SUPPLEMENTARY INFORMATION.

# Synthesis and properties of DNA containing a spore photoproduct analog.

**Eva Bürckstümmer, Thomas Carell***

## 1) SYNTHESIS OF THE SP-ISOMERS **1a/b**

**5'-*O-tert*-butyldimethylsilyl-thymidine (9).** Thymidine **7** (5.00 g, 20.7 mmol) was dissolved in anhydrous DMF. Imidazole (3.09 g, 41.3 mmol) and TBDMSCl (3.42 g, 22.7 mmol) were added and the solution was stirred 6 h at room temperature. The reaction mixture was diluted with $CHCl_3$ (100 mL), washed with water (7 × 100 mL), dried ($MgSO_4$) and the solvent was removed *in vacuo*. Purification by flash chromatography (silica gel, $CHCl_3$/MeOH 20:1) provided **9** (6.35 g, 86 %) as a white solid.

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.07 (*d*, *J* = 1.28 Hz, 6H; Si(C$H_3$)$_2$), 0.88 (*s*, 9H; C(C$H_3$)$_3$), 1.77 (*d*, *J* = 1.10 Hz, 3H; C(5)C$H_3$), 1.90-2.16 (*m*, 2H; C$H_2$(2')), 3.67-3.83 (*m*, 3H; C*H*(4'), C$H_2$(5')), 4.09-4.35 (*m*, 1H; C*H*(3')), 5.27 (*d*, *J* = 4.15 Hz, 1H; C(3)O*H*), 6.17 (*dd*, *J* = 7.59, 6.36 Hz, 1H; C*H*(1')), 7.47 (*s*, 1H; C*H*(6)), 11.32 (*s*, 1H; N*H*); $^{13}$C-NMR (100 MHz, DMSO-$d_6$) δ: -4.98, -4.96, 12.67, 18.48, 26.26, 39.41, 63.76, 70.97, 84.34, 87.27, 109.83, 135.93, 150.82, 164.10; MS (ESI): calc. for $[C_{16}H_{28}N_2O_5Si+H]^+$: 357.1840; found: 357.1838.

**5'-*O-tert*-butyldimethylsilyl-3'-*O-tert*-butyldiphenylsilyl-thymidine (10).** **9** (6.00 g, 16.8 mmol), Imidazole (3.44 g, 50.4 mmol) and TBDPSCl (6.53 mL, 25.2 mmol) were dissolved in anhydrous DMF (40 mL) and the reaction mixture was stirred overnight at room temperature. The reaction mixture was diluted with ethyl acetate (200 mL), washed with saturated aqueous sodium bicarbonate (3 × 200 mL), dried ($MgSO_4$) and the solvent was removed *in vacuo*. Purification by flash chromatography (silica gel, isohexane/ethyl acetate 7:3) provided **10** (10.19 g, 99 %) as white solid.

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: -0.14 (*d*, *J* = 11.13 Hz, 6H; Si(C$H_3$)$_2$), 0.71 (*s*, 9H; C(C$H_3$)$_3$), 1.01 (*s*, 9H; C(C$H_3$)$_3$), 1.69 (*d*, *J* = 0.92 Hz, 3H; C(5)C$H_3$), 1.85-1.96 (*m*, 1H; C$H_{2a}$(2')), 2.11 (*ddd*, *J* = 12.89, 5.48, 1.27 Hz, 1H; C$H_{2b}$(2')), 3.25 (*dd*, *J* = 11.37, 3.36 Hz, 1H; C$H_{2b}$(5')), 3.52 (*dd*, *J* = 11.36, 3.32 Hz, 1H; C$H_{2b}$(5')), 3.92-3.93 (*m*, 1H; C*H*(4')), 4.27-4.29 (*m*, 1H; C*H*(3')), 6.26 (*dd*, *J* = 8.72, 5.58 Hz, 1H; C*H*(1')), 7.31 (*d*, *J* = 1.13 Hz, 1H; C*H*(6)), 7.38-7.69 (*m*, 10H; C$_{ar}$*H*), 11.32 (*s*, 1H; N*H*); $^{13}$C-NMR (100 MHz, DMSO-$d_6$) δ: -5.30, -5.14, 12.63, 18.32, 19.09, 26.09, 27.17, 39.42, 63.33, 74.26, 84.39, 87.41, 110.02, 128.43, 128.45, 130.57, 133.15, 133.21, 135.55, 135.71, 150.81, 164.00; MS (ESI): calc. for [$C_{32}H_{46}N_2O_5Si_2$+Na]$^+$: 617.2837; found: 617.2825.

***N*$^3$-trimethylsilylethoxymethyl-5'-*O-tert*-butydimethylsilyl-3'-*O-tert*-butydiphenylsilyl-thymidine (5).** A solution containing **10** (5.30 g, 8.56 mmol), *i*Pr$_2$NEt (6.10 mL, 34.3 mmol) and SEMCl (2.36 mL, 12.9 mmol) in anhydrous $CH_2Cl_2$ (20 mL) was stirred for 3 d. After 2 d additional *i*Pr$_2$NEt (6.10 mL, 34.3 mmol) and SEMCl (2.36 mL, 12.9 mmol) were added. The reaction mixture was diluted with $CHCl_3$ (30 mL), washed with saturated aqueous sodium bicarbonate (3 × 40 mL), dried ($MgSO_4$) and the solvent was removed *in vacuo*. Flash chromatography (silica gel, isohexane/ethyl acetate 9:1) gave **5** (5.46 g, 85 %) as a colorless oil.

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: -0.12 (*s*, 3H; SiC$H_3$), -0.09 (*s*, 3H; SiC$H_3$), -0.05 (*s*, 9H; Si(C$H_3$)$_3$), 0.74 (*s*, 9H; C(C$H_3$)$_3$), 0.80-0.85 (*m*, 2H; SiC$H_2$CH$_2$O), 1.05 (*s*, 9H; C(C$H_3$)$_3$), 1.77 (*d*, *J* = 0.44 Hz, 3H; C(5)C$H_3$), 1.94 (*ddd*, *J* = 13.60, 10.15, 5.83 Hz, 1H; C$H_{2a}$(2')), 2.18 (*ddd*, *J* = 12.92, 5.52, 1.41 Hz, 1H; C$H_{2b}$(2')), 3.51-3.64 (*m*, 4H; C$H_2$(5'), SiCH$_2$C$H_2$O), 3.93-4.14 (*m*, 1H; C*H*(4')), 4.32-4.33 (*m*, 1H; C*H*(3')), 5.21 (*s*, 2H; OCH$_2$N), 6.31 (*dd*, *J* = 8.45, 5.64 Hz, 1H; C*H*(1')), 7.40-7.67 (*m*, 11H; C$_{ar}$*H*, C*H*(6)); $^{13}$C-NMR (100 MHz, DMSO-$d_6$) δ: -5.92, -5.77, -1.54, 12.66, 17.32, 17.69, 18.47, 25.45, 26.54, 39.72, 62.67,

66.17, 69.50, 73.60, 84.96, 87.06, 108.58, 135.07, 135.08, 150.27, 164.41; MS (ESI): calc. for $[C_{38}H_{60}N_2O_6Si_3+Cl]^-$: 759.3453; found: 759.3455.

**$N^3$-trimethylsilylethoxymethyl-5'-*O*-*tert*-butydimethylsilyl-3'-*O*-*tert*-butydiphenylsilyl-5-bromomethyl-2'-deoxyuridine (4).** The protected thymidine **5** (1.50 g, 1.97 mmol), NBS (774 mg, 4.4 mmol) and dibenzoyl peroxide (15.0 mg, 0.06 mmol) were dissolved in carbon tetrachloride (20 mL). The reaction was heated at 70 °C for 30 min. The reaction was allowed to cool and was filtered through a sintered funnel. The solvent was removed *in vacuo* to yield a crude yellow oil. A quick flash chromatography (silica gel, isohexane/ethyl acetate 9:1) gave **4** (939 mg, 57 %) as a colorless oil which decomposed at room temperature but was stable at -20 °C.

