Supporting information

8-vinyl-deoxyadenosine, an alternative fluorescent nucleoside analogue to 2'-deoxyribosyl-2-aminopurine with improved properties.

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Synthesis: General information.

All nucleoside derivatives were dried twice by coevaporation with dry pyridine before use. Pyridine was distilled and stored over KOH. EtOAc was distilled over CaH_2; CH_2Cl_2 was distilled over P_2O_5, stored over molecular sieves (4Å) and filtered through an activated alumina column before use. Tetrazole was sublimated (P= 0.1 mBar, oil bath = 85°C, caution do not exceed 100°C). $Pd(PPh_3)_4$ was synthesised according to the procedure of Heck (43). Flash chromatography was made on silica gel purchased from Merck (40-63 μm) treated with NEt_3 to prevent dimethoxytrityl degradation. Thin layer chromatography was performed on Merck $60F_{254}$ coated plates. Chromatography of the phosphoramidite was done on silica gel pre-treated as followed: silica gel was washed successively with HCl (1N), water, NH_4OH (10%) and water and finally, dried under reduced pressure. The phosphoramidite **6** was dissolved in CH_3CN (0.1M) (high quality for DNA synthesis, Fluka), stored overnight over activated 3Å molecular sieves (Merck) and filtered through a 0.45μm filter (Millex®-HV, Millipore) before use in oligonucleotide synthesis.

NMR spectra were recorded at 200, 300 or 500 MHz on a Bruker Advance Spectrometer. The NMR chemical shifts are reported in ppm downfield from TMS for ^1H and ^{13}C NMR and 85% H_3PO_4 for ^{31}P NMR. Mass spectra were recorded on an Esquire 3000 Plus equipment, in either positive or negative Electron Spray Ionisation (ESI) mode.

5'-Dimethoxytrityl-8-vinyl -2'-deoxyadenosine (4):

In a Schlenk flask, compound **3** (500 mg, 0.8 mmol) was dissolved in N-methylpyrrolidone (NMP, 2.7 mL). $Pd(PPh_3)_4$ (90 mg, 0.078 mmol) was added and the

reaction mixture was degassed three times before adding tetravinyltin under Ar (290 µL, 1.58 mmol). The reaction was then heated in an oil bath at 110°C and followed by TLC ($CHCl_3$/MeOH/NEt_3 93:5:1). After 1h, the reaction mixture was cooled to room temperature, and EtOAc and H_2O were added. The mixture was filtered over celite and the filtrate was washed twice with H_2O (40 mL), a saturated aqueous solution of $NaHCO_3$ (20 mL), and brine (20 mL), dried over Na_2SO_4, filtered and evaporated. An oily residue was obtained and purified on pretreated silica gel. The product was eluted with CH_2Cl_2/ MeOH (0 →5%). An amorphous solid was obtained in 82% yield (656 mg). Mp:151°C (decomp.). ^{1}H NMR ($CDCl_3$, 300 MHz): 8.1 (1H, s, H_2), 7.38-7.12 (9H, m, DMTr), 7.07-7.01 (1H, dd, J_1= 11 Hz & J_2= 17 Hz, H_{vinyl}), 6.76- 6.72 (4H, m, DMTr), 6.48-6.43 (2H, t, J= 7 Hz, $H_{1'}$ & dd: J_1= 1.5 and J_2= 17 Hz, H_{trans}), 5.93 (2H, s, NH_2), 5.48-5.44 (1H, dd, J_1= 1.5 Hz & J_2= 11 Hz, Hcis), 4.82-4.77 (1H, m, $H_{4'}$), 4.04-4.0 (1H, m, $H_{3'}$), 3.66 (6H, s, OCH_3 DMTr), 3.42-3.2 (3H, $2H_{5'}$ & $H_{2'}$), 2.3- 2.21 (1H, m, $H_{2'}$). ^{13}C NMR ($CDCl_3$, 75.4 MHz): 158.4 (q), 154.9 (q), 152.3 (t), 150.5 (q), 144.6 (q), 135.8 (t), 130.1 (t), 128.3 (t), 128.1 (t), 127.8 (t), 126.8 (t), 124.1 (s), 119.2 (q) 113.6 (t), 85.7 ($_t$), 83.5 ($_t$), 72.1 ($_t$), 63.3 (s), 55.2 (p), 38.5 (s). ESI MS (m/z): 614.0 $[M+ Cl]^-$.

5'-Dimethoxytrityl-6-N,N-dimethylformamidine-8-vinyl-2'-deoxyadenosine (5):

Compound **4** (500 mg, 0.86 mmol) was dissolved in MeOH (3 mL) and N,N-dimethyl-dimethoxyformamide (575 µL, 4.3 mmol) was added. After completion (TLC: $CHCl_3$/ MeOH/ NEt_3 94:5:1), EtOAc was added and the organic phase was extracted with H_2O (15 mL), a saturated aqueous solution of $NaHCO_3$ (30 mL), and brine (15 mL), dried over Na_2SO_4, filtered and evaporated. The resulting product (802 mg, 93%) was pure enough to be used in the next step without further purification. Mp:146°C (decomp.). ^{1}H NMR ($CDCl_3$, 300 MHz): 8.90 (1H, s, N=CH), 8.38 (1H, s, H_2), 7.41-7.07 (9H, m, DMTr), 7.03-6.8 (1H, dd, J_1=

11.2 Hz & J_2= 17.2 Hz, H_{vinyl}), 6.8- 6.7 (4H, m, DMTr), 6.6- 6.5 (2H, J_1= 1.3 Hz & J_2= 17.2 Hz, $H_{1'}$ and H_{trans}), 5.55- 5.50 (1H, dd, J_1= 1.3 Hz & J_2= 11.2 Hz, H_{Cis}), 4.92- 4.87 (1H, m, $H_{4'}$), 4.17- 4.08 (1H, m, $H_{3'}$), 3.7 (6H, s, CH_3 DMTr), 3.43- 3.21 (3H, $H_{5'}$ & $H_{2'}$), 3.16 (3H, s, NCH_3), 3.15 (3H, s, NCH_3), 2.36- 2.30 (1H, m, $H_{2'}$). ^{13}C NMR ($CDCl_3$, 75.4 MHz): 159 (q), 158.4 (q), 158 (t), 152.4 (q), 152 (t), 150 (q), 144.65 (q), 135.8 (t), 130.0 (t), 128.3 (t), 128.1 (t), 127.8 (t), 126.8 (t), 124.5 (s), 124 (q), 113 (t), 85.5 (t), 83.3 (t), 72.2 (t) 63.3 (s), 55.2 (p), 38.6 (p), 35.2 (s), 29.6 (p). ESI MS (m/z): 635.3 $[M+H]^+$, 657.2 $[M+Na]^+$, 680.1 $[M+ 2Na]^+$.

