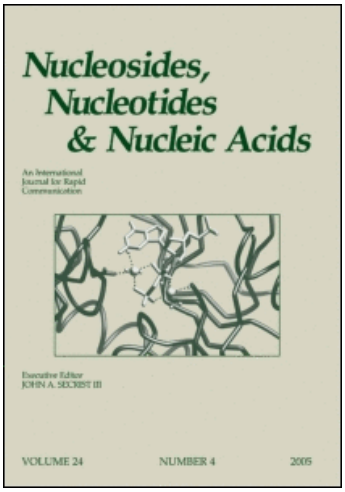

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Synthesis of Deoxycytidine (dC) Building Blocks for Amide-Linked Dimers or Oligomers: Example of a Deoxycytidine-amide-thymidine Dimer

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SYNTHESIS OF DEOXYCYTIDINE (dC) BUILDING BLOCKS FOR AMIDE-LINKED DIMERS OR OLIGOMERS: EXAMPLE OF A DEOXYCYTIDINE-AMIDE-THYMIDINE DIMER

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Abstract: 5'-Azido-3'-carbomethoxymethyl-4-N-benzoyl-2',3',5'-trideoxy-cytidine **17** and 5'-O-t-butyldimethylsilyl-3'-carboxymethyl-4-N-benzoyl-2',3'-dideocytidine **22** were efficiently synthesized from 2'-deoxyuridine via a new method which transformed the uracil heterocycle to 4-N-benzoylcytosine (four steps, 60 % overall yield). The amide-linked deoxycytidine-thymidine dimer analog was synthesized.

INTRODUCTION

Recently, our research has been focused on the synthesis of 3'-carbon-substituted-2',3'-dideoxynucleosides and amide-linked thymidine dimers (TaT) [1, 2]. These backbone modified oligodeoxynucleotides hybridize to RNA as strongly as does natural DNA, with excellent selectivity [2, 3]. They offer promise for potential oligodeoxynucleotide therapy utilizing either antisense [4, 5] and perhaps antigene [6] technologies.

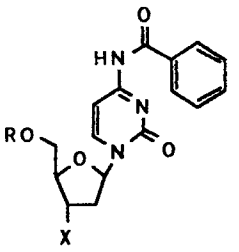
In view of the above results, we decided to develop amide-linked dimers or oligomers containing deoxycytidine (dC). This is particularly important since it is well known that the stability of the binding between the oligonucleotide and its target sequence increases with the proportion of dG-dC base pairs (Watson-Crick hybridization) in the antisense strategy and of triplex binding motif dC^+ x dG-dC (Hoogsteen base triplets) in the antigene methodology [7].

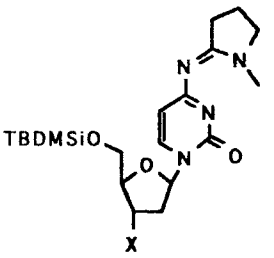
We now wish to report our results in the synthesis of useful deoxycytidine intermediates and dCaT dimer.

RESULTS AND DISCUSSION

Our first target molecule was the 3'-allyl-3'-deoxycytidine derivative of type **4**, which we planned to prepare by adapting a procedure developed by Chu *et al* for the synthesis of the corresponding thymidine derivative [8]. The 5'-position of N-benzoyl-deoxycytidine was first protected either as its t-butyldiphenyl- (**1**) or t-butyldimethylsilyl ether (**2**). Whereas phenoxythiocarbonylation of the former did not proceed to provide a recognizable product using a variety of conditions, **2** could be transformed to its thiocarbonate **3** in 45 % yield. However, tributylallyl tin mediated allylation proceeded in only 7 % yield [9]. The use of N-methyl-2-pyrrolidine amidine as a protecting group of the exocyclic amine function of deoxycytidine **5** for the desired transformation **5** to **7** did not lead to much improved results [10,11,12] We therefore abandoned this approach and decided to use 2'-deoxyuridine (dU) as starting material.

Oxidation of 3'-allyl-2',3'-dideoxyuridine derivative **8** (made in three steps from dU [8]) with $OsO_4/NaIO_4$ gave a 43 % yield of the aldehyde **9** [13] (SCHEME 2). This compound on treatment with PDC/methanol in dry DMF afforded the methyl ester **10** in 56% yield [14].