$^1$H-NMR (600 MHz, $CDCl_3$) δ: -0.09 (*s*, 3H; SiC*H*$_3$), -0.07 (*s*, 3H; SiC*H*$_3$), 0.00 (*s*, 9H; Si(C*H*$_3$)$_3$), 0.80 (*s*, 9H; C(C*H*$_3$)$_3$), 0.97-0.99 (*m*, 2H; SiC*H*$_2$CH$_2$O), 1.09 (*s*, 9H; C(C*H*$_3$)$_3$), 1.77-1.83 (*m*, 1H; C*H*$_{2a}$(2')), 2.37 (*ddd*, *J* = 18.23, 13.10, 5.60 Hz, 1H; C*H*$_{2b}$(2')), 3.16 (*dd*, *J* = 11.58, 2.28 Hz, 1H; C*H*$_{2a}$(5)), 3.64 (*dd*, *J* = 11.38, 2.10 Hz, 1H; C*H*$_{2b}$(5)), 3.66-3.71 (*m*, 2H; SiCH$_2$C*H*$_2$O), 4.03 (*bs*, 1H; C*H*(4')), 4.18 (*d*, *J* = 10.47 Hz, 1H; C*H*$_{2a}$(5')), 4.26 (*d*, *J* = 10.51 Hz, 1H; C*H*$_{2b}$(5')), 4.32-4.33 (*m*, 1H; C*H*(3')), 5.42 (*s*, 2H; OCH$_2$N), 6.49 (*dd*, *J* = 8.82, 5.28 Hz, 1H; C*H*(1')), 7.37-7.42 (*m*, 5H; C$_{ar}$*H*), 7.61-7.65 (*m*, 5H; C$_{ar}$*H*), 7.85 (*s*, 1H; C*H*(6)); $^{13}$C-NMR (150 MHz, $CDCl_3$) δ: -5.57, -5.50, -1.43, 18.10, 19.05, 25.80, 26.08, 26.55, 26.86, 41.87, 63.30, 67.64, 70.25, 74.36, 86.40, 88.42, 110.98, 127.67, 130.03, 130.05, 133.02, 133.28, 135.66, 135.70, 137.86, 150.45, 161.31; MS (MALDI): calc. for $[C_{38}H_{59}BrN_2O_6Si_3+Na]^+$: 827.0; found: 827.1.

**5'-*O*-*tert*-butyldiphenylsilyl-5,6-dihydrothymidine (8).** Thymidine **7** (10.00 g, 0.04 mol) was dissolved in MeOH/water (200 mL, 1:1) and 600 mg $Rh/Al_2O_3$ (5 % Rh) was added. The suspension was stirred under an $H_2$-atmosphere at room temperature for 3 weeks. The reaction

mixture was filtered through celite and the solvent was removed under reduced pressure. The crude product was azeotropically dried three times with pyridine and dissolved in anhydrous DMF (50 mL). Imidazole (6.20 g, 0.09 mol) and TBDPSCl (11.40 mL, 0.05 mol) were added and the reaction mixture was stirred 6 h at room temperature. The reaction mixture was diluted with ethyl acetate (200 mL), washed with saturated aqueous sodium bicarbonate (3 × 200 mL), dried ($MgSO_4$) and the solvent was removed *in vacuo*. Purification by flash chromatography (silica gel, $CHCl_3$/MeOH 20:1) provided **8** (14.00 g, 71 %) as a white solid.

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.91 (*d*, *J* = 6.97 Hz, 3H; C(5)C$H_3$), 1.00 (*s*, 9H; C(C$H_3$)$_3$), 1.89 (*ddd*, *J* = 13.24, 6.57, 3.86 Hz, 1H; C$H_{2a}$(2')), 2.09-2.14 (*m*, 1H; C$H_{2b}$(2')), 2.52-2.57 (*m*, 1H; C*H*(5)), 2.92 (*dd*, *J* = 12.54, 10.67 Hz, 1H; C$H_{2a}$(6)), 3.34 (*dd*, *J* = 12.51, 5.63 Hz, 1H; C$H_{2b}$(6)), 3.66-3.80 (*m*, 3H; C*H*(4'), C$H_2$(5')), 4.24-4.25 (*m*, 1H; C*H*(3')), 5.21 (*d*, *J* = 4.53 Hz, 1H; C(3')O*H*), 6.16 (*t*, *J* = 7.06 Hz, 1H; C*H*(1')), 7.42-7.47 (*m*, 5H; $C_{ar}H$), 7.62-7.65 (*m*, 5H; $C_{ar}H$), 10.22 (*s*, 1H; N*H*); $^{13}$C-NMR (100 MHz, DMSO-$d_6$) δ: 12.82, 19.31, 27.16, 35.00, 37.06, 42.03, 64.54, 70.43, 83.16, 85.60, 128.35, 128.37, 130.34, 130.39, 135.46, 135.60, 153.40, 173.43; MS (ESI): calc. for $[C_{26}H_{34}N_2O_5Si+Na]^+$: 505.2129; found: 505.2121.

**5'-*O*-*tert*-butyldiphenylsilyl-3'-*O*-triethylsilyl-5,6-dihydrothymidine (11).** **8** (4.90 g, 9.70 mmol), Imidazole (2.07 g, 29.1 mmol) and TESCl (2.52 mL, 14.5 mmol) were dissolved in anhydrous DMF (30 mL) and the reaction mixture was stirred overnight at room temperature. The reaction mixture was diluted with $CHCl_3$ (200 mL), washed with saturated aqueous sodium bicarbonate (6 × 150 mL), dried ($MgSO_4$) and the solvent was removed *in vacuo*. Purification by flash chromatography (silica gel, isohexane/ethyl acetate 7:3) provided **11** (5.71 g, 96 %) as a colorless oil.

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.56-0.58 (*m*, 6H; Si(C$H_2$CH$_3$)$_3$), 0.88-0.94 (*m*, 12H; Si(CH$_2$C$H_3$)$_3$, C(5)C$H_3$), 1.01 (*s*, 9H, C(C$H_3$)$_3$), 1.87 (*ddd*, *J* = 13.12, 6.44, 3.58 Hz, 1H;

C$H_{2a}$(2')), 2.21 (*td*, *J* = 13.75, 6.79, 6.79 Hz, 1H; C$H_{2b}$(2')), 2.52-2.60 (*m*, 1H; C*H*(5)), 2.93 (*dd*, *J* = 12.45, 10.61 Hz, 1H; C$H_{2a}$(6)), 3.34 (*dd*, *J* = 12.59, 5.73 Hz, 1H; C$H_{2b}$(6)), 3.63-3.65 (*m*, 1H; C*H*(4')), 3.72-3.74 (*m*, 2H; C$H_2$(5')), 4.37-4.39 (*m*, 1H; C*H*(3')), 6.15 (*t*, *J* = 7.07 Hz, 1H; C*H*(1')), 7.50-7.36 (m, 6H; $C_{ar}H$), 7.65-7.61 (m, 4H; $C_{ar}H$), 10.26 (*s*, 1H; N*H*); $^{13}$C-NMR (100 MHz, DMSO-$d_6$) δ: 4.05, 6.48, 12.27, 18.66, 26.50, 34.38, 36.55, 41.45, 63.35, 71.07, 82.58, 84.94, 127.76, 127.80, 129.82, 129.91, 132.34, 132.54, 134.91, 134.95, 152.85, 172.83; MS (ESI): calc. for $[C_{32}H_{48}N_2O_5Si_2+H]^+$: 597.3175; found: 597.3177.

**$N^3$-trimethylsilylethoxymethyl-5'-*O*-*tert*-butyldiphenylsilyl-3'-*O*-triethylsilyl-5,6-dihydrothymidine (3).** SEM-protection was carried out as described above for **5**. Product **3** (1.98 g, 79 %) was isolated as a colorless oil.