3'-N,N'-diisopropylcyanoethylphosphoramidite-5'-dimethoxytrityl-6-N,N-dimethylformamidine-8-vinyl-2'-deoxyadenosine (6):

Compound **5** (300 mg, 0.473 mmol) was dissolved in dry CH_2Cl_2 (1.4 mL) under Ar. Tetrazole (16.6 mg, 0.28 mmol) was added, followed by a freshly prepared solution of N,N'-diisopropyl-cyanoethyl-phosphorodiamidite (225 µL, 0.71 mmol) in dry CH_2Cl_2 (200 µL). After 4h stirring (TLC: CH_2Cl_2/ EtOAc/ NEt_3 45:45:10), EtOAc and NEt_3 were added and the organic phase was washed with a saturated solution of $NaHCO_3$ and brine; dried over Na_2SO_4, filtered and evaporated. The oily residue obtained was purified on silica gel (CH_2Cl_2/ EtOAc/ NEt_3 45:45:10). The phosphoramidite monomer was dissolved in toluene so that a 35% solution was obtained. The solution was slowly added to a rapidly stirred and cooled solution of hexane (-20°C, 20-25 volumes). The precipitated phosphoramidite **6** (237 mg, 0.284 mmol) was obtained in 60% yield. ^{1}H NMR (CD_2Cl_2, 300 MHz): 8.9 (1H, s, N=CH), 8.3 (1H, s, H_2), 7.4- 7.2 (9H, m, DMTr), 7.1-6.90 (1H, dd, J_1= 11 Hz & J_2= 17 Hz, H_{vinyl}), 6.80- 6.75 (4H, m, DMTr), 6.6- 6.4 (2H, m, $H_{1'}$ and H_{trans}), 5.6- 5.5 (1H, m, H_{Cis}), 5- 4.9 (1H, m, $H_{3'}$), 4.2 (1H, m, $H_{4'}$), 3.8 (6H, s, CH_3 DMTr), 3.7- 3.6 (5H, m, CH_2O, $H_{isopropyl}$, $H_{2'; }H_{5'}$), 3.24- 3.21 (6H, s, CH_3), 2.67- 2.60 (1H, s, $H_{2'}$), 2.58- 2.48 (2H, m, CH_2CN), 1.35- 1.10 (12H, m, CH_3

isopropyl). ^{31}P NMR (200 MHz, CD_2Cl_2): 149.9 and 149.2 ppm. ES MS (m/z): 835.2 $[M+H]^+$.

Solid phase oligonucleotide synthesis.

Three 15 mer sequences (ODN1 to ODN3) differing by the closest neighbors to the nucleoside analogue (N= 8vdA or 2AP) were synthetized in this study. Their sequences are d(CGT TTT **XNX** TTT TGC) with X=T, A, and C for ODN1, ODN2 and ODN3, respectively.

Oligodeoxyribonucleotides containing 8vdA were synthesized on an Applied Biosystem 392 synthesizer using fast-deprotecting phosphoramidite chemistry at a 1 μmol scale. Pac-dA, iPr-Pac-dG, Ac-dC and T phosphoramidites were purchased from Eurogentec Glen Research. The following modifications to the standard procedure were applied. A longer coupling time (35 s) for 8vdA was used to ensure a high coupling yield. Dichloroacetic acid (3%) in acetonitrile was used for removal of the dimethoxytrityl group (DMTr) (32). Phenoxyacetic anhydride (Pac_2O) was used in Cap A (33). Phosphite oxidation was realized with 2-butanone peroxide in CH_2Cl_2 (0.1 M) (36). The 5'-terminal DMTr group of the synthesized ODNs was removed prior to its cleavage from the solid support. Each oligonucleotide was then cleaved and deprotected by treating the solid support with concentrated ammonia (28%) during 4 hours at room temperature. They were further evaporated to dryness and finally purified by HPLC on a DNAPAC™ PA-100 column (9×250 mm) purchased from Dionex. HPLC conditions (50°C): solution A = 4M urea 10% acetonitrile-20mM Mes buffer pH 6.5-1mM $NaClO_4$ and solution B = 400 mM $NaClO_4$; gradient = 85%A-15%B to 35%A-65%B in 50 min at a flow rate of 1ml/min. The oligomers were desalted by gel filtration on Sephadex G25 purchased from Pharmacia. The final oligonucleotide concentration was determined by UV absorption at 260 nm. Prior to mass spectrometry, metallic cations of the oligonucleotides were exchanged with ammonium

(diammonium hydrogen citrate) using C_4 Reverse Phase ZipTip® purchased from Millipore. MALDI TOF MS analysis was realized on a Bruker Biflex III in a linear negative mode. All samples were analysed using 3-hydroxypicolinic acid as matrix (Aldrich).

ODN1: DO_{260nm}= 30 (24%), MS $[M-H]^-$ calc. 4555 found 4556; ODN2 : DO_{260nm}= 28 (20%), MS $[M-H]^-$ calc. 4573 found 4571. ODN3 : DO_{260nm}= 32 (25%), MS $[M-H]^-$ calc. 4525 found 4528;

Nonlabeled (N= A) or 2AP-labeled (N= 2AP) ODN1 to ODN3 oligonucleotides as well as their complementary sequences were purchased by IBA GmBH Nucleic Acids Product Supply (Göttingen, Germany).

Figure 7: HPLC chromatograms of the 8vdA-labeled ODN3 crude product obtained after 4 hours (a) and 24 hours (b) treatment with NH_4OH.

