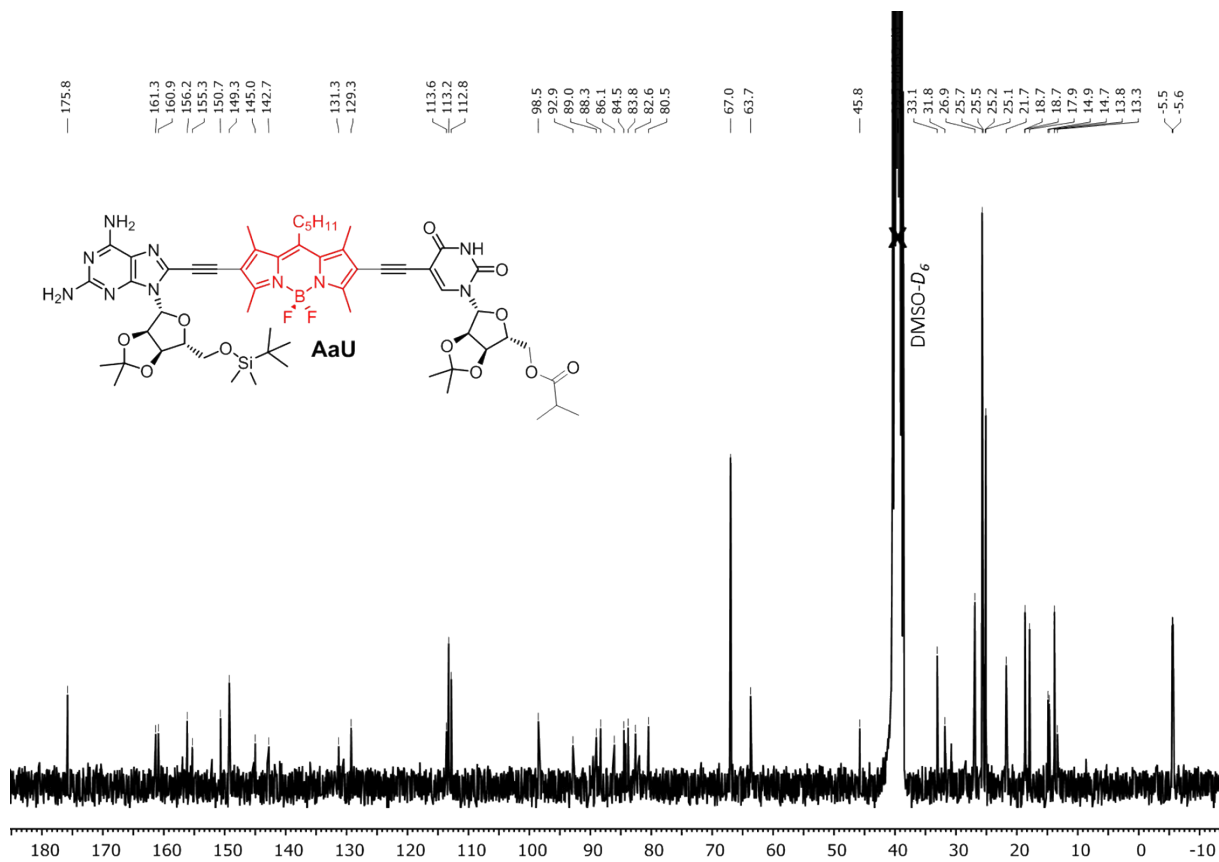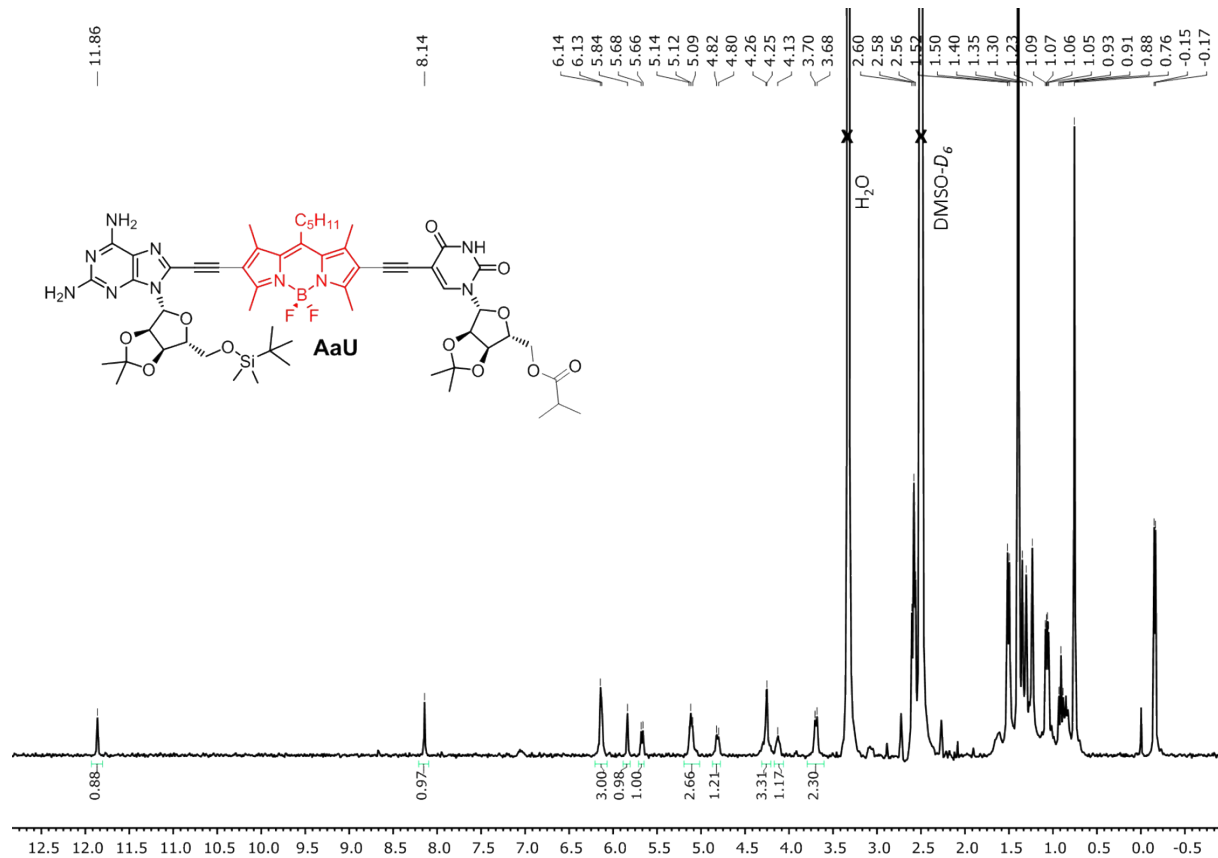180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10

S0C. Photophysical characterization

Fluorescence quantum yields were obtained following the comparative method of Williams *et al*.[3] at room temperature. A solution of anthracene in deoxygenated cyclohexane was used as standard[4] 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.[4] Acceptor species were dissolved in deoxygenated toluene. The emission spectra of the corresponding compounds were measured using an excitation wavelength of 540 nm.

Table S0. Most relevant photophysical parameters of the mononucleosides **C**, **U**, **aC**, **aU**,[5] and dinucleosides **GdC**, **AdU**, **GaC** and **AaU** in toluene.

Compound	λ_{max}^{ab} nm	ε_{max} $M^{-1}cm^{-1}$	λ_{max}^{em} nm	Φ_f
C	331	61700	366, 384	0.25
U	330	40400	368, 389	0.25
aC	564	44700	609	0.64
aU	568	36000	602	0.59
GdC	386	39398	432, 458	0.20
AdU	399	36457	437, 462	0.36
GaC	553	39318	614	0.13
AaU	560	33876	611	0.27

[3] A. T. R. Williams, S. A. Winfield and J. N. Miller, *Analyst*, 1983, **108**, 1067-1071.

