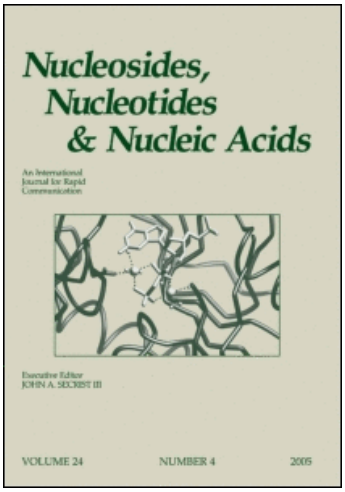

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A Short, Efficient Synthesis of 2'-Deoxypseudoisocytidine Based on Heck-Chemistry

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A Short, Efficient Synthesis of 2′-Deoxypseudoisocytidine Based on Heck-Chemistry

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ABSTRACT

A novel synthesis of 2′-deoxypseudoisocytidine as well as of its phosphoramidite building block for oligonucleotide synthesis is presented. The synthesis is based on Heck-coupling between N-protected pseudoisocytosine and a silyl protected furanoid glycal. With this procedure the corresponding phosphoramidite building block is obtained in 5 steps and an overall yield of 28%.

INTRODUCTION

The *C*-Nucleoside pseudoisocytidine and its 2′-deoxy derivative were first prepared by Watanabe and coworkers in the context of its potential use as a therapeutic agent with antileukemic properties.[1,2] Starting in the early nineties of the last century, the interest in pseudoisocytidine and its 2′O-alkylated derivatives grew due to its function as a neutral replacement for protonated cytidine in triplex forming oligonucleotides designed to bind in the parallel motif to a given double stranded DNA target.[3–5] Current strategies for the synthesis of 2′-deoxypseudoisocytidine

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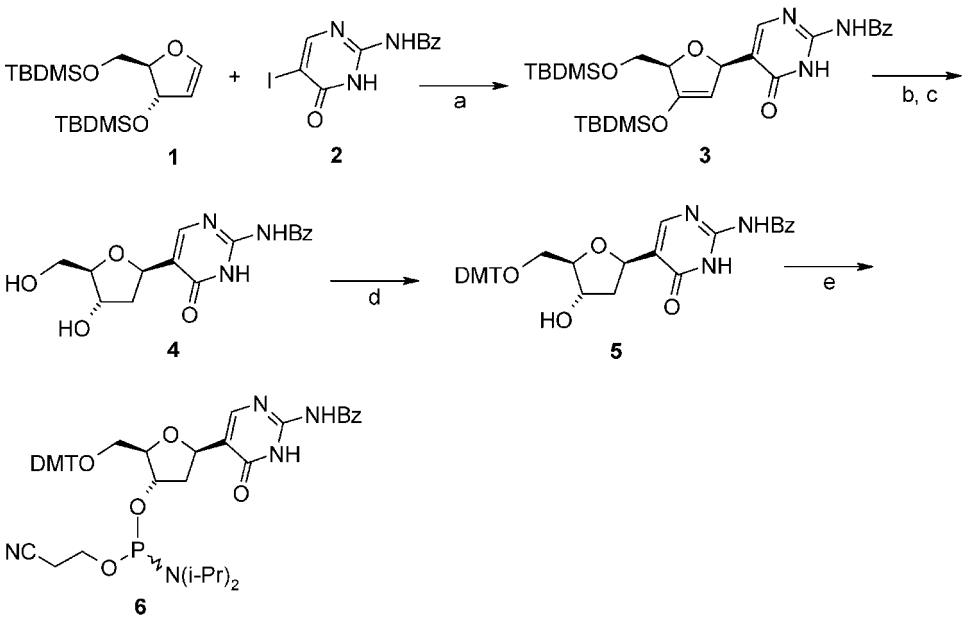
rely on pseudouridine as starting material. Here, the uracil base is converted into isocytosine via a N-methylation-guanidinylation pathway. The 2′-OH function is then removed via standard Barton-McComby reaction. Especially the conditions for remodeling the base require high temperatures in a basic, protic environment, which has been shown in the past to lead to partial isomerization at the pseudoanomeric center. There is clearly need for an improved synthesis.

