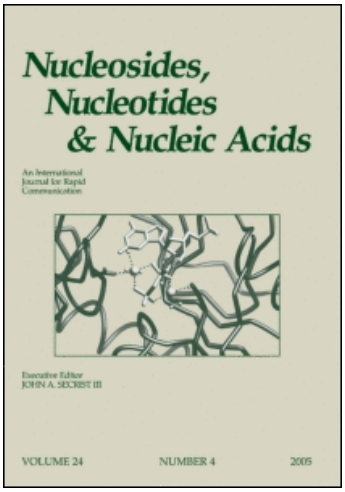

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A Simple Synthesis of *N⁴*-(6-Aminohexyl)-2'-Deoxy-5'-*O*-(4,4'-Dimethoxytrityl)Cytidine

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A SIMPLE SYNTHESIS OF N^4-(6-AMINOHEXYL)-2′-DEOXY-5′-*O*-(4,4′-DIMETHOXYTRITYL)CYTIDINE

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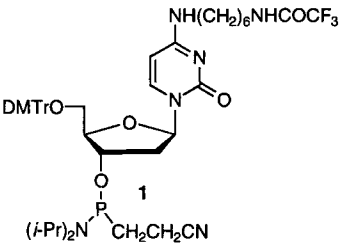
Abstract: The title compound was synthesized by a transamination reaction between N^4-benzoyl-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)cytidine and hexane-1,6-diamine in the presence of 1,5,7-triazabicyclo(4.4.0)dec-5-ene (TBD).

Oligonucleotides labeled with lanthanide chelates are routinely used as tools in research and diagnostic applications as probes for detection of specific nucleic acid sequences. For example, in mixed-phase hybridization assays up to 20 aminohexane-modified deoxycytidine phosphoramidites (**1**) are coupled to the 5′-end of oligonucleotides.[1,2] After deprotection of the modified oligonucleotide, the primary amino functions are labeled with a photoluminescent europium chelate.



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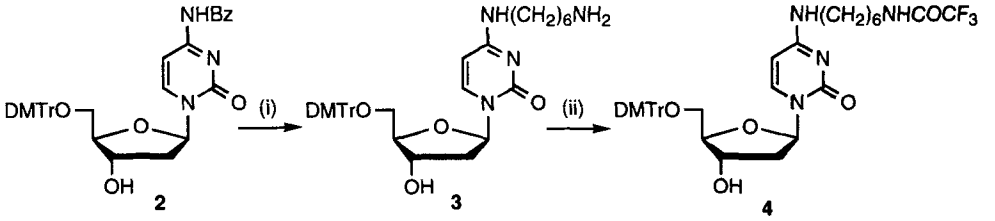
According to the original synthetic strategy,[3,4] the phosphoramidite **1** has been prepared as follows: the hydroxyl groups of 2′-deoxycytidine were protected using 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane and the exocyclic amino function was tosylated. Substitution of the tosylamido group with hexane-1,6-diamine introduced the desired tether. Protection of the linker amino function, desilylation, dimethoxytritylation and phosphitylation completed the reaction sequence. Since several synthetic steps and the use of the expensive 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane to form a transient hydroxyl protecting group are needed, the price of scaling up the experiment is unreasonably high.

Several other methods for cytosine tethering have been reported. For example, Roget *et al.*[5] obtained N^4-(6-aminohexyl)-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)cytidine (**3**) in good yield by allowing 2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-4-thiouridine to react with hexane-1,6-diamine. The 4-thio derivative was, in turn, synthesized in four steps from 2′-deoxyuridine.[6] An alternative approach for tethering involves bisulfite-ion-catalyzed transamination of 2′-deoxycytidine with alkane-α,ω-diamines.[7] The major drawback of this method is the laborious purification procedure of N^4-(ω-alkylamino)-2′-deoxycytidine.[8]

It is well established that transamination of N^4-benzoylated cytosine residues is a serious side reaction when protected oligonucleotides are treated with aqueous primary amines.[9-14] Depending on the nature of the amine and the reaction conditions, the transamination has been reported to occur in up to 40% yield.[13] It is also known that organic solvents and strong bases, such as 1,5,7-triazabicyclo(4.4.0)dec-5-ene (TBD), have an enhancing effect on the transamination reaction.[14] In the present case these observations were exploited in the preparation of the 2′-deoxycytidine derivative **3** (**Scheme**). Accordingly, treatment of commercially available or easily accessible[15] N^4-benzoyl-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)cytidine (**2**) with hexane-1,6-diamine in propan-2-ol in the presence of TBD gave rise to **3**. About 20% of 2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)cytidine, the product of debenzoylation, was formed. The desired product **3** was easily isolated from the reaction mixture in 56% yield by extraction followed by silica gel column chromatography. After protection of the primary amino function of **3** as its trifluoroacetamide, the nucleoside **4** was ready for phosphitylation and incorporation into oligonucleotides.