1 R = t-Bu(Ph)$_2$Si; X = OH

a → **2** R = t-Bu(Ph)$_2$Si; X = OH

a → **3** R = t-Bu(CH$_3$)$_2$Si; X = OC(S)OPh

b → **4** R = t-Bu(CH$_3$)$_2$Si; X = CH$_2$CH=CH$_2$

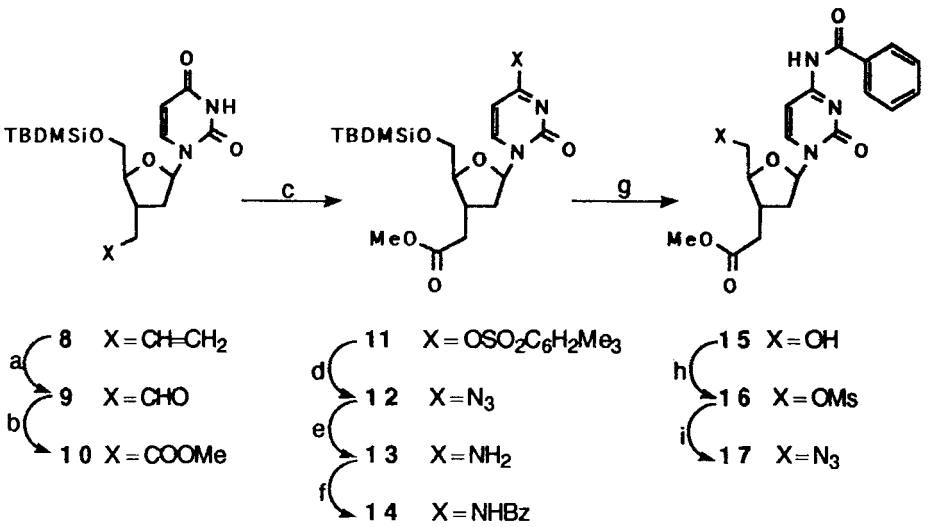
5 X = OH

c → **6** X = OC(S)OPh

d → **7** X = CH$_2$CH=CH$_2$

a: 4 eq. PhOC(S)Cl/4 eq. Py/ 0.1 eq. DMAP/ CH_2Cl_2 *45 %* b: 2 eq. CH_2=$CHCH_2Sn(Bu)_3$/ 0.3 eq. AIBN/ Tol/ 70 °C *7 %* c: 4 eq. PhOC(S)Cl/ 4 eq. Py/ 0.1 eq. DMAP/ CH_2Cl_2 *63 %* d: 2 eq. CH_2=$CHCH_2Sn(Bu)_3$/ 0.3 eq. AIBN/ Tol/ 70 °C *10 %*

SCHEME 1

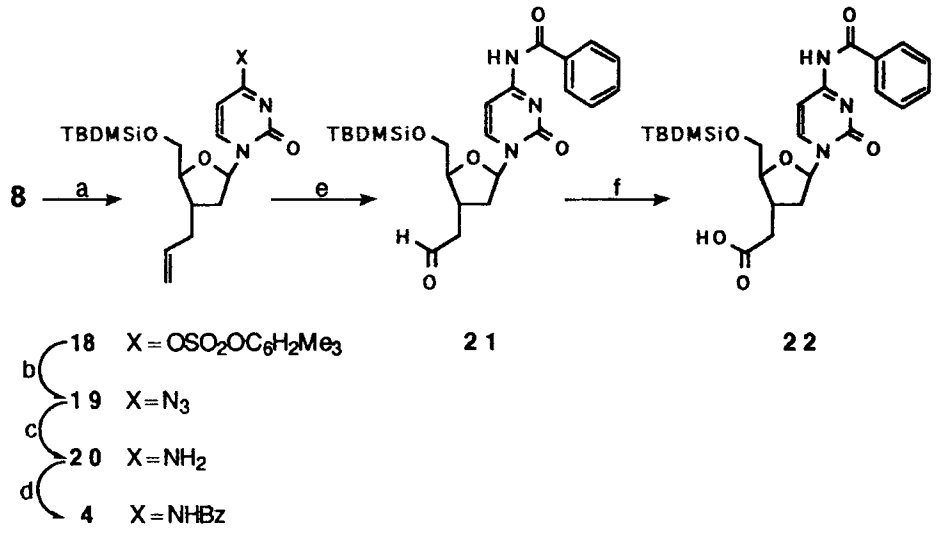


a: 0.4 eq. OsO_4/ 2.5 eq. $NaIO_4$/ Ether/ H_2O *43 %*; b: 6 eq. PDC/ 6 eq. MeOH/ DMF *56 %*; c: 1.5 eq. $C_6H_2Me_3SO_2Cl$/ 0.06 eq. DMAP/ 5 eq. Et_3N/ $C_2H_4Cl_2$; d: 2.5 eq. NaN_3/ DMF *54 %* (two steps); e: 3 eq. $SnCl_2$/ 9 eq. Et_3N/ 12 eq. C_6H_5SH/ CH_3CN *94 %*; f: 5 eq. $C_6H_5C(O)Cl$/ Py *84 %*; g: 3 eq. TBAF/ THF *92 %*; h: 1.5 eq. MsCl/ 2 eq. Et_3N/ CH_2Cl_2 *58 %*; i: 3 eq. NaN_3/ DMF *74 %*

SCHEME 2

Several methods are known to introduce a protected amine in the 4-position of uridine [15,16,17]. We developed our own procedure which does not require any specialized equipment and is particularly easy to carry out. Reaction of dU **10** with mesitylenesulfonyl chloride, followed by displacement of mesitylenesulfonate from the intermediate **11** with sodium azide, using a method described for modified ribonucleosides by Nyilas and Chattopadhyaya [18,19], gave a 54 % isolated yield of the pure product **12**. The azido function was then reduced to the amine,in 94 % yield, by a recently published, highly selective reducing agent $[Sn(SPh)_3]^-$ [20,21,22]. Protection of the exocyclic amine of dC **13** with benzoyl chloride in pyridine [23] afforded 84 % of pure isolated N-benzoyl-dC **14**. Subsequent removal of the t-butyldimethylsilyl group and activation of the primary alcohol obtained with methanesulfonyl chloride gave a 54 % overall yield of the crystalline mesylate **16**. Conversion to the 5'-azide **17** was achieved in 74 % yield by reaction with sodium azide.