$^1$H-NMR (600 MHz, DMSO-$d_6$) δ: -0.01 (*s*, 9H; Si(C$H_3$)$_3$), 0.44-0.73 (*m*, 6H; Si(C$H_2$CH$_3$)$_3$), 0.90-0.97 (*m*, 11H; SiC$H_2$CH$_2$O, Si(CH$_2$C$H_3$)$_3$), 1.07-1.08 (*m*, 9H; C(C$H_3$)$_3$), 1.11 (*d*, *J* = 7.04 Hz, 3H; C(5)C$H_3$), 2.06-2.00 (m, 2H; C*H*(5), C$H_{2a}$(2')), 2.55 (ddd, *J* = 8.81, 7.06, 5.26 Hz, 1H; C$H_{2b}$(2')), 3.19 (*dd*, *J* = 12.82, 8.92 Hz, 1H; C$H_{2a}$(6)), 3.33 (*dd*, *J* = 12.80, 5.22 Hz, 1H; C$H_{2b}$(6)), 3.62 (*dd*, *J* = 9.10, 7.45 Hz, 2H; SiCH$_2$C$H_2$O), 3.72 (*dd*, *J* = 12.20, 3.77 Hz, 1H; C$H_{2a}$(5')), 3.84-3.82 (*m*, 2H; C$H_{2b}$(5'), C*H*(4')), 4.46-4.47 (*m*, 1H; C*H*(3')), 5.22 (*s*, 2H; OC$H_2$N), 6.38 (*t*, *J* = 7.14 Hz, 1H; C*H*(1')), 7.36-7.41 (*m*, 4H; $C_{ar}H$), 7.41-7.47 (*m*, 2H; $C_{ar}H$), 7.59-7.70 (*m*, 4H; $C_{ar}H$); $^{13}$C-NMR (150 MHz, DMSO-$d_6$) δ: -1.42, 4.71, 6.73, 13.30, 18.10, 19.26, 26.94, 35.79, 38.22, 40.80, 63.68, 66.82, 69.80, 71.69, 83.38, 86.41, 127.85, 129.88, 129.99, 132.66, 133.05, 135.41, 135.58, 152.98, 172.62; MS (ESI): calc. for $[C_{38}H_{62}N_2O_6Si_3+Na]^+$: 749.3808; found: 749.3830.

**$N^3$-trimethylsilylethoxymethyl-5'-*O*-*tert*-butyldiphenylsilyl-5-($N^3$-trimethylsilylethoxy-methyl-3'-*O*-*tert*-butyldiphenylsilyl-thymidyl)-5,6-dihydrothymidine (2a/b).** Dihydro-thymidine **3** (177 mg, 0.24 mmol), azeotropically dried with anhydrous toluene, was dissolved

in anhydrous THF (1.5 mL) and cooled to -78 °C. A freshly prepared LDA-solution (diisopropylamine (51.7 µL, 0.37 mmol), BuLi (0.23 mL, 1.60 M in hexane) in anhydrous THF (0.66 mL) at 0 °C for 1 h) was slowly added and the reaction mixture was stirred at -78 °C for 2 h before addition of **4** (200 mg, 0.25 mmol), dissolved in anhydrous THF (2.3 mL). The reaction mixture was stirred at -78 °C for 1.5 h and at 0 °C for 3 h. The reaction was quenched by adding aqueous sodium bicarbonate (15 mL) and the aqueous phase was extracted with $CHCl_3$ (3 × 8 mL). The collected extracts were dried ($MgSO_4$) and the solvent was removed *in vacuo*. Flash chromatography (silica gel-*H*, isohexane/ethyl acetate 7:1) gave the fully protected dimer **12** (310 mg, 88 %) as yellow oil.

For selective deprotection of two OH-groups pTsOH (40.7 mg, 0.21 mmol) and **12** were dissolved in MeOH (40 mL). The reaction mixture was stirred at room temperature for 4 h. The mixture was neutralized with aqueous sodium bicarbonate and the solvent was removed *in vacuo*. The crude product was diluted with aqueous sodium bicarbonate (60 mL) and the aqueous phase was extracted with $CHCl_3$ (3 × 70 mL). The collected organic phases were dried ($MgSO_4$) and the solvent was removed *in vacuo*. An initial purification was achieved by flash chromatography (silica gel-*H*, $CHCl_3$/MeOH 50:1) to give **2a/b** (100 mg, 35 %) as a mixture of diastereomers.

The diastereomers were separated by np-HPLC using a *VP 250/21 Nucleodur 100-5*-column (*Macherey-Nagel*) with a heptane/ethyl acetate gradient (30 → 50 % ethyl acetate in 25 min, 50 → 60 % ethyl acetate in 10 min; flow rate: 15 mL/min; detection wavelength: 250 nm). The mixture was dissolved in heptane/ethyl acetate (4 mL, 4:1) and for each separation 1 mL of the solution was injected through a *rheodyne* valve on the column. After HPLC purification the *S*-isomer **2a** (68.0 mg, 24 %) and the *R*-isomer **2b** (11.0 mg, 4 %) were isolated as white solids.

*S*-isomer **2a**: mp 65-77 °C; IR: 3466*br*, 3072*w*, 2953*m*, 2932*m*, 2859*s*, 1713*s*, 1661*s*, 1589*w*, 1461s, 1428*m*, 1245*m*, 1192*w*, 1087*s*, 1027*w*, 997*w*, 937*w*, 858*m*, 835*s*, 741*m*, 701*s* $cm^{-1}$; $^1$H-

NMR (600 MHz, DMSO-$d_6$) δ: -0.08 (*s*, 9H; Si(C$H_3$)$_3$), -0.06 (*s*, 9H; Si(C$H_3$)$_3$), 0.75-0.81 (*m*, 4H; 2 x SiC$H_2$C$H_2$O), 0.87 (*s*, 3H; C(5A)C$H_3$), 0.99 (*s*, 9H; C(C$H_3$)$_3$), 1.04 (*s*, 9H; C(C$H_3$)$_3$), 1.89-1.93 (*m*, 2H; C$H_{2c}$(2'B), C$H_{2d}$(2'A)), 2.02-2.06 (*m*, 1H; C$H_{2c}$(2'A)), 2.13-2.18 (*m*, 2H; C$H_{2e}$(5), C$H_{2d}$(2'B)), 2.70 (*d*, *J* = 13.76 Hz, 1H; C$H_{2f}$(5)), 3.04 (*d*, *J* = 13.29 Hz, 1H; C$H_{2b}$(6A)), 3.12 (*d*, *J* = 13.22 Hz, 1H; C$H_{2a}$(6A)), 3.23-3.30 (*m*, 1H; C$H_{2a}$(5'B)), 3.39-3.47 (*m*, 3H; SiCH$_2$C$H_2$O(A), C$H_{2b}$(5'B)), 3.51 (*m*, 2H; SiCH$_2$C$H_2$O(B)), 3.65 (*m*, 1H; C$H_{2a}$(5'A)), 3.71-3.72 (*m*, 1H; C*H*(4'A)), 3.76 (*dd*, *J* = 10.75, 3.44 Hz, 1H; C$H_{2b}$(5'A)), 3.97-3.98 (*m*, 1H; C*H*(4'B)), 4.18-4.21 (*m*, 1H; C*H*(3'A)), 4.45-4.46 (*m*, 1H; C*H*(3'B)), 4.91 (*d*, *J* = 9.86 Hz, 1H; OC$H_{2a}$N(A)), 4.99 (*t*, *J* = 5.18 Hz, 1H; C(5')O*H*), 5.04 (*d*, *J* = 9.86 Hz, 1H; OC$H_{2b}$N(A)), 5.13 (*d*, *J* = 3.85 Hz, 2H; OC$H_2$N(B)), 5.23 (*d*, *J* = 4.58 Hz, 1H; C(3')O*H*), 6.25 (*t*, *J* = 7.05 Hz, 1H; C*H*(1'A)), 6.29 (*dd*, *J* = 7.80, 5.99 Hz, 1H; C*H*(1'B)), 7.40-7.47 (*m*, 10H; C$_{ar}$*H*), 7.59-7.65 (*m*, 10H; C$_{ar}$*H*), 7.69 (*s*, 1H; C*H*(6)); $^{13}$C-NMR (150 MHz, DMSO-$d_6$) δ: -1.47, 17.43, 18.69, 19.61, 26.56, 28.53, 31.50, 35.95, 41.06, 44.75, 60.81, 63.91, 65.33, 66.17, 69.41, 69.62, 69.73, 73.65, 83.44, 84.97 (2C), 87.75, 107.54, 127.57, 127.75, 127.83, 134.87, 134.99, 135.10, 137.79, 149.98, 152.96, 162.46, 172.53; MS (ESI): calc. for $[C_{64}H_{92}N_4O_{12}Si_4+Na]^+$: 1243.5681; found: 1243.5700.