EXPERIMENTAL

General. N^4-Benzoyl-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)cytidine (**2**) was either purchased from Sigma or prepared in one pot from 2′-deoxycytidine according to Jones.[15] Methyl trifluoroacetate was prepared by mixing trifluoroacetic acid and anhydrous methanol and collecting the product boiling at 43 °C. No catalyst or external heating was used. Adsorption column chromatography was performed on columns packed with silica gel 60 (Merck). Analytical TLC was conducted on silica gel 60 F_{254} plates (Merck). The



Scheme. (i) Hexane-1,6-diamine and TBD in propan-2-ol, overnight at 60 °C. (ii) Methyl trifluoroacetate in dichloromethane, 1h at rt.

following solvent systems were used as the eluents: *A*: CH_2Cl_2:MeOH 9:1 (v/v); *B*: CH_2Cl_2:MeOH:Et_3N 7:2:1 (v/v/v). NMR spectra were recorded on a Jeol LA-400 spectrometer operating at 399.8 and 376.0 MHz for ^1H and ^{19}F, respectively. The signal of Me_4Si was used as an internal (^1H) and trifluoroacetic acid (^{19}F) as an external reference. Coupling constants are given in Hertz. Mass spectra were recorded on a VG ZabSpec-aoTOF instrument (FAB^+).

N^4-(6-Aminohexyl)-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)cytidine (3). Compound **2** (2.0 g, 3.16 mmol) was dissolved in propan-2-ol (10 mL). Hexane-1,6-diamine (5 g) and TBD (1.3g) were added, and the mixture was stirred overnight at 60 °C. The work up was performed as described by Roget *et al.*[5] with some modifications. Accordingly, the reaction mixture was cooled to room temperature and the solvent was evaporated off *in vacuo*. The residue was dissolved in chloroform (100 mL), extracted with 0.1M NaOH (2x50 mL) and water (5x50 mL). The organic phase was dried ($MgSO_4$) and concentrated. The residue was dissolved dichloromethane and applied onto a silica gel column. The column was eluted first with *eluent A* to remove yellow impurities and 2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)cytidine, and then with *eluent B* to elute the product. Pure fractions were pooled and concentrated to give the title compound as a white foam (1.11 g, 56%). $R_f(A)$: 0.0; $R_f(B)$: 0.40. Its ^1H NMR spectrum was in agreement with that reported in the literature.[5] MS: 629 [M^++H].

2′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-N^4-(6-*N*-trifluoroacetamidohexyl)cytidine (4). Compound **3** (1.5 g, 2.4 mmol) was dissolved in dry dichloromethane (10 mL). Freshly

distilled methyl trifluoroacetate (1 mL) was added and the mixture was stirred for 1h at room temperature. All volatile materials were removed *in vacuo*. Purification on silica gel column chromatography (*eluent A*) yielded the title compound as a white foam (1.56 g, 90%). R_f (*A*) 0.49. ^{1}H NMR ($CDCl_3$): δ 7.78 (1H, d, *J* 7.9, H-6), 7.76 (1H, br, N^4-H); 7.33-7.25 (9H, DMTr), 7.21 (1H, br t, $NHCOCF_3$), 6.83 (4H, d, *J* 8.9, DMTr), 6.32 (1H, t, $J_{1',2'}$ and $J_{1',2''}$ 6.0, H-1´), 5.36 (1H, d, *J* 7.9, H-5), 4.45 (1H, m, H-3´), 4.00 (1H, m, H-4´), 3.78 (6H, s, $2xOCH_3$), 3.47 (2H, dd, $J_{4',5'}$ 3.6 and $J_{5',5''}$ 10.6, H-5´), 3.39 (1H, dd, $J_{4',5'}$ 3.8 and $J_{5',5''}$ 10.6, H-5´´), 3.34 (4H, m, $NHCH_2$ and $CH_2NHCOCF_3$), 2.52 (1H, m, H-2´´), 2.19 (1H, m, H-2´), 1.59 (4H, m, $2xCH_2$), 1.41 (4H, m, $2xCH_2$). ^{19}F NMR ($CDCl_3$): δ -76.56. HRMS Found: 725.3159 [M^++H]. Calcd. for $C_{38}H_{44}F_3N_4O_7$: 725.3162.

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