To the best of your knowledge, compounds **15,16** and **17** are the first 3'-ethyl-substituted-2',3'-dideoxycytidines described which were obtained by direct functionalization of nucleosides. Azide **17** is a good candidate to be a repeating unit

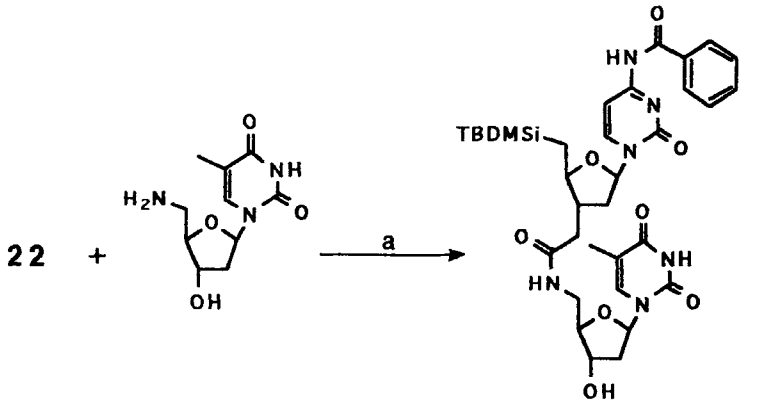


a: 1.5 eq. $C_6H_2(Me)_3SO_2Cl$/ 5 eq. Et_3N/ 0.05 eq. DMAP/ $C_2H_4Cl_2$; b: 5 eq. NaN_3/ DMF *71 %* (two steps); c: 3 eq. $SnCl_2$/ 12 eq. C_6H_5SH/ 9 eq. Et_3N/ CH_3CN *96 %*; d: 5 eq. BzCl/ Py *98 %*; e: 0.05 eq. OsO_4/ 2.5 eq. $NaIO_4$/ Ether/ H_2O *35 %*; f: 1.5 eq. $NaClO_2$/ 1.5 eq. NaH_2PO_4/ 15 eq. CH_2=CHCH(Me)$_2$/ t-BuOH *75 %*

SCHEME 3

with the azide serving as a masked amine, if one were to make amide-linked homooligonucleotides [23,24].

In order, to prepare the 5'-unit of dC-dimers or -oligomers, we synthesized carboxylic acid **22**, using our new method of transformation of uracil to cytosine (SCHEME 3). Allyl derivative **8** was easily and rapidly transformed in the 4-N-benzoyl-dC derivative **4** in 67% overall yield by the sequence described before. In all these reactions, the allyl compounds could be followed on tlc by the specific yellow coloration using anisaldehyde dipping (see experimental section, general methods). Oxidation of the allyl compound **4** with OsO_4/$NaIO_4$ gave a 35 % yield of the aldehyde **21**. Surprisingly, **21** was unstable on tlc (2D tlc), a silica gel column and in a mildly acidic solvent ($CDCl_3$). The only way to purify and characterize it was to crystallise the crude product from hexane/ethyl acetate. Subsequent oxidation of aldehyde **21** to the carboxylic acid derivative **22**, without loosing the t-butyldimethylsilyl group, was achieved in 75 % yield using sodium chlorite under buffered conditions [25,26,27]. Acid **22** obtained was pure enought to be used for the following reaction. The successful



a: 1 eq. BOP/ 1.75 eq. Et_3N/ DMF 69 %

SCHEME 4

coupling (SCHEME 4) of the nucleosides **22** and **23**, obtained in three steps from thymidine [28], was carried out in 69% yield, using the BOP peptide coupling reagent [2, 29]. The structure of the dimer dCaT **24** was confirmed by detailed ^1H and ^{13}C-NMR analysis at 500 and 100 MHz respectively, as well as by FAB mass spectrometry in which an ion at m/e 733 [M+Na^+] was observed.

In summary, the deoxycytidine derivatives **17** and **22** required for the preparation of amide-linked DNA analogues have been efficiently synthesized. Coupling of unit **22** with **23** has been successfully carried out, and **24** is the first example of a deoxycytidine-thymidine amide linked dimer described. The synthetic sequence allows for easy scale-up, and it should therefore be possible to prepare adequate amounts of the monomer for binding studies. The preparation of longer strands, as well as suitably protected shorter fragments activated for incorporation into natural DNA by automated solid-phase methods is ongoing.

EXPERIMENTAL

General Methods.

Melting points were determined on a Gallenkamp block and are uncorrected. Optical rotation measurements were carried out in the indicated solvents employing a Jasco DIP-140 digital polarimeter and a 1-dm cell. Low resolution chemical ionization mass spectra (CI-MS) and fast atom bombardement (FAB-MS) were obtained on an

KRATOS MS 25RFA spectrometer in the direct-inlet mode. High and low resolution FAB mass spectra of compounds 17 and 24 were obtained on a ZAB 2F HS spectrometer in the direct-inlet mode. ^{1}H-NMR spectra were recorded on either a Varian XL-200 or Varian UNITY 500 spectrometer. ^{13}C-NMR spectra were obtained on either a Varian XL-200 or Varian UNITY 500 at 50.4 or 125.7 MHz and the peak assignments were made, in some cases, with the aid of HETCOR, HMQC or HMBC experiments.

All air sensitive experiments were carried out under argon, with freshly dried, distilled solvents. Dichloromethane and 1,2-dichloroethane were distilled from P_2O_5. Pyridine, triethylamine, acetonitrile were distilled from CaH_2. Toluene was dried over sodium wire, N,N-dimethylformamide (DMF) was dried by shaking with KOH followed by distillation at reduced pressure from BaO. Methanol was distilled from magnesium. Thin-Layer Chromatography (tlc) was performed using Kieselgel 60 F_{254} aluminium-backed plates (0.2 mm thickness) and visualized by UV and dipping in different solution A, B or C followed by heating.
Solution A: Ammonium molybdate (2.5 g) and ceric sulfate (1.0 g) in 10% v/v aqueous sulfuric acid (100 mL). Solution B: p-Anisaldehyde (5 mL), sulfuric acid (50 mL), acetic acid (100 mL) and ethanol (850 mL). Solution C: $KMnO_4$ (1.0 g) and water (100 mL).

