Supporting Information

Interplay of Structure, Hydration and Thermal Stability in Formacetal Modified Oligonucleotides: RNA May Tolerate Nonionic Modifications Better than DNA

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Experimental Procedures

Methylene chloride, pyridine, acetonitrile, and toluene were dried by refluxing with CaH_2 followed by distillation. Tetrahydrofuran was distilled from sodium/ benzophenone ketyl. Reactions were carried out in oven (150 ^oC) dried glassware under an atmosphere of dry nitrogen. NMR spectra were recorded either on a Varian Mercury 300 MHz or on a Bruker AM360 spectrometer at ambient temperature. Thin layer chromatography (TLC) was performed on Silacycle 0.25 mm 60 Å silica gel F_{254} plates. Column chromatography was done on SiliaFlash® P60 230-400 mesh silica gel (Silacycle).

Dimer 3 was prepared following the procedure by He and Bischofberger.[1] Uridine donor **1**[2] (399 mg, 586 μmol, 2.0 eq.) and adenosine acceptor **2**[3] (176 mg, 293 μmol, 1.0 eq.) were coevaporated with dry THF (3 × 2.5 mL). *N*-iodosuccinimide (198 mg, 1.88 mmol, 3.0 eq.) was dried under oil pump vaccum (<0.1 mmHg) for 40 minutes, dissolved in dry THF (5 mL) and added to **1** and **2** under nitrogen atmosphere. The solution was cooled to -40 °C and triflic acid (2.0 eq., 586 μmol, 88 mg, 52 μl) was added dropwise. The mixture was stirred for 1.5 h allowing the temperature to gradually rise to -25 °C. Diisopropylethylamine (0.1 ml) was added and the reaction was quenched by addition of saturated aqueous $NaHCO_3$ (50 mL), saturated aqueous $Na_2S_2O_3$ (50 mL) and CH_2Cl_2 (100 mL). After extraction the aqueous phase was washed again with CH_2Cl_2 (30 mL). The organic phases were combined, dried over Na_2SO_4 and concentrated. The light-brown foam purified by silica gel column chromatography (toluene/ethyl acetate, stepwise 1:1) to give 260 mg of dimer **3**, 72% yield. MS (FAB+) *m/z* (M+H) 1332.4 (calc. 1332.5). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 9.54 (s, 1H), 9.43 (s, 1H), 8.78 (s, 1H), 8.23 (s, 1H), 8.04 (d, 2H, J = 7.5 Hz), 7.90 (d, 1H, J = 7.2 Hz), 7.69-7.29 (m, 17 H), 6.26 (d, 1H, J = 5.1 Hz), 5.96 (d, 1H, J = 3.9 Hz), 5.46 (t, 1H, J = 5.1 Hz), 5.35 (dd, 1H, J_A = 2.1 Hz, J_B = 8.4 Hz), 4.82-4.70 (m, 3H), 4.58 (t, 1H, J = 5.1 Hz), 4.26 (t, 1H, J = 4.5 Hz), 4.21 (m, 1H), 4.13 (dd, 1H, J_A = 4.2 Hz, J_B = 8.7 Hz), 3.92-3.72 (m, 4H), 1.10 (s, 9H), 0.89 (s, 9H), 0.83 (s, 9H), 0.05 (s, 3H), 0.02 (s, 3H), -0.02 (s, 3H), -0.14 (s, 3H). ^{13}C-NMR ($CDCl_3$) δ: 164.9, 164.2, 162.9, 152.4, 151.6, 150.2, 149.7, 142.2, 139.6, 135.6, 135.3, 134.2, 133.5, 133.2, 132.7, 132.0, 131.3, 130.2, 130.1, 128.7, 128.1, 128.0, 126.8, 123.9, 102.9, 96.4, 89.4, 86.7, 83.6, 83.1, 75.6, 74.9, 71.6, 67.6, 63.1, 27.0, 25.8, 25.7, 19.3, 18.0, 17.9, -4.4, -4.7, -4.8.

[1] He, G. X.; Bischofberger, N. *Tetrahedron Lett.* **1997**, *38*, 945-948.
[2] Rozners, E.; Strömberg, R. *J.Org.Chem.* **1997**, *62*, 1846-1850.
[3] Zhu, X. F.; Williams, H. J.; Scott, A. I. *Perkin 1* **2000**, 2305-2306.

Dimer 4. Dimer **3** (1.05 g, 0.852 mmol) was dissolved in 1M tetrabutylammonium fluoride (TBAF, 10 mL). The reaction was monitored by TLC (MeOH/CH_2Cl_2, 1:9). After 18 h the reaction was quenched with CH_2Cl_2 (180 mL) and water (100 mL). The aqueous phase was extracted with CH_2Cl_2 (50 mL). Sticky solids formed during work-up (remaining on the surface of separation funnel) were dissolved in 30% MeOH in CH_2Cl_2 and added to the organic solutions. Concentration resulted in a dark-brown residue, which was purified by preparative TLC (10% MeOH/CH_2Cl_2) to give 514 mg of dimer **4** as pale-brown solid, which was contaminated with TBAF in molar ratio ca. 1:1 (estimated yield ca. 0.50 mmol, 59%). Further purification was inefficient and resulted in loss of material. However, the crude product could be used directly in the next step. MS (FAB+) *m/z* (M+H) 765.5 (calc. 766.2). ^{1}H-NMR (d_6-DMSO, 300 MHz) δ: 8.73 (s, 1H), 8.62 (s, 1H), 8.03 (d, 2H, J = 7.2 Hz), 7.93 (d, 1H, J = 8.1 Hz), 7.83 (d, 1H, J = 7.5 Hz), 7.66-7.42 (m, 6H), 6.06 (d, 1H, J = 5.1 Hz), 6.03 (d, 1H, J = 5.7 Hz), 5.68 (d, 1H, J = 8.1 Hz), 5.51 (t, 1H, J = 5.3 Hz), 4.77-4.66 (m, 3H), 4.48 (t, 1H, J = 4.8 Hz), 4.16 (t, 1H, J = 4.2 Hz), 4.07 (m, 2H), 3.77 (dd, 1H, J_A = 5.4 Hz, J_B = 11.1 Hz), 3.69 (dd, 1H, J_A = 3.9 Hz, J_B = 11.1 Hz), 3.57 (m, 2H).

Dimer 5. Crude dimer **4** (490 mg, 0.48 mmol) was coevaporated with dry pyridine (10 mL). *para*-Methoxytrityl chloride (294 mg, 0.95 mmol, 2 eq.) was added in three equal portions each in dry pyridine (20 mL). After each addition the mixture was evaporated and coevaporated twice with dry pyridine (20 mL). Each evaporation took ca. 10 minutes at 35 °C (water bath). The residue was dissolved in CH_2Cl_2 (150 mL) and extracted with saturated aqueous $NaHCO_3$ (50 mL), aqueous phase extracted with CH_2Cl_2 (50 mL), organic phases were dried over Na_2SO_4 and concentrated. Silica gel column chromatography (CH_2Cl_2/MeOH/Et_3N, 94:5:1) yielded 328 mg (0.316 mmol, ca. 66%) of dimer **5**, the overall yield starting from dimer **3** is 39%. MS (FAB+) *m/z* (M+H) 1036.7 (calc. 1038.3). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 10.32 (bs, 1H), 9.58 (bs, 1H), 8.62 (s, 1H), 8.22 (s, 1H), 7.99 (d, 2H, J = 7.2 Hz), 7.80 (t, 2H, J = 8.7 Hz), 7.52-7.17 (m, 18H), 6.82 (d, 2H, J = 9.0 Hz), 6.04 (m, 2H), 5.67 (t, 1H, J = 3.0 Hz), 5.35 (dd, 1H, J_A = 1.2 Hz, J_B = 8.1 Hz), 4.72-4.55 (m, 4H), 4.27 (m, 2H), 4.13 (d, 1H, J = 3.0 Hz), 3.73 (s, 3H), 3.64-3.39 (m, 4H).

H-phosphonate dimer 6. Dimer **5** (265 mg, 255 μmol) was coevaporated with dry pyridine (3 × 5 mL), the residue was dissolved in a mixture of THF (5 mL) and pyridine (2 mL) and cooled to -78 °C. 2-Chlorobenzoyl chloride (37.8 μL of 95% purity, 282 μmol, 1.125 eq.) in THF (0.9 mL) was added dropwise. The reaction mixture was stirred for 90 minutes (TLC MeOH/CH_2Cl_2 1:9) and transferred

via syringe into a pre-cooled (-78 °C) mixture of imidazole (208 mg, 3.06 mmol, 12.0 eq.), PCl_3 (89 μL, 1.02 mmol, 4.0 eq.) and Et_3N (427 μL, 3.06 mmol, 12.0 eq.) in CH_2Cl_2 (5 mL). After 30 minutes the reaction mixture was poured into 2 M triethylammonium bicarbonate (pH <8, 60 mL) and extracted with CH_2Cl_2 (100 mL). The aqueous phase was extracted with CH_2Cl_2 (50 mL), organic solutions were dried and concentrated to give a pale-brown solid. The residue was purified by silica gel column chromatography using a stepwise gradient (10% to 16%) of MeOH in CH_2Cl_2 containing 0.2% of Et_3N. To remove remaining triethylammonium salts the final product was dissolved in CH_2Cl_2 (50 mL) and washed twice with water (10 mL). Evaporation of the organic phase gave 250 mg of H-phosphonate dimer **6**, 73%. MS (FAB+) *m/z* (M+H) 1240.6 (calc. 1240.2). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 9.59 (bs, 1H), 9.32 (bs, 1H), 8.78 (s, 1H), 8.28 (s, 1H), 8.02-7.85 (m, 4H), 7.72 (d, 1H, J = 8.1 Hz), 7.57-7.16 (m, 21H), 6.83 (d, 2H, J = 9.0 Hz), 6.42 (d, 1H, J = 4.8 Hz), 6.19 (d, 1H, J = 4.5 Hz), 5.93 (t, 1H, J = 5.1 Hz), 5.85 (s, 0.5H), 5.67 (t, 1H, J = 4.8 Hz), 5.34 (d, 1H, J = 8.4 Hz), 5.26 (p, 1H, J = 5.1 Hz), 4.79-4.70 (m, 3H), 4.46 (dd, 1H, J_A = 4.2 Hz, J_B = 6.9 Hz), 4.27 (m, 1H), 3.93 (dd, 1H, J_A = 2.7 Hz, J_B = 11.4 Hz), 3.80 (dd, 1H, J_A = 4.2 Hz, J_B = 11.4 Hz), 3.74 (s, 3H), 3.49 (m, 2H), 2.94 (m, 6H), 1.20 (t, 9H, J = 7.5 Hz).

Preparation of solid support for synthesis of formacetal modified RNA. Dimer **5** was converted into mixture of 2' and 3'-O-succinates following our previously reported procedures.[4] Long Chain Alkyl Amino Controlled Pore Glass (LCAA CPG, 100 mg, 6-8 μmol of free amino groups) was placed in a 2.5 mL syringe fitted with glass frit filter and washed twice with dry DMF (1 mL). The succinate mixture (15 mg, 11.8 μmol) was evaporated with dry pyridine (3 × 1 mL) and dissolved in dry DMF (0.8 mL), diisopropylethylamine (100 μL, 575 μmol) and HBTU (4.2 mg 11.2 μmol) were added and the mixture was stirred for 40 min. The mixture was taken in the syringe with LCAA CPG and the syringe was agitated at room temperature for 22 h. The reaction mixture was discarded and the solid support was washed with CH_2Cl_2 (3 × 1 mL) and DMF (3 × 1 mL). Remaining free amino groups were capped with acetic anhydride (0.1 mL) and 2,6-lutidine (0.1 mL) in DMF (0.5 mL) for 30 min. The capping mixture was discarded, the solid support was washed with DMF (3 × 1 mL), methanol (2 × 1 mL), CH_2Cl_2 (3 × 1 mL) and diethyl ether (2 × 1 mL) and finally dried in vacuum. Treatment of small aliquot with 5% dichloroacetic acid in CH_2Cl_2 followed by spectrometric evaluation of the yellow trityl color indicated that the support was loaded with formacetal-linked succinate at 28 μmol/g.

