Nucleosides and Nucleotides

Synthesis of Extended Carbamate and Urea Linked Thymidine Dimers

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SYNTHESIS OF EXTENDED CARBAMATE AND UREA LINKED THYMIDINE DIMERS[1]

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Abstract: Thymidine dimers with extended carbamate and urea linkages were synthesized. The length of the linker is expected to increase flexibility and improve duplex formation after incorporation into oligomer.

INTRODUCTION

The use of modified oligodeoxynucleotides for modulation of gene expression and viral replication has gained increasing attention in the past few years[2-4]. The use of unmodified oligomers is limited due to their instability towards exo and endo nucleases and their inability to penetrate through the cell membrane. Oligomers with modified internucleoside linkages are designed to circumvent the stability and cellular uptake problems. Several reviews of the subject have appeared recently[4-6].
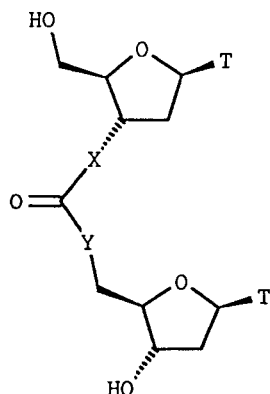
1 X= O, Y= NH

2 X= NH, Y= NH

3 X= O, Y= $NH(CH_2)_2NH$

4 X= NH, Y= $NH(CH_2)_2NH$

5 X= $O(CH_2)_2NH$, Y=NH

1531

Several modifications in which a carbonyl group replaces the phosphoryl group have been introduced. Carbamate analogs (1)[7a,b] have been shown to be stable towards enzymatic degradation but display poor binding. The simple carbamate linkage is calculated[8] to be shorter than a phosphodiester linkage by 0.032 nm, possibly resulting in restricted rotation of the linkage compared to the phosphodiester linkage. Recently Just et al.[9] and De Mesmaeker et al.[10] independently reported the synthesis of simple urea linked thymidine dimers (2) and their incorporation into antisense sequences. Similar poor binding behaviour was observed.

We report here the synthesis of thymidine dimers (3 - 5) with extended carbamate and urea linkages. An increase in the length of the linkage should give more conformational freedom for binding to complimentary base se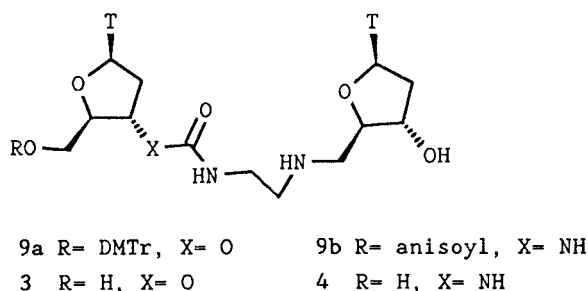quences. Dimer 5 contains a neutral backbone whereas dimers 3 and 4 contain a weakly basic secondary amino group. There are several reports in the literature[11,12] showing that oligomers with cationic groups in the backbone form duplexes with their complimentary oligonucleotides which exhibit unusually high melting temperatures (Tms). Higher Tms were also observed in peptide nucleic acid (PNA) analogs[13,14] containing lysine. Therefore, we anticipate that the presence of a secondary amino group in the linkage, which may carry a positive charge under physiological conditions, should not only enhance cellular uptake but may also improve hybridization to negatively charged oligonucleotides.

CHEMISTRY

Synthesis of dimer 1 requires the 3' modified synthon 8a and the 5' modified synthon 6, both of which have been reported recently[7c,15]. Imidazolide 8a was dissolved in dry pyridine, amine 6 was added, and the solution was stirred at room temperature for 36 hours. After evaporation