*R*-isomer **2b**: mp 66-75 °C; IR: 3412*br*, 3072*w*, 2954*s*, 2930*s*, 2858*s*, 1710*s*, 1664*s*, 1589*w*, 1461*s*, 1428*m*, 1246*m*, 1194*w*, 1085*m*, 1053*m*, 1006*m*, 937*w*, 939*w*, 859*w*, 822*s*, 742*w*, 702*s* cm$^{-1}$; $^1$H-NMR (600 MHz, DMSO-$d_6$) δ: -0.08 (*s*, 9H; Si(C$H_3$)$_3$), -0.06 (*s*, 9H; Si(C$H_3$)$_3$), 0.73-0.81 (*m*, 4H; 2 x SiC$H_2$C$H_2$O), 0.86 (*s*, 3H; C(5A)C$H_3$), 0.99 (*s*, 9H; C(C$H_3$)$_3$), 1.04 (*s*, 9H; C(C$H_3$)$_3$), 1.89-1.92 (*m*, 2H; C$H_{2c}$(2'B), C$H_{2d}$(2'A)), 2.04 (*dd*, *J* = 13.6, 6.9 Hz, 1H; C$H_{2c}$(2'A)), 2.12-2.18 (*m*, 2H; C$H_{2e}$(5), C$H_{2d}$(2'B)), 2.70 (*d*, *J* = 13.8 Hz, 1H; C$H_{2f}$(5)), 3.04 (*d*, *J* = 13.2 Hz, 1H; C$H_{2b}$(6A)), 3.11 (*d*, *J* = 13.2 Hz, 1H; C$H_{2a}$(6A)), 3.11-3.12 (*m*, 1H; C$H_{2a}$(5'B)), 3.42-3.44 (*m*, 3H; SiCH$_2$C$H_2$O(A), C$H_{2b}$(5'B)), 3.65 (*dd*, *J* = 10.7, 4.7 Hz, 2H; SiCH$_2$C$H_2$O(B)), 3.71-3.72 (*m*, 1H; C$H_{2a}$(5'A)), 3.74-3.75 (*m*, 1H; C$H_{2b}$(5'A)), 3.76-3.77 (*m*, 1H; C*H*(4'A)), 3.97-3.98 (*m*, 1H; C*H*(4'B)), 4.19-4.20 (*m*, 1H;

C*H*(3'A)), 4.46-4.47 (*m*, 1H; C*H*(3'B)), 4.90 (*d*, *J* = 9.9 Hz, 1H; OC$H_{2a}$N(A)), 4.99 (*t*, *J* = 5.2 Hz, 1H; C(5'B)O*H*), 5.04 (*d*, *J* = 9.9 Hz, 1H; OC$H_{2b}$N(A)), 5.13 (*d*, *J* = 4.0 Hz, 2H; OC$H_2$N(B)), 5.23 (*d*, *J* = 4.6 Hz, 1H; C(3')O*H*), 6.25 (*t*, *J* = 7.0 Hz, 1H; C*H*(1'A)), 6.29 (*dd*, *J* = 7.8, 6.0 Hz, 1H; C*H*(1'B)), 7.42-7.47 (*m*, 10H; $C_{ar}H$), 7.59-7.69 (*m*, 10H; $C_{ar}H$), 7.69 (*s*, 1H; C*H*(6)); $^{13}$C-NMR (150 MHz, DMSO-$d_6$) δ: -1.48, 17.21, 17.44, 18.53, 18.61, 19.34, 26.46, 26.58, 31.80, 38.80, 40.83, 44.74, 60.81, 64.51, 65.21, 66.14, 69.21, 69.64, 70.25, 73.64, 83.53, 84.98, 84.46, 87.69, 107.59, 127.77, 127.82, 127.84, 129.72, 129.75, 129.92, 132.64, 132.72, 134.96, 134.99, 135.10, 137.88, 150.06, 151.76, 162.46, 173.10; MS (ESI): calc. for $[C_{64}H_{92}N_4O_{12}Si_4+Na]^+$: 1243.5681; found: 1243.5701.

**(5*S*)-*N*$^3$-trimethylsilylethoxymethyl-5'-*O*-*tert*-butyldiphenylsilyl-5-(*N*$^3$-trimethylsilyl-ethoxymethyl-5'-*O*-dimethoxytrityl-3'-*O*-*tert*-butyldiphenylsilyl-thymidyl)-5,6-dihydro-thymidine (6a).** The *S*-isomer **2a** (168 mg, 0.14 mmol), azeotropically dried with pyridine, was dissolved in pyridine (0.8 mL) and stirred at room temperature over molecular sieve (4 Å). Dimethoxytrityltriflat (124 mg, 0.28 mmol) was dissolved in pyridine (0.4 mL) and stirred at room temperature over molecular sieve (4 Å). After 3 h the Dimethoxytrityl-solution was added to **2a** and the reaction mixture was stirred further 6 h. The reaction was quenched by adding methanol (1 mL), filtered and the solvent was removed under reduced pressure. Purification by flash chromatography (silica gel-*H*, $CHCl_3$/MeOH/Pyr 50:1:0.1) provided **6a** (176 mg, 84 %) as a pale yellow, sticky solid.

IR: 3492*br*, 2953*s*, 2859*s*, 1719*s*, 1663*s*, 1509*m*, 1460s, 1428*m*, 1392*w*, 1246*s*, 1182*m*, 1084*s*, 1032*w*, 995*m*, 936*w*, 833*s*, 743*w*, 700*s*, 611*m* cm$^{-1}$; $^1$H-NMR (400 MHz, Acetone-$d_6$) δ: -0.04 (*s*, 9H; Si(C$H_3$)$_3$), -0.02 (*s*, 9H; Si(C$H_3$)$_3$), 0.76-0.93 (*m*, 7H; C(5A)C$H_3$, 2 x SiC$H_2$C$H_2$O), 1.05 (*s*, 9H; C(C$H_3$)$_3$), 1.06 (*s*, 9H; C(C$H_3$)$_3$), 1.93-2.03 (*m*, 2H; C$H_{2a}$(2'B), C$H_{2a}$(2'A)), 2.07-2.12 (*m*, 2H; C$H_{2b}$(2'A), C$H_{2a}$(5)), 2.34 (*ddd*, *J* = 13.46, 5.79, 2.96 Hz, 1H; C$H_{2b}$(2'B)), 2.61 (*d*, *J* = 13.89 Hz, 1H; C$H_{2b}$(5)), 2.99-3.19 (*m*, 4H; C$H_2$(6A), C$H_2$(5'B)), 3.48 (*t*, *J* = 7.81 Hz,

2H; $SiCH_2CH_2O$(A)), 3.57-3.66 (*m*, 2H; $SiCH_2CH_2O$(B)), 3.75 (*s*, 6H; 2 x $OCH_3$), 3.78-3.85 (*m*, 2H; $CH_2$(5'A)), 3.87-3.88 (*m*, 1H; CH(4'A)), 4.21-4.24 (*m*, 1H; CH(4'B)), 4.41-4.46 (*m*, 1H; CH(3'A)), 4.49-4.50 (*m*, 1H; CH(3'B)), 5.00 (*d*, $J = 9.74$ Hz, 1H; $OCH_{2a}N$(A)), 5.05 (*d*, $J = 9.73$ Hz, 1H; $OCH_{2b}N$(A)), 5.26 (*s*, 2H; $OCH_2N$(B)), 6.36-6.41 (*m*, 2H; CH(1'A), CH(1'B)), 6.79-6.81 (*m*, 4H; $C_{ar}H$), 7.17-7.26 (*m*, 6H; $C_{ar}H$), 7.29-7.47 (*m*, 16H; $C_{ar}H$, CH(6B)), 7.13-7.27 (*m*, 4H; $C_{ar}H$), 7.30-7.50 (*m*, 4H; $C_{ar}H$); $^{13}$C-NMR (100 MHz, Acetone-$d_6$) δ: -0.12, 19.54, 19.66, 20.75, 21.74, 28.25, 28.33, 34.27, 40.74, 41.63, 43.34, 47.05, 56.54, 65.72, 66.23, 67.80, 68.68, 71.90, 71.95, 72.34, 75.70, 85.91, 87.35, 88.02, 88.06, 110.35, 114.94, 125.56, 129.64, 129.72, 129.79, 129.93, 129.97, 131.69, 131.71, 131.91, 131.95, 134.89, 135.00, 135.11, 135.12, 137.30, 137.40, 137.49, 137.55, 139.53, 146.75, 152.46, 154.26, 160.60, 164.81, 175.51; MS ($ESI^+$): calc. for $[C_{85}H_{110}N_4O_{14}Si_4+NH_4]^+$: 1540.7434; found: 1540.7580.

**(5*R*)-$N^3$-trimethylsilylethoxymethyl-5'-*O*-*tert*-butyldiphenylsilyl-5-($N^3$-trimethylsilyl-ethoxymethyl-5'-*O*-dimethoxytrityl-3'-*O*-*tert*-butyldiphenylsilyl-thymidyl)-5,6-dihydro-thymidine (6b).** DMT-protection was carried out as described above for **6a**. The *R*-isomer **6b** (110 mg, 100 %) was isolated as a pale yellow, sticky solid.