[4] Sigurdsson, S.; Rozners, E.; Westner, E.; Bizdena, E.; Strömberg, R. *Nucleosides Nucleotides* **1995,** *14,* 875-878.

Synthesis of formacetal modified RNA $(UfA)_6$ was done following our previously reported procedures.[5,6] Long chain alkylamino controlled pore glass support derivatized with dimer **5** (~40 mg, 28 μmol/g, ~1.12 mmol) was placed in a gastight glass syringe (1 mL) equipped with a porous glass filter. All reactions and washings were done by manual intake of an appropriate solvent or reagent as shown in Table S1.

Table S1. Solid phase synthesis protocol

Step	Reagent or solvent	Volume	Time
Coupling	0.1 M H-phosphonate in pyridine, mix with 0.5 M pivaloyl chloride in MeCN	0.1 mL 0.1 mL	3 min
Washing	MeCN CH_2Cl_2	4 × 0.5 mL 4 × 0.5 mL	4 min
Deprotection	3% dichloroacetic acid in CH_2Cl_2	5 × 0.5 mL	2 min
Washing	CH_2Cl_2 MeCN	4 × 0.5 mL 4 × 0.5 mL	4 min

After the assembly of the desired sequence was complete, the support was treated with 2% I_2 in pyridine/water (98:2, 30 min, rt), washed with acetonitrile (5 × 1 mL) and CH_2Cl_2 (4 × 1 mL) and dried. Oligoribonucleotides were cleaved from the support and deprotected using concentrated aqueous ammonia/ethanol (3:1, 8 h, rt) to cleave *N*-benzoyl and 2'-*O*-(2-chlorobenzoyl) groups. Oligonucleotides were purified by reverse phase HPLC as described previously (Figure S1).[7] The composition of the synthesized **$(UfA)_6$** was confirmed by MALDI-TOF mass spectroscopy *m/z* calculated (M-1) 3453.8, *m/z* obtained 3453.5.

[5] Westman, E.; Sigurdsson, S.; Stawinski, J.; Strömberg, R. *Nucleic Acids Sym Ser.* **1994**, *31*, 25. Strömberg, R., Stawinski, J. *Current Protocols in Nucleic Acid Chemistry*, unit 3.4. John Wiley & Sons, Inc.: New York, 2000. Rozners, E.; Renhofa, R.; Petrova, M.; Popelis, Y.; Kumpins, V.; Bizdena, E. *Nucleosides Nucleotides* **1992,** *11,* 1579-1593.

[6] Rozners, E.; Strömberg, R. *J. Org. Chem.* **1997**, *62*, 1846-1850. Rozners, E.; Katkevica, D.; Strömberg, R. *ChemBioChem* **2007**, *8*, 537-545.

[7] Rozners, E.; Moulder, J. *Nucleic Acids Res.* **2004,** *32,* 248-254.

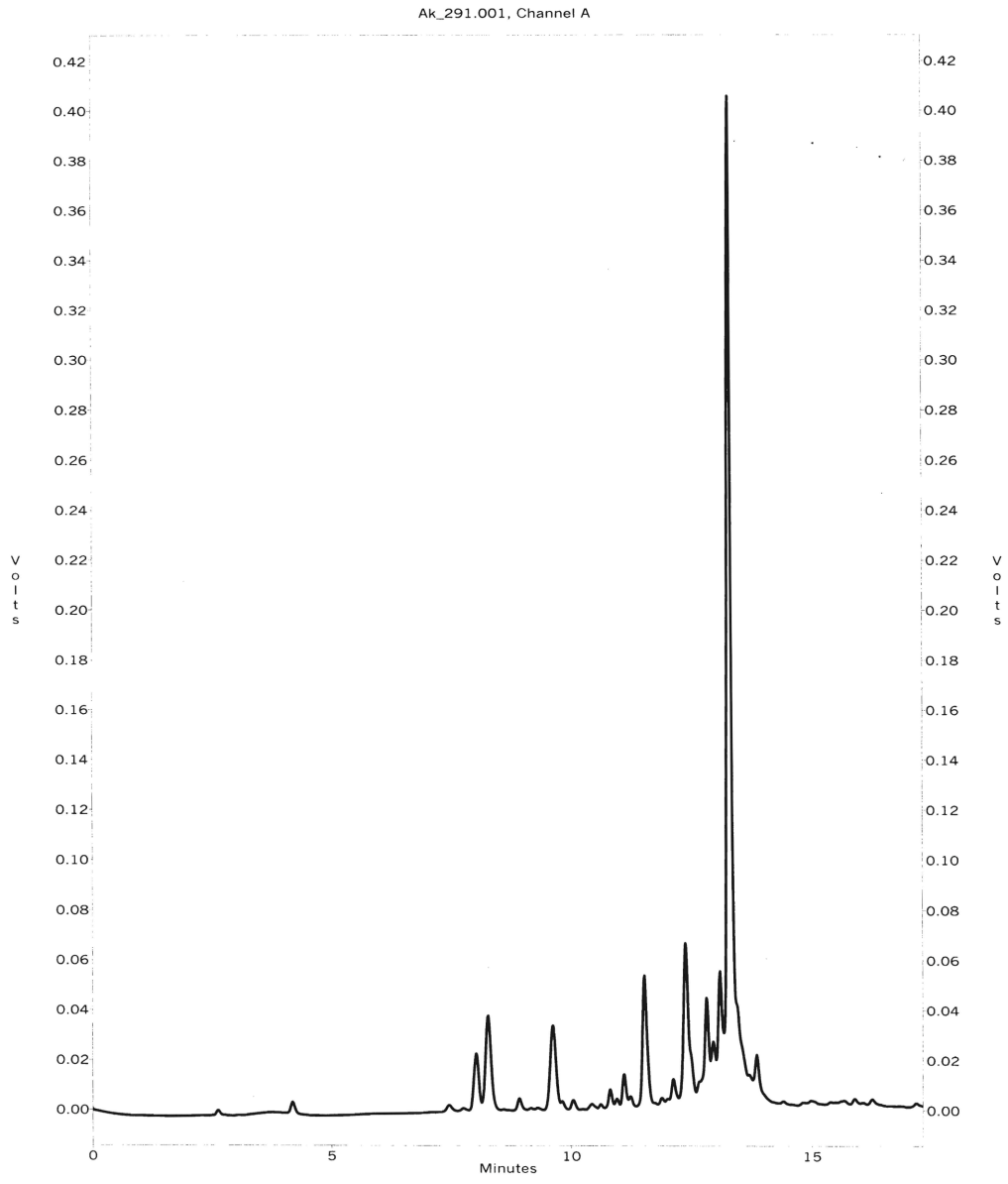


Figure S1. RP HPLC trace of crude synthesis mixture of **(UfA)$_6$**. Conditions: Discovery® HS C18 column (4.6 × 250 mm, 3 μm) at 50 °C, linear gradient of acetonitrile (2-40 % in 40 min) in 50 mM triethylammonium acetate buffer (pH 6.5), flow rate 1 mL/min.

5'-*O*-(*tert*-Butyldiphenylsilyl)-3'-*O*-methylthiomethylthymidine was prepared following the procedure described by Quaedflieg et al.[8] Purification by silica gel column chromatography (hexanes/ethyl acetate, stepwise 9:1 to 4:6) gave the title compound in 76% yield. TLC R_f = 0.56 (hexanes/ethyl acetate, 1:1). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.57 (bs, 1H), 7.69-7.36 (m, 11H), 6.34 (q, 1H), 4.60 (m, 3H), 4.09-3.80 (m, 3H), 2.45 (m, 1H), 2.16-2.04 (m, 1H), 2.11 (s, 3H), 1.66 (s, 3H), 1.10 (s, 9H). ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 170.7, 164.4, 135.8, 135.6, 135.5, 130.4, 130.3, 128.2, 111.4, 85.2, 85.1, 76.3, 73.9, 64.3, 38.1, 27.2, 19.6, 14.0, 12.3.

Thymidine dibutylphosphate 7 was prepared from 5'-*O*-(*tert*-butyldiphenylsilyl)-3'-*O*-methylthiomethylthymidine following the procedure described by Quaedflieg et al.[5] Purification by silica gel column chromatography (methanol in dichloromethane, stepwise 0 to 3%, containing 0.1% of triethylamine) gave **7** in 81% yield. TLC R_f = 0.56 (dichloromethane/methanol, 19:1). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.32 (bs, 1H), 7.70-7.35 (m, 11H), 6.32 (q, 1H), 5.18 (m, 2H), 4.55 (d, 1H), 4.15-3.83 (m, 7H), 2.52 (m, 1H), 2.15 (m, 1H), 1.62 (m, 4H), 1.59 (s, 3H), 1.37 (m, 4H), 1.08 (s, 9H), 0.90 (t, 6H). ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 160.1, 150.6, 135.7, 135.5, 133.3, 132.5, 130.3, 128.2, 111.5, 91.5, 85.1, 84.7, 79.6, 67.9, 64.2, 38.5, 32.5, 32.4, 27.2, 19.6, 18.8, 13.7, 12.2.

Dimer 9 was prepared by coupling thymidine dibutylphosphate (**7**) with *N*-benzoyl-3'-*O*-*tert*-butyldimethylsilyldeoxyadenosine (**8**, prepared as described by Zhu et al.[3]) following the procedures by Quaedflieg et al.[5] Purification by silica gel column chromatography (methanol in dichloromethane, stepwise 0 to 3%) gave dimer **9** in 43% yield. TLC R_f = 0.49 (dichloromethane/methanol, 19:1). MS (ESI+) *m/z* (M+H) 962.0 (calc. 962.4). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.74 (bs, 1H), 9.59 (bs, 1H), 8.79 (s, 1H), 8.29 (s, 1H), 8.05 (d, 2H), 7.65-7.33 (m, 14H), 6.47 (t, 1H), 6.25 (q, 1H), 4.73 (s, 2H), 4.60 (q, 1H), 4.33 (m, 1H), 4.10 (q, 1H), 4.02 (m, 1H), 3.94-3.68 (m, 4H), 2.77 (m, 1H), 2.45 (m, 2H), 2.06 (m, 1H), 1.55 (s, 3H), 1.06 (m, 9H), 0.90 (s, 9H), 0.08 (2s, 6H). ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 165.4, 164.2, 152.7, 151.9, 150.7, 150.0, 142.1, 35.7, 135.5, 135.4, 133.8, 133.1, 132.8, 132.6, 130.3, 128.8, 128.5, 128.2, 124.0, 111.3, 95.8, 86.3, 85.4,, 84.8, 84.7, 78.7, 72.1, 68.2, 64.2, 40.9, 38.9, 27.2, 25.9, 19.6, 18.2, 12.1, -4.4, -4.6.

Dimer 10. Dimer **9** (475 mg, 0.49 mmol) was dissolved in dry THF (8 mL) and TBAF (2 mL) was added. The mixture was stirred for 1 h (TLC MeOH/CH_2Cl_2 1:19) and quenched with saturated aqueous $NaHCO_3$ (1 mL). The mixture was evaporated, dissolved in water (50 mL) and extracted with

[8] Quaedflieg, P. J. L. M.; Timmers, C. M.; van der Marel, G. A.; Kuyl-Yeheskiely, E.; van Boom, J. H. *Synthesis* **1993**, 627.