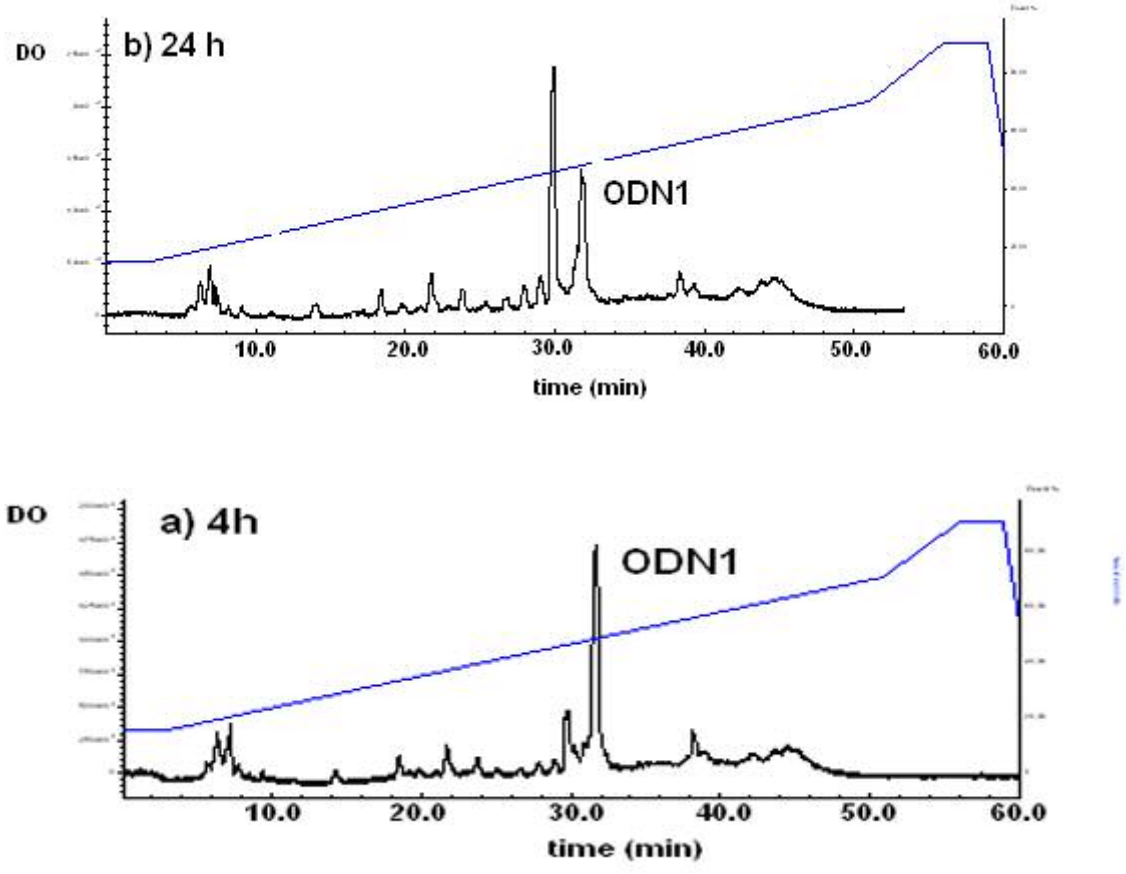
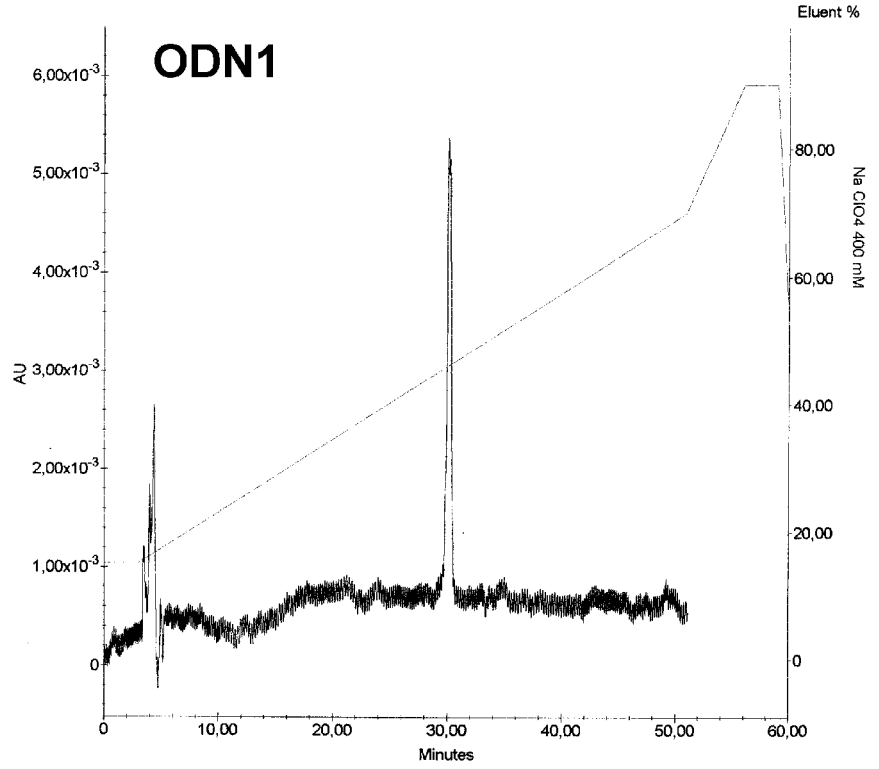
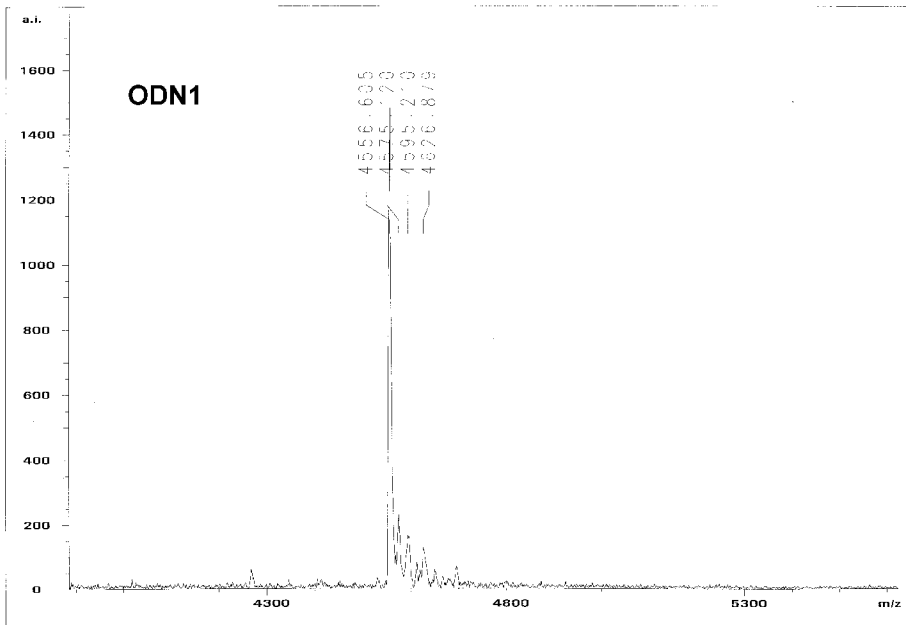
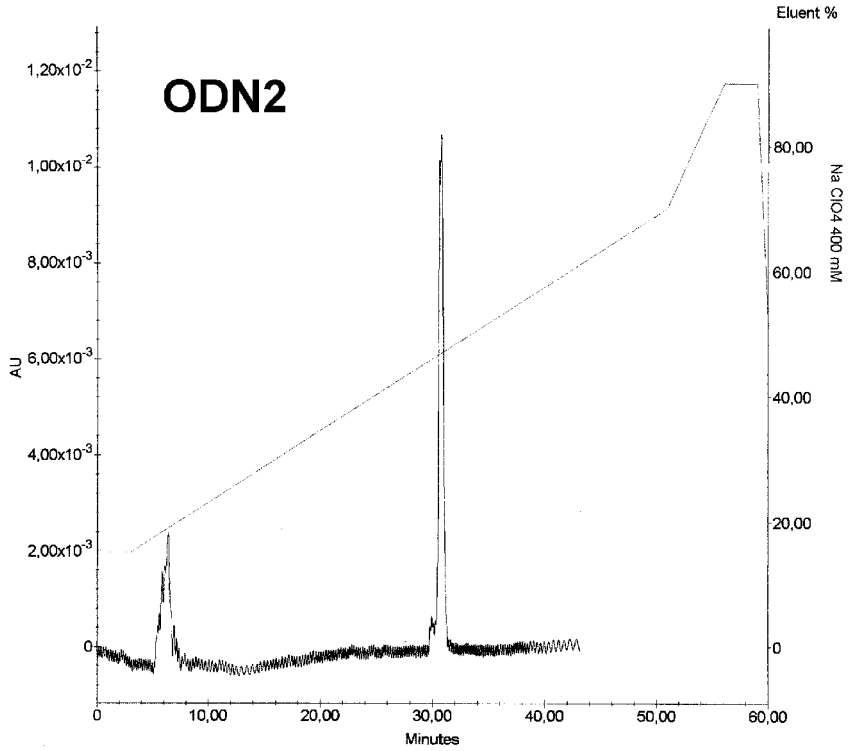
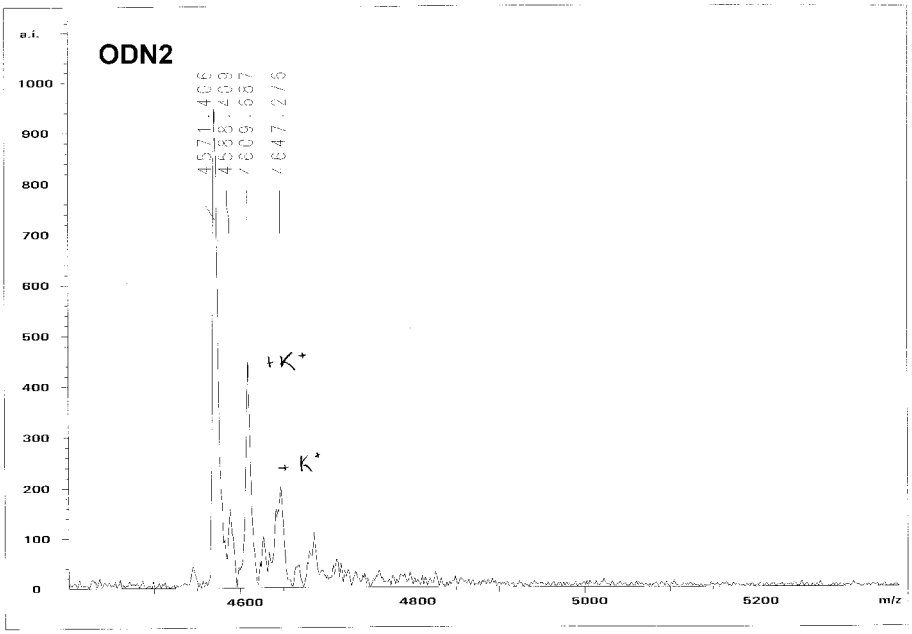