7a R= DMTr, X= OH

7b R= anisoyl, X= N_3

7c R= anisoyl, X= NH_2

8a R= DMTr, X= O

8b R= anisoyl, X= NH

9a R= DMTr, X= O   9b R= anisoyl, X= NH

3 R= H, X= O   4 R= H, X= NH
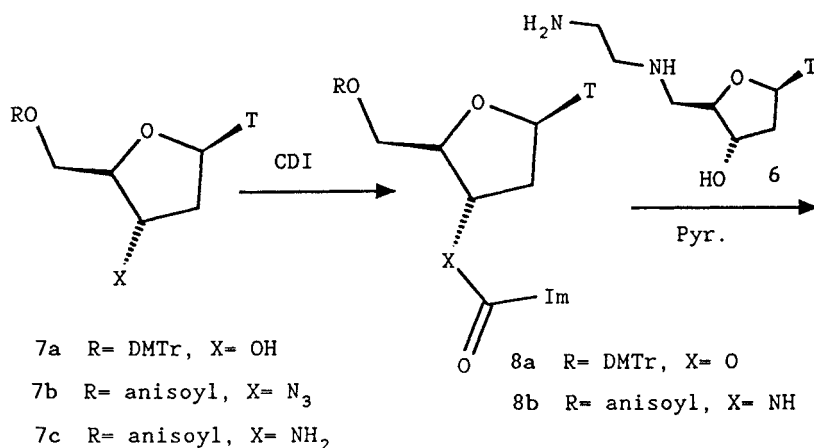
of the solvent, the resulting residue was purified by column chromatography, and compound **9a** was isolated in 42% yield. The dimethoxytrityl group was removed by treatment with 80% aqueous acetic acid at room temperature. Purification by column chromatography gave the deprotected dimer **3** in 70% yield. The proton nmr spectrum of **3** showed a triplet at 7.33 ppm (1H) for the carbamate NH and overlapping broad peaks between 5.20 and 5.23 ppm (2H) corresponding to 5'OH and 3'OH. A peak at 163.2 ppm corresponding to the carbamate carbon was observed in the ^{13}C spectrum.

The synthesis of dimer **4** requires a different 3' modified building block **8b**, which should be prepared from a 5'-protected 3'-amino-3'-deoxythymidine (**7c**). Preparation of this latter compound was accomplished using a variation on a published sequence of reactions[16]. Thymidine was converted

to 3'-anhydro-5'-O-(4-methoxybenzoyl)thymidine by a one pot
reaction with p-methoxybenzoic acid, triphenylphosphine and
DEAD. Ring opening with sodium azide gave 5'-O-anisoyl-
3'-azidothymidine (7b). Attempts to reduce the azido group
by hydrogenation in ethanol resulted in poor yields due to
the poor solubility of 7b in ethanol. Therefore the reaction
was carried out in ethyl acetate, over a 10% palladium on
carbon catalyst at room temperature, and the amine (7c) was
obtained in 82% yield. Compound 7c was dissolved in dry
pyridine and 1,1'-carbonyldiimidazole was added. Excess CDI
was used to avoid the possibility of the formation of a
symmetrical urea. The reaction was quenched with water and
the solvents were removed. The intermediate 8b was not
purified, but instead, after coevaporating several times with
pyridine, compound 6 was added in dry pyridine and the
solution was stirred overnight. The product (9b) was
isolated and the 5'-O-protecting group was removed with
sodium methoxide in methanol. Compound 4 was purified by
column chromatography. The proton spectrum of compound 4
showed a triplet at 6.63 ppm and a doublet at 7.08 ppm for
NHCONHCH$_2$ and NHCONHCH$_2$ respectively.

The synthesis of dimer 5 requires 3'-protected
5'-amino-5'deoxythymidine (10) and 5'-protected 3'-O-
(β-aminoethyl)thymidine (11). The 3'OH group of
5'azido-5'-deoxythymidine[17] was first protected with an
acetyl group to avoid any potential competition during the
later coupling step. Reduction of the azido group to amine
(10) was performed using hydrogen in ethanol over 10%
palladium on carbon at room temperature. Compound 11 was
synthesized by the selective alkylation[18] of the 3' hydroxyl
group using chloroethylamine hydrochloride as the alkylating
agent and potassium hydroxide as the base. Coupling between
10 and 11 was done in the same fashion as for dimer 9b.
Compound 10 was first treated with CDI and later coupled with
compound 11. The fully protected dimer (12) was first shaken
with ammonium hydroxide in methanol. After evaporation of