CH_2Cl_2 (50 mL) and ethyl acetate (50 mL). The aqueous layer was evaporated until 10 mL and purified on C18 reverse-phase silica gel column (27 × 50 mm) eluting with stepwise gradient of methanol (0 to 60 % in 10 % steps) in water. The fractions containing the product were evaporated and purified using silica gel column chromatography (methanol in dichloromethane, stepwise 0 to 12%) to give dimer **10** (150 mg, 50%). TLC R_f = 0.14 (dichloromethane/methanol, 19:1). MS (ESI+) *m/z* (M+H) 609.8 (calc. 610.2). ^{1}H NMR ($CDCl_3$, 360 MHz) δ 11.2 (bs, 2H), 8.75 (s, 1H), 8.63 (s, 1H), 8.05 (m, 2H), 7.96-7.52 (m, 4H), 6.51 (t, 1H), 6.12 (m, 1H), 5.47 (d, 1H), 5.06 (m, 1H), 4.72 (s, 2H), 4.49 (m, 1H), 4.28, (m, 1H), 4.04 (m, 1H), 3.88, (m, 1H), 3.78-3.66 (m, 2H), 3.53 (m, 2H), 2.89 (m, 1H), 2.41 (m, 1H), 2.25-2.06 (m, 2H), 1.76 (s, 3H). ^{13}C NMR ($CDCl_3$, 90.5 MHz) δ 165.7, 163.7, 152.0, 151.6, 150.4, 150.3, 143.0, 135.9, 133.4, 132.4, 128.5, 125.8, 109.5, 94.0, 85.7, 85.0, 83.8, 83.5, 76.8, 70.9, 68.0, 61.2, 36.8, 12.2.

Dimer 11 was prepared using the standard tritylation procedure. Dimer **10** (208 mg, 0.34 mmol) was coevaporated with dry pyridine (3 × 25 mL) and dissolved in dry pyridine (20 mL). Dimethoxytritylchloride (128 mg, 0.38 mmol) was added and the reaction mixture was stirred overnight. Ethanol (2 mL) was added and the mixture was evaporated to syrup. The residue was dissolved in methylene chloride (50 mL) and extracted with saturated aqueous $NaHCO_3$ (50 mL), the organic phase was dried over Na_2SO_4 and concentrated. Silica gel column chromatography (stepwise gradient of MeOH in CH_2Cl_2 containing 0.2% of NEt_3, 0 to 7% in 1% steps) yielded 232 mg (0.255 mmol, 75%) of dimer **11**.). MS (ESI+) *m/z* (M+H) 911.9 (calc. 912.4). ^{1}H NMR ($CDCl_3$, 360 MHz) δ 9.62 (bs, 1H), 8.75 (s, 1H), 8.29 (s, 1H), 8.02 (m, 2H), 7.57-7.16 (m, 13H), 6.80 (m, 4H), 6.50 (t, 1H), 6.27 (m, 1H), 4.73 (m, 2H), 4.57 (m, 1H), 4.35 (m, 1H), 4.22-4.10 (m, 3H), 3.86 and 3.72 (m, 2H), 3.74 (s, 6H), 3.46 and 3.27 (m, 2H), 2.76 (m, 1H), 2.54 (m, 2H), 2.20 (m, 1H), 1.42 (s, 3H).

Dimer 12 was prepared from dimer **11** following the literature procedure[9] and purified by silica gel column chromatography (stepwise gradient of methanol 0 to 5% in dichloromethane containing 2% of triethylamine) gave **12** as a mixture of two diastereomers in 50% yield. TLC R_f = 0.57 and 0.50 (dichloromethane/methanol/triethylamine, 93:5:2). MS (ESI+) did not give the molecular peak of **12**, instead we observed the hydrolysis product (loss of diisopropylamine) *m/z* (M+H) 1028.8 (calc. 1029.4). ^{1}H NMR (CD_3CN, 360 MHz, mixture of diastereomers) δ 9.18 (bs, 1H), 7.98 (2s, 1H), 7.66 (2s, 1H), 7.34 (d, 2H), 7.08 (m, 1H), 6.98-6.94 (m, 2H), 6.85 (m, 2H), 6.76 (m, 3H), 6.65-6.52 (m, 6H),

[9] de Koning, M. C.; Ghisaidoobe, A. B. T.; Duynstee, H. I.; Ten Kortenaar, P. B. W.; Filippov, D. V.; van der Marel, G. A. *Org. Process Res. Dev.* **2006**, *10*, 1238-1245.

6.19 (d, 4H), 5.83 (m, 1H), 5.47 (m, 1H), 4.16-4.06 (m, 3H), 3.77 (m, 1H), 3.63 (m, 1H), 3.60-3.51 (m, 7H), 3.33 (m, 1H), 3.07 (s, 6H), 3.20-2.98 (m, 6H), 2.57 (m, 2H), 2.33-2.23 (m, 1H), 2.02 (t, 3H), 2.02-1.90 (m, 1H), 1.72-1.53 (m, 2H), 1.31 (m, 2H), 0.56 (m, 12 H). ^{31}P NMR (CD_3CN, 121 MHz) δ 149.6 and 149.5.

Synthesis of formacetal modified DNA was done on Expedite 8909 DNA synthesizer following the standard DNA coupling protocol. The coupling yield for dimer **12** was ca. 95%. The modified DNA was cleaved from support and deprotected using standard DNA procedures and purified by reverse-phase HPLC as described above. The composition of the synthesized DNA was confirmed by MALDI-TOF mass spectroscopy: **OL4** *m/z* calculated (M-1) 3345.6, *m/z* obtained 3345.0 and **OL6** *m/z* calculated (M-1) 4013.8, *m/z* obtained 4013.7. The control all DNA **OL5** was purchased from Thermo-Fisher.

Circular Dichroism Spectra were collected on a Jasco 810 spectropolarimeter at room temperature. Oligonucleotide concentrations were 8 μM in 10 mM sodium phosphate (pH 7.4) and 300 mM NaCl. The result is an average of 10 scans.

Table S2. Experimental t_m and thermodynamic data for melting of r(UfA)$_6$ **OL2**.

Experiment	0%	5%	10%	15%	20%	ΔH van't Hoff (cal/mol)	ΔS(eu)	T1	T2	ΔH δα/δT_m (cal/mol)	ΔG
					Ethylene glycol						
er233A	38.5	37.0	35.7	34.5	32.9	84500	245	32.18	44.72	78480	8.5
er233B	38.1	36.9	35.7	34.4		78700	227	31.82	44.64	76656	8.3
er233C	38.2	36.7	36.0	34.8	33.1	84000	244	32.03	44.67	77809	8.3
er233C	38.3	36.8	35.8	34.7	33.1	81800	237	32.06	44.91	76602	8.3
er233D	38.3	36.9	35.9	34.8	33.0	81100	234	31.94	44.91	75864	8.5
er233E	38.3	36.9	35.8	34.5	33.3	82200	238	32.03	44.80	77048	8.4
er233E	38.4	36.7	35.8	34.6	33.2	83800	243	32.19	44.92	77360	8.4
er233E	38.4	37.0	35.8	34.6	33.2	82600	239	32.10	44.82	77374	8.5
er233F	38.3		36.0		32.9	82400	238	32.05	44.79	77232	8.6
Average		**36.9**	**35.8**	**34.6**	**33.1**						
St deviation		**0.1**	**0.1**	**0.1**	**0.1**						
					Glycerol						
er234A	38.1	36.8		35.1	34.3	84500	245	31.89	44.59	77386	8.5
er234B	38.3	36.7	36.2	35.1	34.4	81900	237	32.12	44.71	78151	8.4
er234B	38.3	37.0		35.1	34.5	81500	236	31.92	44.85	76079	8.3
er234B	38.4	36.9	36.3	35.1	34.2	80500	232	31.98	44.92	76052	8.5
er234C	38.2	37.0	36.3	35	34.6	81800	237	31.85	44.62	76959	8.3
er234D	38.2	36.8	36.3	35.1	34.1	81300	235	31.96	44.59	77833	8.4
er234D	38.1	37.0	36.0		34.5	85100	247	31.97	44.61	77779	8.5
er234E			36.2	35.1	34.2						
er234E			36.4		34.4						
er234E		37.1		35.0	34.1						
er234F		36.9			34.2						
er234F		37.2	36.1	34.9	34.4						
Average		**36.9**	**36.2**	**35.1**	**34.3**						
St deviation		**0.2**	**0.1**	**0.1**	**0.2**						
					Acetamide						
er235A/E	38.2	33.6	31.2	28.2	23.7	81600	236	31.83	44.64	76719	8.4
er235A/E	38.1	33.9	31.3	28.2	23.4	81700	236	31.83	44.58	77065	8.5
er235A/E	38.2	33.7	31.3	28.2	23.6	82500	239	31.85	44.71	76442	8.4
er235B/G	38.4	33.6	31.3	28.2	23.5	79200	228	32.02	44.86	76640	8.5
er235B/G	38.1	33.5	31.4	28.1	23.4	83900	243	31.91	44.74	76643	8.5
er235B/G	38.2	33.6	31.4	28.1		82700	240	31.87	44.77	76224	8.3
er235C	38.4	33.7	31.1		23.6	80700	233	32.05	44.82	77058	8.4
er235D	38.3	33.6	31.4			83500	242	32.07	44.81	77242	8.4
er235D	38.4	33.7	30.8			82000	237	32.24	44.79	78450	8.5
er235D	38.4		30.8			81500	235	32.08	44.78	77481	8.6
er235F			31.3	28.2							
Average	**38.3**	**33.7**	**31.2**	**28.2**	**23.5**	**82192**	**238**			**77101**	**8.4**
St deviation	**0.1**	**0.1**	**0.2**	**0.05**	**0.1**	**1554**	**5**			**711**	**0.1**

Table S3. Experimental t_m and thermodynamic data for melting of dCG(TA)$_5$CG **OL5**.

Experiment	0%	5%	10%	15%	20%	ΔH van't Hoff (cal/mol)	ΔS(eu)	T1	T2	ΔH $\delta\alpha/\delta T_m$ (cal/mol)	ΔG
Ethylene glycol											
es063A/062C	46.2	45.5	44.2	42.9	40.9	40900	101.2	27.92	61.65	30302	9.5
es063A/062D	46.7	46.0	44.5	42.9	40.5	35200	84.0	27.96	63.65	28813	9.1
es063A/062E	45.4	44.3		40.9							
es063A/062E		44.3		40.4							
es063B/062E	45.4	43.8	42.9	40.2							
es063C/062E	44.9	43.8	42.6	40.1							
es063D/062F	44.8	44.0	42.5	40.5							
es063D/062F	45.0	44.0		41.0							
es063D/062F	44.7	44.0		40.7							
es063D			43.6								
es063E		44.8	44.1								
Average		**45.2**	**44.1**	**42.8**	**40.6**						
St deviation		**0.4**	**0.3**	**0.2**	**0.3**						
Glycerol											
es062C/063A	46.2	45.6	44.0	43.2		40600	101.0	28.25	60.49	31627	9.3
es062D/063A/C	45.4	45.5	42.6			38700	95.6	28.46	60.55	31803	9.0
es062E/G/063D	44.9	42.5	43.2	40.1							
es062E/G/063D	45.6	43.5	43.7	40.7							
es062E/G/064A	45.6	43.9	43.7	40.2							
es062E/G/064A	45.2	42.8	43.2	40.1							
es062F/064A	46.0		43.6	43.0	40.1	37100	90.3	28.03	61.68	30388	9.1
es062F/064B	46.8		43.6	42.9	40.4	36300	87.4	28.81	63.71	29554	9.2
es062F/064C		43.7	42.9	40.5							
Average		**45.4**	**43.4**	**43.2**	**40.3**						
St deviation		**0.3**	**0.6**	**0.3**	**0.2**						
Acetamide											
es064A		44.3		37.2	34.1						
es064A	46.2	44.0	39.0	36.8	33.8	36500	88.3	28.41	62.82	29856	9.1
es064A	46.0	44.1	40.6	37.5	33.3	36700	88.9	28.24	61.56	30699	9.1
es064A		43.9		37.1	33.2						
es064B	46.3	43.3	39.1	36.6	34.0	39400	97.1	28.84	60.53	32243	9.3
es064C		43.8	40.1	37.2	33.3						
es064C		43.8	40.2	37.5	33.6						
es064C		43.6	40.4	37.7	33.4						
es064C		43.4	40.2	37.7	33.7						
Average	**46.2**	**43.8**	**39.9**	**37.3**	**33.6**	**37933**	**93**			**30587**	**9.2**
St deviation	**0.4**	**0.3**	**0.6**	**0.4**	**0.3**	**2033**	**6**			**1128**	**0.1**

Table S4. Experimental t_m and thermodynamic data for melting of dCG(TfA)$_5$CG **OL6**.