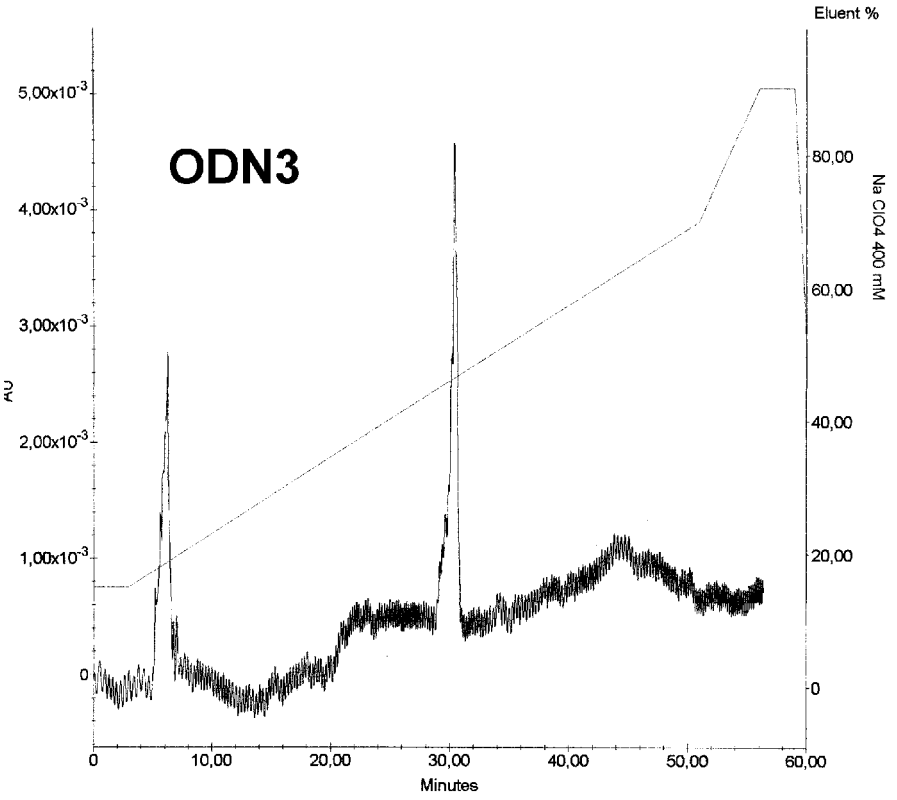
Figure 8: Anion exchange HPLC chromatograms and MALDI-TOF mass spectra of purified ODN1, ODN2 and ODN3

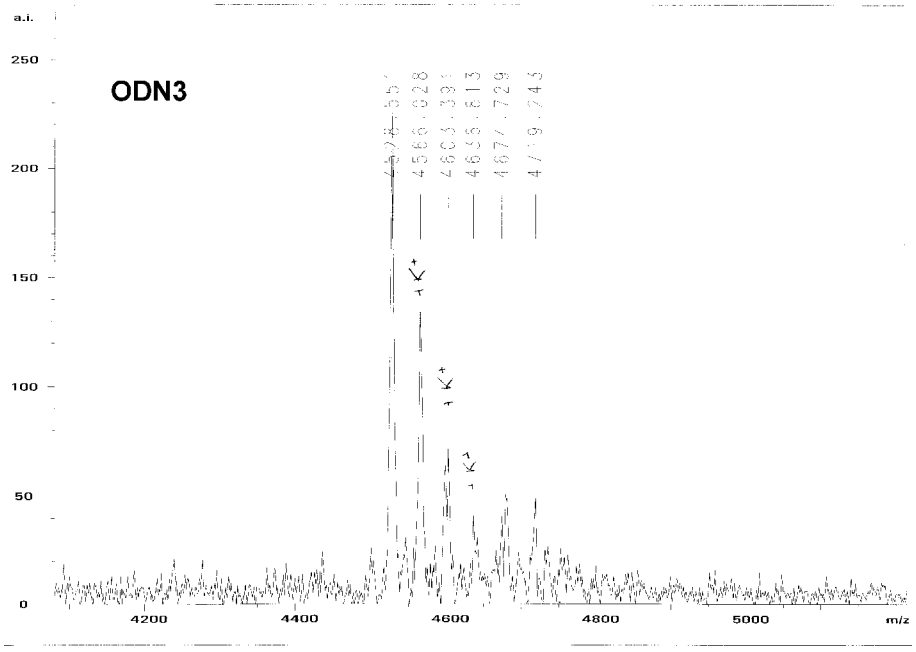





ODN2
Eluent %
Na ClO4 400 mM
AU
1,20x10-2
1,00x10-2
8,00x10-3
6,00x10-3
4,00x10-3
2,00x10-3
0
80,00
60,00
40,00
20,00
0
0
10,00
20,00
30,00
40,00
50,00
60,00
Minutes


ODN2
a.i.
1000
900
800
700
600
500
400
300
200
100
0
4600
4800
5000
5200
m/z
+K+
+K+


ODN3
Eluent %
5,00x10-3
4,00x10-3
3,00x10-3
2,00x10-3
1,00x10-3
0
AU
80,00
60,00
40,00
20,00
0
Na ClO4 400 mM
0
10,00
20,00
30,00
40,00
50,00
60,00
Minutes

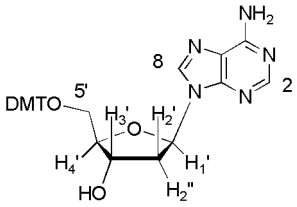

ODN3
a.i.
250
200
150
100
50
0
4566.028
4633.393
4635.813
4667.729
4719.743
4200
4400
4600
4800
5000
m/z

Conformational analysis

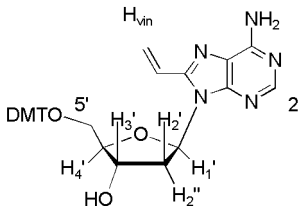
A) Sugar ring conformation analysis

The fraction of the South type conformer was deduced from the graphical method developed by Rinkel and Altona using the sums of J coupling constants (*J. Biomol. Struct. Dyn.* **4**, 621-649 (1987)).