All compounds were shown to be homogeneous by tlc and ^{1}H-NMR.

5'-O-t-Butyldimethylsilyl-3'-(2''-oxoethyl)-2',3'-dideoxyuridine 9

To a solution of **8** (550 mg, 1.50 mmol) in a mixture of ether/water (1/1, 40 mL) and OsO_4 (10 mg, 39 µmol) was added slowly over a period of 20 min $NaIO_4$ (810 mg, 3.79 mmol). After stirring at room temperature for 5 h, the aqueous layer was extracted with ether (50 mL) and the combined organic phases were washed with 5% aq. $NaHCO_3$ (50 mL), brine (50 mL) and dried over $MgSO_4$. After evaporation in vacuo, silica gel column chromatography using hex/AcOEt : 4/6 as eluent gave **9** as a syrup (235 mg, 43%). $[\alpha]_D$ = + 55.2 ° ($CDCl_3$, c = 4.3); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.07 [s, 6H, $Si(CH_3)_2$], 0.88 [s, 9H, $SiC(CH_3)_3$], 2.10-2.41 (m, 2H, H-2', H-2''], 2.52-2.80 [m, 3H, H-3' and CH_2], 3.67-3.76 [m, 2H, H-4', H-5'], 3.96-4.03 [m, 1H, H-5''], 5.64 [d, 1H, J = 8.5 Hz, H-5], 6.08 [dd, 1H, J = 3.4 and 6.7 Hz, H-1'], 8.02 [d, 1H, J = 8.2 Hz, H-6], 9.45 [s large, 1H, NH], 9.74 [s, 1H, CHO]. ^{13}C-NMR ($CDCl_3$, 50.4 MHz) δ -6.41 [CH_3Si], 18.37 [CSi], 25.86 [CH_3], 30.94, 39.43, 45.89 [$C_{2'}$, $C_{3'}$, CH_2], 62.20 [$C_{5'}$], 85.23, 86.10 [$C_{1'}$, $C_{4'}$], 101.74 [C_5], 140.30 [C_6], 150.45 [C_2], 163.66 [C_4], 200.00 [CHO]. LRMS (CI-NH_3) : m/e 369 ([MH^+], 36), 257 (100), 199 (46).

5'-O-t-Butyldimethylsilyl-3'-carbomethoxymethyl-2',3'-dideoxyuridine 10

To a solution of **9** (190 mg, 0.52 mmol) in dry DMF (15 mL) was added dry MeOH (137 µL, 3.38 mmol) and then pyridinium dichromate (PDC, 1.16 g, 3.08 mmol). The solution was stirred for 2 h, filtered over celite and the mixture was purified by silica gel column chromatography using hex/AcOEt : 4/6 as eluent to give **10** as a syrup

(116 mg, 56%). $[\alpha]_D$ = +39.9 ° ($CDCl_3$, c = 1.75). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.08 [s, 6H, $Si(CH_3)_2$], 0.90 [s, 9H, $SiC(CH_3)_3$], 2.13-2.71 [m, 5H, H-2', H-2", H-3' and CH_2], 3.68 [s, 3H, CH_3O], 3.76-3.82 [m, 2H, H-4', H-5'], 3.98-4.04 [m, 1H, H-5"], 5.62-5.67 [dd, 1H, J = 2.1 and 8.2 Hz, H-5], 6.05-6.10 [dd, 1H, J = 3.3 and 6.6 Hz, H-1'], 8.05 [d, 1H, J = 8.2 Hz, H-6], 8.96 [large s, 1H, NH]. ^{13}C-NMR ($CDCl_3$, 50.4 MHz) δ -6.85 [CH_3Si], 18.39 [CSi], 25.85 [CH_3], 33.29, 36.03, 39.54 [C_2', C_3', CH_2], 51.92 [CH_3O], 62.24 [C_5'], 85.21, 85.94 [C_1', C_4'], 101.63 [C_5], 140.40 [C_6], 150.27 [C_2], 163.40 [C_4], 171.81 [CO]. LRMS [CI-NH_3]: m/e 399 ([MH^+], 21), 287 (100), 229 (61).

5'-O-t-Butyldimethylsilyl-3'-carbomethoxymethyl-4-desamino-4-azido-2',3'-dideocytidine 12

To a stirred solution of **10** (100 mg, 0.25 mmol) in dry 1,2-dichloroethane (10 mL) was added, at room temperature under argon, triethylamine (175 µL, 1.26 mmol), 4-dimethylaminopyridine (5 mg, 41 µmol) and mesitylenesulfonyl chloride (82 mg, 0.37 mmol). The reaction mixture was stirred for 4 h and then washed with water (10 mL). The organic layer was separated, dried ($MgSO_4$), filtered and evaporated in vacuo. The intermediate mesitylenesulfonyl derivative **11** was dissolved in DMF (5 mL) and sodium azide (49 mg, 0.62 mmol) was added with stirring under argon. After one night at room temperature, the solvent was evaporated in vacuo. The residue was diluted with AcOEt (10 mL) and washed with water (8 mL). The organic layer was separated, dried ($MgSO_4$), filtered and evaporated in vacuo. The crude product was purified by silica gel column chromatography using hex/AcOEt : 6/4 as eluent. The title compound **12** was obtained as a colourless oil (57 mg, 54%). $[\alpha]_D$ = +69.2 ° ($CDCl_3$, c = 5.7). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.11 [s, 3H, $Si(CH_3)$], 0.13 [s, 3H, $Si(CH_3)$], 0.93 [s, 9H, $SiC(CH_3)_3$], 2.28-2.78 [m, 5H, H-2', H-2", H-3' and CH_2], 3.66 [s, 3H, CH_3O], 3.74-3.94 [m, 2H, H-4', H-5'], 4.08-4.15 [m, 1H, H-5"], 6.30 [dd, 1H, J = 2.7 and 6.5 Hz, H-1'], 6.79 [d, 1H, J = 7.9 Hz, H-5], 8.39 [d, 1H, J = 7.8 Hz, H-6]. ^{13}C-NMR ($CDCl_3$, 50.4 MHz) δ -6.52 [CH_3Si], 18.93 [CSi], 26.36 [CH_3], 33.20, 36.02, 40.43 [C_2', C_3', CH_2], 52.48 [CH_3O], 62.23 [C_5'], 87.27, 87.46 [C_1', C_4'], 93.50 [C_5], 135.30 [C_6], 142.93 [C_4], 151.38 [C_2], 172.05 [CO]. LRMS (CI-NH_3): m/e 424 ([MH^+], 13), 398 (22), 287 (100), 229 (34), 155 (33), 112 (60).