We describe here a novel synthesis of 2′-deoxypseudoisocytidine and its building block for DNA synthesis which is based on Heck-coupling of a N-protected pseudoisocytosine base with a corresponding furanoid glycal. Heck chemistry has successfully been applied in the past for the synthesis of *C*-nucleosides with β-configuration at the pseudoanomeric center using a variety of natural and non-natural bases.[6–9] We recently contributed to this field by providing access to pyrrolidino pseudonucleosides via Heck chemistry.[10]

RESULTS AND DISCUSSION

The synthesis of the N-benzoyl protected 2′-deoxypseudoisocytidine **4** and the corresponding phosphoramidite building block **6** for DNA synthesis is outlined in Sch. 1. The starting materials, more precisely the furanoid glycal **1**[11,12] as well as the halogenated, N-benzoylated pseudoisocytosine **2**[13,14] were obtained as described.



Scheme 1. (a) *N*,*O*-bis(trimethylsilyl)acetamide, $Pd(OAc)_2$, $AsPh_3$, *N*,*N*-diisopropylethylamine, DMF, 80°C, 22 h, (68%); (b) HF-pyridine, THF, rt, 28 h; (c) $NaBH(OAc)_3$, AcOH, MeCN, −15°C → rt, 2 h, (65%); (d) DMT-Cl, DMAP, pyridine, rt, 6 h, (84%); (e) (iPr$_2$N)($NCCH_2CH_2O$)PCl, iPr$_2$NEt, THF, rt, 1 h, (76%).

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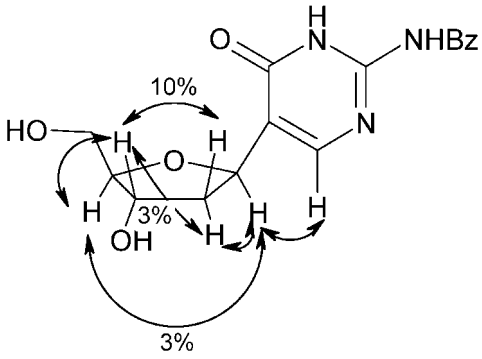

Figure 1. Relevant, mutual NOE signals in compound **4**.

Due to poor solubility of the non silylated base **2**, we decided to follow a one-pot procedure for the Heck coupling step, in which the base is solubilized first via in situ O-silylation, followed by subsequent addition of the glycal **1** and the catalyst. The catalyst system was previously shown to work efficiently in our pyrrolidino-pseudonucleoside synthesis.[10] Intermediate **3** was obtained in a good yield of 68%. The next step involved the desilylation of the hydroxyl functions which was effected by HF-pyridine in THF. The resulting ketone was without further characterization subjected to a diastereoselective reduction with $NaBH(OAc)_3$. A yield of 65% over the two steps was obtained. The relative configurations of the newly installed chiral centers at C(1′) and C(3′) in **4** were rigorously confirmed by ^{1}H-NMR-NOE experiments (Fig. 1). A mutual NOE between H(4′) and H(1′) proves the β-configuration at the pseudoanomeric center, while the strong NOE between H(3′) and H(2′β) is in agreement with the ribo- and not the xylo-configuration of the furanose unit.

The remaining synthesis of the building block **6** for DNA synthesis was finished in two steps and 64% yield via tritylation of **4** and subsequent phosphitylation of **5** according to standard procedures.

We have thus elaborated a novel, mild and stereoselective synthesis of 2′-deoxypseudoisocytidine. Its phosphoramidite derivative **6**, useful for applications in DNA triple helix chemistry, is thus available in 5 steps and an overall yield of 28% starting from the glycal **1** and base **2**. These starting materials in turn can be obtained in two steps each from commercially available compounds.

EXPERIMENTAL PART

General

All experiments were carried out under Ar. Solvents were distilled prior to use. All moisture sensitive reactions were performed in ovendried glassware under Ar. External bath temperatures were used to record all reaction temperatures. Spectral data (NMR, MS) were measured with standard equipment and are indicated in standard format. Flash chromatography (FC) was performed using silica gel with an

average particle size of 40 μm. Thin-layer chromatography (TLC) was performed on silica gel plates (0.25 mm).