12 R_1=DMTr, R_2=Ac
13 R_1=DMTr, R_2=H
 3 R_1=R_2=H
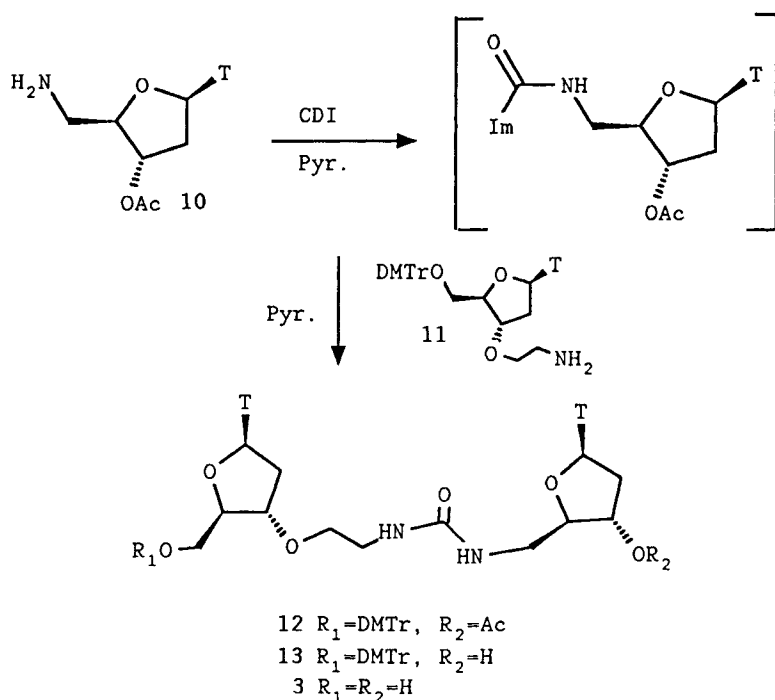
the solvent the residue (13) was stirred with 80% acetic acid. Purification was done by chromatography. The proton NMR of the fully deprotected dimer 5 exhibited a triplet at 6.01 ppm (1H) for $CH_2NHCONHCH_2$-5' and overlapping peaks between 6.10-6.19 ppm (3H) corresponding to H1' and $CH_2NHCONHCH_2$-5' respectively. A triplet at 5.10 ppm (1H) and a doublet at 5.28 ppm (1H) were also observed for 5'OH and 3'OH respectively. The chemical shifts for the sugar protons and carbons for deprotected dimers were assigned from COSY and HETCOR spectra.

EXPERIMENTAL SECTION

NMR data was recorded on a Varian VXR-300 spectrometer and the chemical shifts are reported in parts per million downfield from TMS. Column chromatography was carried out using silica gel (200-400 mesh) 60 A° (Aldrich). Thin layer chromatography (TLC) was performed on pre-coated sheets of

silica gel 250 μM layer (Whatman), and the spots were
visualized using short wavelength (254nm) UV light. Pyridine
was distilled from calcium hydride and stored over 3 A°
molecular sieves (Aldrich). 2-Chloroethylamine hydrochloride
was purchased from Fluka. Other reagents including the
starting material (thymidine) were purchased from Aldrich
chemical company.

5'-O-Anisoyl-3'-azido-3'-deoxythymidine (7b)

2,3'-anhydro-5'-O-anisoylthymidine (3 g, 8.35 mmoL) and
sodium azide (1.12 g, 17.3 mmoL) were suspended in DMF (30
mL) and the mixture was heated in an oil bath at 110 °C for 7
hrs. The homogenous mixture was poured into water (75 mL)
containing 5% aqueous hydrochloric acid (6 mL). The solution
was extracted with ethyl acetate (3 x 20 mL) and the combined
ethyl acetate extracts were washed with water followed by
saturated brine solution. After drying with anhydrous sodium
sulfate the solvent was evaporated *in vacuo* to give a foamy
solid. Yield (2.70 g. 77.1%) ^{1}H NMR (DMSO-d$_6$)ppm: 1.63 (s,
3, CH$_3$), 2.39-2.51 (m, 2, H2'), 3.83 (s, 3, OCH$_3$),
4.12 (m, 1, H4'), 4.41-4.64 (overlapping m, 3, H3', H5'),
6.16 (t, 1, H1'), 7.03, (d, 2, ar-H), 7.94 (d, 2, ar-H), 7.41
(s, 1, H6), 11.37 (br s, 1, NH); ^{13}C NMR (DMSO-d$_6$)ppm: 11.8,
35.7, 55.4, 59.9, 63.3, 80.6, 83.4, 109.9, 114.0, 121.3,
131.3, 135.6, 150.2, 163.3, 163.5, 165.0.

