Supporting Information

Tuning Exciton Coupling of Merocyanine Nucleoside Dimers by RNA, DNA and GNA Double Helix Conformations

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Supporting Information
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Tuning dipolar exciton coupling of merocyanine nucleoside dimers by RNA, DNA and GNA double helix conformations

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Abstract: Exciton coupling between two or more chromophores in a specific environment is a key mechanism associated with color tuning and modulation of absorption energies. This concept is well exemplified by natural photosynthetic proteins, and can also be achieved in synthetic nucleic acid nanostructures. Here we report the coupling of barbituric acid merocyanine (BAM) nucleoside analogues and show that exciton coupling can be tuned by the double helix conformation. BAM is a nucleobase mimic that was incorporated in the phosphodiester backbone of RNA, DNA and GNA oligonucleotides. Duplexes with different backbone constitutions and geometries afforded different mutual dye arrangements, leading to distinct optical signatures due to competing modes of chromophore organization via electrostatic, dipolar, π-π stacking and hydrogen bonding interactions. The realized supramolecular motifs include hydrogen-bonded BAM-adenine base pairs and antiparallel as well as rotationally stacked BAM dimer aggregates with distinct absorption, CD and fluorescence properties.

DOI: 10.1002/anie.2016XXXXX
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1. Materials and Methods

1.1 Materials

All standard chemicals and solvents were purchased from commercial suppliers. Organic solvents were used in pro analysis or for synthesis quality without further purification. Solvents for extraction and column chromatography were purchased in technical quality and distilled prior to use. Dry solvents (dichloromethane, THF, DMF, acetonitrile) were obtained from a solvent purification system (SPS). Nanopure water was obtained from a Sartorius Arium® pro ultrapure water system. Thin layer chromatography (TLC) was performed on silica gel (Kieselgel 60, Merck, 0.063 – 0.200 mm).

1.2 NMR spectroscopy and mass spectrometry

NMR spectra were measured on a Bruker Avance III HD 400 spectrometer at 400 MHz. Chemical shifts (δ) were referenced to the residual solvent signals as internal standards (in ppm; CDCl3: 1H = 7.26, 13C = 77.16, DMSO: 1H = 2.50, 13C = 39.52) or on the unified scale for 31P. Coupling constants (J) were reported in Hz. Multiplets were abbreviated as following: s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Spectral assignments were verified by 2D NMR experiments. High resolution ESI mass spectra were measured on a Bruker micrOTOF-Q III spectrometer. Monoisotopic masses for oligonucleotides were obtained by charge deconvolution of the raw spectra.

2. Synthesis of phosphoramidite building blocks for incorporation of rBAM, dBAM, gBAM

The synthesis of the rBAM phosphoramidite building block for RNA solid-phase synthesis started with a Silyl-Hilbert-Johnson nucleosidation of 2,4,6-tris(trimethylsilyloxy)pyrimidine S1 (prepared from barbituric acid (BA) with hexamethyldisilazane as described[2]) to obtain the protected nucleoside S2. After deprotection of the 2', 3' and 5' positions with sodium methoxide, the free BA riboside (5) was coupled in an aldol condensation reaction with aldehyde 7, which was derived from Fischer’s indole by Vilsmeier-Haack reaction as previously reported[3], to give the merocyanine nucleoside rBAM (2). In situ 2',3'-acetal protection of rBAM was followed by 5'-tritylation to give S6. Then, 2'-silyl protection gave derivative S7, which was finally converted to the rBAM phosphoramidite building block 8 by phosphorylation of the 3'-OH group.

Scheme S1. Reagents and conditions for the synthesis of the rBAM phosphoramidite building block 8 (see also Figure 2a; Intermediates mentioned in the main text are shown with the corresponding main text numbers in addition to the consecutive SI numbers in parentheses). i) SnCl4, DCE, sat. aq. NaHCO3, r. t., 6.5 h; ii) 0.5 M NaOMe, MeOH, r. t., 3 h; iii) EIOH, 70 °C, 21 h; iv) 1. DMF-DMA, pyridine, r. t., 18 h 2. DMT-Cl, pyridine, r. t., 2.5 h; v) 1. DIPEA, tBu2SnCl2, DCE, 70 °C, 40 min. 2. TOM-Cl, r. t., 1.5 h; vi) 2-cyanoethyl-N,N,N-disopropylchlorophosphoramidite, N-ethyl,N,N-dimethylamine, DCM, r. t., 2 h.
Scheme S2. Reagents and conditions for the synthesis of the DNA phosphoramidite building block 9 (see also Figure 2a). i) Hoffer’s chlorosugar, SnCl4, DCE, r.t., 3 h; ii) aldehyde 7, abs. EtOH, 70 °C, 18 h; iii) 1% methanolic NaOH, r.t., 45 min; iv) DMT-Cl, DMAP, pyridine, r.t., 20 h; v) 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite, ethyldimethylamine, r.t., 90 min.

For the synthesis of the dBAM phosphoramidite building block 9, silylated barbituric acid S1 was reacted with Hoffer’s chlorosugar to give an 1:1 anomic mixture of the desired β-anomer 6 and the respective α-anomer. The two anomers were separated by recrystallization from methanol (4) and the β-anomer was further reacted with the aldehyde 7 to give the protected nucleoside 10. Deprotection of the hydroxyl groups gave dBAM (3), followed by 5’-DMT protection to 12 and phosphitylation to afford the dBAM phosphoramidite building block 9.

Scheme S3. Reagents and conditions for the synthesis of the GNA phosphoramidite building block 10. i) barbituric acid, abs. EtOH, 70 °C, 12 h; iii) DMT-(R)-(++)-glycidol, NaH, K2CO3, DMF, 15 h, 110 °C; iv) 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite, ethyldimethylamine, DCM, 2 h, r.t.

The synthesis of the gBAM phosphoramidite building block 10 started with an aldol condensation reaction of aldehyde 7 with barbituric acid to give the free chromophore BAM (1). Afterwards, the DMT-protected (R)-glycidol was attached to one of the barbituric acid nitrogen atoms of BAM by a sodium hydride catalyzed epoxide ring opening reaction. The obtained DMT-protected building block 15 was converted to the GNA phosphoramidite building block 10 by phosphitylation.

2.1 2',3',5'-Tri-O-benzoyl barbituric acid ribonucleoside S2

Tris(trimethylsilyl) barbituric acid S1 (4.62 g, 13.4 mmol, 1.15 eq.) was dissolved under N2-atmosphere in dry DCE (60 mL) and carefully treated with SnCl4 (5.58 g, 22.2 mmol, 1.90 eq.). Afterwards, a solution of 1-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (5.89 g, 11.7 mmol, 1.00 eq.) in DCE (60 mL) was added to the resulting suspension. The reaction mixture was stirred at r.t. for 6.5 h, then poured into sat. aq. NaHCO3 (50 mL) and left to stand overnight. The layers were separated, and the aqueous phase was extracted with DCM (5 x 30 mL). The combined organic phases were dried over Na2SO4, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (DCM/MeOH 98:2) to afford S2 as a slightly yellow foam (4.38 g, 7.66 mmol, 65%).

1H NMR (400 MHz, CDCl3) δ (ppm) = 9.34 (s, 1H, 2-H), 8.09 – 8.00 (m, 2 H, Bz-H), 7.96 (dq, J = 8.2, 1.4 Hz, 2 H, Bz-H), 7.88 – 7.81 (m, 2 H, Bz-H), 7.56 – 7.40 (m, 3 H, Bz-H), 7.39 – 7.29 (m, 4 H, Bz-H), 7.31 – 7.22 (m, 2 H, Bz-H), 6.43 (d, J = 2.0 Hz, 1 H, 1’-H), 6.10 (m, 2 H, Bz-H), 7.56 – 7.40 (m, 3 H, Bz-H), 7.39 – 7.29 (m, 4 H, Bz-H), 7.31 – 7.22 (m, 2 H, Bz-H), 6.43 (d, J = 2.0 Hz, 1 H, 1’-H), 6.10
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1H NMR (400 MHz, DMSO-d$_6$) $\delta$ (ppm) = 11.39 (s, 1 H, 2-H), 5.90 (s, 1 H, 1'-H), 5.01 (sbr, 1 H, OH), 4.92 (sbr, 1 H, OH), 4.56 (sbr, 1 H, OH), 4.35 (s, 1 H, 2'-H), 4.05 (t, $J = 6.5$ Hz, 1 H, 3'-H), 3.66 (td, $J = 6.7$, 3.5 Hz, 1 H, 4'-H), 3.59 (dd, $J = 11.6$, 3.5 Hz, 1 H, 5'-H), 3.39 (dd, $J = 11.6$, 6.6 Hz, 1 H, 5'-H).

13C{1H} NMR (100 MHz, DMSO-d$_6$) $\delta$ (ppm) = 166.70 (C-4, C-6), 150.83 (C-2), 87.64 (C-1'), 83.97 (C-4'), 71.21 (C-2'), 69.76 (C-3'), 62.10 (C-5').

HR-MS (ESI$^+$): Exact mass calculated for C$_{9}$H$_{12}$N$_{2}$NaO$_{7}$ [M+Na$^+$]: 283.05367, found: 283.05389.

2.3 Barbituric acid merocyanine ribonucleoside S5 (BAM, 2)

Aldehyde S4 (600 mg, 2.31 mmol, 1.00 eq.), prepared as previously reported$^5$, and the free barbituric acid ribonucleoside S3 (603 mg, 3.00 mmol, 1.30 eq) were suspended in abs. EtOH (11 mL). The reaction mixture was stirred at 70 °C for 21 h. After removal of the solvent under reduced pressure, the resulting residue was purified by column chromatography on silica gel (DCM/MeOH 95:5) to afford a 1:1 mixture of (E), (E)$^\text{-}$ and (Z), (E)$^\text{-}$-double bond isomers of the pure nucleoside S5 as a dark red solid (625 mg, 1.41 mmol, 61%).