2-Benzoylamino-5-[2′-deoxy-3′,5′-bis-O-(tert-butyldimethylsilyl)-β-D-erythro-pent-2-enofuranosyl]-3H-pyrimidin-4-one (3). To a suspension of **2** (1.83 g, 5.37 mmol) in dry DMF (11 mL) was added dropwise *N*,*O*-bis(trimethylsilyl) acetamide (BSA, 1.66 mL, 6.77 mmol). After 1 h, *N*,*N*-diisopropylethylamine (1.27 mL, 7.39 mmol) and **1** (778 mg, 2.26 mmol) were added to the clear solution. To a solution of triphenylarsine (262 mg, 0.86 mmol) in dry DMF (32 mL) in a separate flask was added $Pd(OAc)_2$ (105 mg, 0.47 mmol). After 30 min, this solution was added dropwise to the first solution and the mixture was heated to 80°C for 22 h. The reaction was quenched by addition of H_2O (15 mL), and most of the solvents were evaporated. The residue was diluted with AcOEt, washed with H_2O, the organic phase separated, dried ($MgSO_4$) and evaporated. FC (AcOEt/hexane 3:7) gave a yellow solid. Crystallization from hexane afforded 857 mg (68%, 1.54 mmol) of **3** as a white solid.

^{1}H-NMR (300 MHz, $CDCl_3$): 0.03, 0.05, 0.16, 0.21 (4 s, 12 H, 4 CH_3-Si); 0.86, 0.92 (2 s, 18H, 2 $(CH_3)_3$C-Si); 3.72 (dd, $J_1 = 3.5$, $J_2 = 11.2$, 1 H, H-C(5′)); 3.85 (dd, $J_1 = 2.0$, $J_2 = 11.2$, 1 H, H-C(5′)); 4.56 (m, 1 H, H-C(4′)); 4.97 (m, 1 H, H-C(2′)); 5.80 (m, 1 H, H-C(1′)); 7.49–7.55 (m, 2 H, Bz); 7.61–7.66 (m, 1 H, Bz); 7.98–8.00 (m, 2 H, Bz); 8.02 (s, 1 H, H-C(6)). ^{13}C-NMR (75 MHz, $CDCl_3$): -5.36, -5.13, -4.89 (3 q, 4 CH_3-Si); 17.99, 18.43 (2 s, 2 $(CH_3)_3C$-Si); 25.53, 25.90 (2 q, 2 $(CH_3)_3$C-Si); 63.59 (t, C(5′)); 78.05, 83.99 (2 d, C(4′), C(1′)); 100.20 (d, C(2′)); 128.03, 128.89 (2 d, Bz); 133.47 (d, C(6)); 150.15 (s, C(3′)). HR-MS (FAB^+, $[M + H]^+$): 558.2833 (calc. 558.2819).

N^4-Benzoyl-2′-deoxypseudoisocytidine (4). A solution of HF-pyridine (70% HF, 0.66 mL, ca. 25 mmol HF) in THF (10 mL) was added dropwise to a solution of **3** (756 mg, 1.36 mmol) in THF (28 mL). In intervals of 10 h, additional portions of HF-pyridine (0.33 mL, ca. 13 mmol HF) were added until the starting material had completely disappeared (TLC control). After 28 h the obtained suspension was diluted with AcOH (8 mL) and evaporated. The residue was dissolved without further purification in a mixture of AcOH (25 mL) and MeCN (25 mL), cooled to −15°C, and $NaBH(OAc)_3$ (718 mg, 3.39 mmol) was added in portions (288 mg followed by 430 mg after 30 min). After 2 h the mixture was distributed between AcOEt and H_2O. The organic layer was dried ($MgSO_4$) and evaporated. FC (CH_2Cl_2/MeOH 9:1) resulted in 289 mg (65%, 0.87 mmol) of **4** as a white solid.