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

[5] M. J. Mayoral, J. Camacho-García, E. Magdalena-Estirado, M. Blanco-Lomas, E. Fadaei, C. Montoro-García, D. Serrano-Molina and D. González-Rodríguez, *Org. Biomol. Chem.*, 2017, **15**, 7558-7565.

S1. DOSY measurements and molecular size estimation

Diffusion NMR experiments were performed with **GdC** solutions in $CDCl_3$ and $CDCl_3/DMSO\text{-}D_6$ mixtures in the presence of equimolar amounts of mesitylene, which served as an internal reference (Figure S1A). The use of an internal reference whose hydrodynamic radius is known, like mesitylene in this case (R_{ref} = 3 Å), allows the estimation of the aggregate size by direct D_0 comparison, using the following equation:[6,7]

$$R_0 = \frac{R_{ref}D_{ref}}{D_0}$$
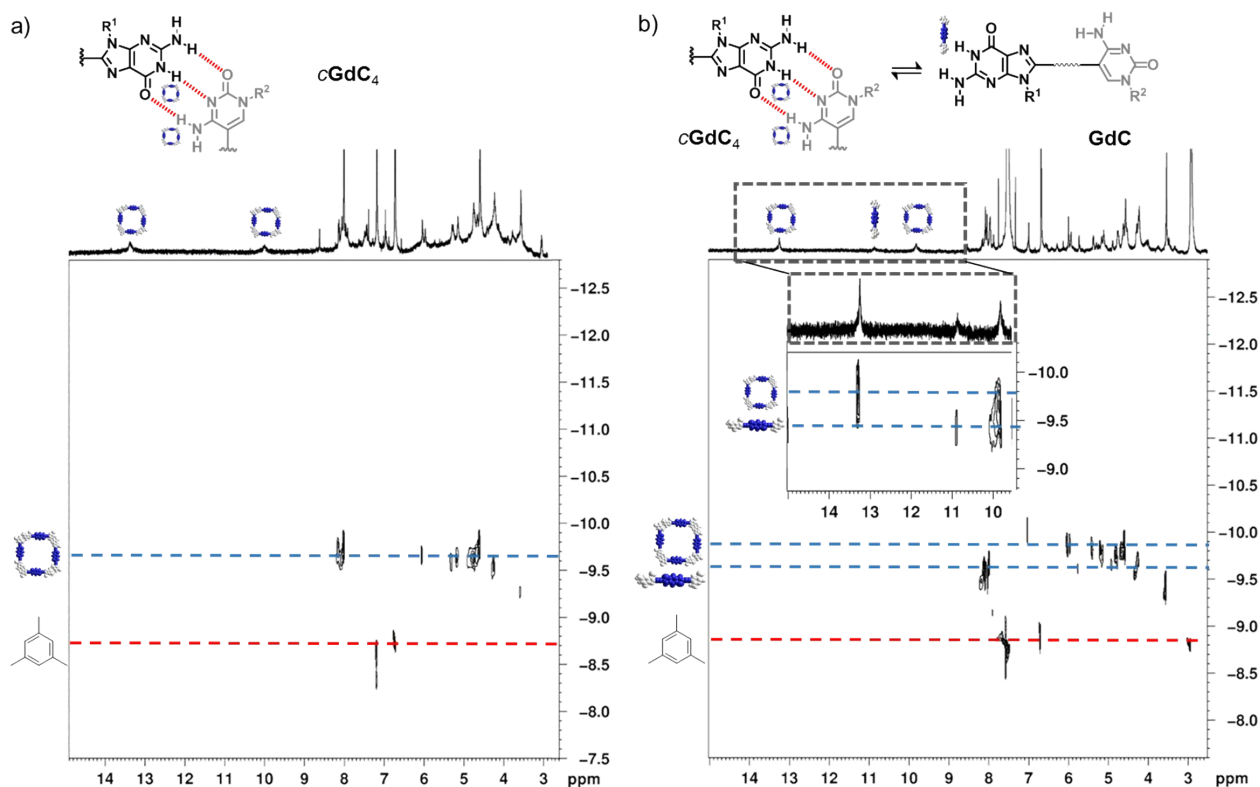
Figure S1A. DOSY NMR spectra of **GdC** (**a**) in $CDCl_3$, 10^{-2} M, 298 K and (**b**) in $CDCl_3$:DMSO-D_6 18% (v/v) 10^{-2} M, 298 K. The average diffusion coefficients measured are indicated in blue for **GdC** and in red for mesitylene, which was employed as an internal reference.

DOSY experiment carried out in $CDCl_3$ (Figure S1A-a) revealed a single diffusing self-assembled species. The characteristic signals for G:C H-bonding (G-amide and C-H amine protons) are found around 13.5 and 10.0 ppm, respectively.[7] The diffusion coefficients obtained from the T1/T2 relaxation curves match an assembly whose hydrodynamic radius agrees with the expected size of a cyclic tetramer, estimated from molecular modelling studies (see Table S1A). However, in $CDCl_3$/DMSO-D_6 82:18 (v/v) the DOSY experiment displayed the presence of two species in slow exchange: the **cGdC4** tetramer, having a lower diffusion coefficient, and the corresponding monomer **GdC4**. In this case the corresponding "solvated" G-H amide signal of the monomer at around 10.8 ppm is also observed (see inset in Figure S1A-b).[7]

[6] (a) P. Timmerman, J.-L. Weidmann, K. A. Jolliffe, L. J. Prins, D. N. Reinhoudt, S. Shinkai, L. Frish and Y. Cohen, *J. Chem. Soc., Perkin Trans.*, 2000, **2**, 2077–2089; b) Y. Cohen, L. Avram and L. Frish, *Angew. Chem. Int. Ed.* 2005, **44**, 520–554.

[7] C. Montoro-García, J. Camacho-García, A. M. López-Pérez, N. Bilbao, S. Romero-Pérez, M. J. Mayoral and D. González-Rodríguez, *Angew. Chem. Int. Ed.*, 2015, **54**, 6780–6784; (b) 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**, 223–227. (c) C. Montoro-García, M. J. Mayoral, R. Chamorro and D. González-Rodríguez, *Angew. Chem. Int. Ed.*, 2017, **56**, 15649 –15653.

Table S1A. Diffusion coefficients and estimated hydrodynamic diameter of **GdC**.

Solvent		Mesitylene	cGdC$_4$	GdC
	D_{ref} (m^{2}s^{-1})[a]	3.10·10^{-11}		
	R_{ref} (Å)	3		
CDCl$_3$	D_0 (m^{2}s^{-1})[a]		4.31·10^{-12}	-
	R_0 (Å)		21.5	-
	Diameter (nm)		**4.3**	
	D_{ref} (m^{2}s^{-1})[a]	2.10·10^{-11}		
	R_{ref} (Å)	3		
CDCl$_3$/DMSO-D$_6$ (18 % vv)	D_0 (m^{2}s^{-1})[a]		2.54·10^{-12}	3.59·10^{-12}
	R_0 (Å)		24.7	16.3
	Diameter (nm)		**4.9**	