1H NMR (400 MHz, DMSO-d$_6$) $\delta$ (ppm) = 10.92, 10.82 (2 s, 1 H, 3-H), 8.48 (2 d, $J = 14.8$, 1 H, 7-H), 7.59 (d, $J = 7.4$ Hz, 1 H, 16-H), 7.54 – 7.38 (m, 3 H, 8-H, 13-H, 14-H), 7.29 – 7.21 (m, 1 H, 15-H), 6.08 (s, 1 H, 1'-H), 5.02 (2 d, $J = 5.1$, 1 H, 2'-OH), 4.82 (2 d, $J = 6.6$, 1 H, 3'-OH), 4.60 (t, $J = 6.4$, 1 H, 5'-OH), 4.47 (q, $J = 5.0$ Hz, 1 H, 2'-H), 4.15 – 4.07 (m, 1 H, 3'-H), 3.70-3.65 (m, 1 H, 4'-H), 3.64 – 3.57 (m, 1 H, 5'-H), 3.56 (2 s, 3 H, 18-H), 3.48 – 3.39(m, 1 H, 5'-H), 1.67 – 1.59 (m, 6 H, 11-H, 11'-H).

13C{1H} NMR (100 MHz, DMSO-d$_6$) $\delta$ (ppm) = 177.20 (C-9), 176.97 (C-9), 163.74 (C-4, C-6), 163.03 (C-4, C-6), 162.85 (C-4, C-6), 162.59 (C-4, C-6), 150.52 (C-7), 149.84 (C-7), 142.64 (C-17), 140.76 (C-12), 128.39 (C-14), 124.77 (C-15), 122.27 (C-16), 111.13 (C-13), 98.32 (C-8), 97.93 (C-8), 87.83 (C-1'), 84.09 (C-4'), 71.31 (C-2'), 70.15 (C-3'), 62.46 (C-5'), 48.75 (C-10), 48.72 (C-10), 31.05 (C-18), 28.14 (C-11, C-11'), 28.02 (C-11, C-11').

HR-MS (ESI$^+$): Exact mass calculated for C$_{22}$H$_{25}$N$_{3}$NaO$_{7}$ [M+Na$^+$]: 466.15847, found: 466.15779.
The barbituric acid merocyanine ribonucleoside S5 (331 mg, 746 µmol, 1.00 eq.) was dissolved in dry pyridine (10 mL) under N2-atmosphere and DMF-DMA (222 mg, 1.87 mmol, 2.51 eq.) was added. The mixture was stirred at r. t. for 17.5 h. Afterwards, the solvent was removed in vacuum and the residue re-dissolved in dry pyridine (10 mL) under N2-atmosphere. DMT-Cl (278 mg, 0.821 mmol, 1.10 eq.) was added and the mixture was stirred at r. t. for 2.5 h. After addition of MeOH (5 mL) the reaction mixture was stirred for additional 20 min. The solvent was removed under reduced pressure and the crude product was purified via column chromatography on silica gel (DCM/MeOH 95:5 + 4% NEt3) to obtain S6 as a red foam (401 mg, 538 µmol, 72%).

**1H NMR** (400 MHz, CDCl3) δ (ppm) = 8.71 (s, 1 H, 3-H), 8.64 – 8.55 (m, 1 H, 7-H), 7.55 (2 d, J = 14.6, 1 H, 8-H), 7.40 – 7.08 (m, 16 H, DMT-H, 13-H, 14-H, 15-H), 7.06 – 6.97 (m, 1 H, 16-H), 6.80 – 6.74 (m, 4 H, DMT-H), 6.39 (2 d, J = 2.4, 1 H, 1'-H), 4.02 – 3.92 (m, 1 H, 4'-H), 3.79 (s, 3 H, 18-H), 3.75 – 3.69 (m, 6 H, DMT-OMe-H), 3.50 – 3.34 (m, 2 H, 5'-H), 1.73 – 1.58 (m, 6 H, 11-H, 11'-H).

**13C{1H} NMR** (100 MHz, CDCl3) δ (ppm) = 178.00 (C-9), 177.79 (C-9), 164.02 (Carbonyl-C), 163.51 (Carbonyl-C), 163.27 (Carbonyl-C), 163.24 (Carbonyl-C), 158.69, 158.39, 152.56 (C-7), 151.64 (C-7), 150.39, 150.34, 149.74 (C-7), 147.46, 145.18, 145.14, 142.65 (C-17), 139.59 (C-12), 136.43, 136.39, 136.34, 136.26, 130.31, 130.26, 129.25, 128.53, 128.44, 127.95, 127.88, 127.82, 127.17, 126.68, 125.10, 123.92, 122.25 (C-13), 113.25 (DMT-O-Me-H), 113.12 (DMT-O-Me-C), 110.23, 100.59, 100.55, 99.41 (C-8), 99.10 (C-8), 88.85 (C-1'), 88.23 (C-1'), 86.24, 86.20, 82.18 (C-4'), 81.52, 72.77 (C-2'), 71.38 (C-3'), 71.35 (C-3'), 64.78 (C-5'), 55.36 (C-53, C-55), 55.27 (C-53, C-55), 55.25 (C-18), 49.33 (C-10), 31.06, 28.85 (C-11, C-11').

**HR-MS** (ESI+): Exact mass calculated for C43H43N3NaO9 [M+Na]+: 768.28915, found: 768.28953.

Merocyanine ribonucleoside S6 (602 mg, 807 µmol, 1.00 eq.) and DIPEA (415 mg, 3.21 mmol, 3.98 eq.) were dissolved in dry DCE under N2-atmosphere. tBu2SnCl2 (294 mg, 968 µmol, 1.20 eq.) was added and the mixture was heated to 70 °C and stirred for 40 min at this temperature. The reaction mixture was cooled to r. t., TOM-Cl (216 mg, 968 µmol, 1.20 eq.) was added and the reaction mixture was stirred at this temperature for 1.5 h and then for 15 min at 80 °C. The solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica gel (ethyl acetate/n-hexane 3:1 + 4% NEt3). The pure product S7 was obtained as a red foam (200 mg, 215 µmol, 26%), along with an equal amount of the undesired 3'-silylated compound which both were identified by 1H-1H COSY NMR.

**1H NMR** (400 MHz, CDCl3) δ (ppm) = 8.65 (2 d, J = 14.6, 1 H, 7-H), 7.57 (2 d, J = 14.6, 1 H, 8-H), 7.52 – 7.45 (m, 2 H, DMT-H), 7.41-7.32 (m, 7 H, 14-H, 16-H, DMT-H), 7.28 – 7.18 (m, 3 H, DMT-H), 7.18 – 7.10 (m, 1 H, 13-H), 7.08 – 7.00 (m, 1 H, 15-H), 6.81 – 6.73 (m, 4 H, DMT-H), 6.43 (s, 1 H, 1'-H), 5.10 (dd, J = 4.8, 1.8 Hz, 1 H, 19-H), 4.98 (dd, J = 4.8, 2.6 Hz, 1 H, 19-H), 4.91 – 4.80 (m, 1 H, 2'-H), 4.57 – 4.46 (m, 1 H, 3'-H), 4.02 – 3.91 (m, 1 H, 4'-H), 3.75 (2 s, 6 H, DMT-O-Me-H), 3.52 (s, 3 H, 18-H), 3.45 – 3.28 (m, 2 H, 5'-H), 3.02 – 2.93 (m, 1 H, 3'-OH), 1.77 – 1.59 (m, 6 H, 11-H, 11'-H), 1.15 – 1.00 (m, 21 H, 20-H, 21-H).

**13C{1H} NMR** (100 MHz, CDCl3) δ (ppm) = 177.82 (C-9), 177.68 (C-9), 163.04 (C-5), 162.79 (C-2, C-4, C-6), 158.38 (DMT-C), 152.76 (C-7), 145.37 (DMT-C), 145.31 (DMT-C), 142.69 (17-C), 141.15 (DMT-C), 136.62 (DMT-C), 136.56 (DMT-C), 130.38 (DMT-C), 130.32...
2.6 5'-O-(4,4'-Dimethoxytrityl)-2'-O-tri-isopropylsilyloxymethyl barbituric acid merocyanine ribonucleoside 3'-(2-cyanoethyl-N,N-di-isopropyl phosphoramidite S8

The protected ribonucleoside S7 (150 mg, 161 µmol, 1.00 eq.) was dissolved in dry DCM under N₂-atmosphere. Me₂NEt (0.208 mg, 1.61 mmol, 10.0 eq.) and CEP-Cl (57.0 mg, 242 µmol, 1.50 eq.) were added to the mixture which was then stirred at r. t. for 2 h. The solvent was removed in vacuum. Purification of the crude product by column chromatography on silica gel (ethyl acetate/n-hexane 3:1 + 1% NEt₃) yielded the target compound S8 as a red foam (141 mg, 125 µmol, 77%).

1H NMR (400 MHz, CDCl₃) δ (ppm) = 8.72 – 8.60 (m, 1 H, 7-H), 7.64 – 7.42 (m, 3 H, 8-H), 7.41 – 7.30 (m, 7 H, DMT-H), 7.29 – 7.18 (m, 3 H), 7.19 – 7.11 (m, 1 H), 7.09 – 7.01 (m, 1 H), 6.83 – 6.70 (m, 1 H, DMT-H), 6.53 – 6.39 (m, 1 H, 1'-H), 5.08 – 5.03 (m, 1 H, 21-H), 5.02 – 4.89 (m, 1 H, 21-H), 4.78 – 4.46 (m, 1 H, 1H), 4.22-4.07 (m, 1H), 3.80 – 3.70 (m, 8 H, DMT-OMe-H), 3.59 – 3.45 (m, 4 H, 18-H, 24-H), 3.45-3.34 (m, 1H), 3.32 – 3.18 (m, 1H), 2.66 (td, J = 7.0, 6.5, 2.2 Hz, 1 H, 25-H), 2.34 (td, J = 6.8, 4.6 Hz, 1 H, 25-H), 1.75 – 1.68 (m, 6 H, 11-H, 11'-H), 1.17 – 0.95 (m, 32 H, 21-H, 23-H).

13C{1H} NMR (100 MHz, CDCl₃) δ (ppm) = 158.24, 151.50 (C-7), 145.14, 142.61, 140.99, 136.40, 130.25, 128.41, 127.62, 126.46, 124.83, 122.15, 112.92 (DMT-C), 109.87, 98.66 (C-8), 89.40 (C-19), 85.91, 81.71, 60.40, 55.17 (C-65-C-67), 55.15 (DMT-OMe-C), 49.06, 42.95, 30.95 (C-18), 28.79 (C-11, C11'), 24.67 (C-22, C-22'), 24.59 (C-22, C-22'), 21.07, 20.23 (C-25), 19.99 (C-25), 17.86 (C-21), 17.82 (C-21), 17.78 (C-21), 14.20, 12.01 (C-20), 11.99 (C-20), 11.97 (C-20), 11.89 (C-20).