Experiment	0%	5%	10%	15%	20%	ΔH van't Hoff (cal/mol)	ΔS(eu)	T1	T2	ΔH $\delta\alpha/\delta T_m$ (cal/mol)	ΔG
Ethylene glycol											
er289A	30.2	30.3	29.2	27.5	26.8	32300	80.6	16.45	43.68	34168	7.3
er289B		30.1	29.1	27.7							
er289B		29.9	28.9	27.7							
er289B		30.0	28.7	27.6							
er289B		30.2	29.3	27.8							
er289C	30.8	30.7	29.6	28.0	27.0	33800	85.3				7.3
er289C	30.4	30.7	29.6	28		33200	83.3	16.17	43.56	33922	7.4
er289D	30.8	29.7	29	27.2	26.5	30900	75.6	16.27	43.54	34081	7.5
er289D		30.5	28.7	28.1	26.9						
Average		**30.2**	**29.1**	**27.7**	**26.8**						
St deviation		**0.4**	**0.3**	**0.3**	**0.2**						
Glycerol											
egmg011A	30.3	30.4	30.2			37000	95.8	16.83	43.17	35312	7.3
egmg011A		30.3									
egmg011B	30.0	30.7	29.2			33300	83.7	16.29	43.32	34362	7.3
egmg011B	30.5	30.8	30.1			32000	79.4	16.45	43.47	34410	7.4
egmg011B	30.6	30.7	29.9		28.8	32700	81.7	16.42	45.39	32285	7.4
egmg011B		30.1		28.7							
egmg011C	30.7	30.1	29.6		28.4	31000	76	16.48	43.15	34830	7.4
egmg011D		29.6		28.3							
egmg011E		30.1	28.8	29							
egmg011E			29.5	28.3							
egmg011F	30.0	30.3	29.7	29.0	28.2	32400	81.0	16.51	42.46	35722	7.3
egmg011G		30.0	28.4	28.2							
egmg011G		30.2	28.6	28.6							
egmg011G		30.2	28.8	28.4							
egmg011G		29.7	28.3	28.3							
Average		**30.5**	**29.9**	**28.8**	**28.5**						
St deviation		**0.3**	**0.3**	**0.4**	**0.3**						
Acetamide											
er288A/289A	30.1		27.9		24.7	33300	83.8				7.3
er288A/289B		27.8		24.9							
er288A/289B	30.3		27.5		25.2	34200	86.9				7.2
er288B/289B	30.8	30.6			25	31300	77				7.4
er288B/289B	30.4	27.6		24.8							
er288C	30.5	29.9	27.3	26.5	25.4	33100	82.9	16.26	44.5	33009	7.4
er288C/289C	30.7	29.8	27.2	26.3	24.5	31800	78.7	16.25	44.24	33276	7.4
er288D		29.6	27.1	26.7	25.1						
er288D		30.2	27.3	26.4	24.8						
er288D		30.1	27.2	26	24.9						
er288D		30.5	26.9	26.1							
er288E			27.1	26.3							
Average	**30.4**	**30.1**	**27.4**	**26.3**	**24.9**	**32820**	**82**			**34125**	**7.4**
St deviation	**0.3**	**0.4**	**0.3**	**0.2**	**0.3**	**1524**	**5**			**999**	**0.1**

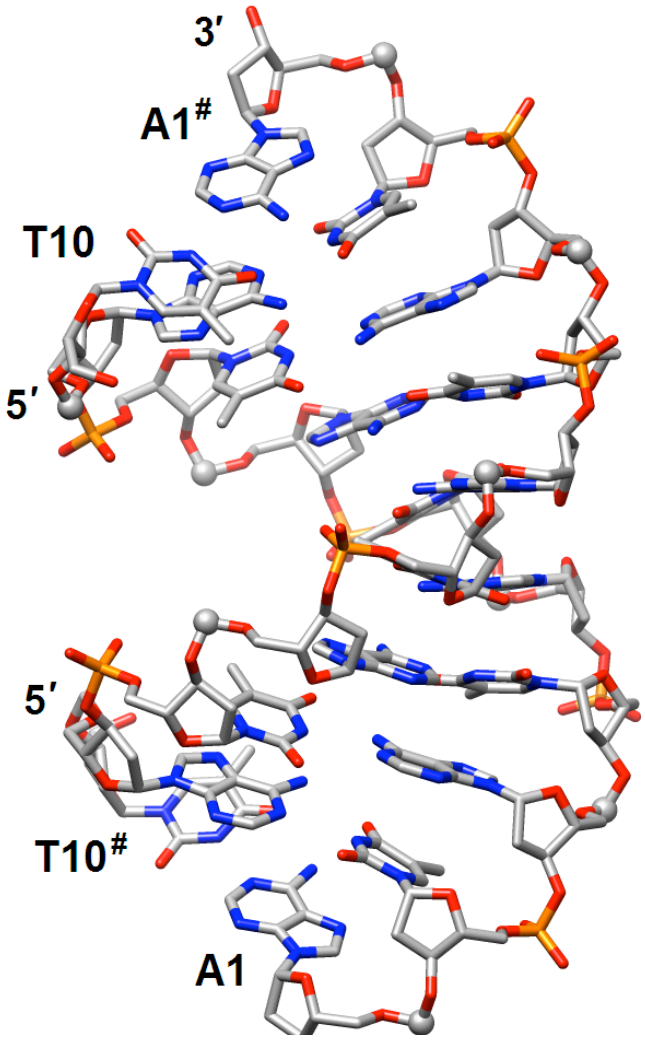
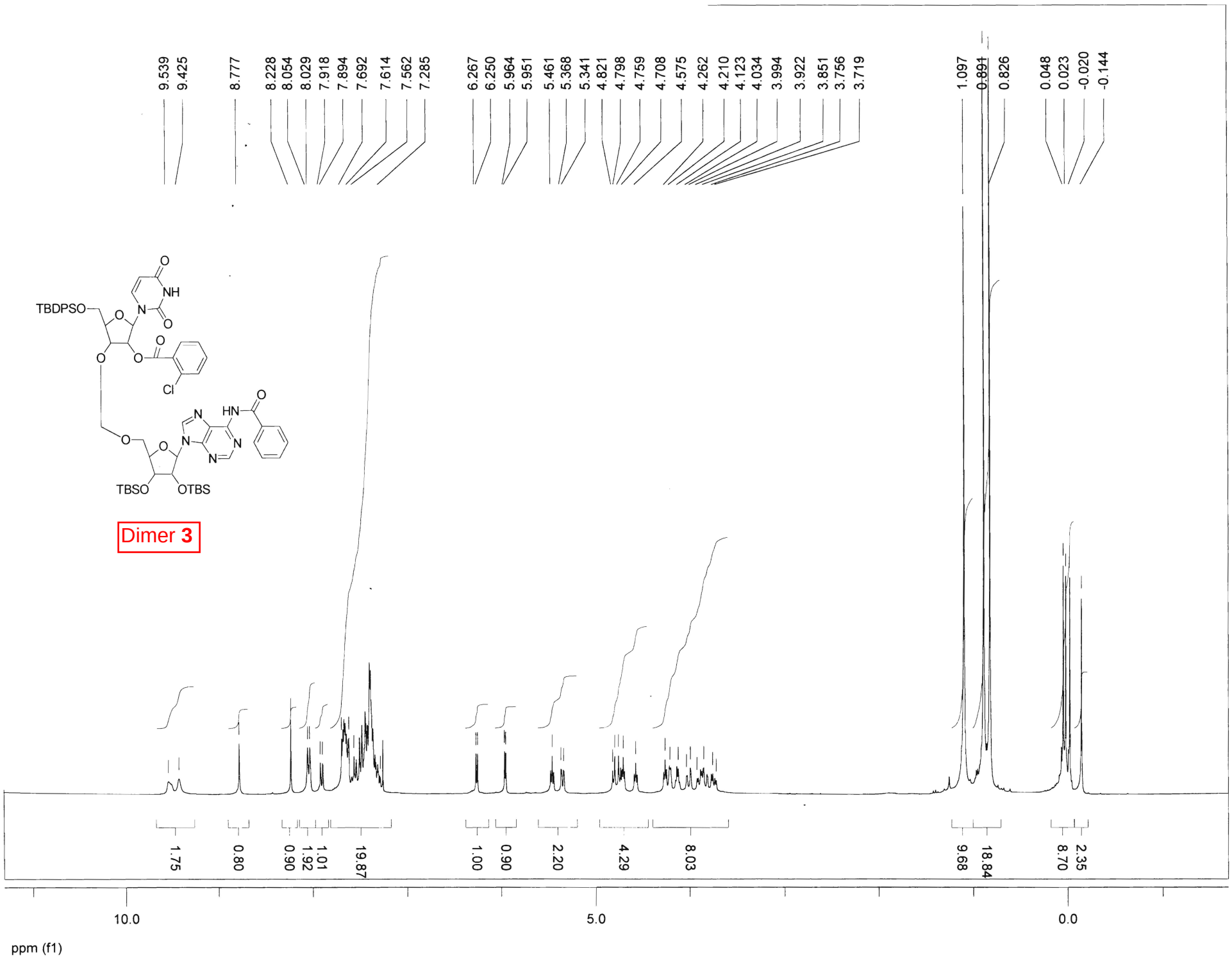