IR: 3390*br*, 2954*s*, 2862*s*, 1718*s*, 1660*s*, 1507*m*, 1461*s*, 1425*m*, 1390*w*, 1244*s*, 1181*m*, 1082*s*, 1030*w*, 993*m*, 935*w*, 832*s*, 743*w*, 701*s*, 610*m* $cm^{-1}$; $^1$H-NMR (400 MHz, Acetone-$d_6$) δ: -0.03 (*s*, 9H; $Si(CH_3)_3$), -0.02 (*s*, 9H; $Si(CH_3)_3$), 0.80-0.90 (*m*, 4H; 2 x $SiCH_2CH_2O$), 0.93 (*s*, 3H; C(5A)$CH_3$), 1.05 (*s*, 9H; $C(CH_3)_3$), 1.06 (*s*, 9H; $C(CH_3)_3$), 1.84-1.92 (*m*, 1H; $CH_{2a}$(2'B)), 2.06-2.10 (*m*, 1H; $CH_{2a}$(5)), 2.11-2.12 (*m*, 1H; $CH_{2a}$(2'A)), 2.35 (*ddd*, $J = 13.48$, 5.99, 3.09 Hz, 1H; $CH_{2b}$(2'A)) 2.56-2.66 (*m*, 1H; $CH_{2b}$(2'B)), 2.85 (*bs*, 1H; $CH_{2b}$(5)), 3.09 (*dd*, $J = 10.29$, 4.92 Hz, 1H; $CH_{2a}$(5'B)). 3.18 (*dd*, $J = 10.30$, 4.20 Hz, 1H; $CH_{2b}$(5'B)), 3.27 (*d*, $J = 13.34$ Hz, 1H; $CH_{2a}$(6A)), 3.41 (*d*, $J = 13.35$ Hz, 1H; $CH_{2b}$(6A)), 3.53 (*t*, $J = 7.85$ Hz, 2H; $SiCH_2CH_2O$(A)), 3.60-3.65 (*m*, 2H; $SiCH_2CH_2O$(B)), 33.71 (*dd*, $J = 7.80$, 3.73 Hz, 2H;

C*H*$_2$(5'A)), 3.76 (*s*, 6H; 2 x OC*H*$_3$), 4.12-4.13 (*m*, 1H; C*H*(4'A)), 4.18-4.19 (*m*, 1H; C*H*(4'B)), 4.50-4.57 (*m*, 2H; C*H*(3'B), C*H*(3'A)), 5.05 (*d*, *J* = 9.69 Hz, 1H; OC*H*$_{2a}$N(A)), 5.13 (*d*, *J* = 9.69 Hz, 1H; OC*H*$_{2b}$N(A)), 5.27 (*s*, 2H; OC*H*$_2$N(B)), 6.19-6.48 (*m*, 2H; C*H*(1'A), C*H*(1'B)), 6.70-6.90 (*m*, 4H; C$_{ar}$*H*), 7.26-7.17 (*m*, 6H; C$_{ar}$*H*), 7.29-7.47 (*m*, 16H; C$_{ar}$*H,* C*H*(6B)), 7.60-7.67 (*m*, 4H; C$_{ar}$*H*), 7.71-7.78 (*m*, 4H; C$_{ar}$*H*); $^{13}$C-NMR (100 MHz, Acetone-$d_6$) δ: -0.14, 19.54, 19.66, 20.75, 21.74, 28.25, 28.29, 34.27, 40.74, 41.63, 43.34, 47.05, 56.55, 65.60, 66.78, 67.67, 68.63, 71.55, 71.94, 73.36, 87.43, 87.79, 88.22, 89.47, 110.35, 114.95, 125.71, 129.72, 129.77, 129.78, 129.97, 131.69, 131.71, 131.91, 131.93, 134.89, 135.00, 135.11, 135.12, 137.43, 137.49, 137.51, 137.54, 139.53, 146.75, 152.46, 154.26, 160.61, 164.81, 175.51; MS (ESI$^+$): calc. for $[C_{85}H_{110}N_4O_{14}Si_4+H]^+$: 1523.7168; found: 1523.7312.

**(5*S*)-*N*$^3$-trimethylsilylethoxymethyl-5'-*O*-*tert*-butyldiphenylsilyl-3'-*O*-*β*-cyanoethyl-*N,N*-diisopropylaminophosphanoxy-5-(*N*$^3$-trimethylsilylethoxymethyl-5'-*O*-dimethoxytrityl-3'-*O*-*tert*-butyldiphenylsilyl-thymidyl)-5,6-dihydrothymidine (1a).** The *S*-isomer **6a** (120 mg, 0.08 mmol) and Diisoproplyamine (54 µL, 0.32 mmol) was dissolved in THF (0.6 mL), degassed 3 times and cooled to 0 °C. CEDCl (33 µL, 0.12 mmol) was added at 0 °C and the solution was stirred for 5 h. The temperature was allowed to go up to room temperature. The solvent was removed under reduced pressure. Purification by flash chromatography (deactivated silica gel, isohexane/ethyl acetate 3:1) provided **1a** (100 mg, 73 %) as a white solid.

$^{31}$P-NMR (200 MHz, $CDCl_3$) δ: 149.30, 149.52; $^1$H-NMR (600 MHz, $CDCl_3$) δ: -0.04 (*s*, 9H; Si(C*H*$_3$)$_3$), -0.01 (*s*, 9H; Si(C*H*$_3$)$_3$), 0.85-0.97 (*m*, 7H; C(5A)C*H*$_3$, 2 x SiC*H*$_2$CH$_2$O), 1.00-1.07 (*m*, 9H, C(C*H*$_3$)$_3$), 1.11-1.15 (*m*, 9H, C(C*H*$_3$)$_3$), 1.32-1.42 (*m*, 6H, NCH(C*H*$_3$)$_2$), 1.73-1.84 (*m*, 6H, NCH(C*H*$_3$)$_2$), 1.92-2.04 (*m*, 1H, C*H*$_{2a}$(2'A)), 2.10-2.15 (*m*, 2H, C*H*$_{2a}$(2'B), C*H*$_{2b}$(2'A)), 2.22-2.33 (*m*, 2H, C*H*$_{2b}$(2'B), C*H*$_{2a}$(5)), 2.49-2.62 (*m*, 2H, C*H*$_{2a}$CN, C*H*$_{2b}$(5)),