[a] The diffusion coefficients were measured at 298 K in a 3-mm NMR tube and using mesitylene as internal reference
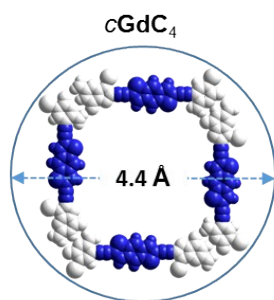
We also performed similar experiments for **AaU**, in which both the base pair and the central dye are different. In this case, in analogy with previous dilution NMR experiments with **AU**,[7] the ^{1}H NMR spectrum in CDCl$_3$ shows a slow equilibrium between cAaU$_4$ and a small amount of monomer **AaU**, which is at the same time in fast equilibrium with small open oligomers (Figure S1B). Despite this sample is not ideal for DOSY experiments, the calculated diffusion coefficients are again in good agreement with those the size expected for a tetramer, estimated from molecular modelling studies (Table S1B).
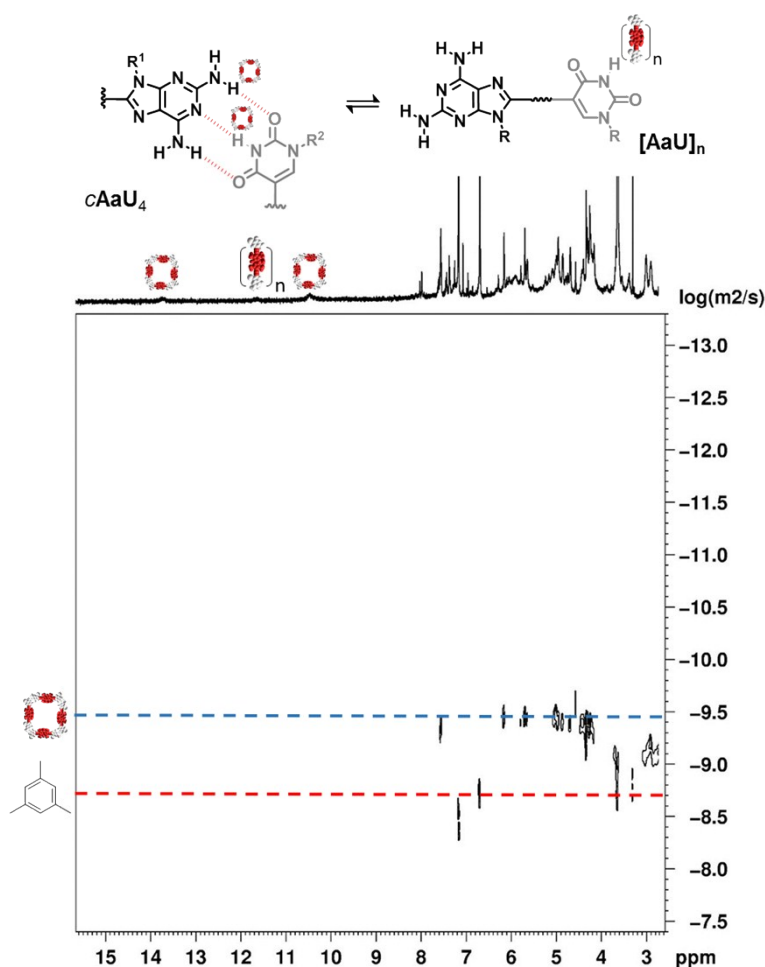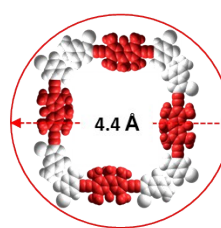

Figure S1B. DOSY NMR spectrum of **AaU** in CDCl$_3$, 10^{-2} M, 298 K. The average diffusion coefficients measured are indicated in blue for **AaU** and in red for mesitylene, which was employed as an internal reference.

Table S1B. Diffusion coefficients and estimated hydrodynamic diameter of **AaU**.

c**AaU**$_4$

4.4 Å

Solvent		Mesitylene	cAaU$_4$
CDCl$_3$	D_{ref} (m^{2}s^{-1})[a]	4.63·10^{-11}	
	R_{ref}(Å)	3	
	D_0 (m^{2}s^{-1})[a]		6.59·10^{-12}
	R_0 (Å)		21.1
	Diameter (nm)		**4.2**

[a] The diffusion coefficients were measured at 298 K in a
3-mm NMR tube and using mesitylene as internal reference

S2. Temperature-dependent emission and CD experiments

Diverse CD and emission measurements at different concentration and as a function of the temperature were also performed for **GdC** (Figure S2A), **GaC** (Figure S2B), **AdU** (Figure S2C), and **AaU** (Figure S2D).