31P NMR (162 MHz, CDCl₃) δ (ppm) = 149.25, 149.20, 148.99.

HR-MS (ESI⁺): Exact mass calculated for C₆₂H₈₂N₅NaO₁₁PSi [M+Na]+: 1154.54099, found: 1154.54192.

2.7 3',5'-Di-O-toluoyl barbituric acid 2'-deoxyribonucleoside S9

Under nitrogen atmosphere, silylated barbituric acid S1 (5.82 g, 16.9 mmol, 1.22 eq.) in 80 mL of dry 1,2-dichloroethane was added to a solution of Hoffer's chlorosugar (5.40 g, 13.9 mmol, 1.00 eq.) in 80 mL of the same solvent. SnCl₄ (0.36 mL, 3.48 mmol, 0.25 eq.) in 25 mL of dry 1,2-dichloroethane was added and the reaction mixture was stirred at r. t. for 3 h. Afterwards, the solvent was removed in vacuum and the crude product was purified by column chromatography on silica gel (DCM/methanol 98:2 to 95:5) to yield the product as a mixture of two anomers. The obtained white foam was recrystallized from methanol, where the desired β-anomer precipitated upon cooling while the α-anomer stayed in solution. The protected β-nucleoside S9 was obtained as white solid (1.65 g, 3.44 mmol, 20%).

1H NMR (400 MHz, CDCl₃) δ (ppm) = 8.20 (s, 1 H, 3-H), 7.96 – 7.88 (m, 4 H, Tol-H), 7.27 – 7.14 (m, 4 H, Tol-H), 6.68 (dd, J = 8.5, 5.0 Hz, 1 H, 1'-H), 5.77 (dt, J = 8.0, 5.3 Hz, 1 H, 3'-H), 4.70 – 4.54 (m, 2 H, 5'-H), 4.48 (dt, J = 6.7, 4.8 Hz, 1 H, 4'-H), 3.67 (s, 2 H, 5-H), 3.15 (ddd, J = 14.1, 8.1, 5.0 Hz, 1 H, 1'-H), 2.49 – 2.35 (m, 7 H, 2'-H, Tol-Me-H);
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13C{1H} NMR (100 MHz, CDCl3): δ (ppm) = 166.42 (Tol-C), 165.99 (Tol-C), 164.98 (C-6), 163.56 (C-4), 149.25 (C-2), 144.21 (Tol-C), 143.74 (Tol-C), 129.82 (Tol-C), 129.76 (Tol-C), 129.18 (Tol-C), 129.02 (Tol-C), 127.03 (Tol-C), 126.60 (Tol-C), 82.42 (C'-4), 82.34 (C'-1').

HR-MS (ESI+): m/z calc. (C25H24N2NaO8 ,[M+Na]+): 503.14393, found: 503.14304.

2.8 3',5'-Di-O-toluoyl barbituric acid merocyanine 2'-deoxyribonucleoside S10

The protected barbituric acid deoxyribonucleoside S9 (1.00 g, 2.08 mmol, 1.00 eq.) and aldehyde S4 (503 mg, 2.50 mmol, 1.20 eq.) were dissolved in 12 mL of abs. EtOH and heated to 70 °C for 16 h. Then, the reaction mixture was cooled to r. t. and the solvent was removed in vacuum. Column chromatography on silica gel (DCM/MeOH 98:2) yielded the merocyanine nucleoside S10 as an orange foam (1.35 g, 2.03 mmol, 98%).

1H NMR (400 MHz, CDCl3): δ (ppm) = 8.66 (2 d, J = 14.6, 1 H, 7-H), 8.25 (2 s, 1 H, 3-H), 8.00 – 7.89 (m, 4 H, Tol-H), 7.61 (t, J = 14.2 Hz, 1 H, 8-H), 7.38 – 7.34 (m, 2 H, 14-H, 15-H), 7.26 – 7.11 (m, 6 H, 13-H, Tol-H), 7.11 – 7.05 (m, 1 H, 6-H), 5.89 (dd, J = 8.3, 4.2 Hz, 1 H, 3'-H), 4.78 – 4.59 (m, 2 H, 5'-H), 4.54 – 4.45 (m, 1 H, 4'-H), 3.57 (2 s, 3 H, 18-H), 3.36 – 3.24 (m, 1 H, 2'-H).

13C{1H} NMR (100 MHz, CDCl3): δ (ppm) = 177.97 (C-9), 177.68 (C-9), 166.60 (Tol-C), 166.12 (Tol-C), 164.03 (C-4), 163.40 (C-6, C-2), 163.20 (C-6, C-2), 163.02 (C-6, C-2), 152.60 (C-7), 151.79 (C-7), 150.19 (C-2), 144.03 (Tol-C), 144.00 (Tol-C), 143.54 (Tol-C), 141.14 (C-12), 129.97 (Tol-C), 129.88 (Tol-C), 129.21 (Tol-C), 128.57 (Tol-C), 127.12 (C-14), 127.12 (C-14), 125.11 (C-13), 125.05 (C-13), 122.29 (C-15), 122.29 (C-15), 110.20 (C-16), 110.15 (C-16), 99.31 (C-8), 98.80 (C-8), 82.34 (C-1', C-4'), 81.90 (C-1', C-4'), 75.74 (C-3'), 65.21 (C-5'), 49.33 (C-10), 49.27 (C-10), 35.79 (C-2'), 35.69 (C-2'), 31.23 (C-18), 28.96 (C-11'), 28.87 (C-11'), 21.82 (Tol-Me-C), 21.77 (Tol-Me-C).

HR-MS (ESI+): m/z calc. (C38H37N3NaO8 ,[M+Na]+): 686.24783, found: 686.25147.

2.9 Barbituric acid merocyanine 2'-deoxyribonucleoside S11

The protected nucleoside S10 (1.00 g, 1.51 mmol, 1.00 eq.) was dissolved in 45 mL of 1% methanolic NaOH and stirred at r. t. for 45 min. Afterwards, the solvent was removed in vacuum and the crude material was purified by column chromatography on silica gel (DCM/MeOH 95:5 to 8:2) to give the desired product S11 as an orange solid (466 mg, 1.09 mmol, 73%).

1H NMR (400 MHz, DMSO-d6): δ (ppm) = 10.86, 10.76 (2 s, 1 H, 3-H), 8.47 (2 d, J = 14.8, 1 H, 7-H), 7.59 (d, J = 7.5 Hz, 1 H, 16-H), 7.52 – 7.37 (m, 3 H, 8-H, 13-H, 14-H), 7.32 – 7.18 (m, 1 H, 15-H), 6.56 (ddd, J = 8.2, 6.3, 4.7 Hz, 1 H, 1'-H), 5.07 (d, J = 5.1, 1 H, 3'-OH), 4.64 – 4.52 (m, 1 H, 5'-OH), 4.37 – 4.25 (m, 1 H, 3'-H), 3.70 – 3.57 (m, 2 H, 4'-H, 5'-H), 3.55 (2 s, 3 H, 18-H), 3.51 – 3.41 (m, 1 H, 5'-H), 2.71 (ddd, J = 13.3, 7.6, 6.2, 1.6 Hz, 1 H, 2'-H), 1.91 (td, J = 8.2, 4.1 Hz, 1 H, 2'-H), 1.67 – 1.56 (m, 6 H, 11-H, 11'-H).

13C{1H} NMR (100 MHz, DMSO-d6): δ (ppm) = 177.10 (C-9), 176.84 (C-9), 163.81 (C-4, C-6), 163.20 (C-4, C-6), 162.00 (C-4, C-6), 150.51 (C-7), 150.40 (C-7), 149.79 (C-7), 142.66 (C-17), 140.74 (C-12), 128.39 (C-13), 124.72 (C-15), 122.27 (C-16), 111.07 (C-14), 99.84 (C-5), 99.77 (C-5), 98.23 (C-5), 97.87 (C-8), 87.18 (C-4'), 81.15 (C-1'), 80.51 (C-1'), 71.18 (C-3'), 62.40 (C-5'), 48.72 (C-10), 48.68 (C-10), 37.08 (C-2'), 31.03 (C-18), 28.17 (C-11, C-11'), 28.13 (C-11, C-11'), 28.03 (C-11, C-11');

HR-MS (ESI+): m/z calc. (C38H37N3NaO9,[M+Na]+): 450.16410, found: 450.16580.
The unprotected nucleoside **S11** (400 mg, 936 µmol, 1.00 eq.) and DMAP (5.7 mg, 47 µmol, 0.05 eq.) were dissolved in dry pyridine (15 mL) under nitrogen atmosphere. The reaction mixture was cooled to 0 °C and DMT-Cl (476 mg, 1.40 mmol, 1.50 eq.) was added. After warming up to r. t., the reaction mixture was stirred at this temperature for 20 h. The solvent was removed in vacuum and the crude product was purified by column chromatography on silica gel (DCM/MeOH 100:0 to 97:3) to give compound **S12** as an orange foam (533 mg, 732 µmol, 78%).

**^1H NMR** (400 MHz, DMSO-**d**6): δ (ppm) = 10.82, 10.72 (2 s, 1 H, 3'-H), 8.60 – 8.38 (m, 1 H, 7'-H), 7.60-7.53 (m, 1 H, 13'-H), 7.50 – 7.32 (m, 5 H, 8-H, DMT-H), 7.31 – 7.19 (m, 7 H, DMT-H, 14'-H), 7.20 – 7.12 (m, 1 H), 6.87 – 6.76 (m, 4 H, DMT-H), 6.61 (dd, J = 9.1, 4.2 Hz, 1 H, 1'-H), 5.05 (dd, J = 5.5, 3.7 Hz, 5 H, 14'-H), 4.32 – 4.24 (m, 1 H, 3'-H), 3.85-3.78 (m, 1 H, 4'-H), 3.72 – 3.63 (m, 6 H, DMT-OMe-H), 3.49 (2 s, 3 H, 18-H), 3.30 – 3.21 (m, 1 H, 5'-H), 3.04 (td, J = 9.5, 3.2 Hz, 1 H, 5'-H), 2.68 – 2.54 (m, 1 H, 2'-H), 2.09 – 1.95 (m, 1 H, 2'-H), 1.66 – 1.51 (m, 6 H, 11-H, 11'-H).