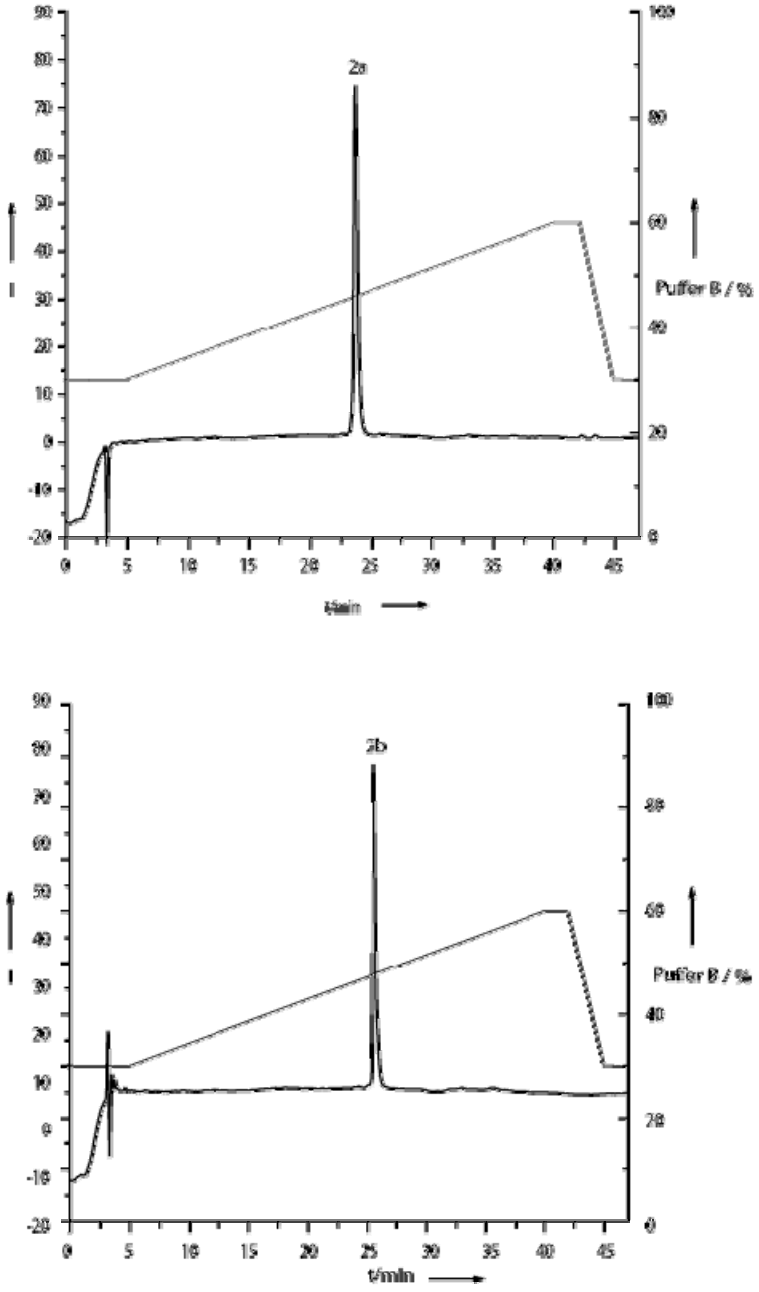
2.96-3.05 (*m*, 2H, C$H_{2a}$(6A), C$H_{2b}$CN), 3.08-3.16 (*m*, 3H, N(C$H$(C$H_3$)$_2$)$_2$, C$H_{2b}$(6A)), 3.39-3.45 (*m*, 1H, C$H_{2a}$(5'B)), 3.47-3.53 (*m*, 3H, SiCH$_2$C$H_2$O(A), C$H_{2b}$(5'B)), 3.58-3.66 (*m*, 4H, SiCH$_2$C$H_2$O(B), OC$H_2$CH$_2$CN), 3.75 (*s*, 6H; 2 x OC$H_3$), 3.83-3.84 (*m*, 1H, C$H_{2a}$(5'A)), 4.04-4.11 (*m*, 2H, C$H_{2b}$(5'A), C$H$(4'A)), 4.12-4.19 (*m*, 2H, C$H$(3'A), C$H$(4'B)), 4.32-4.42 (*m*, 1H, C$H$(3'B)), 4.98-5.04 (*m*, 1H, OC$H_{2a}$N(A)), 5.09 (*d*, $J$ = 9.63 Hz, 1H, OC$H_{2b}$N(A)), 5.24-5.32 (*m*, 2H, OC$H_2$N(B)), 6.30-6.42 (*m*, 2H, C$H$(1'A), C$H$(1'B)), 6.65-6.75 (*m*, 4H, C$_{ar}H$), 7.09-7.20 (*m*, 6H, C$_{ar}H$), 7.24-7.45 (*m*, 16H, C$_{ar}H$, C$H$(6)), 7.51-7.60 (*m*, 4H, C$_{ar}H$), 7.60-7.71 (*m*, 4H, C$_{ar}H$); $^{13}$C-NMR (150 MHz, $CDCl_3$) δ: -1.39, -1.37, 17.59, 18.04, 18.15, 19.02, 19.23, 22.41, 22.53, 26.46, 26.87, 32.52, 38.72, 40.24, 41.87, 41.93, 43.25, 46.35, 55.15, 60.11, 63.73, 63.75, 66.57, 67.40, 70.24, 70.33, 71.01, 73.72, 84.08, 85.52, 86.33, 86.39, 108.38, 113.07, 116.59, 116.77, 123.68, 126.81, 127.88, 128.02, 128.04, 129.81, 129.83, 129.97, 129.98, 130.00, 132.72, 133.06, 133.13, 135.56, 135.61, 135.68, 139.48, 144.63, 150.62, 152.77, 158.42, 163.05, 172.97; MS (ESI): calc. for $[C_{94}H_{127}N_6O_{15}PSi_4+NH_4]^+$: 1740.8512; found: 1740.8615.

**(5*R*)-*N*$^3$-trimethylsilylethoxymethyl-5'-*O*-*tert*-butyldiphenylsilyl-3'-*O*-*β*-cyanoethyl-*N*,*N*-diisopropylaminophosphanoxy-5-(*N*$^3$-trimethylsilylethoxymethyl-5'-*O*-dimethoxytrityl-3'-*O*-*tert*-butyldiphenylsilyl-thymidyl)-5,6-dihydrothymidine (1b).** Phosphoramidite-synthesis was carried out as described above for **1a**. The *R*-isomer **1b** (101 mg, 89 %) was isolated as a white solid.

$^{31}$P-NMR (200 MHz, $CDCl_3$) δ: 149.44, 149.47; $^1$H-NMR (600 MHz, $CDCl_3$) δ: -0.04 (*s*, 9H; Si(C$H_3$)$_3$), -0.01 (*s*, 9H; Si(C$H_3$)$_3$), 0.86-0.95 (*m*, 7H; C(5A)C$H_3$, 2 x SiC$H_2$CH$_2$O), 1.04 (*s*, 9H; C(C$H_3$)$_3$), 1.06 (*s*, 9H; C(C$H_3$)$_3$), 1.12-1.26 (*m*, 12H; N(CH(C$H_3$)$_2$)$_2$), 1.16-1.28 (*m*, 2H; C$H_{2a}$(2'B), C$H_{2a}$(2'A)), 1.92-1.94 (*m*, 1H; C$H_{2a}$(5)), 2.21-2.30 (*m*, 1H; C$H_{2b}$(2'A)), 2.36-2.40 (*m*, 1H; C$H_{2b}$(2'B)), 2.49-2.58 (*m*, 1H; C$H_{2b}$(5)), 2.61-2.72 (*m*, 1H; C$H_{2a}$CN), 2.99-3.03 (*m*, 1H; C$H_{2b}$CN), 3.01 (*dd*, $J$ = 10.17, 4.54 Hz, 1H; C$H_{2a}$(5'B)), 3.10-3.15 (*m*, 1H; C$H_{2b}$(5'B)),