^{1}H-NMR (300 MHz, CD_3OD): 2.09 (ddd, $J_1 = 5.9$, $J_2 = 10.1$, $J_3 = 12.9$, 1 H, Hβ-C(2′)); 2.33 (ddd, $J_1 = 1.8$, $J_2 = 5.9$, $J_3 = 12.9$, 1 H, Hα-C(2′)); 3.68 (dd, $J_1 = 4.6$, $J_2 = 11.8$, 1 H, H-C(5′)); 3.73 (dd, $J_1 = 4.1$, $J_2 = 11.8$, 1 H, H-C(5′)); 3.96–4.00 (m, 1 H, H-C(4′)); 4.36–4.38 (m, 1 H, H-C(3′)); 5.12 (dd, $J_1 = 5.9$, $J_2 = 9.9$, 1 H, H-C(1′)); 7.56–7.61 (m, 2 H, Bz); 7.68–7.72 (m, 1 H, Bz); 7.98 (s, 1 H, H-C(6)); 8.05–8.08 (m, 2 H, Bz). Difference-NOE (500 MHz, CD_3OD): 2.09 (Hβ-C(2′)) → 2.33 (11.05%, Hα-C(2′))/4.36–4.38 (10.32%, H-C(3′))/7.98 (0.9%, H-C(6)); 2.33 (Hα-C(2′)) → 2.09 (8.42%, Hβ-C(2′))/4.36–4.38 (3.22%, H-C(3′))/5.12 (7.20%, H-C(1′)); 3.64-3.76 (H_2-C(5′)) → 3.96–4.00 (5.62%, H-C(4′))/4.36–4.38 (3.21%, H-C(3′))/7.98 (0.52%, H-C(6)); 3.96–4.00 (H-C(4′)) → 3.64–3.76 (3.95%, H_2-C(5′))/4.36–4.38

(2.21%, H-C(3′))/5.12 (2.75%, H-C(1′)); 4.36–4.38 (H-C(3′)) → 2.09 (4.86%, Hβ-C(2′))/2.33 (1.17%, Hα-C(2′))/3.64–3.76 (2.49%, H_2-C(5′))/3.96–4.00 (2.85%, H-C(4′)); 5.12 (H-C(1′)) → 2.33 (4.83%, Hα-C(2′))/3.96–4.00 (2.98%, H-C(4′))/7.98 (3.94%, H-C(6)); 7.98 (H-C(6)) → 2.09 (0.84%, Hβ-C(2′))/3.64–3.76 (0.79%, H_2-C(5′))/5.12 (4.96%, H-C(1′)). ^{13}C-NMR (75 MHz, CD_3OD): 42.09 (t, C(2′)); 64.33 (t, C(5′)); 74.74, 76.74 (2 d, C(1′), C(3′)); 89.2 (d, C(4′)); 129.66, 130.11 (2 d, Bz); 134.67 (d, C(6)). HR-MS (FAB^+, $[M+H]^+$): 332.1261 (calc. 332.1246).

N^4-Benzoyl-5′-O-(dimethoxytrityl)-2′-deoxypseudoisocytidine (5). To a solution of **4** (76 mg, 0.23 mmol) in dry pyridine (1 mL) was added 4,4′-dimethoxytrityl chloride (94 mg, 0.27 mmol) and DMAP (2.8 mg, 0.02 mmol). Additional portions of 4,4′-dimethoxytrityl chloride (39 mg, 0.11 mmol, each) were added in intervals of 1 h until no more starting material was detected by TLC (total reaction time of 6 h). The solution was diluted with AcOEt, washed with water, the organic phase dried ($MgSO_4$) and evaporated. The residue was purified by FC (CH_2Cl_2/MeOH 2%, conditioned with 1% Et_3N) to give 121 mg (84%, 0.19 mmol) of **5** as a white foam.

^{1}H-NMR (300 MHz, $CDCl_3$): 1.93 (ddd, $J_1 = 6.3$, $J_2 = 9.2$, $J_3 = 13.2$, 1 H, Hβ-C(2′)); 2.48 (ddd, $J_1 = 2.9$, $J_2 = 6.3$, $J_3 = 13.2$, 1 H, Hα-C(2′)); 3.22 (dd, $J_1 = 5.3$, $J_2 = 9.9$, 1 H, H-C(5′)); 3.31 (dd, $J_1 = 4.4$, $J_2 = 9.9$, 1 H, H-C(5′)); 3.78 (s, 6 H, 2 OCH_3); 4.04 (m, 1 H, H-C(4′)); 4.39 (m, 1 H, H-C(3′)); 5.17 (dd, $J_1 = 6.1$, $J_2 = 9.0$, 1 H, H-C(1′)); 6.81–6.84 (m, 4 H, DMT); 7.18–7.33 (m, 7 H, DMT); 7.42–7.45 (m, 2 H, DMT); 7.48–7.53 (m, 2 H, Bz); 7.59–7.64 (m, 1 H, Bz); 7.81 (s, 1 H, H-C(6)); 7.93-7.96 (m, 2 H, Bz). ^{13}C-NMR (75 MHz, $CDCl_3$): 41.16 (t, C(2′)); 55.20 (q, 2 OCH_3); 64.30 (t, C(5′)); 73.85, 74.19 (2 d, C(1′), C(3′)); 85.49 (d, C(4′)); 113.13, 126.81, 127.82, 127.92 (4 d, DMT); 128.15, 128.95 (2 d, Bz); 130.02, 130.04 (2 d, DMT); 133.57 (d, C(6)); 135.93, 144.73, 158.49 (3 s, DMT). HR-MS (FAB^+, $[M+H]^+$): 634.2525 (calc. 634.2553).