5'-O-t-Butyldimethylsilyl-3'-carbomethoxymethyl-2',3'-dideoxycytidine 13

To a stirred solution of anhydrous stannous chloride (56 mg, 0.29 mmol) and thiophenol (121 µL, 1.18 mmol) in dry acetonitrile (2 mL) was added dropwise under

argon a solution of **12** (42 mg, 99 µmol) in dry acetonitrile (1 mL). After 24 h, the solvents were evaporated in vacuo and the residue was diluted with dichloromethane (5 mL). This organic layer was washed with 1N aq. NaOH (2 mL) and the aqueous layer reextracted twice with dichloromethane (3 mL). All organic layers were washed with brine (10 mL), dried ($MgSO_4$) and filtered. Evaporation and purification by silica gel column chromatography (CH_2Cl_2/MeOH : 95/5) gave title compound as a white powder (37 mg, 94%). $[\alpha]_D$ = +52.0 ° ($CDCl_3$, c = 3.7). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.08 [s, 6H, $Si(CH_3)_2$], 0.90 [s, 9H, $SiC(CH_3)_3$], 2.15-2.63 [m, 5H, H-2', H-2'', H-3' and CH_2], 3.65 [s, 3H, CH_3], 3.74-4.04 [m, 2H, H-4', H-5'], 4.15-4.21 [m, 1H, H-5''], 5.64 [d, 1H, J = 7.4 Hz, H-5], 6.06-6.11 [dd, 1H, J = 3.1 and 6.3 Hz, H-1'], 8.08 [d, 1H, J = 7.4 Hz, H-6].

5'-O-t-Butyldimethylsilyl-3'-carbomethoxymethyl-4-N-benzoyl-2',3'-dideoxy cytidine 14

To a solution of **13** (35 mg, 88 µmol) in dry pyridine (1 mL) was added dropwise, under stirring and argon, benzoyl chloride (51 µL, 440 µmol). After 2 h, the pyridine was evaporated and the residue dissolved in AcOEt (10 mL). This organic layer was washed with 0.5 N aq. HCl solution (10 mL) and then brine (5 mL). The organic phase was separated, dried ($MgSO_4$), filtered and evaporated in vacuo. The crude product was purified by silica gel column chromatography with CH_2Cl_2/MeOH : 98/2 as eluent to give **14** as a thick colourless syrup (37 mg, 84%). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.13 [s, 3H, $Si(CH_3)$], 0.14 [s, 3H, $Si(CH_3)$], 0.94 [s, 9H, $SiC(CH_3)_3$], 2.25-2.65 [m, 5H, H-2', H-2'', H-3, and CH_2], 3.67 [s, 3H, CH_3], 3.71-3.88 [m, 2H, H-4', H-5'], 4.06-4.14 [m, 1H, H-5''], 6.11 [m, 1H, H-1'], 7.40-7.61 [m, 4H, 3 $H_{arom.}$ and H-5'], 7.91-7.96 [m, 2H, 2 $H_{arom.}$], 8.08-8.13 [large d, 1H, J = 8.5 Hz, NH], 8.60 [d, 1H, J= 7.3 Hz, H-6]. LRMS (FAB-NBA): m/e 524 ([M+Na^+], 4), 502 ([MH^+], 12), 287 (36), 216 (100), 165 (59), 155 (88).

3'-Carbomethoxymethyl-4-N-benzoyl-2',3'-dideoxycytidine 15

To s solution of **14** (35 mg, 70 µmol) in dry THF (2 mL) was added dropwise under argon and stirring a 1M solution of tetrabutylammonium fluoride (TBAF) in THF (0.21 mL, 210 µmol). After 3 h, the solvent was evaporated in vacuo. The residue was chromatographed on a silica gel column using CH_2Cl_2/MeOH : 5/0.2 as eluent to give **15** as a white solid (25 mg, 92%). ^{1}H-NMR ($CDCl_3$ + few drops MeOD, 200 MHz) δ 2.37-2.85 (m, 5H, H-2', H-2'', H-3', CH_2), 3.68-4.18 (m, 3H, H-5', H-5'', H-4'), 3.79 (s, 3H, CH_3O), 6.25 (m, 1H, H-1'), 7.54-7.88 (m, 4H, H-5, 3 $H_{arom.}$), 8.00-8.17 (m, 2H, 2 $H_{arom.}$) 8.86 (d, 1H, J = 7.5 Hz, H-6).