In the case of **GdC** or **GaC**, which form more stable assemblies, the characteristic spectroscopic features of the cyclic tetramers (emission maxima at ca. 530 nm (c**GdC$_4$**) and 623 (c**GaC$_4$**) and Cotton effects at the absorption maxima) remain virtually invariable in toluene, and only start to display the typical monomer features at high temperatures and/or concentrations below $5 \cdot 10^{-6}$ M (see for instance Figure S2A-d, measured at a concentration of 1.0×10^{-6} M, too low for CD measurements).
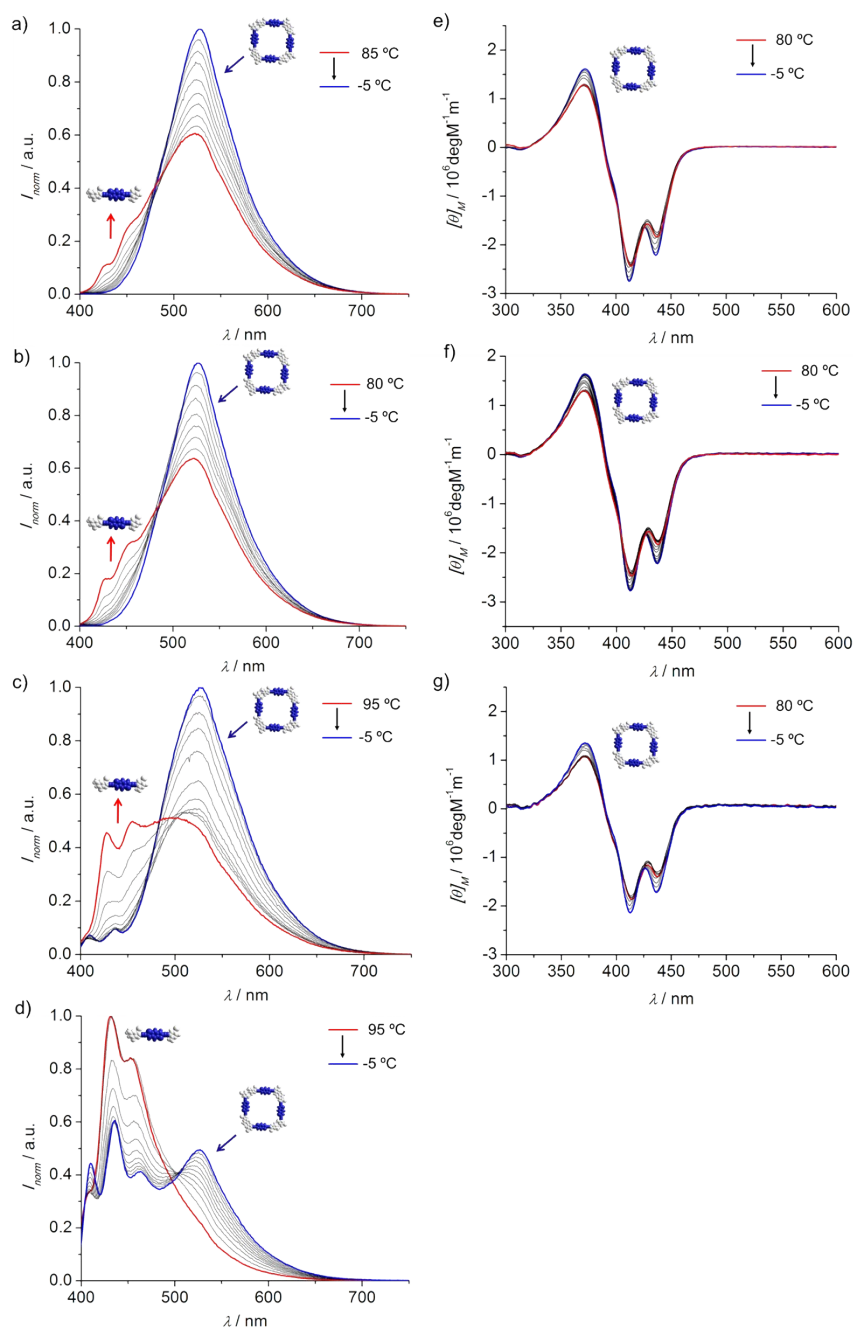
Figure S2A. Temperature-dependent emission spectra of **GdC** in toluene at **a**) 2.0×10^{-5} M, **b**) 1.0×10^{-5} M, **c**) 5.0×10^{-6} M and **d**) 1.0×10^{-6} M (λ_{exc} = 385 nm). Temperature-dependent CD spectra of **GdC** in toluene at **e**) 2.0×10^{-5} M, **f**) 1.0×10^{-5} M and **g**) 5.0×10^{-6} M.
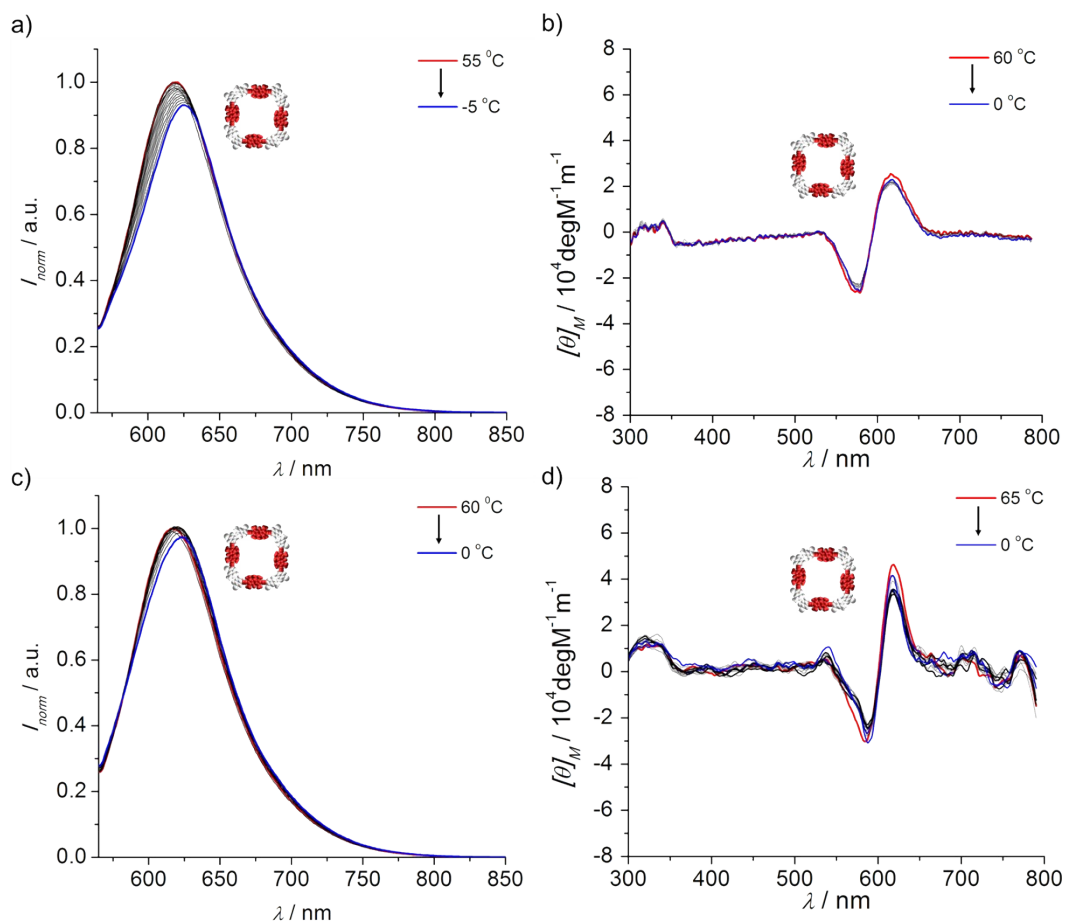
Figure S2B. Temperature-dependent emission spectra of **GaC** in toluene at (**a**) 1.0×10^{-5} M and (**c**) 5.0×10^{-6} M (λ_{exc} = 555 nm). Temperature-dependent CD spectra of **GaC** in toluene at (**b**) 1.0×10^{-5} M and (**d**) 5.0×10^{-6} M.