**^13C{^1H} NMR** (100 MHz, DMSO-**d**6): δ (ppm) = 176.84 (C-9), 176.69 (C-9), 163.07, 162.80, 162.66, 157.99 (DMT-C), 157.90 (DMT-C), 149.65 (C-7), 145.27, 145.24, 142.66, 140.70, 135.90 (DMT-C), 135.84 (DMT-C), 135.79, 129.86, 129.80, 128.38, 127.80, 127.69, 126.54, 124.67, 123.95, 122.26 (C-13), 113.06 (DMT-C), 113.01 (DMT-C), 111.00, 100.03 (C-5), 98.08 (C-8), 85.44 (C-4'), 85.21 (C-4'), 71.40 (C-3'), 54.99 (DMT-OMe-C), 54.96 (DMT-OMe-C), 54.93 (DMT-OMe-C), 54.90 (DMT-OMe-C), 48.63 (C-10), 48.60 (C-10), 37.96 (C-2'), 37.96 (C-2'), 28.16 (C-11, C-11'), 28.09 (C-11, C-11').

**HR-MS** (ESI+): *m/z* calc. (C_{60}H_{68}N_{12}O_{15}S_{2}Na^{+}) [M+Na^+] +: 729.3052, found: 729.3039.

The DMT-protected nucleoside **S12** (400 mg, 537 µmol, 1.00 eq.) was dissolved in dry DCM (9 mL) under nitrogen atmosphere. DIPEA (0.80 mL, 4.30 mmol, 8.00 eq.) and CEP-Cl (191 mg, 806 µmol, 1.50 eq.) were added dropwise and the reaction mixture was stirred at r. t. for 1.5 h. After removal of the solvent under reduced pressure, the crude product was purified by column chromatography on silica gel (ethyl acetate/hexane 90:10 to 80:20+2% NEt3) to obtain the phosphoramidite building block **S13** as an orange foam (436 mg, 473 µmol, 88%).

**^1H NMR** (400 MHz, CDCl_3): δ (ppm) = 8.73-8.53 (m, 1 H, 7-H), 7.90 (s, 1 H, 3-H), 7.62 – 7.45 (m, 3 H, 8-H, 14-H, DMT-H), 7.27 – 7.18 (m, 3 H), 7.18 – 7.09 (m, 1 H), 7.04 (d, J = 8.0 Hz, 1 H, 13-H), 6.86 – 6.72 (m, 5 H, 1'-H, DMT-H), 4.85 – 4.71 (m, 0.5 H, 3'-H), 4.72 – 4.60 (m, 0.5 H, 3'-H), 4.17 – 4.04 (m, 1 H, 4'-H), 3.82 – 3.69 (m, 7 H, 19-H, DMT-OMe-H), 3.34 – 3.23 (m, 3 H, 14'-H), 2.99 – 2.80 (m, 1 H, 2'-H), 2.63 – 2.54 (m, 1 H, 20-H), 2.42 – 2.35 (m, 1 H, 20-H), 2.28 (dd, J = 13.3, 5.8, 4.5 Hz, 1 H, 2'-H), 1.75 – 1.58 (m, 6 H, 11-H, 11'-H), 1.19 – 1.10 (m, 9 H, 23-H), 1.00 (dd, J = 6.8, 3.2 Hz, 3 H, 23-H).

**^13C{^1H} NMR** (100 MHz, CDCl_3): δ (ppm) = 177.57 (C-9), 177.40 (C-9), 162.98 (C-4, C-6), 162.80, 162.66, 162.50 (C-13), 158.24 (C-7), 151.44 (C-7), 149.91, 149.81, 149.15, 148.89, 148.54, 148.09, 147.64, 147.37, 141.00 (C-12), 136.54 (DMT-C), 136.45 (DMT-C), 136.40 (DMT-C), 136.36 (DMT-C), 130.25, 130.22, 130.16, 128.45, 128.39, 127.62, 127.59, 126.52, 126.46, 124.88, 122.15, 117.61 (C-21), 112.90 (DMT-OMe-C), 109.94 (C-13), 100.97, 98.94 (C-8), 98.66 (C-8), 85.95 (DMT-C), 85.92 (DMT-C), 85.90 (DMT-C), 85.00 (C-4'), 84.87 (C-4'), 84.82 (C-4'), 81.72 (C-1'), 81.55 (C-1'), 81.24 (C-1'), 74.38 (C-3'), 74.32 (C-3'), 74.21 (C-3'), 73.78 (C-3'), 73.73 (C-3'), 73.53 (C-3'), 64.52 (C-5'), 64.27 (C-5'), 58.53 (C-19), 58.35 (C-19), 58.16 (C-19), 55.18 (DMT-OMe-C), 55.15 (DMT-OMe-C), 55.12 (DMT-OMe-C), 49.11 (C-10), 49.07 (C-10), 43.20 (C-18), 43.18 (C-18), 43.14 (C-18), 43.12 (C-18), 43.08 (C-18), 43.06 (C-18), 43.01 (C-18), 42.99 (C-18).
Aldehyde S4 (143 mg, 710 µmol, 1.00 eq.) and barbituric acid (100 mg, 870 µmol, 1.22 eq.) were suspended in abs. ethanol and heated to 70 °C overnight. After cooling to r. t., the precipitate was filtered off and washed with ethanol (2x5 mL). Column chromatography on silica gel (dichloromethane/methanol 4:1) yielded the product BAM (1), S14 as red solid (135 mg, 430 µmol, 61%). Analytical data were consistent with those previously reported.[6]

1H NMR (400 MHz, DMSO-d6): δ (ppm) = 10.64 (d, J = 1.9 Hz, 1 H, 1'-H or 3'-H), 10.53 (d, J = 2.0 Hz, 1 H, 1'-H or 3'-H), 8.43 (d, J = 14.5 Hz, 1 H, 7'-H), 7.60 – 7.54 (m, 1 H, 9-H), 7.46 – 7.34 (m, 3 H, 6-H, 7-H, 8'-H), 7.25 – 7.18 (m, 1 H, 8-H), 3.51 (s, 3 H, 12-H), 1.62 (s, 6 H, 10-H, 11-H).

13C{1H} NMR (100 MHz, DMSO-d6): δ (ppm) = 176.6 (C-2), 165.0 (C-6'), 164.6 (C-4'), 151.3 (C-2'), 149.7 (C-7'), 143.2 (C-5), 141.0 (C-4), 128.8 (C-5'), 124.8 (C-7), 122.7 (C-9), 111.2 (C-8), 101.0 (C-6), 97.8 (C-8'), 48.9 (C-12), 46.1 (C-3), 28.6 (C-10, C-11); HR-MS (ESI+): m/z calc. (C17H17N3NaO3, [M+Na]+): 334.1162, found: 334.1159.

2.13 DMT-protected gBAM building block S15

Compound S14 (800 mg, 2.57 mmol, 1.43 eq.) and barbituric acid (250 mg, 870 µmol, 1.22 eq.) were suspended under N2-atmosphere in anhydrous DMF (8 mL) and stirred for 2 h. DMT-protected (+)-(R)-glycidol (prepared following a standard protocol) (572 mg, 1.80 mmol, 1.00 eq.) in dry DMF (16 mL) was added and the reaction mixture was heated to 110 °C for 15 h. The reaction mixture was cooled to room temperature and filtered over celite. The solvent was removed in vacuum and the residue was purified by column chromatography (packed with acetone/hexane 1:1 + 2% NEt3, eluted with acetone/hexane 3:2 + 2% NEt3) to yield the product S15 as orange foam (251 mg, 360 µmol, 20%). The low yield is due to the formation of a double-substituted side product which was separated from the target compound by column chromatography.

1H NMR (400 MHz, CDCl3): δ (ppm) = 8.66 (d, J = 14.6 Hz, 1 H, 7-H), 7.59 (2 d, J = 14.5, 1 H, 8-H), 7.52 – 7.15 (m, 17 H, DMT-H), 7.09-7.04 (s, 1 H), 6.89 – 6.75 (m, 6 H, DMT-H), 4.26 (dd, J = 13.9, 9.1 Hz, 1 H, 22-H), 4.20 – 4.03 (m, 2 H, 21-H, 22-H), 3.78 (d, J = 0.9 Hz, 6 H, DMT-OMe-H), 3.56 (2 s, 3 H, 19-H), 3.31 – 3.14 (m, 2 H, 20-H), 2.31 (s, 2 H), 1.72 (s, 6 H, 11-H, 12-H);

13C{1H} NMR (100 MHz, CDCl3): δ (ppm) = 177.74 (C-9), 177.59 (C-9), 165.60 (C-4, C-6), 164.85 (C-4, C-6), 162.54, 158.41 (DMT-C), 158.38 (DMT-C), 152.36 (C-7), 151.61 (C-7), 151.51 (C-7), 144.94, 144.89, 144.86, 142.55 (C-18), 140.99 (C-13), 136.11 (DMT-C), 136.08 (DMT-C), 130.12, 130.06, 130.00, 128.47, 128.23, 128.16, 128.05, 127.90, 127.79, 126.71, 124.99, 122.16, 113.17 (DMT-C), 113.08 (DMT-C), 110.02 (C-15), 100.88, 100.81, 99.06 (C-8), 98.65 (C-8), 85.92 (DMT-C), 70.51 (C-21), 70.46 (C-21), 66.89 (C-20), 66.15 (C-20), 65.73 (C-20), 65.64 (C-20), 62.54, 55.20 (DMT-OMe-C), 49.16 (C-10), 45.41, 44.81 (C-22), 44.04 (C-22), 36.49, 31.43 (C-19), 28.84 (C-11, C-12);
HR-MS (ESI+): m/z calc. (C41H41N3NaO7, [M+Na]+): 710.2837, found: 710.2837.
The DMT-protected glycidol merocyanine S15 (160 mg, 230 µmol, 1.00 eq.) was dissolved in anhydrous DCM (4 mL) under nitrogen atmosphere. Me₂NEt (0.26 mL, 2.33 mmol, 10.0 eq.) and CEP-Cl (83.0 mg, 350 µmol, 1.50 eq.) were added dropwise and the reaction mixture was stirred at room temperature for 2 h. After the reaction was completed, the solvent was removed in vacuum and the crude product was purified by column chromatography on silica gel (ethyl acetate/hexane 8:2 + 2% NEt₃). The product S16 was obtained as bright orange foam (187 mg, 210 µmol, 91%).