3'-Amino-5'-O-anisoyl-3'-deoxythymidine (7c)

Azide (7b) (1 g, 2.78 mmol) was dissolved in ethyl
acetate (150 mL), and 10% Pd/C (0.7 g) was added, and the
mixture was shaken under 40 psi H$_2$ for 36 hrs. TLC showed no
starting material. The solution was filtered through celite
and the solution was concentrated to dryness, on which the
amine (7c) was obtained as a solid. Yield 0.93 g (82.7%) m.p
128-132 °C; ^{1}H NMR (CD$_3$OD)ppm: 1.61 (s, 3, CH$_3$) , 2.27-2.36
(m, 2, H2'), 3.62 (m, 1, H4'), 3.85 (s, 3, OCH$_3$), 4.01 (
m, 1, H3'), 3.99-4.70 (dd, 2, H5'), 6.19 (t, 1, H1'), 7.00 (
d, 2, ar-H), 7.42 (s, 1, H6), 7.99 (d, 2, ar-H); ^{13}C NMR

(CD$_3$OD)ppm: 12.4, 41.3, 52.3, 56.1, 64.5, 86.0, 86.1, 111.4, 115.0, 123.1, 132.7, 137.3, 152.1, 165.4, 166.3, 167.5

5'-Dimethoxytritylthymidinyl-(3'-N(β)-carbamoyl)-5'-(β-aminoethyl)amino-5'-deoxythymidine (9a).

Imidazolide **8a** (2.0 g, 3.12 mmol) and amine **6** (0.98 g, 3.44 mmol) were dissolved in dry pyridine and evaporated *in vacuo* to dryness. The residue was redissolved in pyridine (10 mL) and the mixture was stirred at room temperature for 36 hrs. The solvent was removed and the resulting glass, on purification by chromatography (0-10% MeOH in CH$_2$Cl$_2$), gave compound **9a** (1.12 g, 42%). R$_f$ 0.38 (20% MeOH in CHCl$_3$ v/v). ^{1}H NMR (DMSO-d$_6$)ppm: 1.37 (s, 3, CH$_3$), 1.77 (s, 3, CH$_3$), 1.97-2.41 (overlapping m, 4, 2 x H2'), 2.60 (m, 2, CONHCH$_2$CH$_2$), 2.72 (m, 2, H5''), 3.09 (m, 2, CONHCH$_2$CH$_2$), 3.17-3.22 (m, 2, H5'), 3.71-3.72 (overlapping s, m, 7, 2 x OMe, H4''), 4.02 (m, 1, H4'), 4.12 (m, 1, H3''), 5.24 (m, 1, H3'), 6.14 (m, 1, H1'), 6.21 (m, 1, H1'), 6.87-7.32 (m, 13, ar-H), 7.52 (s, 1, H6), 7.57 (s, 1, H6), 11.30 (br, 2, 2 x NH); ^{13}C NMR (DMSO-d$_6$)ppm: 11.5, 12.1, 36.5, 39.1, 39.4, 49.0, 51.1, 54.8, 55.0, 64.0, 71.1, 83.4, 83.5, 83.5, 85.5, 86.0, 109.5, 109.8, 113.2, 126.7, 127.6, 127.8, 129.6, 135.0, 135.2, 136.1, 144.4, 150.3, 150.4, 155.3, 158.1, 163.5, 163.6

Thymidinyl-(3'-N(β)-carbamoyl)-5'-(β-aminoethyl)amino-5'-deoxythymidine (3).