For **AdU** or **AaU**, on the contrary, the cyclic tetramers are not formed quantitatively within the measured concentration range, and are dissociated rapidly by increasing temperature and/or decreasing concentration.
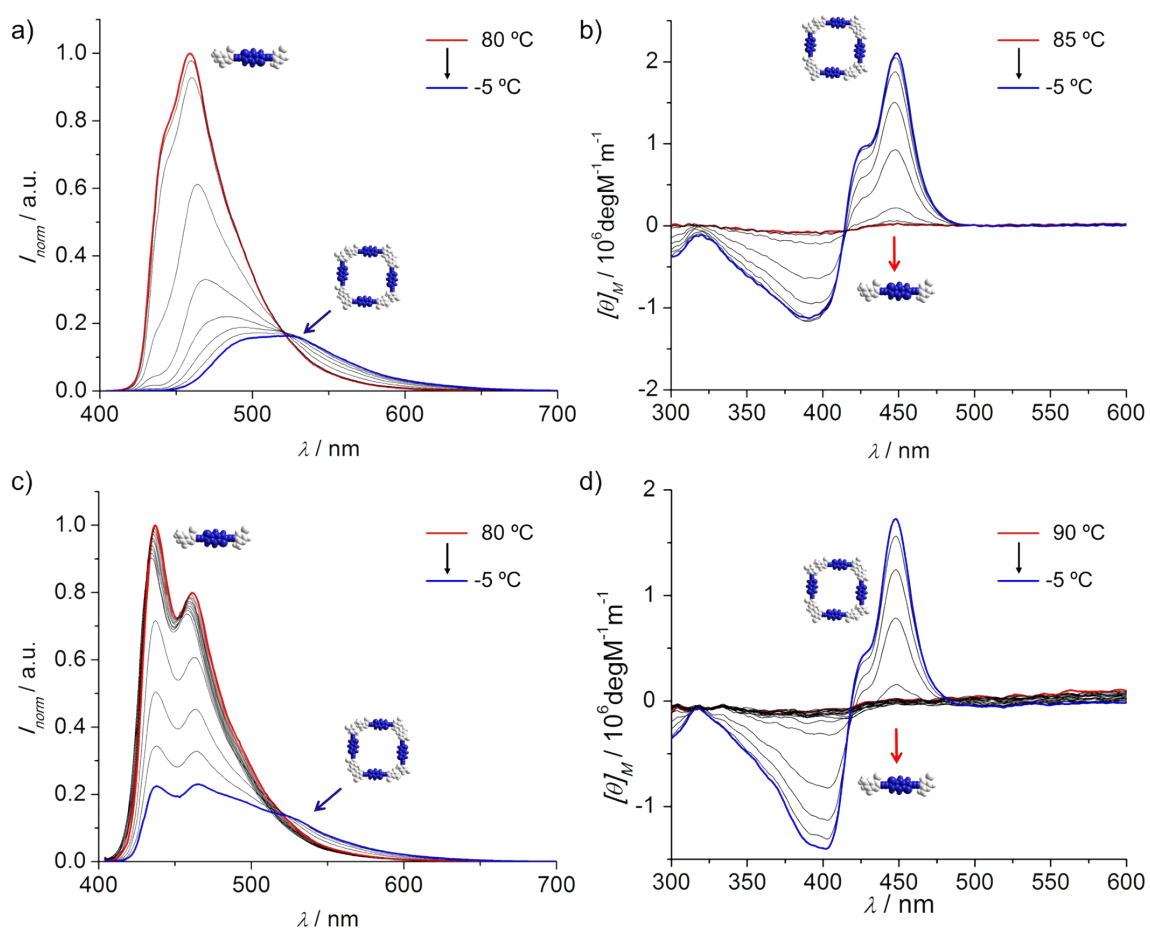
Figure S2C. Temperature-dependent emission (**a**,**c**) and CD (**b**,**d**) spectra of **AdU** in toluene at 1.0×10^{-4} M (**a**,**b**) and 1.4×10^{-5} M (**c**,**d**).
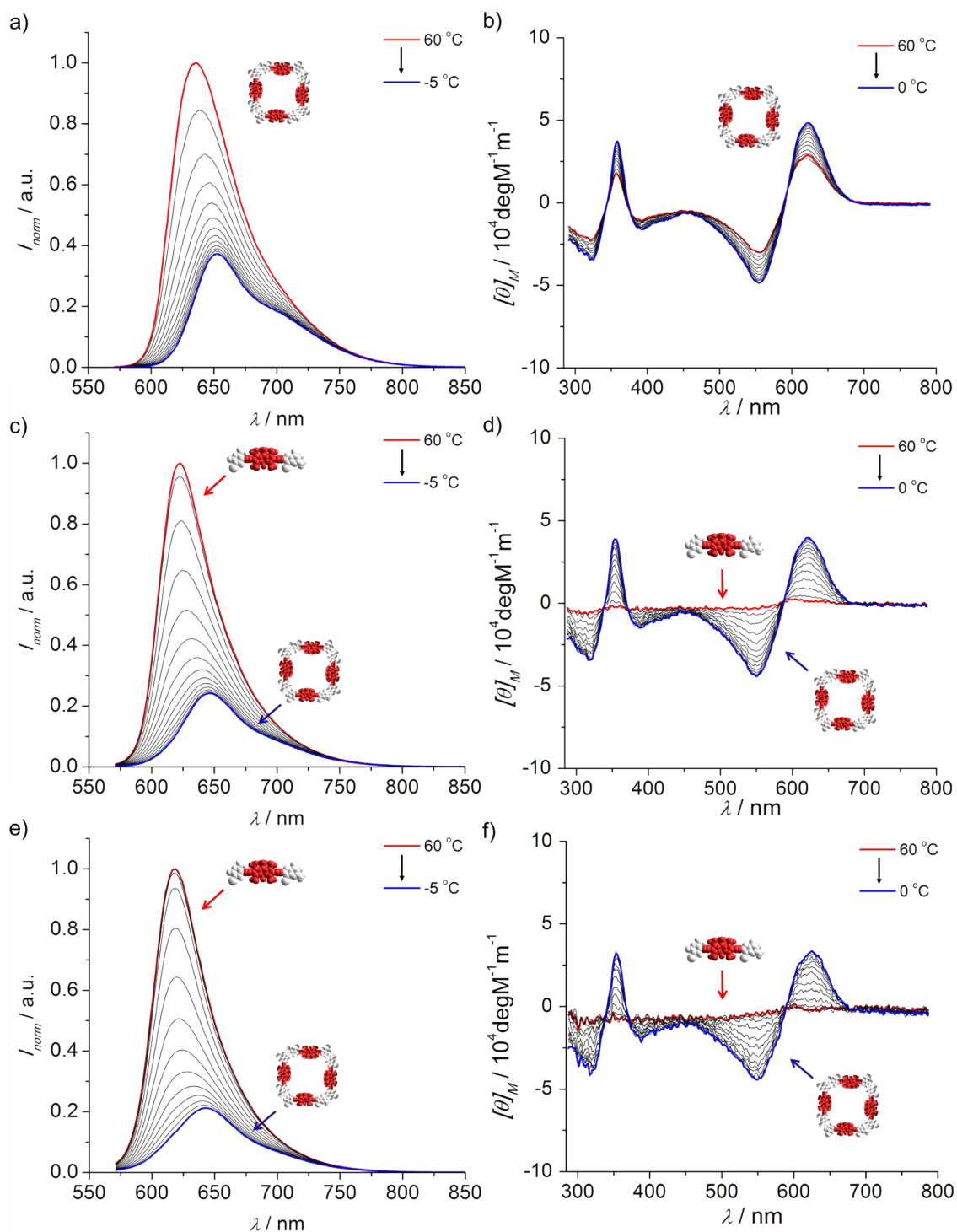
Figure S2D. Temperature-dependent emission (λ_{exc} = 561 nm) (**a**,**c**,**e**) and CD (**b**,**d**,**f**) spectra of **AaU** in toluene at 2.4x10⁻⁴ M (**a**,**b**) 1.0x10⁻⁴ M (**c**,**d**) and 5.0x10⁻⁵ M (**e**,**f**).
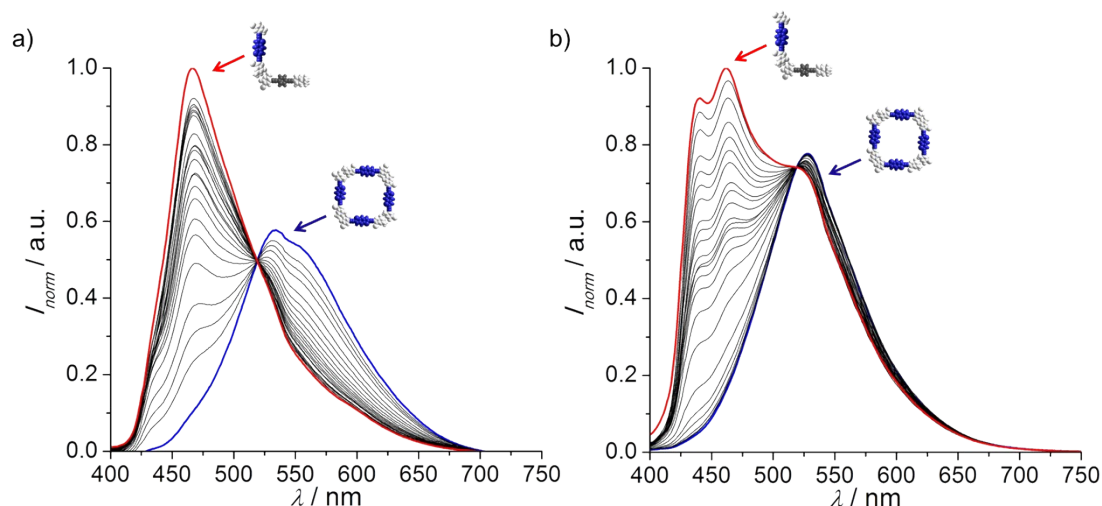
S3. Denaturation experiments in toluene