1H NMR (400 MHz, CDCl₃): δ (ppm) = 8.71 – 8.49 (m, 1 H, 7-H), 7.59-7.46 (m, 1 H, 8-H), 7.46 – 7.07 (m, 13 H, 14-H, 15-H, 16-H, DMT-H), 7.02 – 6.95 (m, 1 H, 17-H), 6.80 – 6.69 (m, 4 H, DMT-H), 4.47 – 4.20 (m, 2H), 4.19 – 3.79 (m, 1H), 3.71 (2 s, 6 H, DMT-OMe-H), 3.53 – 3.44 (m, 3 H, 36-H), 3.43 – 3.09 (m, 1H), 3.07 – 2.97 (m, 1H), 2.72 – 2.59 (m, 1H), 2.57 – 2.46 (m, 1H), 2.37 – 2.27 (m, 1H), 1.91 (s, 1H), 1.71 – 1.58 (m, 6 H, 11-H, 12-H), 1.24 – 0.94 (m, 12 H, 37-H, 38-H);

13C{1H} NMR (100 MHz, CDCl₃): δ (ppm) = 177.48 (C-9), 177.30 (C-9), 177.19 (C-9), 177.14 (C-9), 164.70 (C-4, C-6), 164.42 (C-4, C-6), 164.06 (C-4, C-6), 163.70 (C-4, C-6), 163.53 (C-4, C-6), 163.36 (C-4, C-6), 163.25 (C-4, C-6), 158.66 (DMT-C), 158.43 (DMC-C), 152.30 (C-7), 151.99 (C-7), 151.15, 150.95, 150.91, 145.02, 144.98, 142.74, 141.03 (13-C), 136.35, 136.32, 136.25, 136.22, 136.20, 130.27 (DMT-C), 130.24 (DMT-C), 130.19 (DMT-C), 128.52, 128.40, 128.34, 127.82, 127.80, 126.74, 126.70, 124.88, 124.83, 122.22, 117.97, 117.88, 117.79, 113.30 (DMT-C), 113.12 (DMT-C), 113.08 (DMT-C), 110.01 (C-17), 109.93 (C-17), 101.43, 101.37, 101.33, 98.82 (C-8), 98.21 (C-8), 86.13, 86.02, 77.48, 77.16, 76.84, 69.80, 69.69, 65.51, 56.57, 58.47, 58.26, 58.20, 58.08, 55.29 (DMT-OMe-C), 49.18 (C-10), 49.10 (C-10), 45.41, 45.35, 43.47, 43.31, 43.18, 43.04, 42.94, 42.47, 28.89 (C-11, C-12), 24.86 (C-37, C-38), 24.78 (C-37, C-38), 24.71 (C-37, C-38), 24.64 (C-37, C-38), 24.56 (C-37, C-38), 24.47 (C-37, C-38), 23.08, 23.06, 23.00, 22.97, 20.16, 20.01, 1.99.

31P{1H} NMR (162 MHz, CDCl₃): δ (ppm) = 149.27, 149.23, 149.16, 149.12;

HR-MS (ESI+): m/z calc. (C₉₀H₆₃N₁₅NaO₁₈P, [M+Na]+): 910.39152, found: 910.39136.

3. Synthesis and purification of oligonucleotides

Automated solid phase synthesis was carried using an Applied Biosysiana ABI 392 DNA/RNA synthesizer on a 0.6 µmol scale. The following solutions were used: 0.25 M ethylthiotetrazole (ETT) in dry acetonitrile as activator, 3% dichloro acetic acid in 1,2-dichloroethane for detritylation of RNA oligonucleotides and 3% trichloro acetic acid in 1,2-dichloroethane for DNA and GNA oligonucleotides, 20 mM iodine in THF/water/pyridine 66/12/22 for oxidation, pyridine/acetic anhydride/THF 10/10/80 as Cap A solution and 16% NMI in THF as Cap B solution. DNA and RNA syntheses were carried out in DMT-off mode while GNA oligonucleotides were synthesized in DMT-on mode to prevent degradation during alkaline deprotection.[7] RNA and DNA oligonucleotides were prepared on commercially available CPG solid supports using 70 mM solutions for standard phosphoramidites and 100 mM solutions for self-made phosphoramidites. Coupling times were 2 min for DNA, and 4 min for RNA and GNA. GNA oligonucleotides were prepared on a 3'-phosphate solid support.

RNA oligonucleotides were cleaved from the solid support and deprotected by incubation with aqueous ammonia/abs. ethanol 3/1 overnight at 37 °C. Afterwards, the solid support was filtered off and the solvent was removed in vacuum. The crude samples were redissolved in 1 M TBAF solution in THF (500 µL) and shaken overnight at r. t. After addition of 1 M Tris-HCl pH 8.0 (500 µL), the THF was removed in vacuum and the oligonucleotide samples were desalted by size exclusion chromatography using an Äkta start purification system with three HiTRAP Desalting columns (each 5 mL volume) from GE Healthcare. The desalted oligonucleotides were eluted with water and a flow rate of 2 mL/min. The water was removed under reduced pressure and the samples were redissolved in 600 µL of nanopure water.

DNA oligonucleotides were cleaved from the solid support and deprotected by incubation with 1 mL of aqueous ammonia overnight at 37 °C. After filtration and removal of the solvent in vacuum, the crude oligonucleotides were redissolved in 600 µL of nanopure water.

GNA oligonucleotides were cleaved from the solid support and deprotected by incubation with 1 mL of aqueous ammonia overnight at 37 °C. After filtration and removal of the solvent under reduced pressure, the crude oligonucleotides were dissolved in 600 µL nanopure water.
for 30 min at r. t., 3 M NaCl solution was added to a final NaCl concentration of 300 mM. The crude oligonucleotides were then precipitated with absolute ethanol (70% final concentration) and the dried pellets were redissolved in 600 µL of nanopure water.

PAGE purification of the crude oligonucleotides was performed on 300x200x0.7 mm denaturing polyacrylamide gels (20% acrylamide/bisacrylamide 19:1, 7 M urea) with 1 M TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.3). Gels were run at 35 W constant power for 2.5 h. The oligonucleotides were visualized by UV shadowing on a TLC plate. After excision from the gel, the gel pieces were soaked in TEN buffer (10 mM Tris-HCl, 0.1 mM EDTA, 300 mM NaCl, pH 8.0) for oligonucleotide extraction. The pure oligonucleotides were precipitated with absolute ethanol (70% final concentration).

Crude as well as pure oligonucleotides were analyzed by anion exchange HPLC. A Dionex DNA Pac PA200 column (2x250 mm) with buffer A (25 mM Tris-HCl, 6 M urea, pH 8.0) and buffer B (25 mM Tris, 0.5 M NaClO4, 6 M urea, pH 8.0) was used for this quality control.

Samples were run with a gradient of 0-48% B over 12 column volumes with a flow rate of 0.5 mL/min and UV detection at 260 nm. The oven temperature was set to 60 °C. Pure oligonucleotides were additionally analyzed by HR-ESI mass spectrometry, and the mono-isotopic masses were obtained by deconvolution of the raw spectra.

4. Spectroscopic characterization

4.1 UV/vis spectroscopy

UV/vis absorbance spectra were recorded on a JASCO V-770 spectrophotometer with a PAC-743 cell changer and a Julabo F250 temperature controlling device. 5 µM samples were prepared in 1x phosphate buffer (100 mM NaCl, 10 mM phosphate, pH 7.0) and measured in semi-micro quartz cuvettes with a path length of 10 mm. Temperature-dependent spectra were recorded in a temperature range of 20 to 80 °C in steps of 10 °C. Spectra were measured from 200 to 700 nm with a spectral bandwidth of 2 nm. The response was set to 0.06 s. A scan speed of 1000 nm/min with a data interval of 0.5 nm was used.

4.2 Thermal UV melting curves

Thermal UV melting curves from 1, 2, 5, 10 and 20 µM samples in phosphate buffer (100 mM NaCl, 10 mM phosphate, pH 7.0) were recorded at a VARIAN CARY 100 Bio spectrophotometer equipped with a 6x6 Multicell Block Peltier Series II cell changer and a VARIAN CARY Temperature Controller. For sample concentrations of 1, 2 and 5 µM, 500 µL final sample volumes were prepared and measured in 10 mm semi-micro quartz cuvettes. For sample concentrations of 10 and 20 µM, 300 µL final sample volumes were prepared and measured in 1 mm cuvettes. Prior to measurement, the samples were overlaid with silicon oil to prevent evaporation during heating. Five temperature ramps with a heating/cooling rate of 0.5 °C/min between 10 and 90 °C at 250, 260, 280 and 460 nm were collected. The melting curves were fit to a two-state transition model with upper and lower baselines to determine Tm as previously reported,[12] and thermodynamic parameters were obtained from van’t Hoff analysis, plotting 1/Tm versus ln[c]ext.

4.3 CD and CPL spectroscopy

CD spectra from 5 µM samples in 1x phosphate buffer (100 mM NaCl, 10 mM phosphate, pH 7.0) were recorded on a JASCO P-1020 polarimeter with a sodium light source (λ = 589 nm) in a range between 200 and 600 nm. For the measurements, 10 mm semi-micro quartz cuvettes were used.

CPL spectra for 1 µM samples in 1x phosphate buffer (100 mM NaCl, 10 mM phosphate, pH 7.0) were recorded on a customized JASCO CPL-300/J-1500 hybrid spectrometer in a range between 600 and 200 nm. 10 mm semi-micro quartz cuvettes were used for measurements.

4.4 Fluorescence spectroscopy

Fluorescence spectra were recorded on a Jasco FP-8300 spectrofluorometer equipped with a FCT-817S cell changer and a Julabo F12 temperature control device. 1 µM samples were prepared in 1x phosphate buffer (100 mM NaCl, 10 mM phosphate, pH 7.0) and measured in Hellma ultra-micro quartz cuvettes (10x2 mm). Spectra were recorded with an excitation wavelength of 420 nm (measured emission range 440-700 nm) and an emission wavelength of 540 nm (measured excitation range 200-520 nm). Excitation and emission bandwidth were both set to 5 nm. Spectra were recorded with 0.1 s response, a PMT voltage of 400 V with 0.5 nm data interval and a scan speed of 500 nm/min.