5'-O-Mesyl-3'-carbomethoxymethyl-4-N-benzoyl-2',3'-dideoxycytidine 16

To a solution of **15** (23 mg, 59 µmol) and triethylamine (17 µL, 122 µmol) in dry dichloromethane (1 mL) was added dropwise, at 0°C under argon and stirring, methanesulfonyl chloride (7 µL, 90 µmol). After 2 h, the mixture was diluted with CH_2Cl_2 (10 mL) and washed with aq. HCl 0.5 N (10 mL). The organic layer was dried ($MgSO_4$) and evaporated in vacuo. The crude product was purified by silica gel column chromatography with CH_2Cl_2/MeOH : 5/0.2 as eluent to provide **16** as a white solid (16 mg, 58%); m.p. (dec.) 207 °C. ^1H NMR ($CDCl_3$, 200 MHz) δ 2.24-2.67 [m, 5H, H-2', H-2", H-3', and CH_2], 3.25 [s, 3H, CH_3SO_2], 3.59 [s, 3H, CH_3O], 4.02-4.09 [m, 1H, H-4'], 4.49-4.54 [m, 2H, 2 H-5'], 6.03 [m, 1H, H-1'], 7.38 [d, 1H, J = 7.2 Hz, H-5], 7.46-7.62 [m, 3H, 3 $H_{arom.}$], 7.97-8.01 [m, 2H, 2 $H_{arom.}$], 8.20 [d, 1H, J = 7.4 Hz, H-6].

5'-Azido-3'-carbomethoxymethyl-4-N-benzoyl-2',3',5'-trideoxycytidine 17

To a solution of **16** (12 mg, 26 µmol) in dry DMF (1 mL) was added at room temperature, under argon, sodium azide (5 mg, 77 µmol). The reaction was stirred at 80 °C for 12 h and the DMF was evaporated in-vacuo. The residue was diluted with ethyl acetate (10 mL) and washed with water (5 mL). The organic layer was dried ($MgSO_4$) and evaporated. Purification by silica gel column chromatography with CH_2Cl_2/MeOH: 5/0.2 as eluent gave **17** as a white solid (8 mg, 74%). ^1H-NMR ($CDCl_3$, 200 MHz) δ 2.31-2.58 [m, 5H, H-2', H-2", H-3' and CH_2], 3.59-3.99 [m, 3H, H-5', H-5" and H-4'], 3.70 [s, 3H, CH_3O], 6.11 [dd, 1H, J = 4.1 and 6.0 Hz, H-1'], 7.47-7.66 [m, 4H, 3$H_{arom.}$ and H-5], 7.90-7.94 [m, 2H, 2$H_{arom.}$], 8.30 [d, 1H, J = 7.6 Hz, H-6]. LRMS [FAB-NBA]: m/e 847([2M+Na^+], 4), 824 ([2M+H^+], 6), 435 ([M+Na^+), 16], 413 ([M+H^+], 47), 216 (100). HRMA [FAB-glycerol]: m/e calcd. for $C_{19}H_{21}N_6O_5$ [MH^+]: 413.1575; found: 413.1573.

5'-O-t-Butyldimethylsilyl-3'-C-allyl-4-azido-2',3'-dideoxyuridine 19

Azide **19** was obtained using the experimental procedure described for compound **12** as a colourless oil (71 % yield) after purification by silica gel column chromatography using ether/hex: 1/1 as eluent. $[\alpha]_D$ = + 84.2° ($CDCl_3$, c = 3.2). ^1H-NMR ($CDCl_3$, 500 MHz) δ 0.14 [s, 3H, $Si(CH_3)$], 0.16 [s, 3H, $Si(CH_3)$], 0.96 [s, 9H, $SiC(CH_3)_3$], 2.07-2.15 [m, 1H, H-2'], 2.22-2.29 [m, 1H, H-2"], 2.30-2.37 [m, 2H, CH_2], 2.39-2.46 [m, 1H, H-3'], 3.80 [m, 1H, H-5'], 3.86 [m, 1H, H-4'], 4.18 [m, 1H, H-5"], 5.02-5.14 [m, 2H, CH_2 allyl], 5.66-5.80 [m, 1H, CH allyl], 6.30 [m, 1H, H-1'], 6.80 [d, 1H, J = 7.8 Hz, H-5], 8.48[d,1H, J = 7.8 Hz, H-6]. ^{13}C-NMR ($CDCl_3$, 125.7 MHz) δ -5.68 [CH_3Si], -5.57 [CH_3Si], 18.29 [CSi], 25.72 [CH_3], 34.90 [C_2'], 35.19 [CH_2], 39.90 [C_3'], 61.53 [C_5'], 86.93 [C_1'], 87.27

[$C_{4'}$], 92.59 [C_5], 117.33 [CH_2 allyl], 134.66 [CH allyl], 134.82 [C_6], 142.28 [C_4], 150.78 [C_2]. LRMS (FAB-NBA): m/e 414 ([M+Na^+], 2), 392 ([MH^+], 8), 334 (10), 255 (100).