Figure S3A. Normalized fluorescence emission changes (λ_{exc} = 385 nm, T = 298 K, toluene) observed in the titration of **GdC** with increasing amounts of **C**, (**a**) [**GdC**] = $1.0 \cdot 10^{-4}$ M, [**C**] = $1.4 \cdot 10^{2}$ M and (**b**) [**GdC**] = $1.0 \cdot 10^{-5}$ M, [**C**] = $4.0 \cdot 10^{-4}$ M.
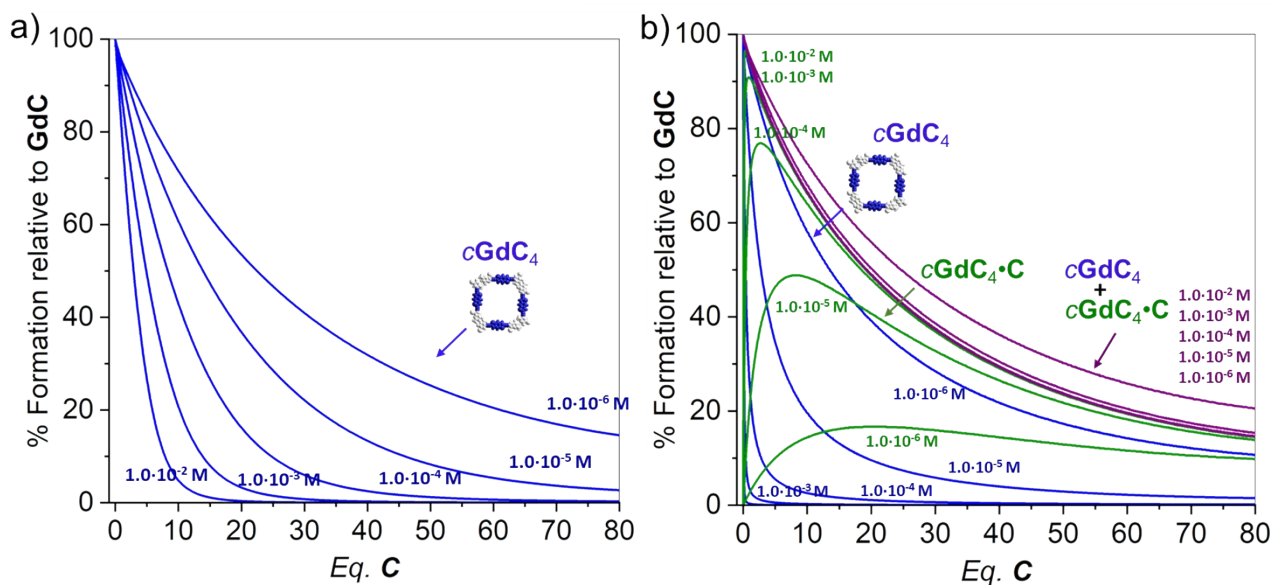
Figure S3B. Simulated speciation profiles of the degree of c**GdC**$_4$ cyclic tetramer association as a function of the equivalents of complementary **C** pyrimidine mononucleoside at different overall concentrations **a**) without considering peripheral binding and **b**) taking into account peripheral binding. The curves represent the relative abundance of c**GdC**$_4$ cycles (blue), peripherally bound c**GdC**$_4 \cdot$**C** cycles (green) and the sum of both species (purple). As can be noted from Fig. S3Bb, the sum of these cyclic species shows a weaker dependence on overall concentration.
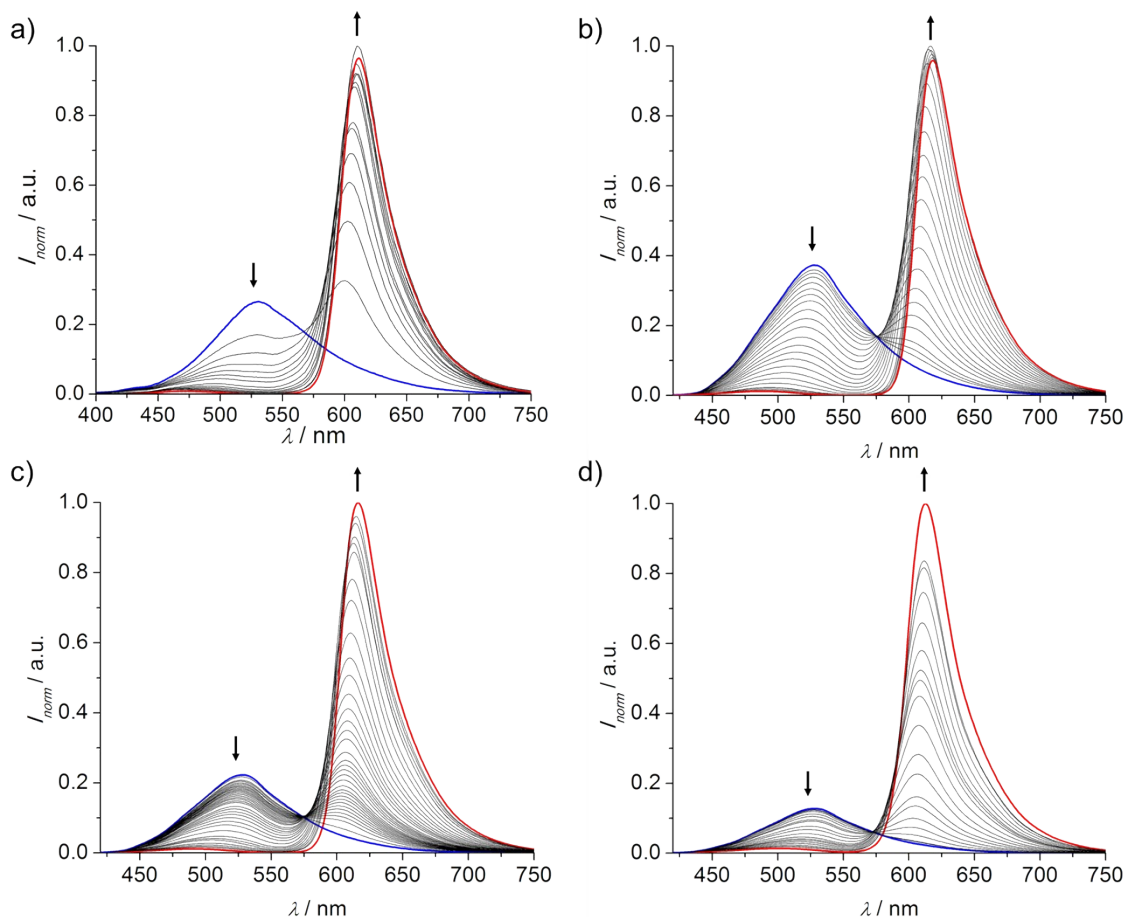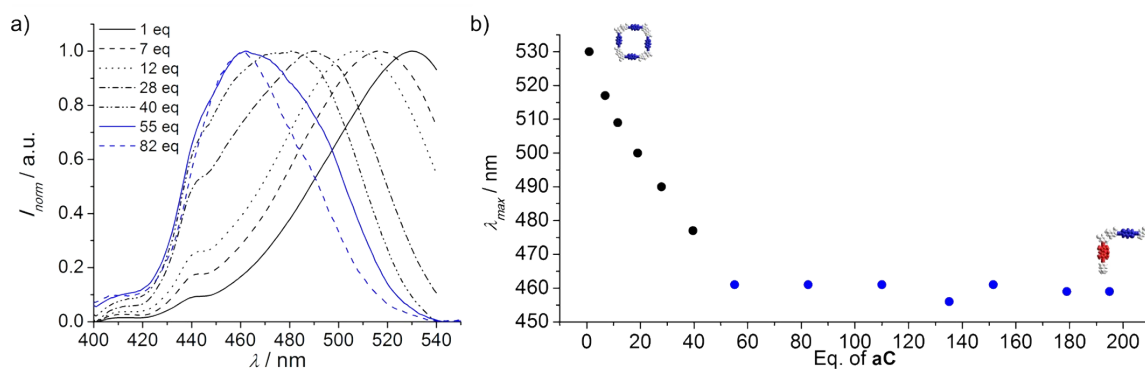
Figure S3C. Normalized fluorescence emission changes observed in the titration of **GdC** with increasing amounts of **aC** in toluene (up to 8 equivalents of **aC**). **a**) ([**GdC**] = $1.0 \cdot 10^{-4}$ M, [**aC**] = $2.0 \cdot 10^{-4}$ M, **b**) [**GdC**] = 2.0×10^{-5} M, [**aC**] = 2.0×10^{-4} M, **c**) [**GdC**] = 1.0×10^{-5} M, [**aC**] = 1.0×10^{-4} M and **d**) [**GdC**] = 5.0×10^{-6} M, [**aC**] = 5.0×10^{-5} M (T = 298 K, λ_{exc} = 385 nm).