Absolute fluorescence quantum yield of fluorescein (0.1 µM in 100 mM NaCl, 10 mM phosphate, pH 7.0) was conducted with an ILF-835 integrating sphere in a Jasco 6808-H150A liquid cell (1x10x250 mm side length). Spectra were recorded in an emission range between 420 and 750 nm with an excitation wavelength of 435 nm, excitation and emission bandwidths were 2.5 nm, data interval 0.2 nm, scan speed 100 nm/min, response 1 s, PMT voltage 450 V. For relative quantum yield determination, a dilution series (1, 2, 5 µM) of the analyte in 1x phosphate buffer (100 mM NaCl, 10 mM phosphate, pH 7.0) was prepared. Fluorescence measurements were conducted in Hellma ultra-micro quartz cuvettes (10x2 mm). spectra were recorded with excitation at 435 nm, emission range 440 to 750 nm, excitation and emission bandwidths 2.5 nm, scan speed 200 nm/min, data interval 0.2 nm, response time 1 s, PMT voltage 300 V. After fluorescence measurement, the samples were transferred into VARIAN semi-micro quartz cuvettes with a path length of 10 mm for absorbance measurements.
Fluorescence lifetimes were determined by TCSPC using a Horiba DeltaFlex spectrometer with a DeltaDiode excitation source (DD-405L (λex 408 nm) or DD-485L (λex 477 nm), under magic angle conditions (54.7°) of polarization filters. 1 µM samples in 1x phosphate buffer (100 mM NaCl, 10 mM phosphate, pH7.0) were measured in Hellma ultra-micro quartz cuvettes (10×2 mm). The emission bandwidth was adjusted for a stop rate of close to 2%. The photon arrival time was set to 4096 channels (0.026 ns per channel), and data was collected until 10,000 counts had accumulated in the peak channel. The instrument response function was recorded from only buffer by setting the emission monochromator to the peak wavelength of the excitation source. Fluorescence decay curves were analyzed by iterative reconvolution fitting with the instrument response function using the algorithm implemented in Horiba DAS6 (version 6.8).

5. Theoretical calculations

Simulation of absorption spectra

The absorption spectra for the merocyanine dimer were calculated employing a Holstein-type Hamiltonian\[^8\] with the two-particle approximation\[^9\] following the procedure as previously described.\[^10\] A collinear assignment of the chromophores was assumed with a long-range Coulomb coupling \( J_{\text{Coul}} \) between the transition dipole moments of the chromophores. First, the monomer spectrum was calculated to adjust the parameters. Accordingly, best agreement with the experimental absorption spectrum of the monomer was obtained with a transition energy of \( E_g = 21400 \text{ cm}^{-1} \) and a vibrational frequency of \( \omega_0 = 1200 \text{ cm}^{-1} \) assuming a one-effective mode. Furthermore, a Huang-Rhys factor of \( \lambda_2 = 0.74 \) has been used, which determines the ratio between the 0–1 and 0–0 absorption bands of the vibronic progression. For the dimer spectrum, a Coulomb coupling of \( J_{\text{Coul}} = 1100 \text{ cm}^{-1} \) has been applied. Furthermore, the Huang-Rhys factor had to be decreased to \( \lambda_2 = 0.61 \) in order to obtain best agreement with the experimental dimer spectrum. The lower Huang-Rhys factor compared to the monomer can be rationalized by the polarizability effect of the adjacent chromophore within the dimer stack, which puts the chromophores closer to the cyanine limit compared to the monomeric chromophore,\[^11\] leading to a lower effective Huang-Rhys factor. Additionally, a “gas-to-crystal” shift of \(-150 \text{ cm}^{-1}\) was applied to adjust the position of the absorption maximum with the experimental spectrum. For all simulated spectra, a maximum number of 7 vibrational quanta has been used for each chromophore.

The absorption spectra were simulated by

\[
A(E) = \sum_{\epsilon} E_{\epsilon} |\Psi_\epsilon|\bar{\mu}_{\epsilon g}|\Psi_\epsilon >|^2 \exp(- (E - E_{\epsilon g})^2 / \sigma^2
\]

(1)

Here, \( A(E) \) is the absorption at photon energy \( E \) and \( E_{\epsilon g} \) is the transition energy between the ground state with wave function \( \Psi_\epsilon \) and the respective excited state \( \Psi_\epsilon > \). \( \sigma \) is the band-width with \( 2\sqrt{\ln(2)}\sigma \) being the full width at half height. A value of \( \sigma = 830 \text{ cm}^{-1} \) and \( \sigma = 520 \text{ cm}^{-1} \) has been used for the simulation of the monomer and dimer spectrum, respectively. According to equation (1), Gaussian line shape functions are used to simulate the spectra. In addition, \( \bar{\mu}_{\epsilon g} \) is the transition dipole moment operator and defined as

\[
\bar{\mu}_{\epsilon g} = \mu_{\epsilon g} \sum_{n=1,2} |n > < g | + |g > < n|
\]

(2)

with the transition dipole moment \( \mu_{\epsilon g} \) of the monomeric chromophore. \( |n > \) means that chromophore \( n \) is electronically excited, whereas \( |g > \) indicates that all chromophores are in the electronic ground state.

Geometry optimization

Geometry optimization for the two geometrical stereoisomers of merocyanine derivative S17 (in which the ribose/deoxyribose/glycidol substituent is abbreviated as a methyl group) have been performed in order to estimate the energy difference between the two isomers. Toward this goal, the structures were optimized at the level of density functional theory employing the long-range correct CAM-B3LYP-D3\[^{[12]}\] functional together with the def2-SVP\[^{[13]}\] basis set as implemented in the Gaussian 09\[^{[14]}\] package. Accordingly, the \( E \)-isomer is only slightly higher in energy than the respective \( Z \)-isomer with an energy difference of 0.70 kJ/mol.

The overlay of the two isomers shows that they have almost equal steric demand and their dipole moments are essentially overlapping. For these reasons the two (experimentally nearly unseparable) isomers are considered as equal in the analysis of the dimer aggregates.
### Table S1. Sequences of RNA, DNA and GNA oligonucleotides, isolated yields of purified oligonucleotides, and HR-ESI mass spec data.

| Number (paper) | 5'-sequence-3' yield (nmol) | Chemical formula m/z calculated (g/mol) | m/z found (g/mol) |
|----------------|----------------------------|------------------------------------------|-------------------|
| RS1 GAUGAUAGCUAG | 114 C₁₁₆H₁₄₂N₄₉O₈₁P₁₁ | 3857.56124 | 3857.57503 |
| RS2 CUAGCUAUCAuC | 51 C₁₁₂H₁₄₁N₄₀O₈₃P₁₁ | 3714.51558 | 3714.52176 |
| RS3 CUAGCUAUCAuC | 138 C₁₂₉H₁₅₅N₅₀O₈₂P₁₁ | 3913.61530 | 3913.61359 |
| RS4 GAUGAXAGCUAG | 59 C₁₂₉H₁₅₅N₅₀O₈₂P₁₁ | 4056.66096 | 4056.64911 |
| RS5 CUAGCUAUCAuC | 150 C₁₂₉H₁₅₅N₅₀O₈₂P₁₁ | 3913.61530 | 3913.62010 |
| RS6 CUAGCUAUCAuC | 145 C₁₂₉H₁₅₅N₅₀O₈₂P₁₁ | 3890.58808 | 3890.59383 |
| RS7 GAUGAUCCUAG | 75 C₁₁₇H₁₄₁N₄₉O₈₁P₁₁ | 3833.55001 | 3833.54554 |
| RS8 GAUGAUCCUAG | 125 C₁₁₇H₁₄₁N₄₉O₈₁P₁₁ | 3873.55616 | 3873.56620 |
| RS9 GAUGAUCCUAG | 66 C₁₁₇H₁₄₁N₄₉O₈₁P₁₁ | 3834.53403 | 3834.52933 |
| GS1 GATGATGCTAG | 199 C₉₅H₁₂₅N₄₉O₆₀P₁₂ | 3283.50932 | 3283.51387 |
| GS2 CTAGCTATCATC | 124 C₉₂H₁₂₆N₄₀O₆₂P₁₂ | 3154.47931 | 3154.48223 |
| GS3 CTAGCXATCATC | 108 C₁₀₄H₁₃₇N₄₁O₆₃P₁₂ | 3339.56282 | 3339.54139 |
| GS4 GATGAXGCTAG | 101 C₁₀₇H₁₃₆N₅₀O₆₁P₁₂ | 3468.59338 | 3468.58403 |
| GS5 CTAGCXATCATC | 128 C₁₀₄H₁₃₇N₄₁O₆₃P₁₂ | 3339.56282 | 3339.55835 |
| GS6 CTAGCTXTATCAT | 163 C₁₁₄H₁₄₉N₄₁O₇₁P₁₂ | 3763.72327 | 3763.72978 |
| GS7 GATGAGACGTAG | 103 C₉₆H₁₂₄N₅₀O₆₂P₁₂ | 3268.50911 | 3268.52156 |
| GS8 GATGAGACGTAG | 162 C₉₆H₁₂₄N₅₀O₆₂P₁₂ | 3308.51525 | 3308.53476 |
| GS9 GATGAAAGCTAG | 100 C₉₄H₁₂₄N₅₀O₆₂P₁₂ | 3292.52034 | 3292.54139 |
| DS1 GATGATAGCTAG | 249 C₁₁₈H₁₄₈N₄₉O₇₀P₁₁ | 3707.66977 | 3707.69796 |
| DS2 CTAGCTATCATC | 162 C₁₁₆H₁₄₉N₅₀O₇₁P₁₁ | 3578.63975 | 3578.65536 |
| DS3 CTAGCTATCATC | 211 C₁₁₈H₁₄₉N₅₀O₇₁P₁₁ | 3763.72327 | 3763.69956 |
| DS4 GATGAXGCTAG | 103 C₁₀₄H₁₃₇N₄₁O₆₃P₁₂ | 3339.56282 | 3339.55835 |
| DS5 CTAGCTXTATCAT | 206 C₁₂₈H₁₆₀N₄₁O₇₂P₁₁ | 3763.72327 | 3763.72978 |
| DS6 CTAGCTXTATCAT | 194 C₁₂₈H₁₆₀N₄₁O₇₂P₁₁ | 3754.71170 | 3754.71066 |
| DS7 GATGACGTAG | 173 C₁₁₉H₁₄₉N₅₀O₇₁P₁₁ | 3683.65853 | 3683.68621 |
| DS8 GATGACGTAG | 193 C₁₁₉H₁₄₉N₅₀O₇₁P₁₁ | 3723.66468 | 3723.66893 |
| DS9 GATGAGGTAG | 214 C₁₁₉H₁₄₉N₅₀O₇₁P₁₁ | 3698.65820 | 3698.70495 |
| DS10 GATGATGCCTAG | 157 C₁₁₉H₁₄₈N₄₉O₇₀P₁₁ | 3707.66922 | 3707.66874 |