Compound **9a** (0.7 g, 0.819 mmol) was dissolved in 80% acetic acid (10 mL) and the solution was stirred at room temperature. The progress of the reaction was monitored by TLC and detritylation was complete after 3 hrs. Acetic acid was removed *in vacuo* and the residue was chromatographed, eluting with 5-20% methanol in chloroform, on which compound **3** was obtained as a glass. The product was washed with anhydrous ether (10 mL) and dried. Yield 0.32 g (71%); R$_f$ 0.08 (20% MeOH in CHCl$_3$); ^{1}H NMR (DMSO-d$_6$)ppm: 1.78 (overlapping s, 6, 2 x CH$_3$), 2.12-2.19 (overlapping m, 4, 2 x H2'), 2.63 (m, 2, H5''), 2.71 (dd, 2, H5'), 3.10 (m, 2,

CONHCH$_2$CH$_2$), 3.22 -3.48 (br, 1, NH), 3.62 (m, 2,
CONHCH$_2$CH$_2$), 3.75 (m, 1, H4''), 3.93 (m, 1, H4'), 4.25 (m,
1, H3''), 5.11 (m, 1, H3'), 5.20-5.23 (overlapping br, 2,
5'OH, 3'OH), 6.11-6.28 (overlapping t, 2, 2 x H1'), 7.33 (t,
1, NH carbamate), 7.74 (overlapping s, 2, 2 x H6), 11.3 (br,
2, 2 x NH); ^{13}C NMR (DMSO-d$_6$)ppm: 12.1, 12.2, 36.7, 38.6,
49.0, 50.8, 61.3, 71.1, 74.6, 83.4, 83.5, 84.8, 85.5, 109.4,
109.6, 135.7, 136.1, 150.4, 150.4, 155.4, 163.2, 163.6

N-(5'-Anisoyl-3'-deoxythymidin-3'-yl)-N'-(β-(5'-deoxy-thymidin-5'-yl)amino)ethylurea (9b).

Amine **7c** (0.6 g, 1.59 mmol) was dissolved in pyridine
and evaporated. This process was repeated twice. It
was again redissolved in pyridine (10 mL),
1,1'-carbonyldiimidazole (0.32 g, 2 mmol) was added, and the
solution was stirred for 16 hours. Water (3 mL) was added,
and the mixture was shaken for 5 minutes. Solvents were
removed on rotary evaporator. The residue was dissolved in
pyridine and evaporated. This process was again repeated
three times. The residue was taken in dry pyridine (10 mL),
amine **6** was added, and the solution was stirred for 30 hrs.
The solvent was removed and the residue was purified on a
silica gel column eluted with a gradient of 5-15% methanol in
chloroform, on which compound **9b** was isolated. Yield 0.158 g
(15.8%). R$_f$ 0.21 (20% MeOH in CHCl$_3$ v/v); ^{1}H NMR(DMSO-d$_6$)ppm:
1.63 (s, 3, CH$_3$), 1.78 (s, 3, CH$_3$), 2.05-2.50 (overlapping
m, 4, H2', H2''), 2.58-2.69 (m, 2, CONHCH$_2$CH$_2$), 2.71-2.82 (
m, 2, H5''), 3.06-3.10 (m, 2, CONHCH$_2$CH$_2$), 3.75 (m, 1,
H4''), 3.83 (s, 3, OCH$_3$), 3.98 (m, 1, H4''), 4.11-4.19 (m,
1, H3''), 4.31-4.38 (m, 1, H3'), 4.53 (m, 2, H5'), 6.01 (t,
1, NHCONHCH$_2$-), 6.12-6.23 (overlapping m, 2, 2 x H1'), 6.55 (
d, 1, NHCONHCH$_2$-), 7.06 (d, 2, ar-H), 7.44 (s, 1, H6), 7.58
(s, 1, H6), 7.92 (d, 2, ar-H); ^{13}C NMR (DMSO-d$_6$)ppm: 11.8,
12.0, 37.3, 39.1, 39.4, 49.6, 51.3, 55.4 64.3, 71.2, 79.1,
82.5, 83.4, 83.5, 85.6, 109.5, 109.7, 135.7, 136.1, 114.0,
121.5, 131.2, 157.6, 150.2, 150.3, 163.2, 163.5, 163.6, 165.1

N-(3'-Deoxythymidin-3'-yl)-N'-(β-(5'-deoxythymidin-5'-yl)-amino)ethylurea (4).