Proton numbering



5'-DMT-dA



4

Sums of J coupling

Compound	$\Sigma_{1'}$	$\Sigma_{2'}$	$\Sigma_{2''}$	$\Sigma_{3'}$
4	13.4	27.4	24.7	15.5
5'-DMT-dA	13.0	26.4	24.2	14.1

Sums of J coupling constants Σ in Hz determined by ^{1}H NMR (500MHz, CD_2Cl_2) at 300K: $\Sigma_{1'} = J_{1'2'} + J_{1'2''}$, $\Sigma_{2'} = J_{1'2'} + J_{2'3'} + J_{2'2''}$, $\Sigma_{2''} = J_{1'2''} + J_{2''3'} + J_{2'2''}$, $\Sigma_{3'} = J_{2'3'} + J_{2''3'} + J_{3'4'}$

Figure 9a: 1D ^{1}H NMR, COSY and NOESY spectra of 5'-DMT-dA

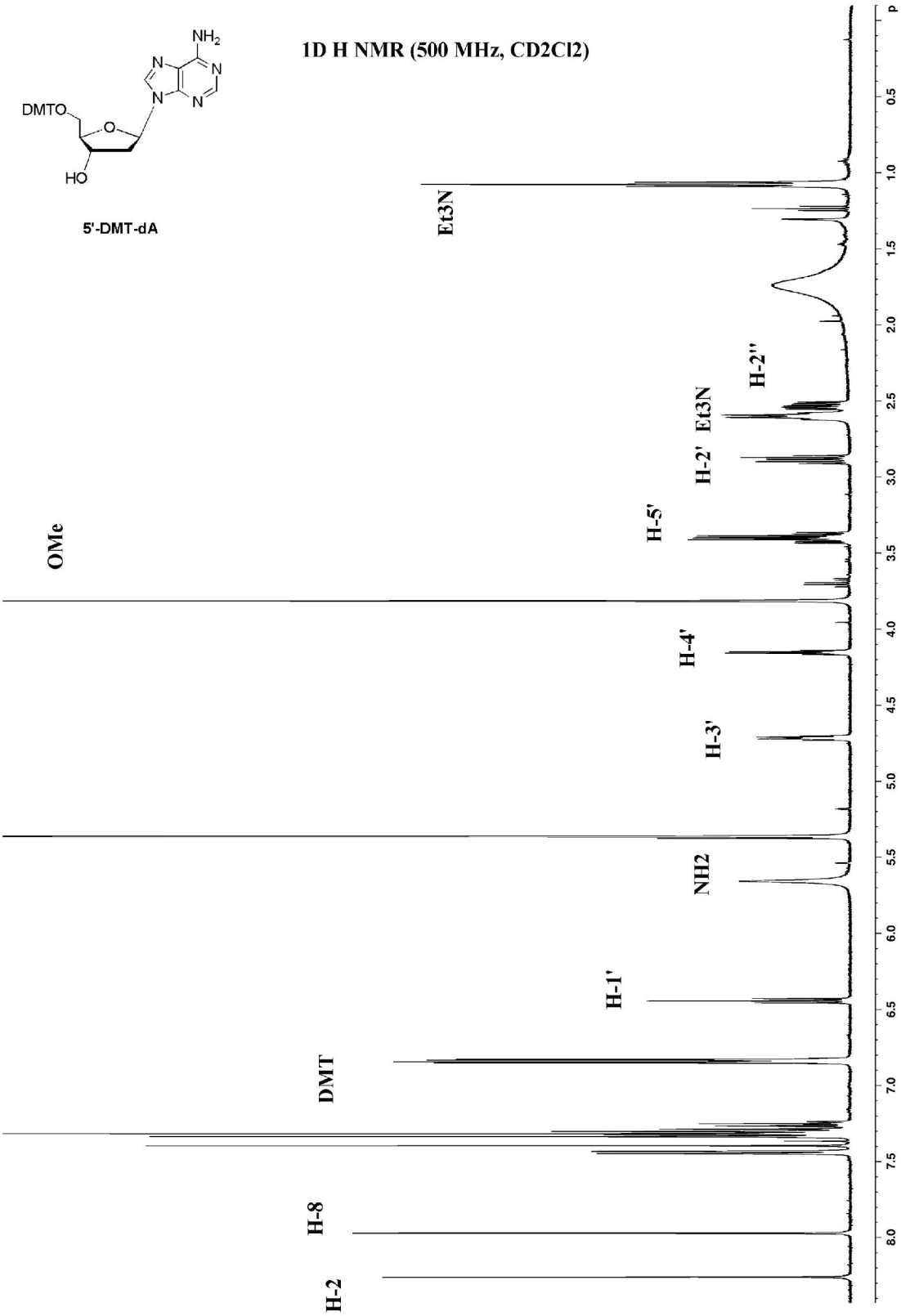
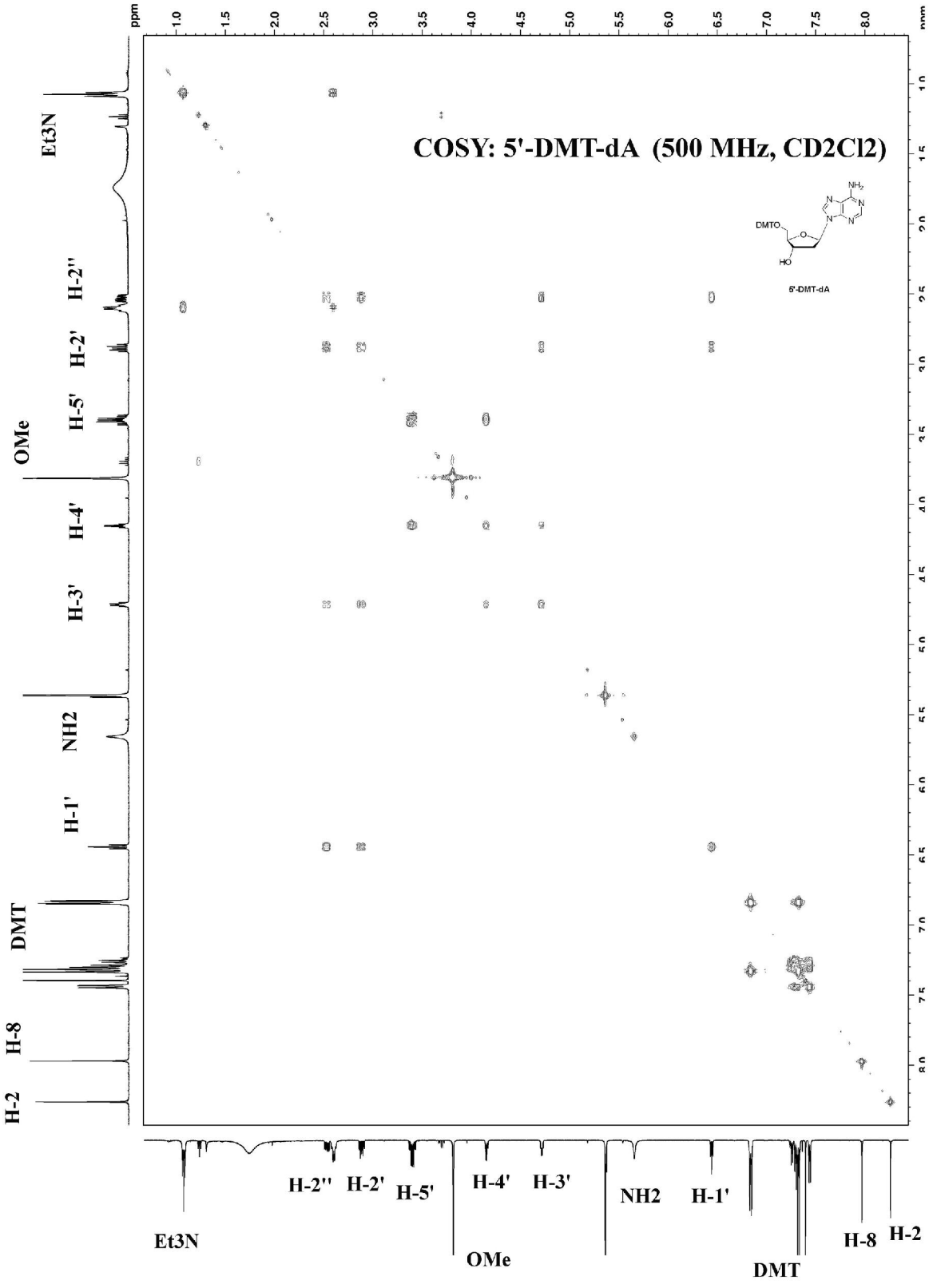
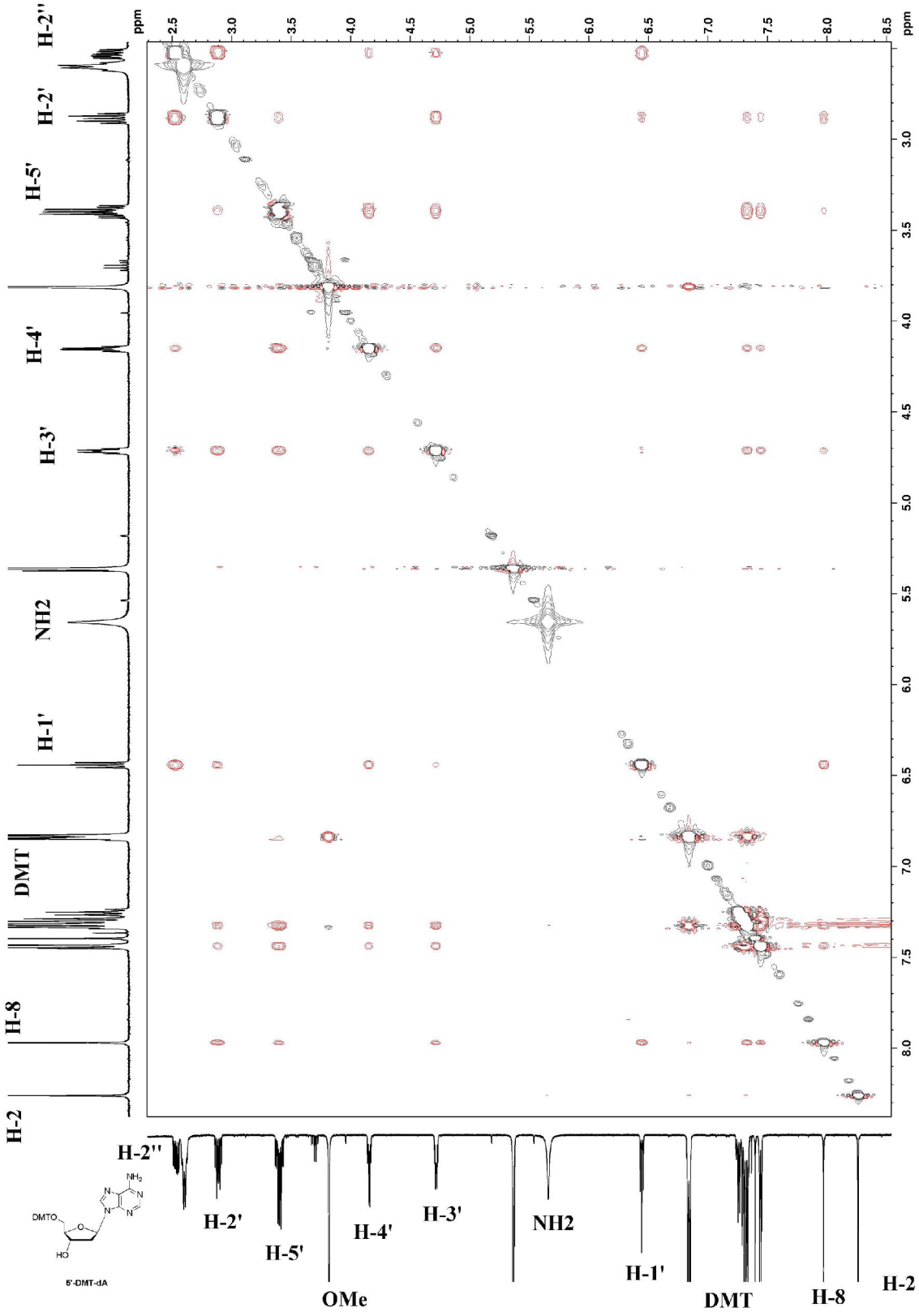