### Table S2. Thermal melting data for RNA duplexes

| ss number | 5'-NNN-3' | ds RNA | Tm (°C) | ΔTm (°C) | ΔH (kcal/mol) | ΔS (cal/molK) | ΔG (kcal/mol) |
|-----------|-----------|--------|---------|----------|---------------|---------------|---------------|
| R0 | GAUGAUAGCUAG CUACAUAGCUAC | 51.6 / 52.6 / 54.4 / 55.2 / 56.8 | -- | -119 | -337 | -18.4 |
| R0 | GAUGAUAGCUAG CUACAUAGCUAC | 35.3 / 36.3 / 38.2 / 39.6 / 40.6 | -16.2 | -105 | -312 | -12.3 |
| R0 | GAUGAUAGCUAG CUACAUAGCUAC | 35.0 / 36.4 / 38.1 / 39.2 / 41.0 | -16.3 | -98.7 | -291 | -11.9 |
| R0 | GAUGAUAGCUAG CUACAUAGCUAC | 31.2 / 32.6 / 34.7 / 36.1 / 37.5 | -19.7 | -89.8 | -266 | -10.6 |
| R0 | GAUGAUAGCUAG CUACAUAGCUAC | 33.4 / 34.6 / 36.8 / 38.1 / 39.7 | -17.6 | -93.4 | -276 | -11.3 |
| R0 | GAUGAUAGCUAG CUACAUAGCUAC | 31.3 / 33.0 / 35.0 / 36.3 / 38.0 | -19.4 | -89.1 | -264 | -10.5 |
| R0 | GAUGAUAGCUAG CUACAUAGCUAC | 32.7 / 34.9 / 35.9 / 36.8 / 37.8 | -18.5 | -118 | -357 | -12.0 |
| R0 | GAUGAUAGCUAG CUACAUAGCUAC | 32.4 / 33.5 / 36.0 / 37.6 / 39.7 | -18.4 | -77.3 | -224 | -10.4 |
| R0 | GAUGAUAGCUAG CUACAUAGCUAC | 35.3 / 36.5 / 39.2 / 39.8 / 42.2 | -15.2 | -86.5 | -252 | -11.5 |
Table S3. Thermal melting data for DNA duplexes

| ds DNA | $T_m$ (°C) 1/2/5/10/20 µM | $\Delta T_m$, 5µM vs D0 | $\Delta H$ (kcal/mol) | $\Delta S$ (cal/molK) | $\Delta G$ (kcal/mol) |
|--------|----------------------|-------------------|----------------|-----------------|----------------|
| DS1    | GATGATAGCTAG         | 40.0 / 41.3       | -86.9         | -248            | -12.9          |
| DS2    | CTACTATCGATC         | 41.3 / 43.6       |               |                 |                |
| DS3    | GATGATAGCTAG         | 30.0 / 30.4       | -79.0         | -232            | -9.9           |
| DS7    | CTACTATCGATC         | 30.2 / 31.2       | -78.6         | -231            | -9.9           |
| DS8    | GATGATAGCTAG         | 28.2 / 29.9       | -76.1         | -224            | -9.3           |
| DS9    | CTACTATCGATC         | 28.1 / 29.8       | -70.4         | -205            | -9.3           |
| DS4    | GATGATAGCTAG         | 30.5 / 32.3       | -74.0         | -215            | -10.0          |
| DS5    | CTACTATCGATC         | 33.2 / 35.5       | -76.6         | -221            | -10.8          |
| DS4    | GATGATAGCTAG         | 36.0 / 37.9       | -73.7         | -209            | -11.3          |

Table S4. Thermal melting data for GNA duplexes

| ds GNA | $T_m$ (°C) 1/2/5/10/20 µM | $\Delta T_m$, 5µM vs G0 | $\Delta H$ (kcal/mol) | $\Delta S$ (cal/molK) | $\Delta G$ (kcal/mol) |
|--------|----------------------|-------------------|----------------|-----------------|----------------|
| GS1    | GATGATAGCTAG         | 62.8 / 64.5       | -89.5         | -238            | -18.6          |
| GS2    | CTACTATCGATC         | 64.5 / 67.0       |               |                 |                |
| GS1    | GATGATAGCTAG         | 58.2 / 59.3       | -5.1          | -97.6           | -18.4          |
| GS9    | GATGATAGCTAG         | 60.3 / 61.9       | -93.7         | -252            | -18.6          |
| GS7    | GATGATAGCTAG         | 52.3 / 53.4       | -11.9         | -92.2           | -16.4          |
| GS8    | GATGATAGCTAG         | 54.0 / 55.1       | -9.3          | -82.7           | -16.0          |
| GS1    | GATGATAGCTAG         | 52.7 / 54.1       | -10.5         | -91.0           | -16.4          |
| GS4    | GATGATAGCTAG         | 54.9 / 56.5       | -7.8          | -116            | -19.4          |
| GS4    | GATGATAGCTAG         | 58.5 / 60.0       | -4.3          | -88.5           | -17.6          |
| GS4    | GATGATAGCTAG         | 51.3 / 52.9       | -11.6         | -86.8           | -15.7          |
SUPPORTING INFORMATION

7. Supporting Figures

Figure S1. a) $^1$H-NMR spectra of double BAM-modified DNA duplex D2b, showing imino proton region of H-bonded base pairs. Conditions: 200 µM DNA in 10 mM Na-phosphate buffer pH 7.0, 100 mM NaCl. b) Hyperchromicity at 460 nm of D2a, D2b and D2c. Melting curves measured at 5 µM DNA in 10 mM Na-phosphate buffer pH 7.0, 100 mM NaCl, heating rate 0.5°C/min.

Figure S2. a) Absorption spectra of BAM (extinction coefficient $\varepsilon = 4.1 \times 10^4$ L·mol$^{-1}$·cm$^{-1}$, dipol moment $\mu = 7.7$ D) at increasing pH (left), and intensities at 435 nm and 473 nm plotted versus pH (right), giving a $pK_a$ of 10.7 by global fitting according to the Henderson-Hasselbalch equation. Conditions: 1 µM BAM in 30 mM Britton-Robinson buffer of the respective pH. b) Normalized absorption spectra for 5 µM BAM in various solvents as given in the legend. Protic and aprotic solvents are shown in red and blue colors, respectively. c) Polarity dependence of BAM absorption in water/1,4-dioxane mixtures of different ratios. Insert: linear relationship between the dielectric constant of the solvent mixture and the absorption intensity at 464 nm.
Figure S3. a) Fluorescence enhancement of BAM upon incorporation into oligonucleotides. a) Excitation and emission spectra of free BAM nucleobase surrogate (magenta) in comparison to single-stranded RNA RS5 containing rBAM (red), and the duplex R1a (green), containing adenine opposite to rBAM (see Table S2 for sequences). b) Excitation and emission spectra of free BAM nucleobase surrogate (magenta, same as in a) in comparison to single-stranded DNA DS3 containing dBAM, and the duplex D1a, containing adenine opposite to dBAM (see Table S3 for sequences). d) Temperature dependence of folding-induced fluorescence enhancement shows that emission intensity ($\lambda_{ex} = 420$ nm) increases upon cooling below the melting temperature of the duplex, reflecting the rigidification of the assembly upon hybridization.

Figure S4. Quantum yield determination of a) single-modified duplexes R1 (orange, $\phi_f = 1.6\%$), D1 (blue, $\phi_f = 2.9\%$) and G1 (green, $\phi_f = 0.7\%$), and b) double modified duplex D2c relative to fluorescein (c) in 10 mM Na-phosphate buffer pH 7.0 (fluorescein $\phi_f = 67\%$; absolute quantum yield determined in integration sphere). Absorbance (left) and emission spectra (right) were recorded at 1 µM, 2 µM and 5 µM (shown) duplex concentration. Quantum yield of the free BAM chromophore was also measured, and resulted in an estimate of $\phi_f < 0.3\%$. 
Figure S5. Fluorescence lifetime (by TCSPC) of duplex D2c (left column, three pairs of excitation and emission wavelengths as indicated in the inset), single-labeled duplexes R1, D1, G1 (middle column, $\lambda_{ex} = 477$ nm, $\lambda_{em} = 492$ nm), and single-stranded DNA DS6 (right, $\lambda_{ex} = 477$ nm, $\lambda_{em} = 492$ nm). Sample (red), instrument response function (black), triexponential fit (blue). Amplitude average lifetimes for D2c see Table S5, for R1, D1, G1 and DS6 the lifetimes are too short and cannot be determined reliably (< 0.1 ns).

Table S5. Fitting results of lifetime measurements of D2c

|     | $A_1$ | $\tau_1$ [ns] | $A_2$ | $\tau_2$ [ns] | $A_3$ | $\tau_3$ [ns] | $<\tau>$ [ns] |
|-----|-------|--------------|-------|--------------|-------|--------------|--------------|
| D2c | 0.93  | 0.15         | 0.06  | 0.83         | 0.004 | 3.28         | 0.20         |
| D2c | 0.81  | 0.18         | 0.16  | 1.21         | 0.024 | 3.45         | 0.43         |
| D2c | 0.91  | 0.20         | 0.08  | 1.83         | 0.007 | 4.74         | 0.35         |
| D2c | 0.73  | 0.32         | 0.24  | 1.67         | 0.023 | 4.73         | 0.80         |
Figure S6. Temperature-dependent fluorescence excitation and emission spectra of double-BAM containing duplexes D2b, R2a and R2c. Conditions: 1 µM duplex in 10 mM Na-phosphate buffer, pH 7.0, 100 mM NaCl, λ_{ex} = 420 nm, λ_{em} = 540 nm. 5°C temperature steps from 20°C (purple) to 60°C (red).