5'-O-t-Butyldimethylsilyl-3'-C-allyl-2',3'-dideoxycytidine 20

Cytidine **20** was obtained in 96 % yield, using the reduction experiment described for **13**, followed by a purification on a silica gel column chromatography using CH_2Cl_2/MeOH: 95/5 as eluent. $[\alpha]_D$ = +80.9 ° ($CDCl_3$, c = 1.5). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.09 [s, 6H, 2(CH_3)Si], 0.91 [s, 9H, SiC(CH_3)$_3$], 2.00-2.30 [m, 5H, H-2', H-2'', H-3', CH_2], 3.70-3.75 [m, 2H, H-4', H-5'], 4.01-4.07 [m, 1H, H-5''], 4.97-5.07 [m, 2H, CH_2 allyl], 5.60 [d, 1H, J = 7.3 Hz, H-5], 5.68-5.72 [m, 1H, CH allyl], 6.04 [m, 1H, H-1'], 8.19 [d, 1H, J = 7.3 Hz, H-6]. ^{13}C-NMR ($CDCl_3$, 50.4 MHz) δ -5.05 [CH_3Si], -4.92 [CH_3Si], 18.91 [CSi], 26.34 [CH_3], 35.61 [$C_{2'}$], 35.98 [CH_2], 40.48 [$C_{3'}$], 62.44 [$C_{5'}$], 86.52 [$C_{1'}$], 87.02 [$C_{4'}$], 93.37 [C_5], 117.34 [CH_2 allyl], 135.98 [CH allyl], 142.52 [C_6], 156.30 [C_2], 165.97 [C_4]. LRMS (FAB-NBA): m/e 388 ([M+Na^+], 40), 366 ([MH^+], 33), 308 (16), 255 (80), 134 (100).

5'-O-t-Butyldimethylsilyl-3'-C-allyl-4-N-benzoyl-2',3'-dideoxycytidine 4

Deoxycytidine **4**, obtained as a thick oil from **20** after silica gel chromatography (CH_2Cl_2/MeOH: 97/3), as described for **14**, solidified from hexane; yield 98 %. $[\alpha]_D$ = +51.2 ° ($CDCl_3$, c = 1.4). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.02 [s, 3H, $SiCH_3$], 0.04 [s, 3H, $SiCH_3$], 0.97 [s, 9H, SiC(CH_3)$_3$], 2.07-2.39 [m, 5H, H-2', H-2'', H-3', CH_2], 3.76-3.82 [m, 2H, H-4', H-5'], 4.11-4.17 [m, 1H, H-5''], 5.02-5.11 [m, 2H, CH_2 allyl], 5.66-5.80 [m, 1H, CH allyl], 6.11 [m, 1H, H-1'], 7.47 [d, 1H, J = 7.6 Hz, H-5], 7.52-7.66 [m, 3$H_{arom.}$], 7.99-8.15 [m, 2$H_{arom.}$], 8.72 [d, 1H, J = 7.6 Hz, H-6]. ^{13}C-NMR ($CDCl_3$, 50.4 MHz) δ -4.87 [CH_3Si], -4.50 [CH_3Si], 18.49 [CSi], 25.94 [CH_3], 34.66, 35.21, 39.92 [$C_{2'}$, $C_{3'}$, CH_2], 61.64 [$C_{5'}$], 86.96 [$C_{4'}$], 87.24 [$C_{1'}$], 95.83 [C_5], 117.14 [CH_2 allyl], 128.05, 128.29, 128.85, 130.13 [$CH_{arom.}$], 133.13 [CH allyl], 135.22 [C_6], 145.90 [$C_{arom.}$], 154.63 [C_2], 162.56 [C_4], 171.04 [CO]. LRMS (FAB-NBA): m/e 492 ([M+Na^+], 2), 470 ([MH^+), 7), 255 (26), 238 (15), 216 (100).

5'-O-t-Butyldimethylsilyl-3'-(2''-oxoethyl)-4-N-benzoyl-2',3'-dideoxycytidine 21

To a solution of **4** (9 mg, 19 µmol) in a mixture of ether/water (1/1, 1 mL) and OsO_4 (1 mg, 4 µmol) was added slowly over a period of 10 min $NaIO_4$ (10 mg, 47 µmol). After stirring at room temperature for 3 h, the aqueous layer was washed with ether (5 mL) and the combined organic phases were then washed with 5 % aqueous $NaHCO_3$ (8 mL), brine (5 mL) and dried over $MgSO_4$. After evaporation of the solvent

in vacuo, the crude mixture (grey solid) was recrystalized from hex/AcOEt (7/3) to give **21** as white solid, m.p. (dec.) = 165 °C, in 35 % yield. ^{1}H-NMR (acetone, 500 MHz) δ 0.17 [s, 3H, CH_3Si], 0.18 [s, 3H, CH_3Si], 0.98 [s, 9H, $SiC(CH_3)_3$], 2.24-2.30 [m, 1H, H-2'], 2.41-2.45 [m, 1H, H-2''], 2.63-2.66 [m, 2H, CH_2], 2.74-2.79 [m, 1H, H-3'], 3.92-3.95 [m, 2H, H-4', H-5'], 4.14-4.17 [m, 1H, H-5''], 6.03 [m, 1H, H-1'], 7.55-7.66 [m, 4H, H-5, 3 $H_{arom.}$], 8.16-8.18 [m, 2H, 2 $H_{arom.}$], 8.59 [d, 1H, J = 7.3 Hz, H-6], 9.77 [s, 1H, CHO]. ^{13}C-NMR ($CDCl_3$, 125.7 MHz) δ -6.93 [CH_3Si], 17.37 [CSi], 24.84 [CH_3], 29.98, 38.86, 44.46 [$C_{2'}$, $C_{3'}$, CH_2], 61.28 [$C_{5'}$], 86.04 [$C_{1'}$, $C_{4'}$], 92.30 [C_5], 127.62, 128.09, 132.29 [$CH_{arom.}$], 133.23 [$C_{arom.}$], 144.44 [C_6], 153.78 [C_2], 162.19 [C_4], 166.35 [CO benzoyl], 200.04 [CHO]. LRMS (FAB-NBA): m/e 484 ([M+Na$^+$], 1), 472 ([MH$^+$], 4), 257 (15), 238 (12), 216 (100).