Figure 9 : spectra of 5’-DMT-dA



NOESY, 5'-DMT-dA (500 Mhz, CD2Cl2)



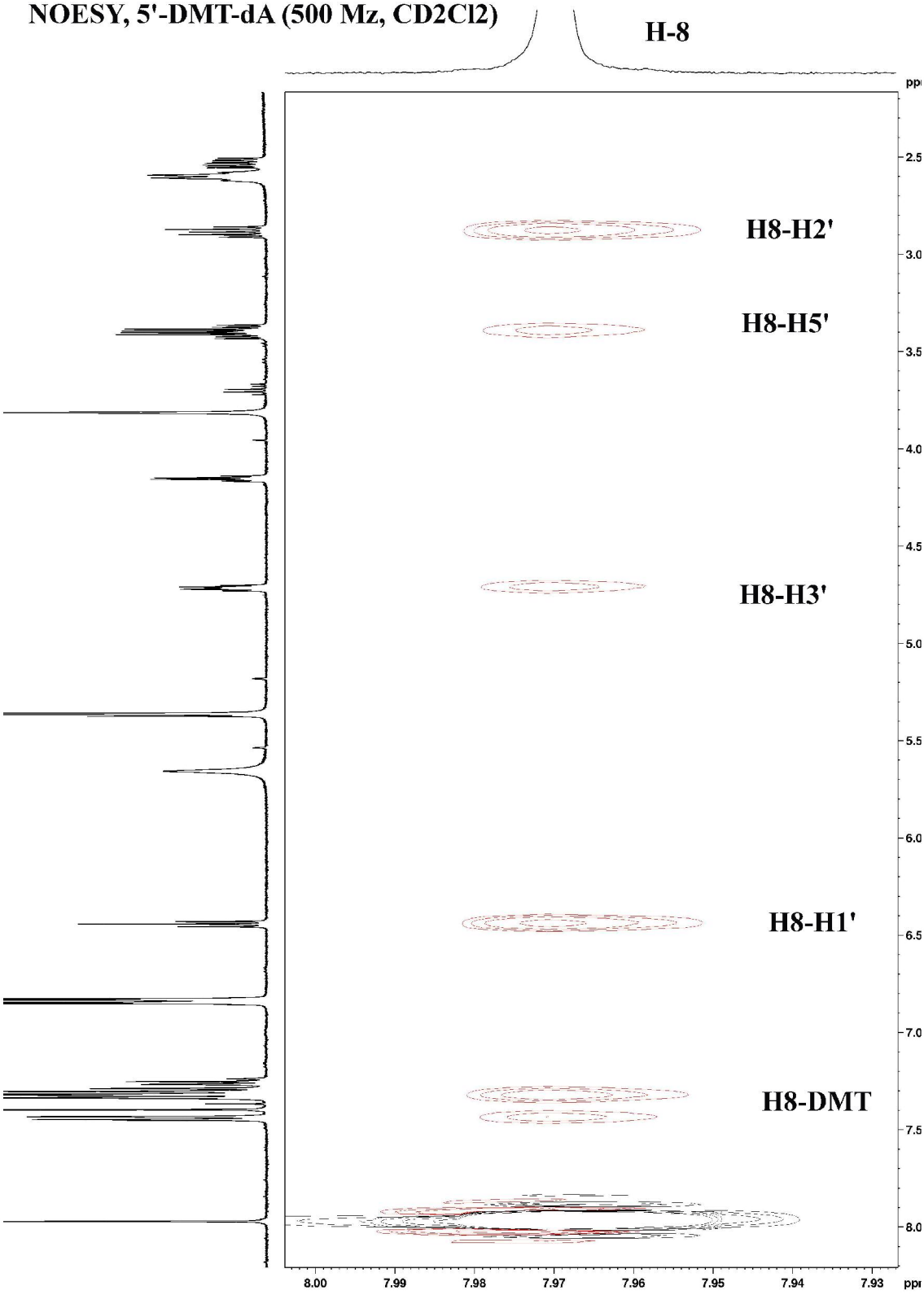
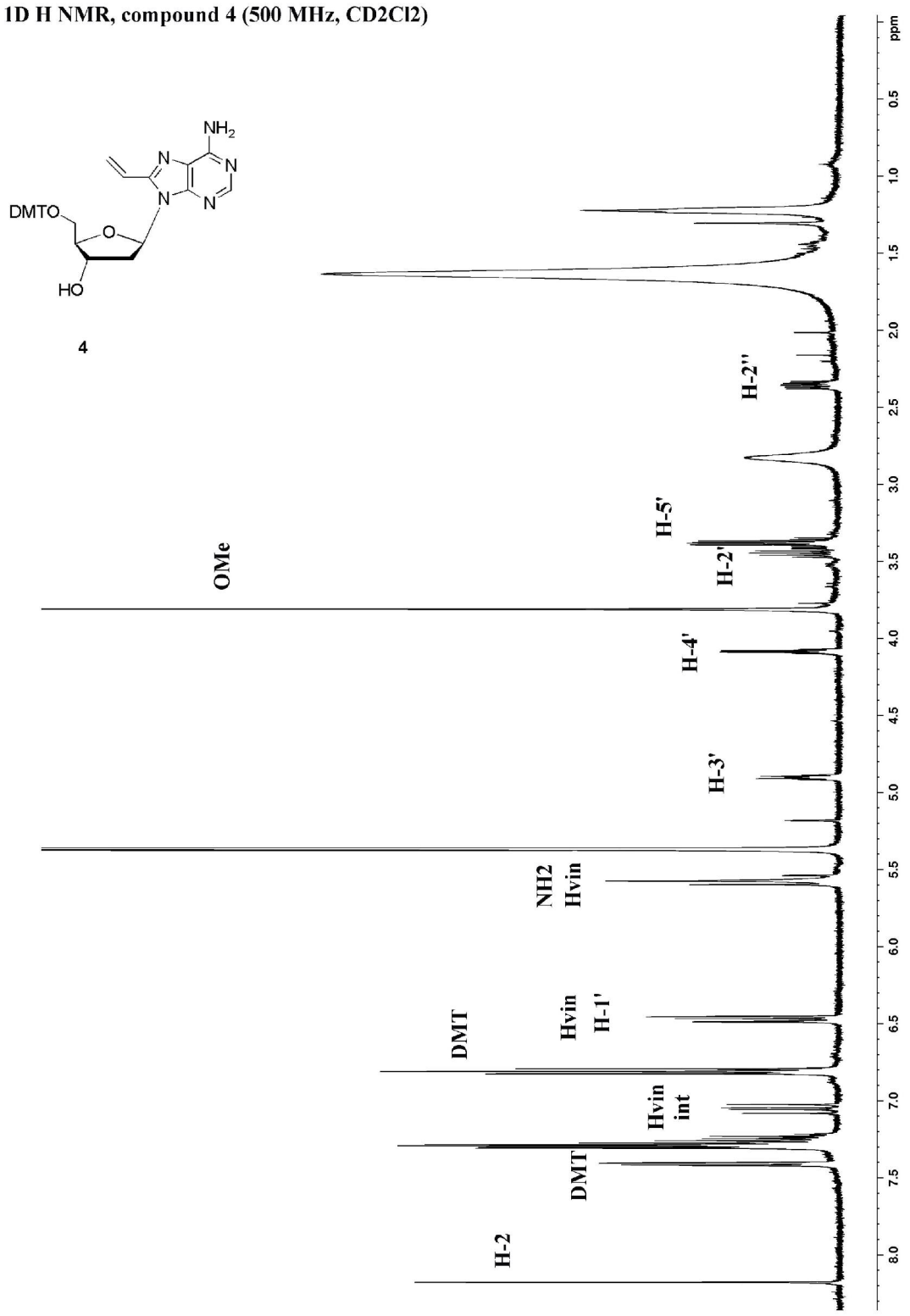
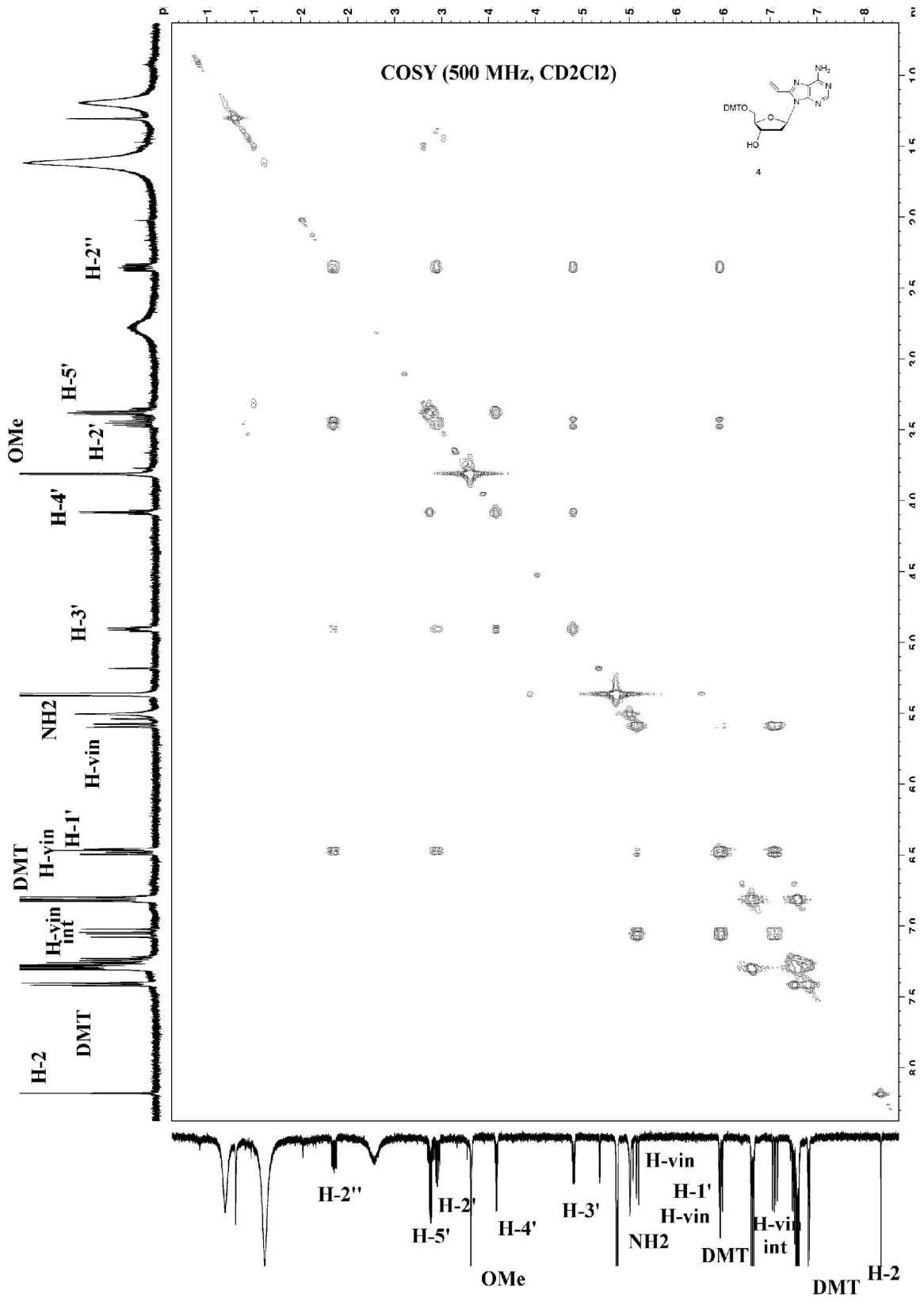