Figure S2. Modeled duplex [d(T**f**ApT**f**ApT**f**ApT**f**ApT**f**ApT**f**A)]$_2$ (**OL4**). The formacetal carbon is highlighted with a sphere and the hash symbol indicates the complementary strand. The model was built manually in Turbo-Frodo using the T4**f**A5 dimer co-ordinates from the crystal structure of the sequence **OL7**. The energy minimization was carried out using the program MOE (MOE 2008.10; The Chemical Computing Group Inc.) with parameters from the *AMBER* force-field.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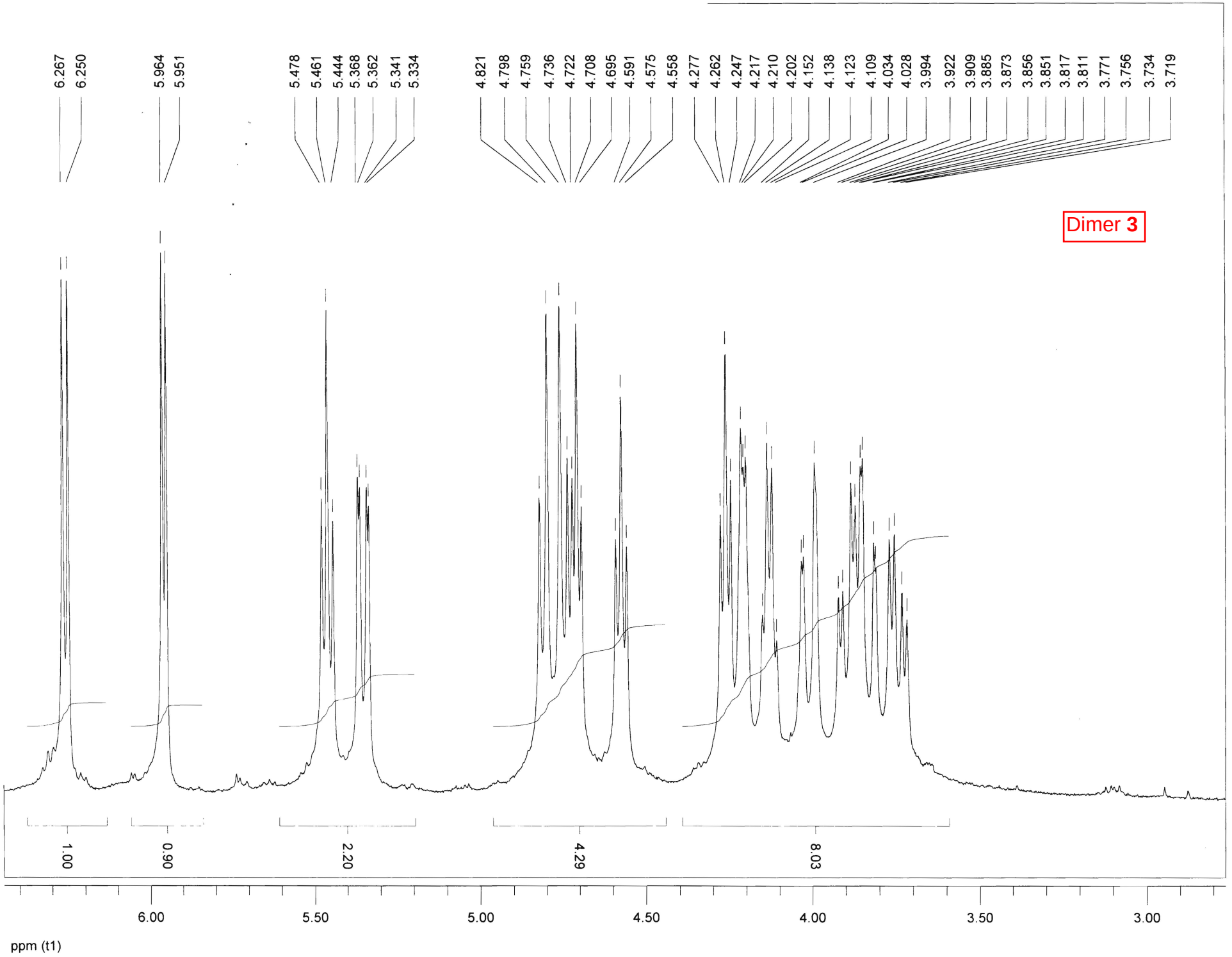
8.228
8.054
8.029
7.918
7.894
7.692
7.614
7.562
7.285
6.267
6.250
5.964
5.951
5.461
5.368
5.341
4.821
4.798
4.759
4.708
4.575
4.262
4.210
4.123
4.034
3.994
3.922
3.851
3.756
3.719
1.097
0.891
0.826
0.048
0.023
-0.020
-0.144
TBDPSO
NH
HN
Cl
TBSO
OTBS
Dimer 3
1.75
0.80
0.90
1.92
1.01
19.87
1.00
0.90
2.20
4.29
8.03
9.68
18.84
8.70
2.35
10.0
5.0
0.0
ppm (f1)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5.341
5.334
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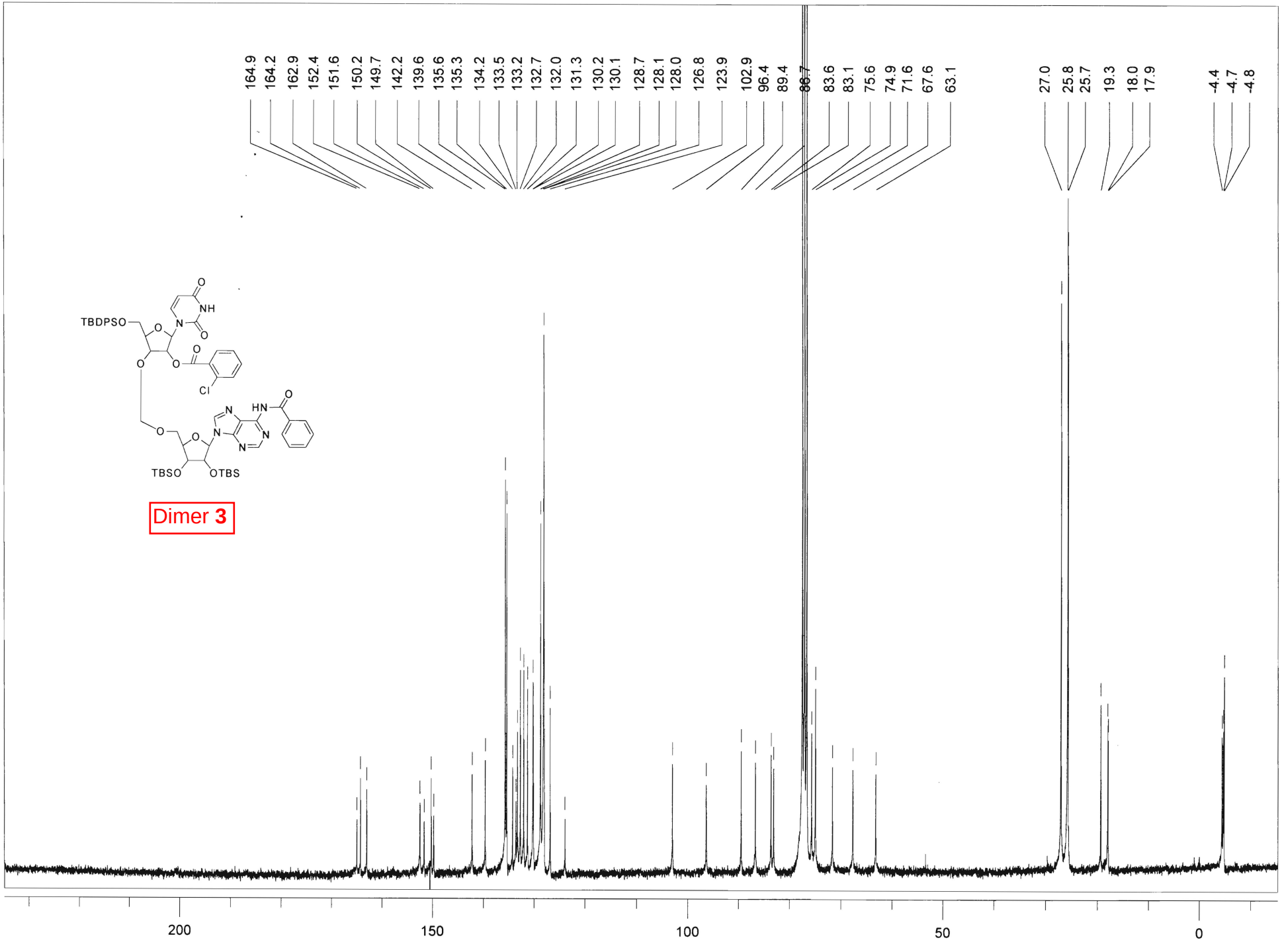
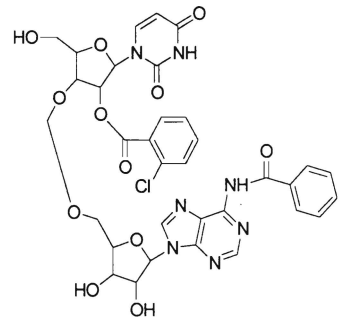
4.798
4.759
4.736
4.722
4.708
4.695
4.591
4.575
4.558
4.277
4.262
4.247
4.217
4.210
4.202
4.152
4.138
4.123
4.109
4.034
4.028
3.994
3.922
3.909
3.885
3.873
3.856
3.851
3.817
3.811
3.771
3.756
3.734
3.719
Dimer 3
1.00
0.90
2.20
4.29
8.03
6.00
5.50
5.00
4.50
4.00
3.50
3.00
ppm (t1)


164.9
164.2
162.9
152.4
151.6
150.2
149.7
142.2
139.6
135.6
135.3
134.2
133.5
133.2
132.7
132.0
131.3
130.2
130.1
128.7
128.1
128.0
126.8
123.9
102.9
96.4
89.4
86.7
83.6
83.1
75.6
74.9
71.6
67.6
63.1
27.0
25.8
25.7
19.3
18.0
17.9
-4.4
-4.7
-4.8
TBDPSO
NH
HN
Cl
TBSO
OTBS
Dimer 3
200
150
100
50
0
ppm (t1)

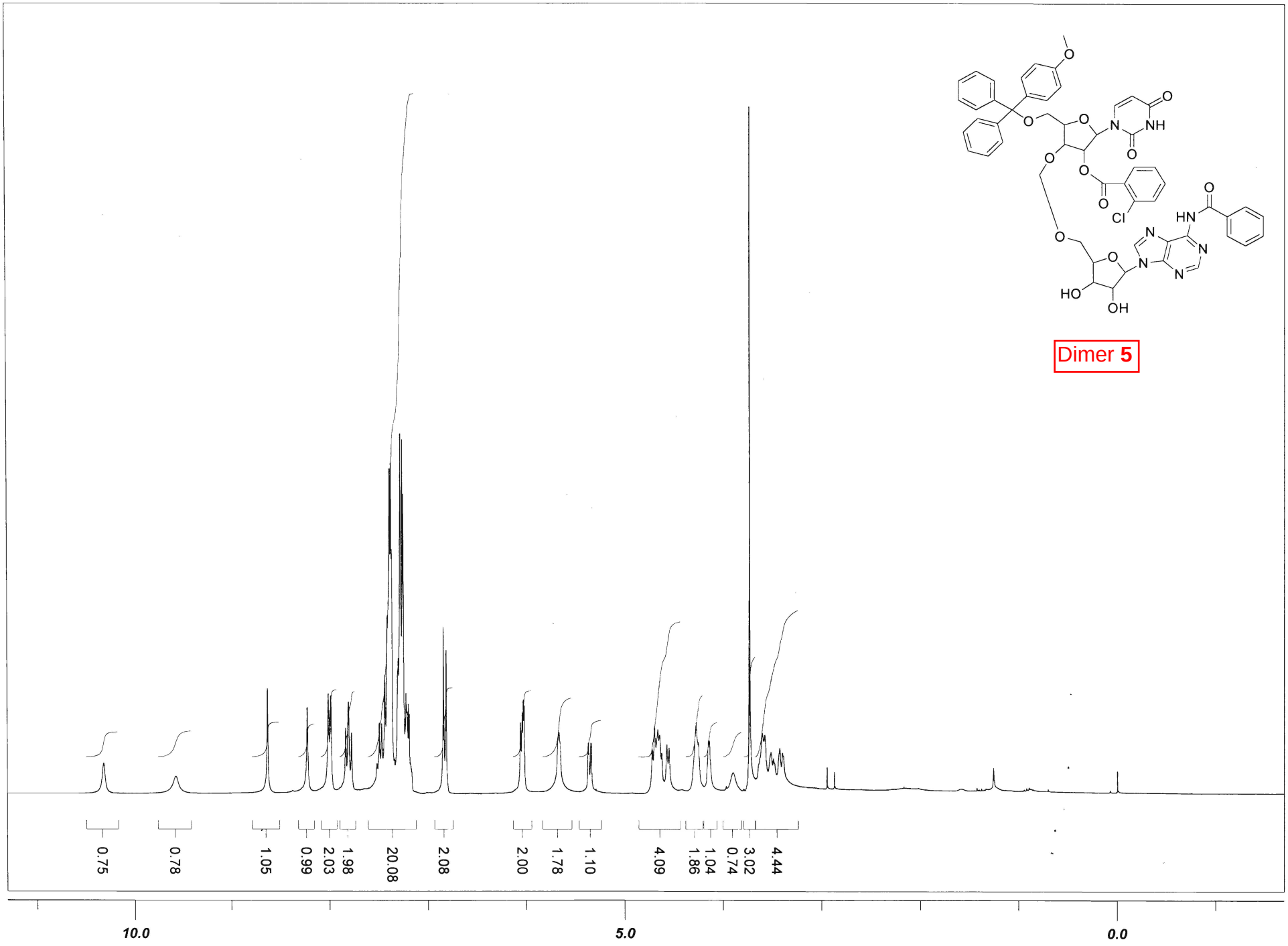


Dimer **4**

0.36 0.87 0.85 1.91 1.06 1.00 6.34 2.00 1.07 1.06 1.04 2.92 0.96 0.96 1.94 1.99 1.91