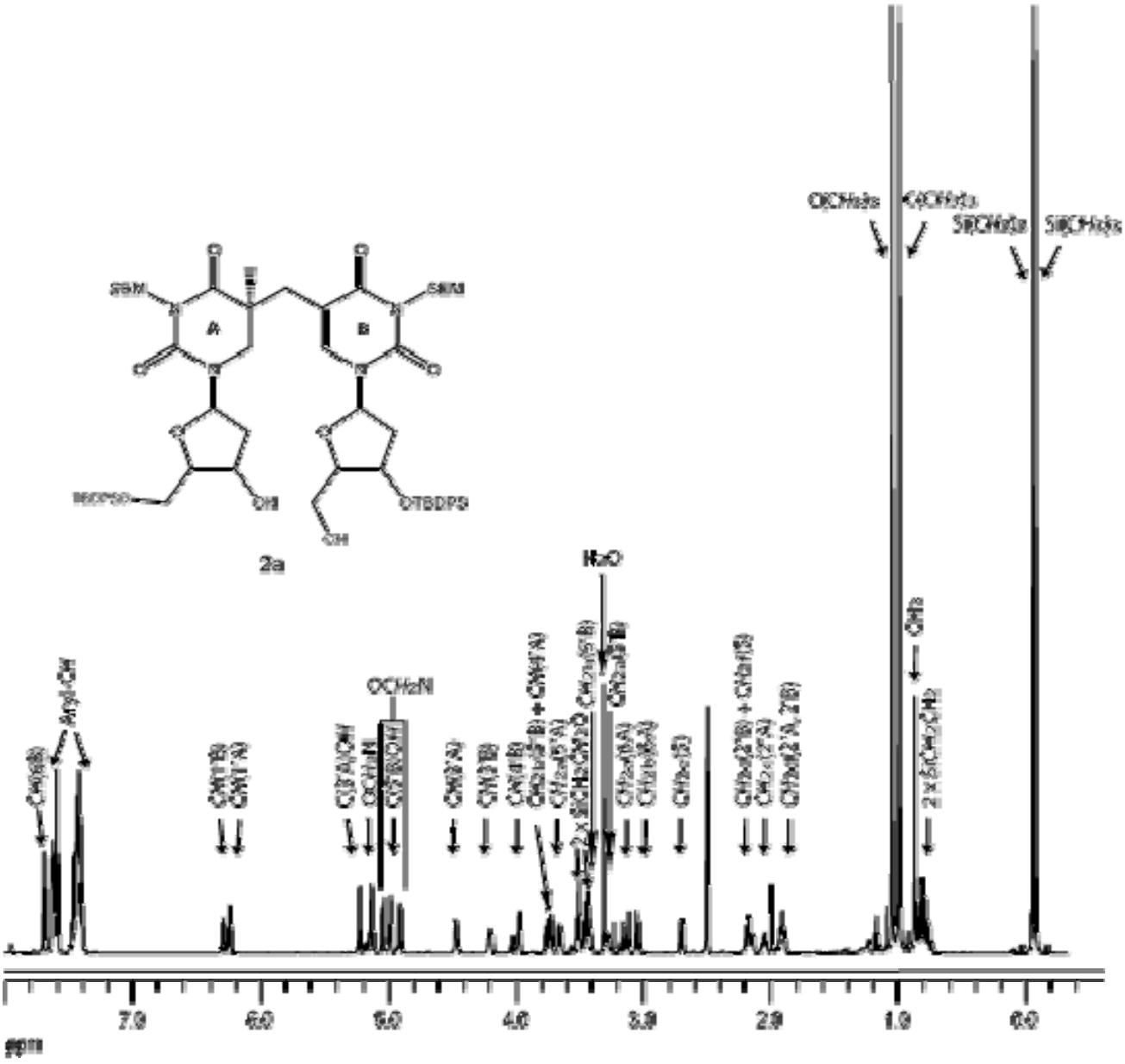
3.17-3.27 (*m*, 2H; N(C*H*($CH_3$)$_2$)$_2$), 3.37-3.45 (*m*, 1H; C$H_{2a}$(6A)), 3.55-3.59 (*m*, 3H; C$H_{2b}$(6A), Si$CH_2$C$H_2$O(A)), 3.59-3.70 (*m*, 6H; C$H_2$(5'A), Si$CH_2$C$H_2$O(B), OC$H_2$$CH_2$CN), 3.74 (*s*, 6H; 2 x OC$H_3$), 4.02-4.04 (*m*, 2H; C*H*(4'B), C*H*(4'A)), 4.40-4.42 (*m*, 1H; C*H*(3'A)), 4.50-4.65 (*m*, 1H; C*H*(3'B)), 4.98 (*d*, *J* = 9.67 Hz, 1H; OC$H_{2a}$N(A)), 5.16 (*d*, *J* = 9.63 Hz, 1H; OC$H_{2b}$N(A)), 5.30 (*d*, *J* = 3.99 Hz, 2H; OC$H_2$N(B)), 6.34-6.39 (*m*, 2H; C*H*(1'A), C*H*(1'B)), 6.65-6.75 (*m*, 4H, $C_{ar}$*H*), 7.23-7.30 (*m*, 6H; $C_{ar}$*H*), 7.30-7.44 (*m*, 16H; $C_{ar}$*H,* C*H*(6)), 7.53-7.60 (*m*, 4H, $C_{ar}$*H*), 7.64-7.70 (*m*, 4H, $C_{ar}$*H*); $^{13}$C-NMR (150 MHz, $CDCl_3$) δ: -0.14, 18.15, 19.01, 19.63, 20.20, 20.62, 22.37, 22.61, 24.55, 26.84, 32.88, 38.38, 40.27, 41.77, 42.14, 43.21, 45.09, 55.15, 60.36, 63.81, 64.29, 66.45, 67.37, 70.04, 70.38, 71.01, 73.66, 81.39, 85.61, 85.67, 86.32, 108.70, 113.08, 116.94, 117.48, 123.67, 126.78, 127.78, 127.83, 128.06, 129.11, 129.80, 129.92, 129.97, 130.01, 133.11, 133.14, 133.15, 135.59, 135.62, 135.64, 135.68, 139.48, 144.64, 150.69, 152.22, 158.44, 163.00, 173.27; MS (ESI): calc. for $[C_{94}H_{127}N_6O_{15}PSi_4+NH_4]^+$: 1740.8512; found: 1740.8535.

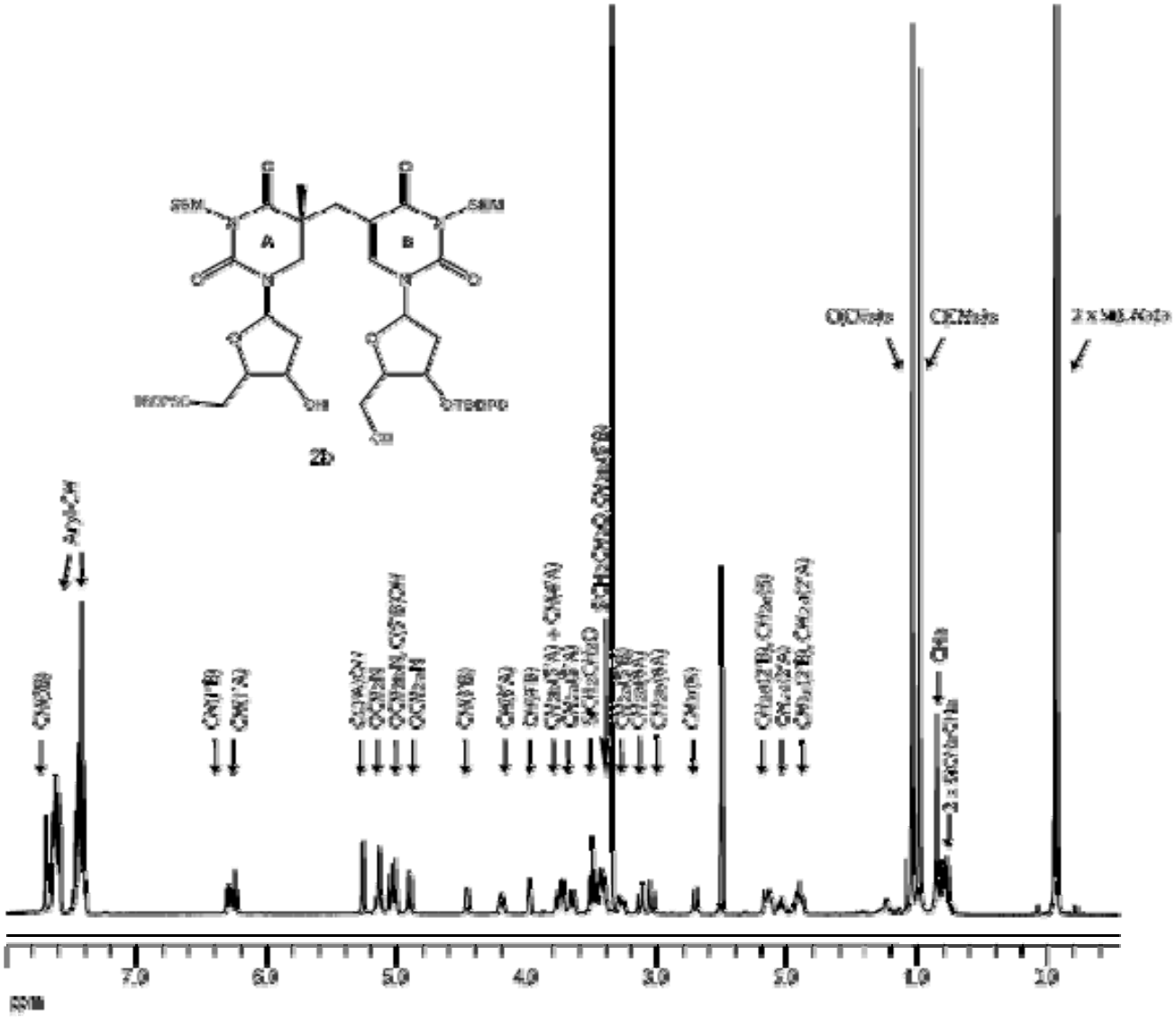
2) HPLC-CHROMATOGRAMS FOR THE SEPARATED DIASTEREOMERS **2a** AND **2b**




Analytical HPLC-chromatograms of the separated diastereomers **2a** and **2b**. *np*-HPLC: Heptane (A), Ethyl acetate (B); gradient: 30 → 50 % B in 25 min, 50 → 60 % B in 10 min; column: *CC 250/4 Nucleodur 100-3*; flow rate: 0.7 mL/min; detection: 250 nm; retention times: $R_f$ (**2a**) 23.65 min, $R_f$ (**2b**) 25.45 min.