5'-O-t-Butyldimethylsilyl-3'-carboxymethyl-4-N-benzoyl-2',3'-dideoxycytidine 22

To a solution of **21** (5 mg, 11 μmol) in t-BuOH (1 mL) was added 2-methylbut-2-ene (16 μL, 190 μmol), $NaClO_2$ (2 mg, 18 μmol) and NaH_2PO_4 (2 mg, 18 μmol, dissolved in 0.5 mL of water). After stirring at room temperature overnight, t-BuOH was evaporated at room temperature. Water (3 mL) was then added to the residue, and the aqueous phase was extracted with ethyl acetate (10 mL and 5 mL). The combined organic phase was washed with brine (5 mL) and dried over $MgSO_4$. After evaporation of the solvent in vacuo, the product was pure enough (tlc, NMR ^{1}H and ^{13}C) for further reaction, yield 75 % (4 mg). ^{1}H-NMR (acetone, 500 MHz) δ 0.18 [s, 3H, CH_3Si], 0.19 [s, 3H, CH_3Si], 0.98 [s, 9H, $SiC(CH_3)_3$], 2.35-2.77 [m, 5H, H-2', H-2'', H-3', CH_2], 3.92-3.98 [m, 2H, H-4', H-5'], 4.14-4.17 [m, 1H, H-5''], 6.03-6.05 [m, 1H, H-1'], 7.40-7.67 [m, 4H, H-5, $H_{arom.}$], 8.15-8.17 [m, 2H, 2 $H_{arom.}$], 8.62 [d, J = 7.3 Hz, H-6], 10.35 [large s, 1H, COOH]. ^{13}C-NMR (Acetone, 125.7 MHz) δ -6.93 [CH_3Si], 17.37 [CSi], 24.84 [CH_3], 31.85, 34.19, 44.46 [$C_{2'}$, $C_{3'}$, CH_2], 61.28 [$C_{5'}$], 86.04 [$C_{1'}$, $C_{4'}$], 94.92 [C_5], 127.62, 128.09, 132.29 [$CH_{arom.}$], 133.21 [$C_{arom.}$], 144.10 [C_6], 153.78 [C_2], 162.19 [C_4] 167.33 [CO benzoyl], 192.03 [COOH].

Dimer 3'-dCaT-5' 24

To a solution of acid **22** (4 mg, 8 μmol) and amine **23** (2 mg, 8 μmol) in dry DMF (1 mL) was added triethylamine (2 μL, 14 μmol) and then benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP, 4 mg, 9 μmol). After stirring 2 h at room temperature, DMF was evaporated; ethyl acetate (10 mL) was then added to the residue. This organic layer was washed with water (5 mL), brine (5 mL) and dried with $MgSO_4$. The solid crude product, obtained after evaporation in vacuo of

the solvent, was purified by a silica gel column chromatography using CH_2Cl_2/MeOH: 93/7 as eluent. The title compound was obtained as a white solid (4 mg, 69 %). ^{1}H-NMR (MeOD, 500 MHz) δ 0.17 [s, 3H, CH_3Si], 0.18 [s, 3H, CH_3Si], 0.98 [s, 9H, $SiC(CH_3)_3$], 1.82 [s, 3H, CH_3], 2.17-2.44 [m, 6H, 2 H-2', 2 H-2'', CH_2], 2.65-2.72 [m, 1H, H-3'$_{dC}$], 3.30-3.37 [m, 2H, H-4'$_T$, H-5'$_T$], 3.54-3.58 [m, 1H, H-5''$_T$], 3.85-3.93 [m, 2H, H-5'$_{dC}$, H-4'$_{dC}$], 4.12-4.15 [m, 1H, H-5''$_{dC}$], 4.21-4.22 [m, 1H, H-3'$_T$], 5.99 [m, 1H, H-1'$_{dC}$], 6.18 [m, 1H, H-1'$_T$], 7.50 [s, 1H, H-6$_T$], 7.53-7.64 [m, 4H, H-5$_{dC}$, 3 $H_{arom.}$], 7.99-8.01 [m, 2H, 2 $H_{arom.}$], 8.73 [d, 1H, J = 7.3 Hz, H-6$_{dC}$]. ^{13}C-NMR (MeOD, 125.7 MHz) δ -8.79 [CH_3Si], 8.96 [CH_3T], 17.84 [CSi], 23.44 [CH_3], 30.92, 35.12, 36.99, 37.46 [CH_2, C$_{3'dC}$, C$_{2'dC}$, C$_{2''T}$], 39.33 [C$_{5''T}$], 45.87 [C$_{4''T}$], 59.88 [C$_{5'dC}$], 70.16 [C$_{3''T}$], 83.24 [C$_{4'dC}$], 84.17 [C$_{1''T}$], 85.11 [C$_{1'dC}$], 94.45 [C$_{5dC}$], 108.47 [C$_{5T}$], 126.22, 126.69, 130.89 [$CH_{arom.}$], 135.10 [$C_{arom.}$], 143.51 [C$_{6dC}$], 149.11 [C$_{6T}$], 150.35 [C$_{2T}$], 154.72 [C$_{2dC}$], 161.73 [C$_{4dC}$], 163.13 [C$_{4T}$], 171.07 [CO benzoyl], 173.68 [CO, amide]. LRMS (FAB-NAB): m/e 755 ([M-H$^+$ +2 Na$^+$], 6), 733 [(M+Na$^+$], 35), 238 (100). HRMS (FAB-glycerol): m/e calcd. for $C_{34}H_{46}N_6O_9Si$, Na [MNa$^+$]: 733.2992; found: 733.2993.

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