10.0 5.0 0.0

ppm (t1)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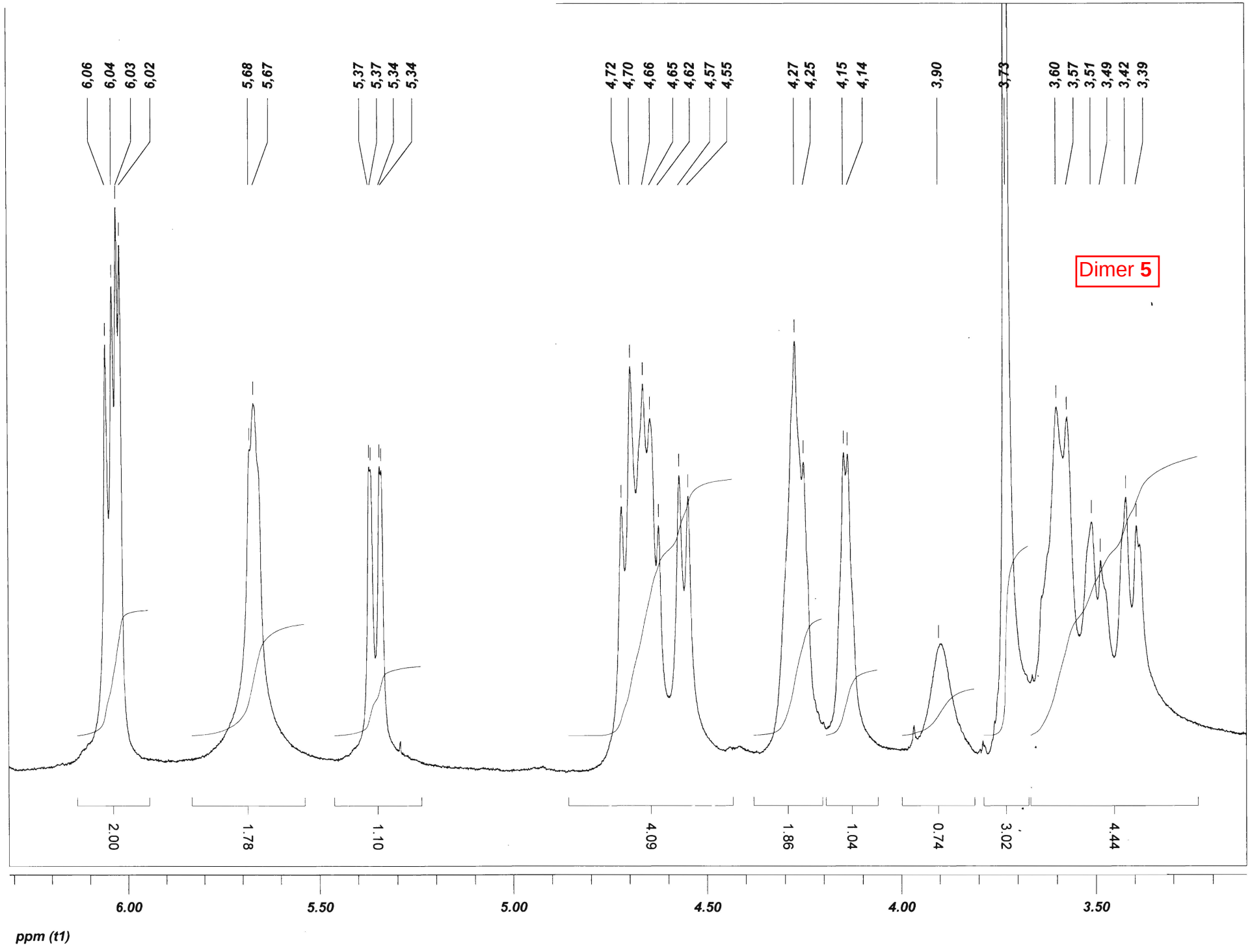
0.78
1.05
0.99
2.03
1.98
20.08
2.08
2.00
1.78
1.10
4.09
1.86
1.04
0.74
3.02
4.44
10.0
5.0
0.0
ppm (t1)

Dimer **5**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6,04
6,03
6,02
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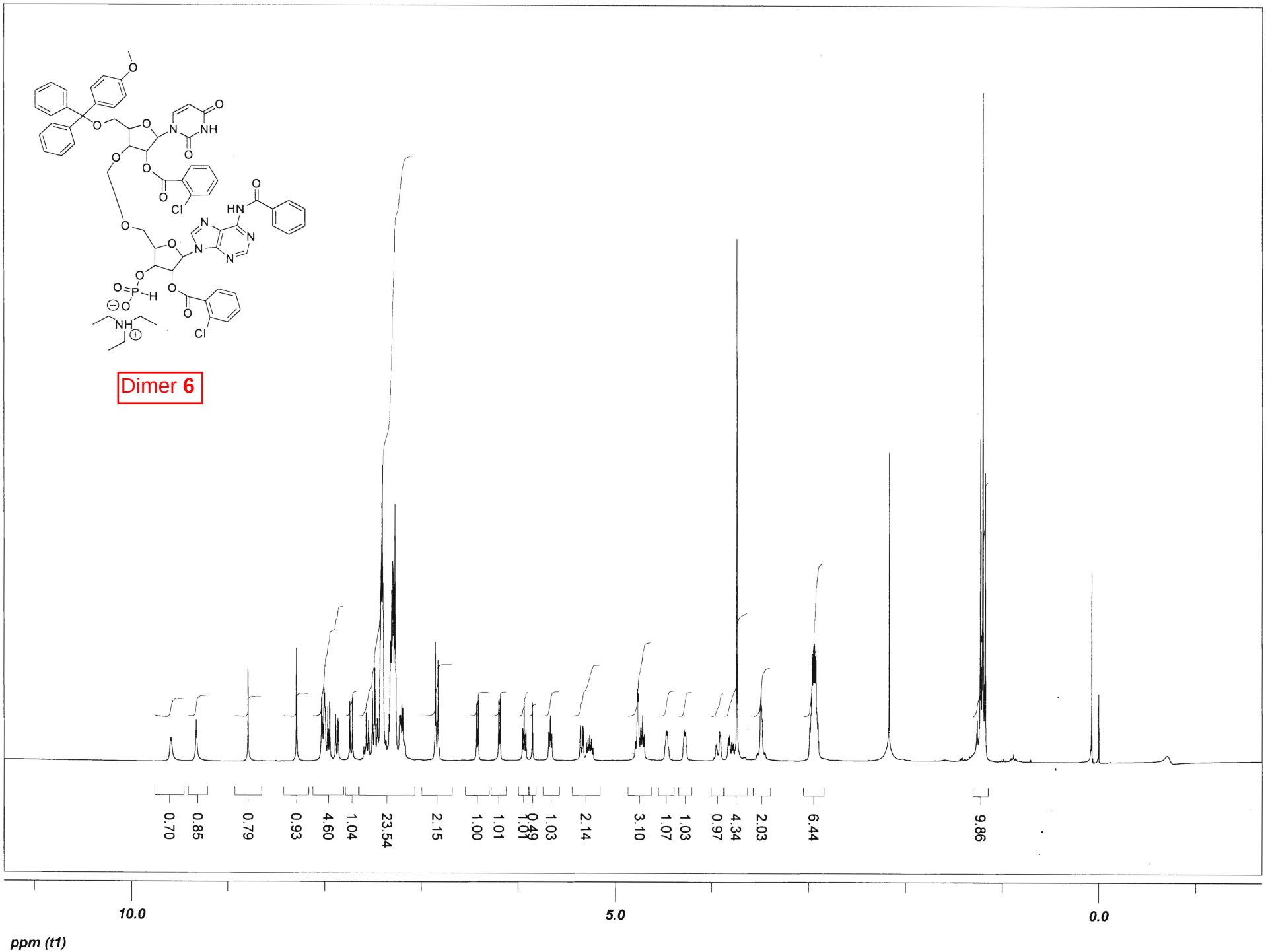
5,67
5,37
5,37
5,34
5,34
4,72
4,70
4,66
4,65
4,62
4,57
4,55
4,27
4,25
4,15
4,14
3,90
3,73
3,60
3,57
3,51
3,49
3,42
3,39
Dimer 5
2.00
1.78
1.10
4.09
1.86
1.04
0.74
3.02
4.44
6.00
5.50
5.00
4.50
4.00
3.50
ppm (t1)


Dimer 6
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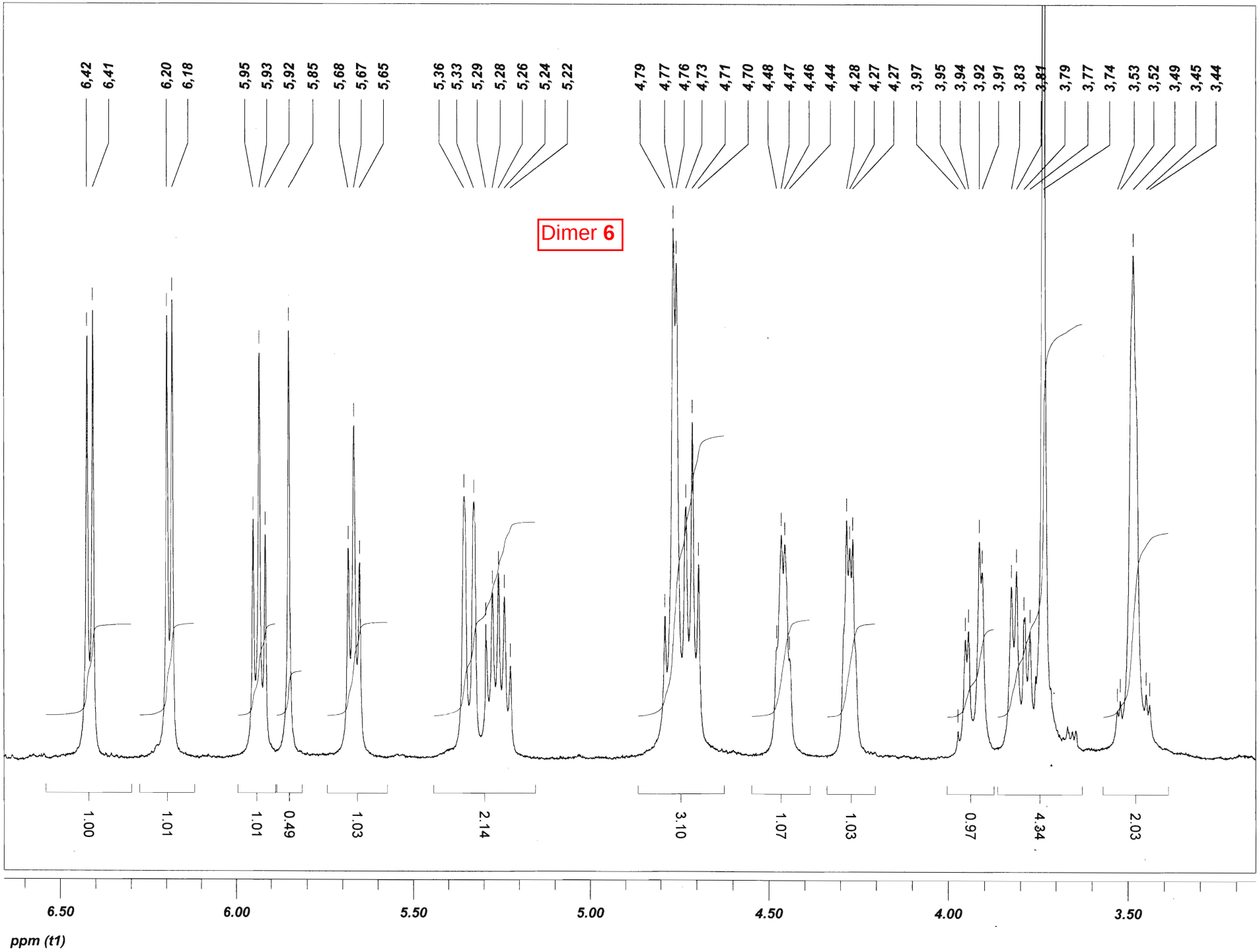
0.85
0.79
0.93
4.60
1.04
23.54
2.15
1.00
1.01
1.03
2.14
3.10
1.07
1.03
0.97
4.34
2.03
6.44
9.86
10.0
5.0
0.0
ppm (t1)