3) $^{1}$H-NMR SPECTRA FOR THE SEPARATED DIASTEREOMERS **2a** AND **2b**

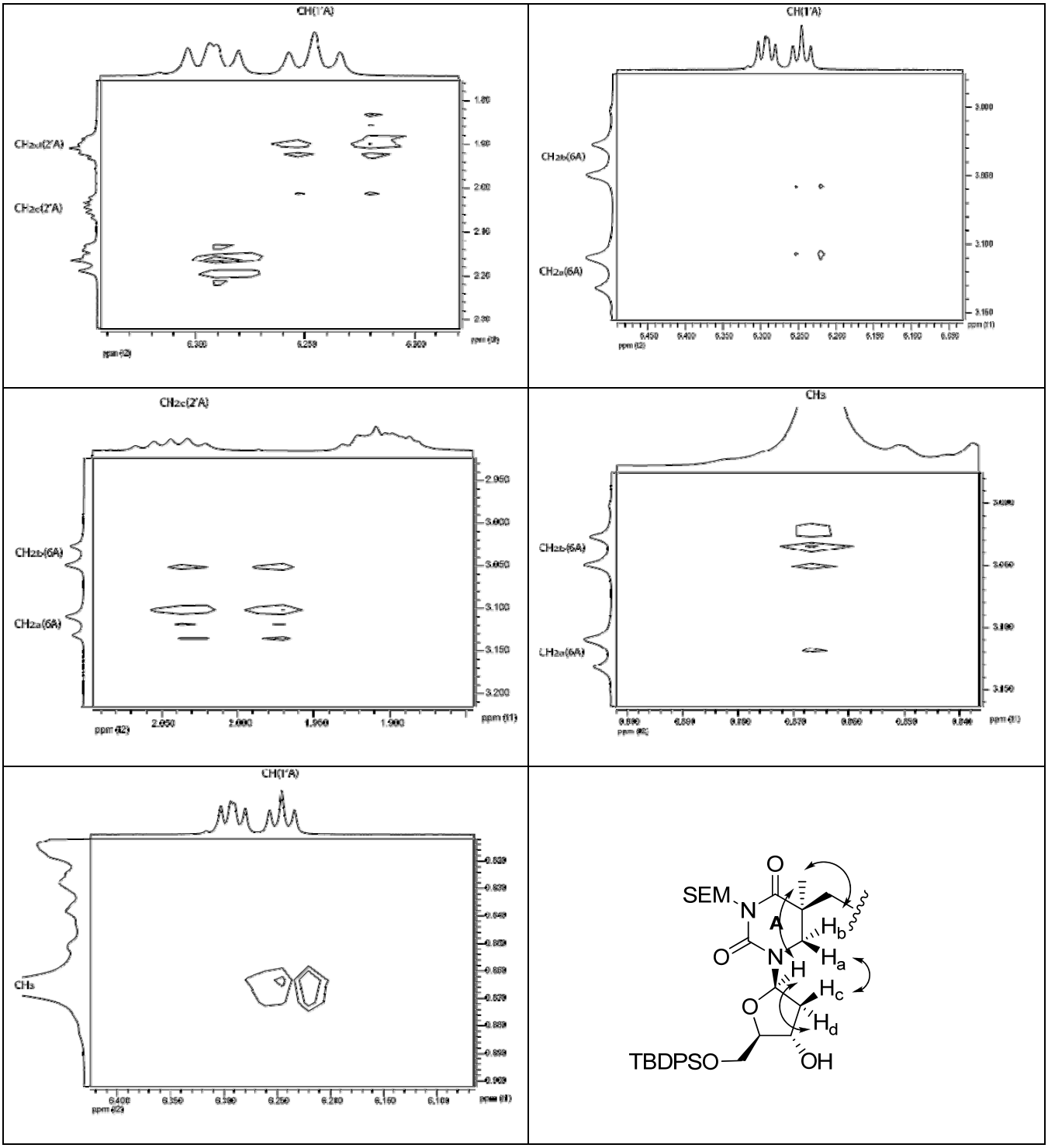





$^{1}$H-NMR spectrums of the diastereomers **2a** and **2b** in DMSO-$d_6$ (600 MHz).

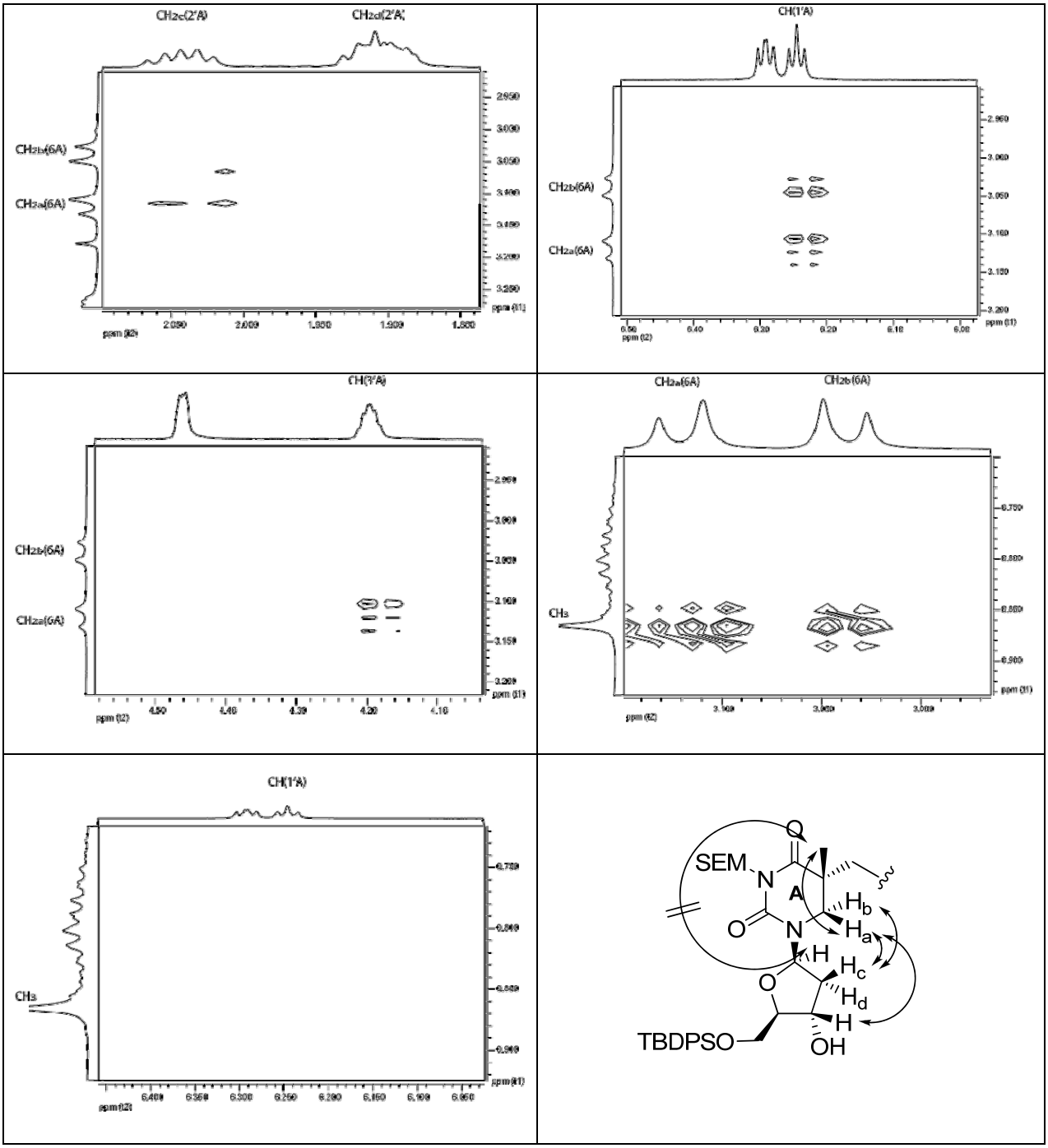
4) NOE DATA FOR THE ISOMERS **2a/b**

Isomer **2a**




NOESY crosspeaks for isomers **2a** in DMSO-$d_6$ (600 MHz).

Isomer **2b**




NOESY crosspeaks for isomers **2b** in DMSO-$d_6$ (600 MHz).