Figure S3D. a) Normalized fluorescence emission changes observed in the titration of **GdC** with increasing amounts of **aC** in toluene (up to 200 equivalents of **aC**; [**GdC**] = 2.5×10^{-6} M, [**aC**] = 1.0×10^{-3} M, T = 298 K, λ_{exc} = 385 nm. **b**) Plot of the emission maximum of **GdC** as a function of the equivalents of **aC** added.
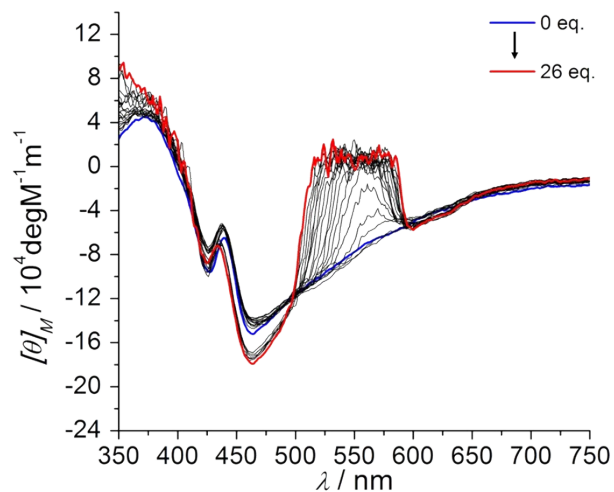
Figure S3E. CD signal changes (λ_{exc} = 385 nm, T = 298 K, toluene) observed in the titration of **GdC** with increasing amounts of **aC** ([**GdC**] = 1.0·10^{-4} M, [**dC**] = 7.0·10^{-3} M). Absorption saturation from the excess of **aC**/**aU** molecules in the **GdC**/**adU** absorption region produces a considerable distortion of the CD spectra,
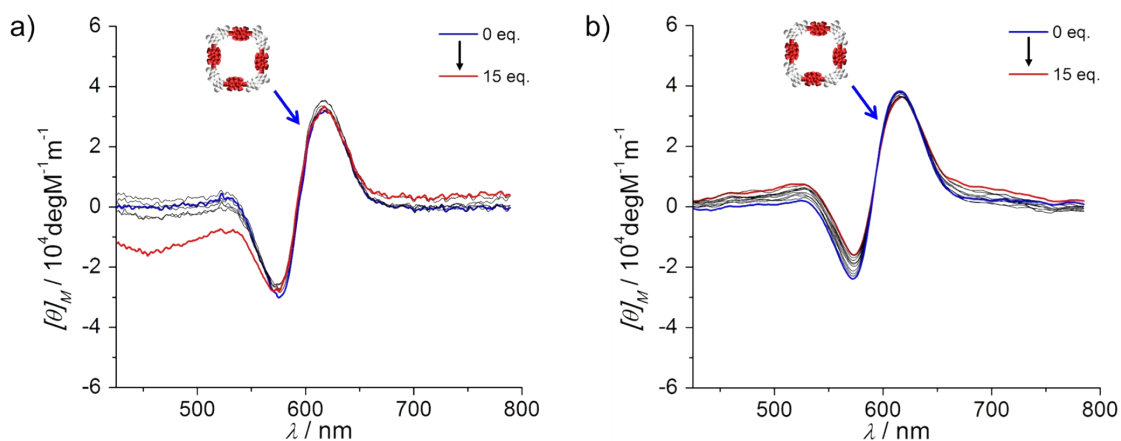

Figure S3F. CD signal changes (λ_{exc} = 385 nm, T = 298 K, toluene) observed in the titration of **GaC** with increasing amounts of **dC**, (**a**) [**GaC**] = 1.0·10^{-4} M, [**dC**] = 7.0·10^{-3} M and (**b**) [**GaC**] = 1.0·10^{-5} M, [**dC**] = 1.4·10^{-3} M.

Calculation of K_T and/or K_p from the spectroscopic changes experienced by **GdC+aC** and **AdU+aU** in the corresponding competition experiments was performed by using the software *ReactLab™ EQUILIBRIA* which is developed and commercialized by *Jplus Consulting Pty Ltd (http://jplusconsulting.com/)*. It allows for the global fitting of multiwavelength spectroscopic data to chemical reaction schemes, and determines all equilibrium constants in the underlying mechanism. 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.

The whole spectra are fitted by this program in all cases, but only a few selected wavelengths are plotted in Figures S3G and S3H. Three equilibrium constants were considered: K_a (the reference association constant between complementary nucleosides in toluene), which was calculated before[5] and fixed at the values shown in Table 1 in the text; K_T (the cyclotetramerization equilibrium constant), which can be calculated by the program or fixed at the values determined from the dilution experiments and shown in Table 1, and K_p (the apparent peripheral binding constant between macrocycles and mononucleosides), which was calculated by the program. The program provides the calculated spectra of all the species involved for additional assessment. It also provides the corresponding speciation curves from the calculated data, but those are usually more conveniently plotted with the *HYSS* software for practical reasons.
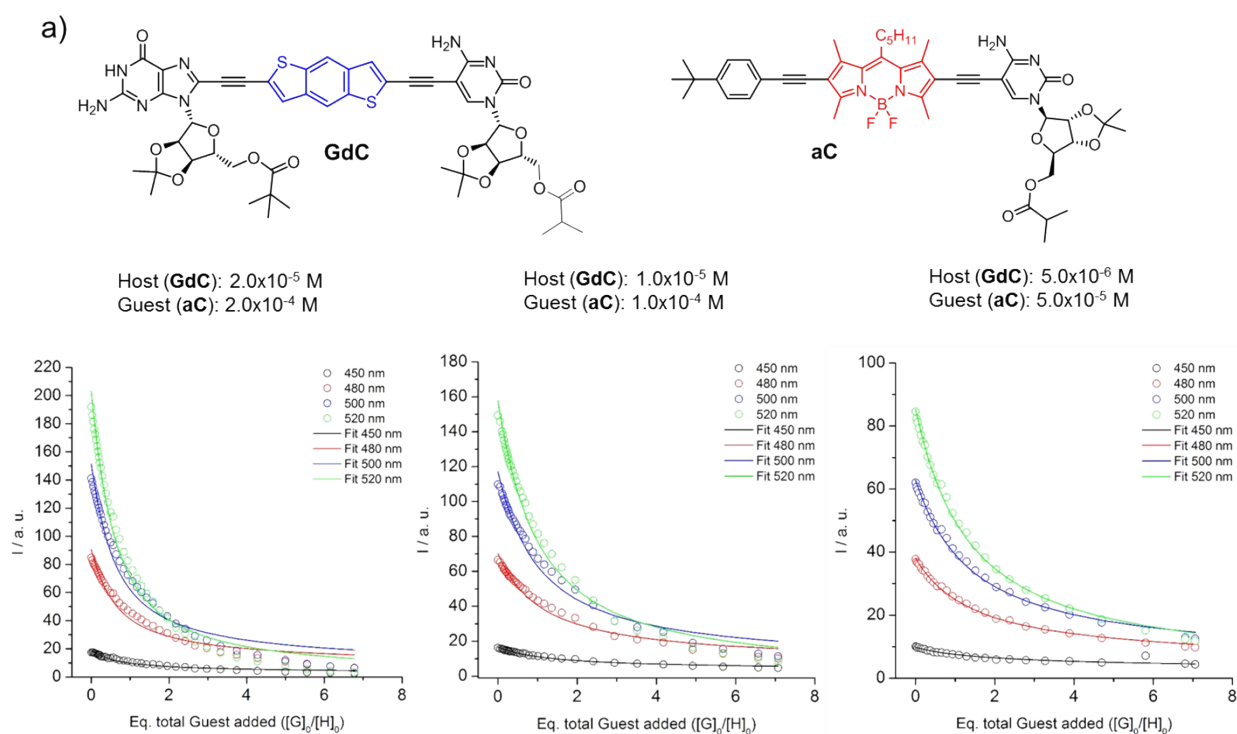