N^4-Benzoyl-5′-O-(dimethoxytrityl)-2′-deoxypseudoisocytidine-3′-O-(2-cyanoethyl-N,N-diisopropyl) phosphoramidite (6). To a solution of **5** (107 mg, 0.17 mmol) in dry THF (4 mL) was added Et*i*Pr_2 (145 μL, 0.84 mmol) followed by 2-cyanoethyl diisopropylchlorophosphoramidite (94 μL, 0.42 mmol). After 70 min, 5% aq. $NaHCO_3$ was added and the mixture extracted twice with AcOEt. The combined organic layers were dried ($MgSO_4$) and evaporated. FC (AcOEt/hexane 6:4, conditioned with 1% Et_3N) gave 107 mg (76%, 0.13 mmol) of **6** as a white foam.

^{1}H-NMR (300 MHz, $CDCl_3$): 1.06–1.33 (m, 12 H, 4 CH_3-C); 1.86–1.96 (m, 1 H, Hβ-C(2′)); 2.45 (t, J = 5.0, 1 H, -CH_2CN); 2.55–2.67 (m, 2 H, Hα-C(2′), -CH_2CN); 3.21–3.29 (m, 2 H, H-C(5′)); 3.778, 3.785 (2 s, 6 H, 2 OCH_3); 3.50–3.86 (m, 4 H, 2 H-C(CH_3)$_2$, OCH_2); 4.18 (m, 1 H, H-C(4′)); 4.49 (m, 1 H, H-C(3′)); 5.16 (m, 1 H, H-C(1′)); 6.80–6.84 (m, 4 H, DMT); 7.18–7.34 (m, 7 H, DMT); 7.43–7.45 (m, 2 H, DMT); 7.49–7.53 (m, 2 H, Bz); 7.60–7.63 (m, 1 H, Bz); 7.83, 7.86 (2 s,br, 1 H, H-C(6)); 7.93–7.95 (m, 2 H, Bz). ^{13}C-NMR (75 MHz, $CDCl_3$): 20.16, 20.34 (2 dt, $J_{(C,P)} = 6.68$, OCH_2CH_2CN); 24.53, 24.58, 24.63, 24.68 (4 q, 2 (CH_3)$_2$CH); 29.68 (t, C(2′)); 43.10 (dd, $J_{(C,P)} = 5.80$, (CH_3)$_2$CH); 43.27 (dd, $J_{(C,P)} = 6.07$, (CH_3)$_2$CH); 55.20, 55.21 (2 q, 2 OCH_3); 58.25, 58.32 (2 dt, $J_{(C,P)} = 18.19$, OCH_2CH_2CN); 63.80, 63.95 (2 t, C(5′)); 74.14, 74.20 (2 d, C(1′)); 75.19, 75.55 (2 dd, $J_{(C,P)} = 17.29$,

C(3′)); 85.03, 85.25 (2 dd, $J_{(C,P)} = 4.86$, C(4′)); 86.07 (s, C(5′)-O-*C*); 113.07 (d, DMT); 117.50, 117.63 (2 s, CN); 126.75, 126.79, 127.78, 127.82 (4 d, DMT); 128.19, 128.25, 129.03 (3 d, Bz); 130.07, 130.11 (2 d, DMT); 133.65 (d, C(6)); 135.97, 144.75, 158.42 (3 s, DMT). ^{31}P-NMR (161.9 MHz, $CDCl_3$): 149.04, 149.56. HR-MS (FAB^+, $[M + H]^+$): 834.3635 (calc. 834.3631).

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