The protected dimer **9b** (0.2 g, 0.29 mmol) was dissolved in methanol, sodium methoxide in methanol (4 mL) was added, and the mixture was stirred for 24 hrs. Water (10 mL) was added and the solution was concentrated to dryness. The residue was dissolved in water and neutralized with 50% aqueous acetic acid. The solution was concentrated to dryness. The residue was dissolved in the minimum amount of methanol, applied to a silica gel column, and eluted with a gradient of 10-20% methanol in chloroform. Yield 0.07 g (43.75%); R_f 0.08 (20% MeOH in $CHCL_3$ v/v); ^1H NMR (DMSO-d_6)ppm: 1.65 (s, 3, CH_3), 1.77 (s, 3, CH_3), 2.02-2.18 (overlapping m, 4, H2', H2''), 2.50 (m, 2, CONHCH$_2$C$\underline{H}_2$), 2.66 (m, 2, H5''), 2.97 (m, 2, CONHC$\underline{H}_2$CH$_2$), 3.22 (br, 1, H4'), 3.45-4.08 (overlapping m, 4, H5', H4', H3''), 4.12 (br, 1, H3'), 6.09-6.22 (m, 2, H1', H1''), 6.63 (t, 1, NHCON$\underline{H}$CH$_2$), 7.08 (d, 1, N$\underline{H}$CONHCH$_2$), 7.60 (s, 1, H6), 7.77 (s, 1, H6); ^{13}C NMR (DMSO-d_6)ppm: 12.2, 37.6, 39.1, 39.4, 49.5, 61.0, 71.8, 83.3, 85.3, 109.1, 109.6, 136.1, 150.5, 150.6, 158.1, 163.9.

3'-O-Acetyl-5'-amino-5'-deoxythymidine (10).

5'-azido-5'-deoxythymidine (0.34 g, 1.29 mmoL) was dissolved in pyridine (5 mL) and acetic anhydride (0.4 mL, 4.23 mmoL) was added and the mixture was stirred for 15 hrs. The reaction was quenched with water (1 mL) and the pyridine was evaporated. The residue was dissolved in chloroform and washed with 5% sodium bicarbonate, followed by water, and saturated brine solution. The organic layer was separated and on evaporation 3'-O-acetyl-5'-azido-5'-deoxythymidine was obtained. Yield (0.36 g, 91%) m.p. 115-117 °C. The acetyl derivative (0.36 g, 1.16 mmoL) was taken in absolute ethanol (30 mL) and 10% palladium on activated carbon (0.21 g) was added. The solution was shaken under hydrogen (40 psi) at room temperature for 12 hrs. The solution was filtered through a celite pad, the solvent was evaporated, and

compound (10) was obtained as a glass. Yield (0.28 g,
85.1%); ^{1}H NMR (DMSO-d$_6$)ppm: 1.79 (s, 3, CH$_3$), 2.06 (s, 3,
COCH$_3$), 2.20-2.36 (m, 2, H2'), 2.78-2.80 (m, 2, H5'),
3.83-3.87 (m, 1, H4'), 5.18-5.20 (m, 1, H3'), 6.13 (t, 1,
H1'), 7.77 (s, 1, H6); ^{13}C NMR (DMSO-d$_6$)ppm: 12.0, 20.7,
35.8, 43.1, 74.6, 83.4, 84.8, 109.7, 136.0, 150.4, 163.6,
169.9.