6,42
6,41
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6,18
5,95
5,93
5,92
5,85
5,68
5,67
5,65
5,36
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5,28
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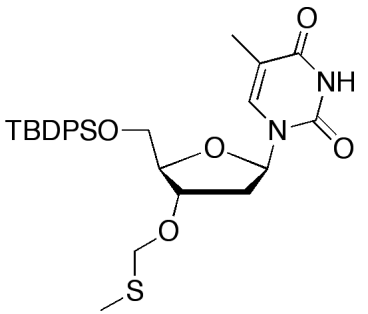
5,24
5,22
4,79
4,77
4,76
4,73
4,71
4,70
4,48
4,47
4,46
4,44
4,28
4,27
4,27
3,97
3,95
3,94
3,92
3,91
3,83
3,81
3,79
3,77
3,74
3,53
3,52
3,49
3,45
3,44
Dimer 6
1.00
1.01
1.01
0.49
1.03
2.14
3.10
1.07
1.03
0.97
4.34
2.03
6.50
6.00
5.50
5.00
4.50
4.00
3.50
ppm (t1)

TBDPS dT 3'-OCH2OSMe

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-300 "nmr300"

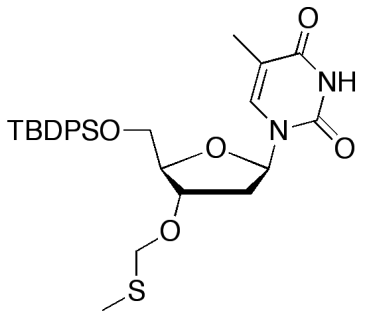
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 64.3 degrees
Acq. time 1.994 sec
Width 3898.6 Hz
64 repetitions
OBSERVE H1, 299.9504945 MHz
DATA PROCESSING
FT size 16384
Total time 3 min, 18 sec



TBDPS dT 3'-OCH2OSMe

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-300 "nmr300"

PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 64.3 degrees
Acq. time 1.994 sec
Width 3898.6 Hz
64 repetitions
OBSERVE H1, 299.9504945 MHz
DATA PROCESSING
FT size 16384
Total time 3 min, 18 sec

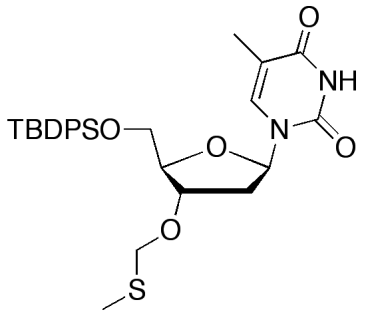


TBDPS dT 3'-OCH2-SMe

Pulse Sequence: s2pul

Solvent: CDCl3
Ambient temperature
Mercury-300 "nmr300"

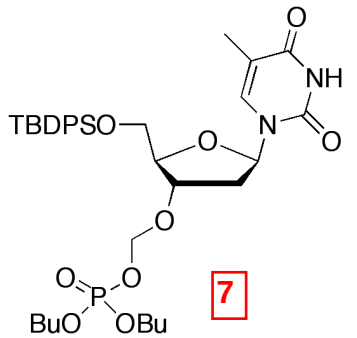
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 56.6 degrees
Acq. time 1.738 sec
Width 18867.9 Hz
1456 repetitions
OBSERVE C13, 75.4225938 MHz
DECOUPLE H1, 299.9519364 MHz
Power 39 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 2.0 Hz
FT size 131072
Total time 84 hr, 3 min, 29 sec



STANDARD 1H OBSERVE

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-300 "nmr300"

PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 64.3 degrees
Acq. time 1.994 sec
Width 3898.6 Hz
32 repetitions
OBSERVE H1, 299.9504945 MHz
DATA PROCESSING
FT size 16384
Total time 1 min, 39 sec

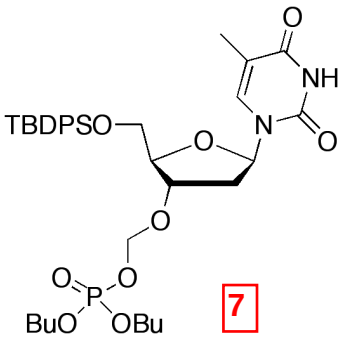


7

STANDARD 1H OBSERVE

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-300 "nmr300"

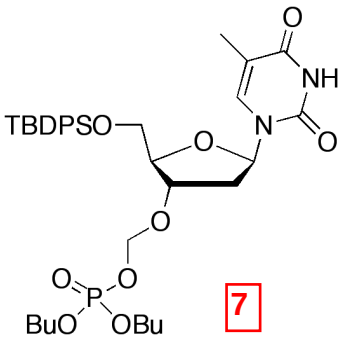
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 64.3 degrees
Acq. time 1.994 sec
Width 3898.6 Hz
32 repetitions
OBSERVE H1, 299.9504945 MHz
DATA PROCESSING
FT size 16384
Total time 1 min, 39 sec



7

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-300 "nmr300"

PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 56.6 degrees
Acq. time 1.738 sec
Width 18867.9 Hz
656 repetitions
OBSERVE C13, 75.4225938 MHz
DECOUPLE H1, 299.9519364 MHz
Power 39 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 8 hr, 25 min, 10 sec



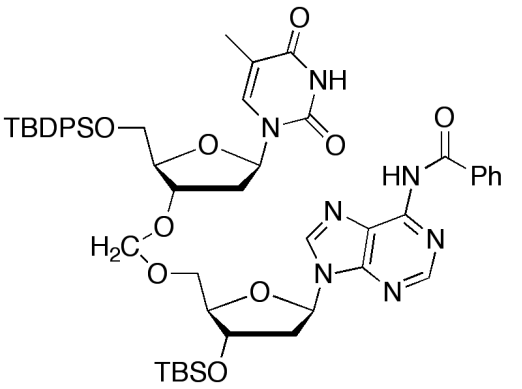
7

5'-TBDPSO-dT-3'-OCH2O-5'-dA(Bz)-3'-OTBS

Pulse Sequence: s2pul

Solvent: CDCl3
Ambient temperature
Mercury-300 "nmr300"

PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 64.3 degrees
Acq. time 1.994 sec
Width 3898.6 Hz
64 repetitions
OBSERVE H1, 299.9504945 MHz
DATA PROCESSING
FT size 16384
Total time 3 min, 18 sec



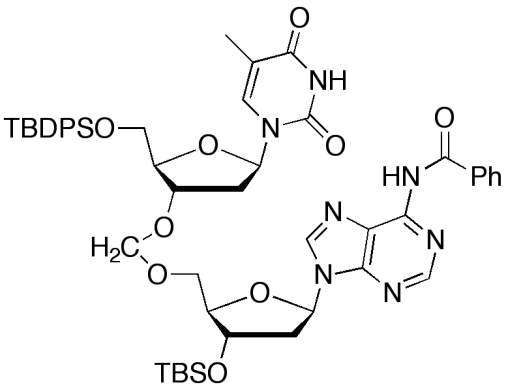
Dimer **9**

5'-TBDPSO-dT-3'-OCH2O-5'-dA(Bz)-3'-OTBS

Pulse Sequence: s2pul

Solvent: CDCl3
Ambient temperature
Mercury-300 "nmr300"

PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 64.3 degrees
Acq. time 1.994 sec
Width 3898.6 Hz
64 repetitions
OBSERVE H1, 299.9504945 MHz
DATA PROCESSING
FT size 16384
Total time 3 min, 18 sec



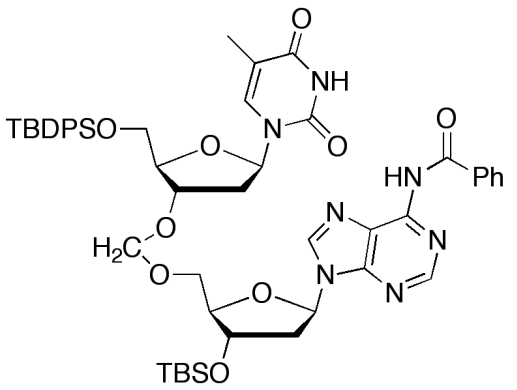
Dimer **9**

5'-TBDPSO-dT-3'-OCH2O-5'-dA(Bz)-3'-OTBS

Pulse Sequence: s2pul

Solvent: CDCl3
Ambient temperature
Mercury-300 "nmr300"

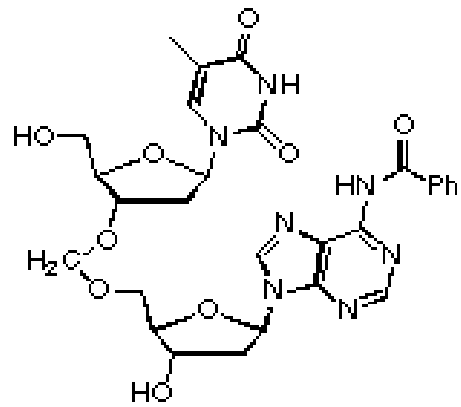
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 56.6 degrees
Acq. time 1.738 sec
Width 18867.9 Hz
2528 repetitions
OBSERVE C13, 75.4225938 MHz
DECOUPLE H1, 299.9519364 MHz
Power 39 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 3.0 Hz
FT size 131072
Total time 84 hr, 3 min, 29 sec



Dimer **9**

deprotected dimer in DMSO-d6
83 mg

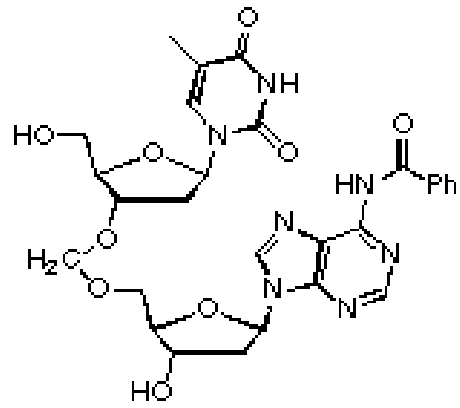
Label	Assigned Value
1	1.82
2	0.95
3	0.88
4	1.96
5	3.72
6	1.00
7	1.01
8	0.90
9	0.95
10	1.98
11	0.96
12	0.98
13	0.89
14	0.90
15	1.97
16	1.69
17	3.56
18	0.88
19	0.86
20	1.87
21	2.78



Dimer **10**

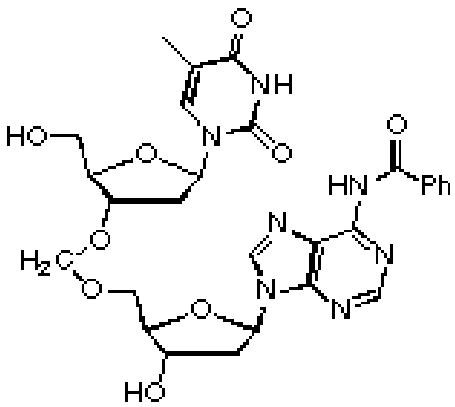
deprotected dimer in DMSO-d6
83 mg

Label	Assigned Value
1	1.82
2	0.95
3	0.88
4	1.96
5	3.72
6	1.00
7	1.01
8	0.90
9	0.95
10	1.98
11	0.96
12	0.98
13	0.89
14	0.90
15	1.97
16	1.69
17	3.56
18	0.88
19	0.86
20	1.87
21	2.78



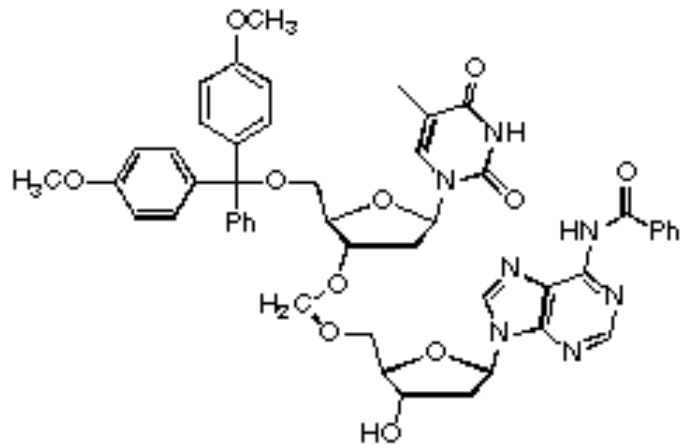
Dimer **10**

C-13 NMR er_1_274
deprotected dimer in DMSO-d6



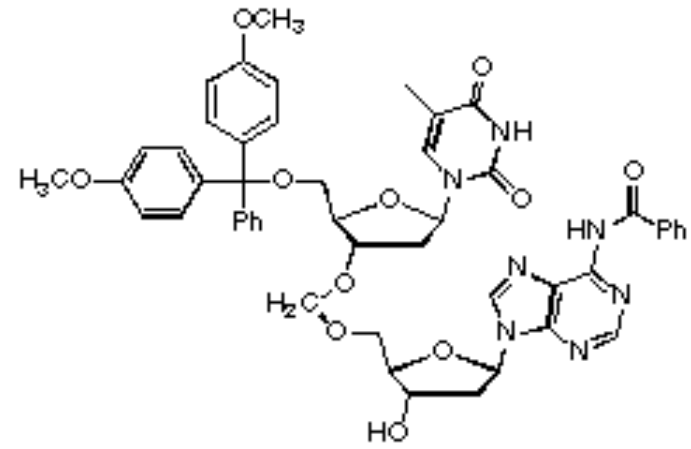
Dimer **10**

Label	Assigned Value
1	0.65
2	1.03
3	1.07
4	2.21
5	0.44
6	15.54
7	4.47
8	1.00
9	1.06
10	2.12
11	0.97
12	1.02
13	3.04
14	0.91
15	7.68
16	1.02
17	0.94
18	1.26
19	0.92
20	1.94
21	0.97
22	3.59