NOESY, 5'-DMT-dA (500 Mz, CD2Cl2)
H-8
H8-H2'
H8-H5'
H8-H3'
H8-H1'
H8-DMT
8.00
7.99
7.98
7.97
7.96
7.95
7.94
7.93

Figure 10 : 1D ^{1}H NMR, COSY and NOESY spectra of compound **4**




COSY (500 MHz, CD2Cl2)
DMTO
HO
NH2
4
H-2
DMT
H-vin
int
H-vin
DMT
H-1'
H-vin
NH2
H-3'
H-4'
H-2'
H-5'
OMe
H-2''
H-2''
H-5'
H-2'
OMe
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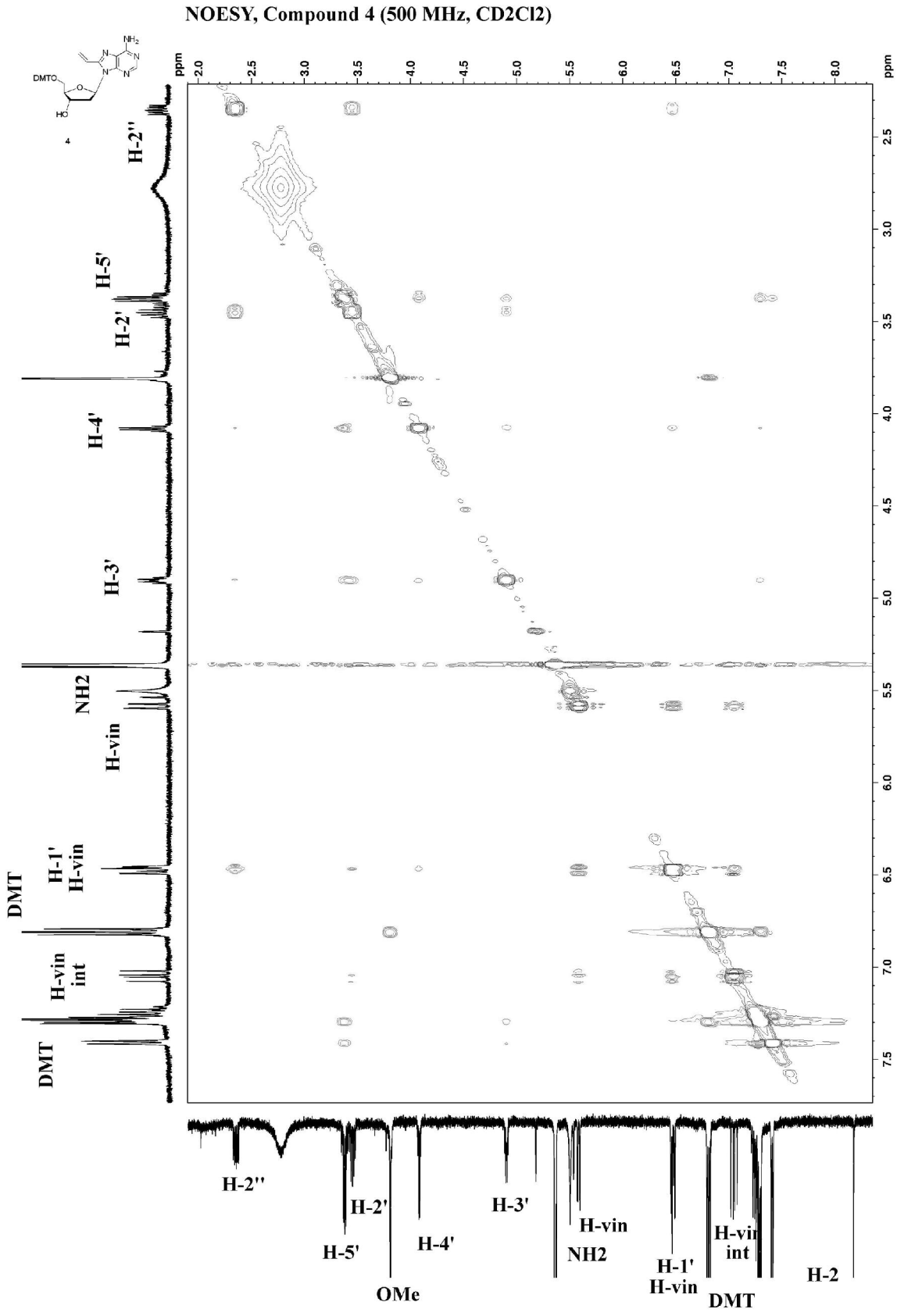
NH2
H-vin
H-1'
H-vin
DMT
H-vin
int
DMT
H-2


NOESY, Compound 4 (500 MHz, CD2Cl2)
DMTO
HO
NH2
4
H-2''
H-5'
H-2'
H-4'
H-3'
NH2
H-vin
DMT
H-1'
H-vin
H-vin
int
DMT
ppm
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
H-2''
H-5'
H-2'
OMe
H-4'
H-3'
NH2
H-vin
H-1'
H-vin
DMT
H-vin
int
H-2

NOESY, Compound 4 (500 MHz, CD2Cl2)