Figure S3G. Fitting of the emission data of **GdC**+**aC** (at 3 different concentrations) in toluene by the ReactLab™ EQUILIBRIA software.[8]

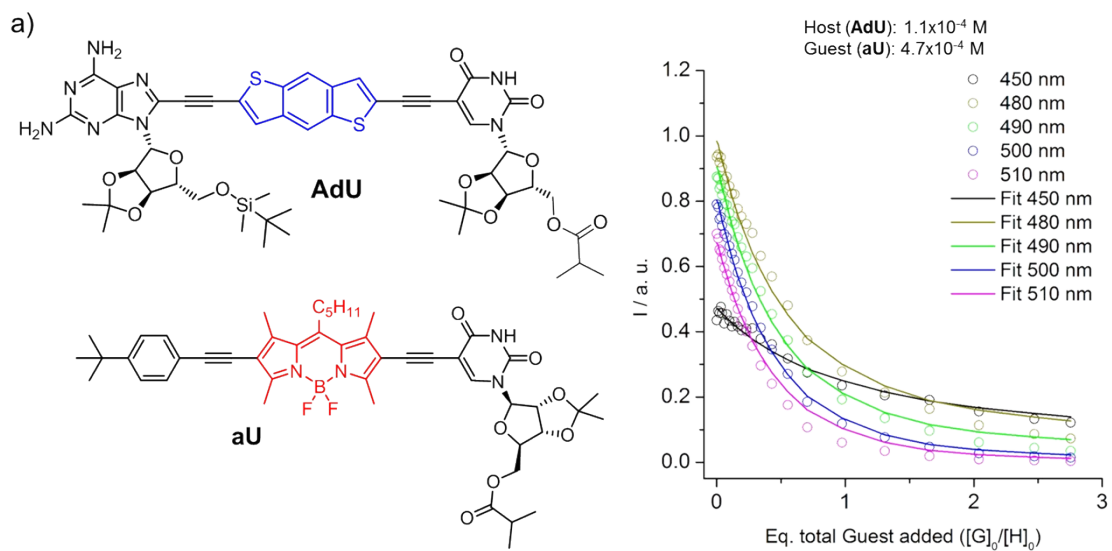[8] ReactLab™ EQUILIBRIA, Jplus Consulting Pty Ltd.

Figure S3H. Fitting of the emission data of (**a**) **AdU**+**aU** in toluene by the ReactLab™ EQUILIBRIA software.[8]
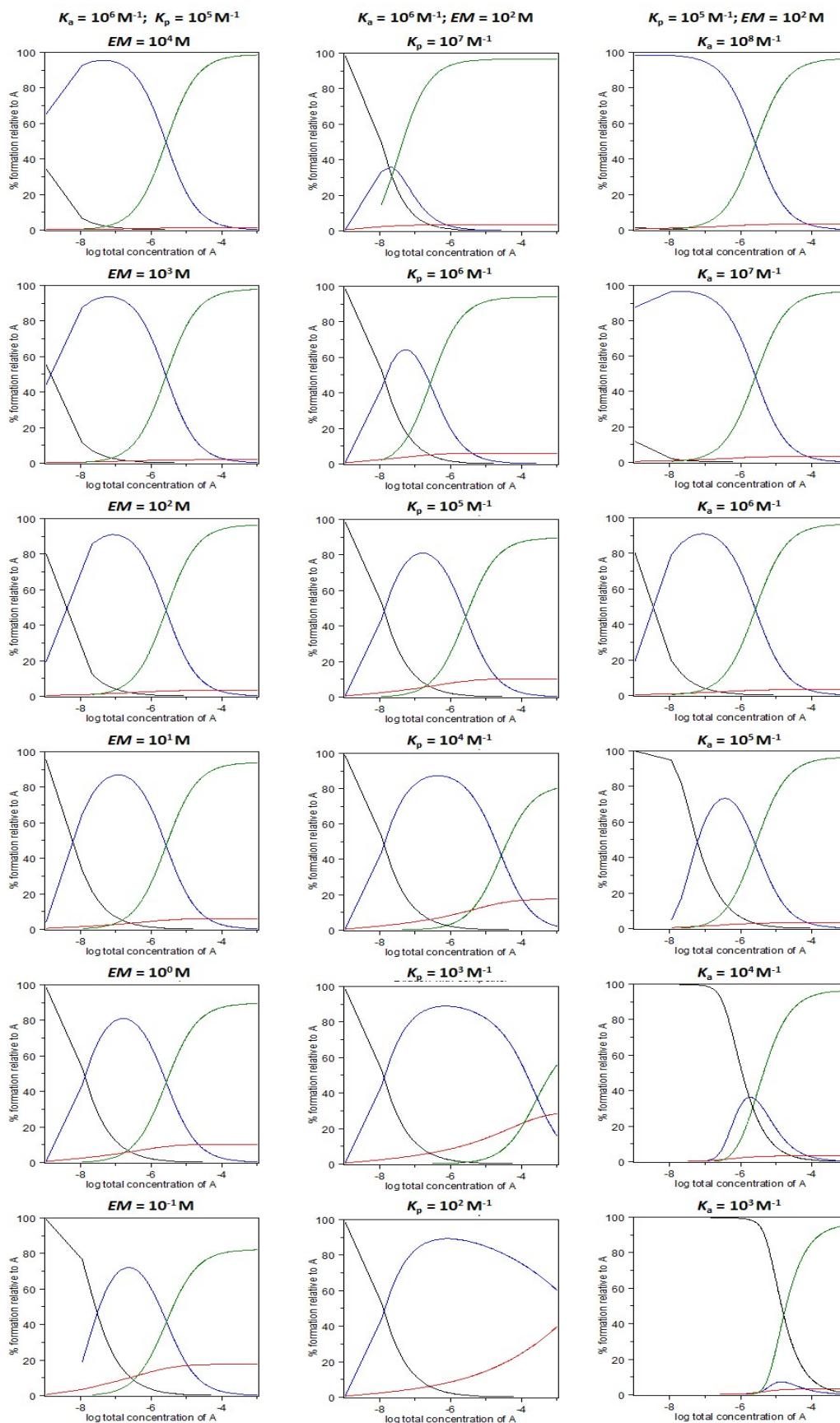
Figure S4. Speciation profiles of a 1:1 **GdC** + **aC** mixture showing the relative distribution of $c\textbf{GdC}_4\cdot\textbf{aC}$ (green), $c\textbf{GdC}_4$ (blue), **GdC·aC** (red) and **GdC** (black) species at different values of: 1) (left row) *EM*, at fixed K_a and K_p; 2) (middle row) K_p, at fixed K_a and *EM*; and 3) (right row) K_a, at fixed K_p and *EM*. The overall concentration at which $[c\textbf{GdC}_4\cdot\textbf{aC}] = [c\textbf{GdC}_4]$ depends on K_p, but not on K_a and *EM*. The overall concentration at which $[c\textbf{GdC}_4] = [\textbf{GdC}]$ depends on K_a and *EM*, but not on K_p. On the other hand, the degree of participation of **GdC·aC** in the overall equilibria depends on K_p and *EM*, but not on K_a.