Dimer 11

Dimer 11

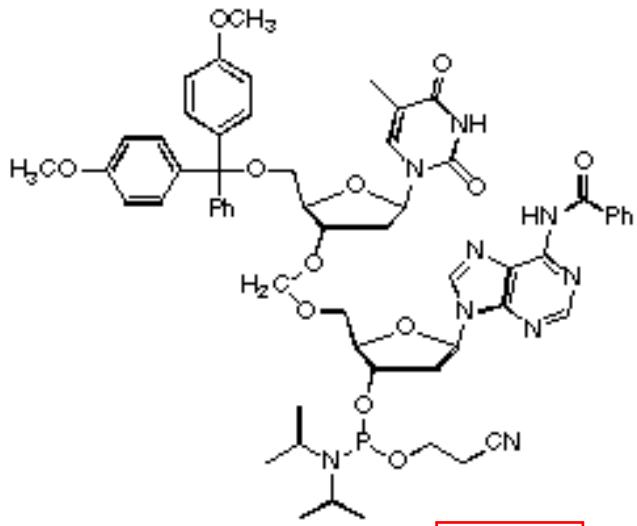


Label	Assigned Value
1	0.65
2	1.03
3	1.07
4	2.21
5	0.44
6	15.54
7	4.47
8	1.00
9	1.06
10	2.12
11	0.97
12	1.02
13	3.04
14	0.91
15	7.68
16	1.02
17	0.94
18	1.26
19	0.92
20	1.94
21	0.97
22	3.59

H-1 in acetonitrile-d3

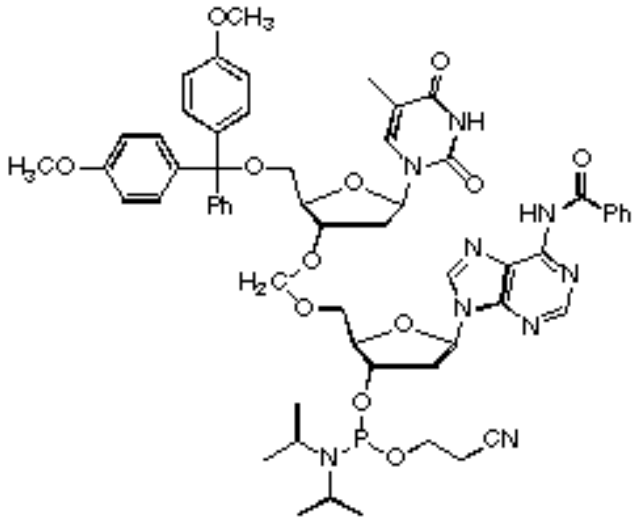
ppm

Label	Assigned Value
1	1.01
2	0.90
3	2.12
4	0.93
5	1.90
6	1.87
7	2.68
8	7.04
9	4.08
10	1.00
11	0.92
12	2.96
13	0.97
14	2.79
15	0.86
16	12.30
17	0.76
18	1.78
19	0.98
20	3.16
21	3.47
22	1.98
23	0.76
24	28.10
25	5.95



Dimer **12**

Label	Assigned Value
1	1.01
2	0.90
3	2.12
4	0.93
5	1.90
6	1.87
7	2.68
8	7.04
9	4.08
10	1.00
11	0.92
12	2.96
13	0.97
14	2.79
15	0.86
16	12.30
17	0.76
18	1.78
19	0.98
20	3.16
21	3.47
22	1.98
23	0.76
24	28.10
25	5.95

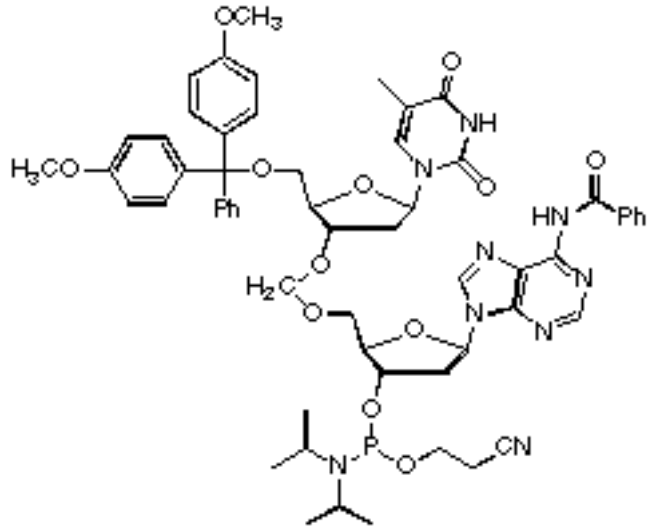


Dimer **12**

H-1 in acetonitrile-d3

ppm

P-31 SHIFTS REL. TO 85% H3PO4 (: = 0 PPM)



Dimer **12**

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ERER1104.P1D
AU PROG:
 ZG.AU

SF     121.497
SF2    121.497
SY  121.0
O1   75699.848
SI   65536
TD   65536
SW  125000.000
SW2 1.25000E5
HZ/PT    3.815

PW       5.0
RD       1.000
AQ        .262
NS    3328

DE       8.2
O2    6313.000
DP   20H CPD

LB       1.000
HZ/CM 971.985
PPM/CM   8.000
SR   75692.22

D1    5.0000000
RD    1.0000000
PW         5.00
DE         8.20
NS       3328
DS          0
NE       1000
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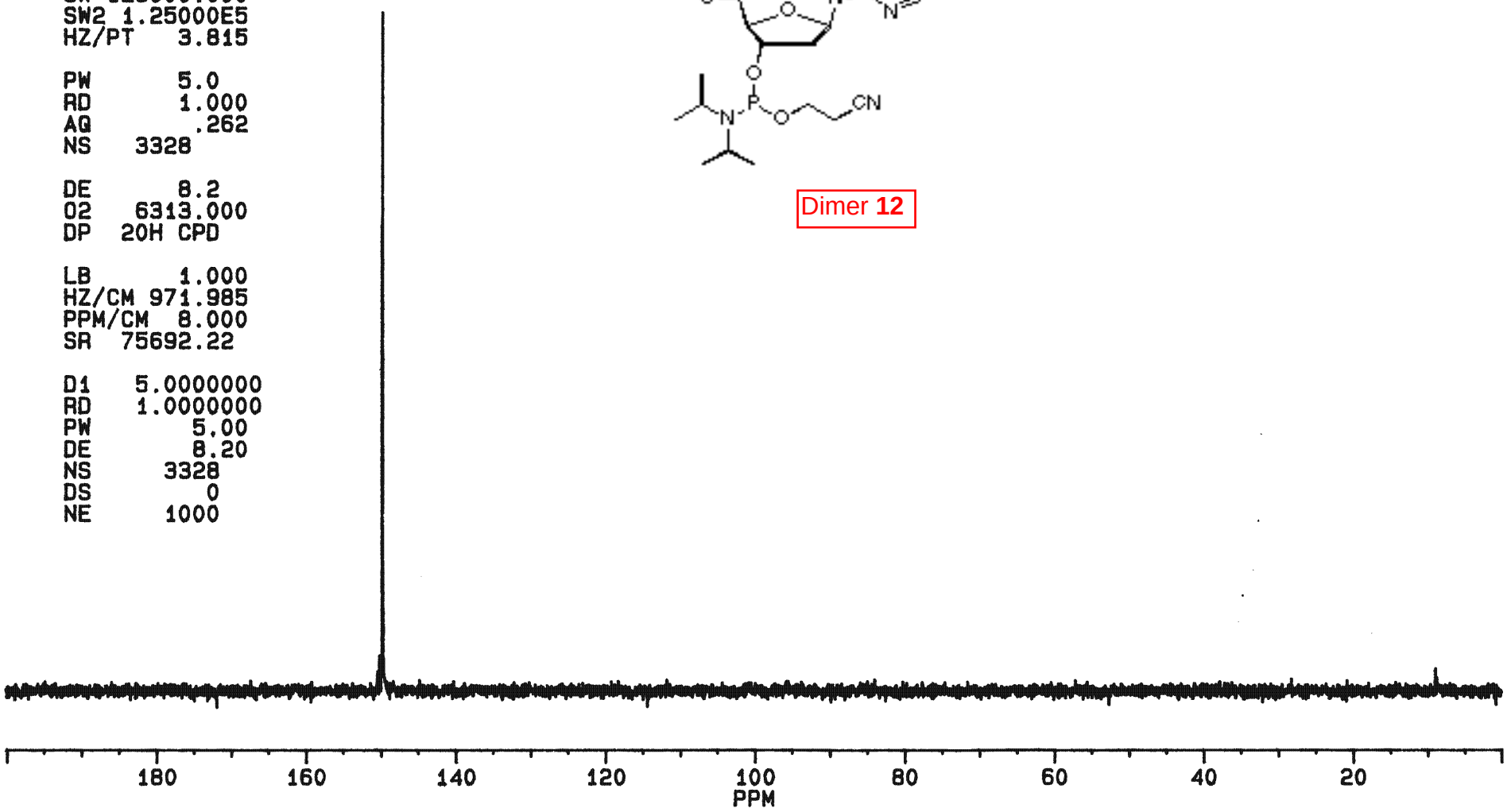


P-31 SHIFTS REL. TO 85% H3P04 (: = 0 PPM)

BRUKER

PPM

150.241
149.955
149.577
149.485

ERER1104.P1D
AU PROG:
ZG.AU

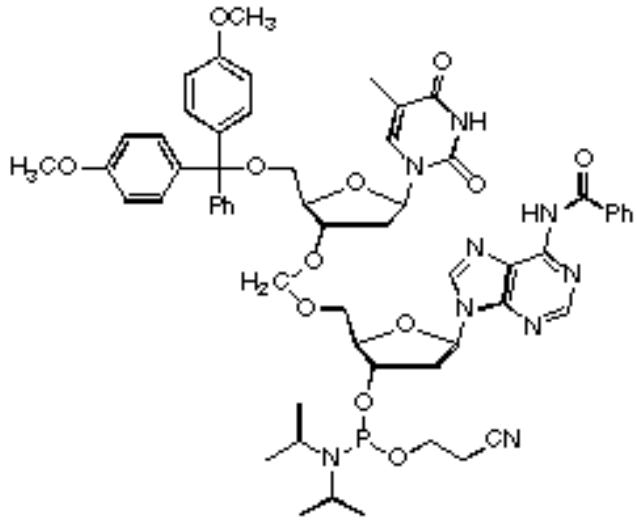
SF 121.497
SF2 121.497
SY 121.0
O1 75699.848
SI 65536
TD 65536
SW 125000.000
SW2 1.25000E5
HZ/PT 3.815

PW 5.0
RD 1.000
AQ .262
NS 3328

DE 8.2
O2 6313.000
DP 20H CPD

LB 1.000
HZ/CM 48.523
PPM/CM .399
SR 75692.22

D1 5.0000000
RD 1.0000000
PW 5.00
DE 8.20
NS 3328
DS 0
NE 1000



Dimer **12**