Figure S7. a) Fluorescence excitation and emission spectra of D2c recorded at different wavelength (green: ex 420, em 540; blue: ex 435, em 505; red: ex 450, em 500; black: ex 470, em 490 nm). Matrix scan of double BAM-modified DNA duplex D2c (b) and the single BAM-modified DNA single strand DS6 (c). Conditions: 1 µM each DNA strand in 10 mM Na-phosphate buffer pH 7.0, 100 mM NaCl, 20°C.
Figure S8. a) FRET pair with 2-aminopurine as donor and dBAM as acceptor. Inset shows overlap of donor emission spectrum and acceptor absorbance. b) Fluorescence spectra of DNA samples shown on the right. 1 µM duplex in 10 mM Na-phosphate buffer, pH 7.0, 100 mM NaCl, $\lambda_{em} = 320$ nm. c) integrated residual donor intensity (left, 335-460 nm), and FRET emission (460-600 nm).
8. Supporting Data

8.1 Melting curves and van’t Hoff plots for UV melting analyses.

For all duplex structures, the hyperchromicity at 260 nm for the final heating ramp and the corresponding van’t Hoff plot are shown. The standard deviation for the estimated melting temperatures is ±0.5 °C.

R0
RS1
5' - GAUGAUAGCUAG - 3'
RS2
3' - CUACUAUCGAUC - 5'

R1a
RS1
5' - GAUGAUAGCUAG - 3'
RS3
3' - CUACUAUCGAUC - 5'

R1a'
RS4
5' - GAUGAXAGCUAG - 3'
RS2
3' - CUACUAUCGAUC - 5'

R1b
RS7
5' - GAUGAUCGUAG - 3'
RS3
3' - CUACUAUCGAUC - 5'
R1c  RS8  5' - GAUGAUGCUAG - 3'
RS3  3' - CUACUXCGAUC - 5'

R1d  RS9  5' - GAUGAUUGCUAG - 3'
RS3  3' - CUACUXCGAUC - 5'

R2a  RS4  5' - GAUGAXAGCUAG - 3'
RS3  3' - CUACUXCGAUC - 5'

R2b  RS4  5' - GAUGAXAGCUAG - 3'
RS5  3' - CUAXAUCGAUC - 5'
**SUPPORTING INFORMATION**

R2c  RS4  5' - GAUGAXAGCUAG - 3'
RS6  3' - CUACUXACGAUC - 5'

![Graph 1](image1)

D0  DS1  5' - GATGATAGCTAG - 3'
DS2  3' - CTACTATCGATC - 5'

![Graph 2](image2)

Dla  DS1  5' - GATGATAGCTAG - 3'
..... DS3  3' - CTACTAXCGATC - 5'

![Graph 3](image3)
**SUPPORTING INFORMATION**

**D1b** DS7  5' – GATGATGCTAG – 3'
DS3  3' – CTACTAXCGATC – 5'

**D1c** DS8  5' – GATGATGCTAG – 3'
DS3  3' – CTACTAXCGATC – 5'

**D1d** DS9  5' – GATGATGCTAG – 3'
DS3  3' – CTACTAXCGATC – 5'

**D2a** DS4  5' – GATGATGCTAG – 3'
DS3  3' – CTACTAXCGATC – 5'

---

![Graphs showing melting temperature (Tm) vs. log concentration (ln c)](image)

- **D1b**
- **D1c**
- **D1d**
- **D2a**

---

![Graphs showing 1000/Tm vs. log concentration (ln c)](image)

- **D1b**
- **D1c**
- **D1d**
- **D2a**

---

Table showing data points for different concentrations (1 µM, 2 µM, 5 µM, 10 µM, 20 µM) and their corresponding Tm values.
**SUPPORTING INFORMATION**

**D2b**  
**DS4** 5’ - GATGAXAGCTAG - 3’  
**DS5** 3’ - CTACXATCGATC - 5’

**D2c**  
**DS4** 5’ - GATGAXAGCTAG - 3’  
**DS6** 3’ - CTACTXTCGATC - 5’

**G0**  
**GS1** 5’ - GATGATAGCTAG - 3’  
**GS2** 3’ - CTACXATCGATC - 5’

**Glα**  
**GS1** 5’ - GATGATAGCTAG - 3’  
**GS3** 3’ - CTACTXCGATC - 5’
SUPPORTING INFORMATION

G2a  GS4  5' - GATGAXAGCTAG - 3'
      GS3  3' - CTACTAXCGATC - 5'

G2b  GS4  5' - GATGAXAGCTAG - 3'
      GS5  3' - CTACXATCGATC - 5'

G2c  GS4  5' - GATGAXAGCTAG - 3'
      GS6  3' - CTACXCTCGATC - 5'

\[ \frac{1000}{T_m} \ln c_{tot} \]

\[ \begin{align*}
\text{T}[^\circ C] & \quad \text{Hyp260} \\
-13.5 & \quad 3.00 \\
-13.0 & \quad 3.02 \\
-12.5 & \quad 3.04 \\
-12.0 & \quad 3.06 \\
-11.5 & \quad 3.08 \\
-11.0 & \quad 3.10 \\
-10.5 & \quad 3.12 \\
-10.0 & \quad 3.14 \\
\end{align*} \]
8.2 NMR spectra

Figure S7. $^1$H-NMR spectrum (400 MHz, CDCl$_3$) of compound S2.

Figure S8. $^{13}$C-NMR spectrum (100 MHz, CDCl$_3$) of compound S2.
Figure S9. $^1$H-NMR spectrum (400 MHz, DMSO-$d_6$) of compound S3.

Figure S10. $^{13}$C-NMR spectrum (100 MHz, DMSO-$d_6$) of compound S3.
Figure S11. $^1$H-NMR spectrum (400 MHz, CDCl$_3$) of compound S4.

Figure S12. $^{13}$H-NMR spectrum (100 MHz, CDCl$_3$) of compound S4.
Figure S13. $^1$H-NMR spectrum (400 MHz, DMSO-d$_6$) of compound S5 (rBAM, 2).

Figure S14. $^{13}$C-NMR spectrum (100 MHz, DMSO-d$_6$) of compound S5 (rBAM, 2).
Figure S15. $^1$H-NMR spectrum (400 MHz, CDCl$_3$) of compound S6.

Figure S16. $^{13}$C-NMR spectrum (100 MHz, CDCl$_3$) of compound S6.
Figure S17. $^1$H-NMR spectrum (400 MHz, CDCl$_3$) of compound S7.

Figure S18. $^1$H,$^1$H-COSY-NMR spectrum (400 MHz, CDCl$_3$) of compound S7.
Figure S19. $^{13}$C-NMR spectrum (100 MHz, CDCl$_3$) of compound S7.

Figure S20. $^1$H-NMR spectrum (400 MHz, CDCl$_3$) of compound S8.
Figure S21. $^{13}$C-NMR spectrum (100 MHz, CDCl$_3$) of compound S8.

Figure S22. $^{31}$P-NMR spectrum (162 MHz, CDCl$_3$) of compound S8 (rBAM-PA, 8).
Figure S23. $^1$H-NMR spectrum (400 MHz, CDCl$_3$) of compound S9.

Figure S24. $^{13}$C-NMR spectrum (100 MHz, CDCl$_3$) of compound S9.
Figure S25. $^1$H-NMR spectrum (400 MHz, CDCl$_3$) of compound S10.

Figure S26. $^{13}$C-NMR spectrum (100 MHz, CDCl$_3$) of compound S10.
Figure S27. $^1$H-NMR spectrum (400 MHz, DMSO-$d_6$) of compound S11 (dBAM, 3).

Figure S28. $^{13}$C-NMR spectrum (100 MHz, DMSO-$d_6$) of compound S11 (dBAM, 3).
Figure S29. $^1$H-NMR spectrum (400 MHz, DMSO-$d_6$) of compound S12.

Figure S30. $^{13}$C-NMR spectrum (100 MHz, DMSO-$d_6$) of compound S12.
Figure S31. $^1$H-NMR spectrum (400 MHz, CDCl$_3$) of compound S13.

Figure S32. $^{13}$C-NMR spectrum (100 MHz, CDCl$_3$) of compound S13.
Figure S33. $^{31}$P-NMR spectrum (162 MHz, CDCl$_3$) of compound S13 (dBAM-PA, 9).

Figure S34. $^1$H-NMR spectrum (400 MHz, DMSO-$d_6$) of compound BAM, 1 (S14).
Figure S35. $^{13}$C-NMR spectrum (100 MHz, DMSO-$d_6$) of compound BAM, 1 (S14).

Figure S36. $^1$H-NMR spectrum (400 MHz, CDCl$_3$) of compound S15.
Figure S37. $^{13}$C-NMR spectrum (100 MHz, CDCl$_3$) of compound S15.

Figure S38. $^1$H-NMR spectrum (400 MHz, CDCl$_3$) of compound S16.
Figure S39. $^{13}$C-NMR spectrum (100 MHz, CDCl₃) of compound S16.

Figure S40. $^{31}$P-NMR spectrum (162 MHz, CDCl₃) of compound S16 (gBAM-PA, 10).
Figure S41. HPLC traces for pure RNA oligonucleotides: a) RS1, b) RS2, c) RS3, d) RS4, e) RS5, f) RS6, g) RS7, h) RS8, i) RS9. Anion exchange HPLC, Dionex, DNAPac PA200, 2x250mm, 60°C.
Figure S42. HPLC traces for pure DNA oligonucleotides: a) DS1, b) DS2, c) DS3, d) DS4, e) DS5, f) DS6, g) DS7, h) DS8, i) DS9. Anion exchange HPLC, Dionex, DNAPac PA200, 2x250mm, 60°C.
Figure S43. HPLC traces for pure GNA oligonucleotides: a) GS1, b) GS2, c) GS3, d) GS4, e) GS5, f) GS6, g) GS7, h) GS8, i) GS9. Anion exchange HPLC, Dionex, DNAPac PA200, 2x250mm, 60°C.
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