**N-β-(5'-Dimethoxytritylthymidin-3'-yl)ethyl-N'-(3'-O-acetyl-
5'-deoxythymidin-5'-yl)urea (12).**

Amine **10** (0.65 g, 2.29 mmol) was dissolved in dry
pyridine and evaporated twice. It was again dissolved in
pyridine (15 mL), 1,1'-carbonyldiimidazole was added, and the
solution was stirred at room temperature for 24 hours. Water
(1 mL) was added and stirred for 15 minutes. The solvent was
removed and the residue was dissolved in pyridine and again
evaporated. This process was repeated twice. Finally it was
taken in pyridine (15 mL), compound **11** (1.34 g, 2.29 mmol)
was added and stirring continued for 36 hours. Pyridine was
removed on rotary evaporator and the residue was applied to a
silica gel column and eluted with a gradient of 0-5% methanol
in chloroform. Appropriate fractions were collected and on
evaporation, compound **12** was isolated in the form of glass.
Yield 1.35 g (65.8%); R$_f$ 0.87 (20% MeOH in CHCL$_3$ v/v); ^{1}H NMR
(DMSO-d$_6$)ppm: 1.45 (s, 3, CH$_3$), 1.80 (s, 3, CH$_3$), 2.04 (s,
3, COCH$_3$), 2.22-2.28 (overlapping m, 4, H2', H2''), 3.14-3.27
(overlapping m, 4, H5', H5''), 3.36-3.39 (m, 4, OC$\underline{H}_2$C$\underline{H}_2$NH$_2$),
3.73 (s, 6, 2 x OCH$_3$), 3.92 (m, 1, H4'), 4.01 (m, 1,
H4''), 4.18 (m, 1. H3'), 5.13 (m, 1, H3''), 6.05-6.22
(overlapping m, 4, H1', H1'', N$\underline{H}$CON$\underline{H}$), 6.89-7.52 (overlapping
m, s, 14, H6, ar-H), 11.37 (overlapping s, 2, NH); ^{13}C NMR
(DMSO-d$_6$)ppm: 11.6, 12.0, 20.7, 35.4, 36.1, 40.8 54.9, 63.1,
68.2, 74.5, 79.1, 79.3, 82.7, 82.9, 83.6, 83.7, 85.9, 109.6,
109.8, 126.7, 127.8, 129.6, 135.1, 135.2, 135.5, 135.8,
144.6, 150.3, 150.3, 157.9, 158.1, 163.5, 163.6, 169.8

N-β-(3'-Deoxythymidin-3'-yl)ethyl-N'-(5'-deoxythymidin-5'-yl) urea (5).

Compound **12** (0.85 g, 0.947 mmol) was dissolved in methanol (20 mL) and 1,4-dioxane (5 mL) and ammonium hydroxide (5 mL) was added, and the solution was stirred overnight. The solvents were removed, the residue was dissolved in chloroform, and the solution was washed with 5% sodium bicarbonate and then with water. Chloroform was evaporated and the resulting glass was dissolved in 80% acetic acid (10 mL) and stirred for 10 hrs. Acetic acid was removed on rotary evaporator, the resulting residue was triturated in water, and the solid was separated by filtration. The filtrate was evaporated to dryness and the product was purified by column chromatography. On eluting with a gradient of 5-15% methanol in chloroform, compound **5** was isolated. Yield 0.22 g (42.3%); R_f 0.40 (20% MeOH in $CHCL_3$ v/v); ^1H NMR (DMSO-d_6)ppm: 1.77 (s, 3, CH_3), 1.80 (s, 3, CH_3), 2.04-2.18 (overlapping m, 4, H2', H2''), 3.14-3.17 (overlapping m, 4, H5'', $OCH_2C\underline{H}_2NH$-), 3.34-3.43 (m, 2, $OC\underline{H}_2CH_2NH$-), 3.55-3.58 (m, 2, H5'), 3.69 (m, 1, H4''), 3.90 (m, 1, H4'), 4.05-4.12 (overlapping m, 2, H3', H3''), 5.10 (t, 1, 5'OH), 5.28 (d, 1, 3'OH), 6.01 (t, 1, $CH_2NHCON\underline{H}CH_2$-5'), 6.10-6.19 (overlapping m, 3, $CH_2N\underline{H}CONHCH_2$-5', H1', H1''), 7.49 (s, 1, H6), 7.69 (s, 1, H6), 11.30 (s, 2, NH); ^{13}C NMR (DMSO-d_6)ppm: 12.0, 12.2, 36.3, 38.4, 39.2, 41.5, 48.5, 61.4, 68.1, 71.0, 79.2, 83.5, 83.7, 84.5, 85.5, 109.4, 109.6, 135.8, 135.9, 150.3, 157.9, 163.6, 163.6

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