JOC Article

A Short Path Synthesis of [^{13}C/^{15}N] Multilabeled Pyrimidine Nucleosides Starting from Glucopyranose Nucleosides

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The synthesis of fully [^{13}C/^{15}N] labeled pyrimidine nucleosides has been achieved from ^{13}C-glucose and labeled nucleobases. The reaction scheme leads directly to the protected nucleosides without the need for the inversion of configuration of C-3 of ^{13}C-glucose. This was achieved by an oxitative ring-opening reaction removing the carbon with the wrong configuration.

Introduction

Full determination of the tertiary structure of DNA or RNA molecules by NMR techniques needs two sorts of information: the conformation of each nucleotide unit and the spatial proximity between the nucleotidyl protons. Such information is available from 2D- and 3D-NMR experiments[1] for smaller oligonucleotides, but it is difficult to collect this information for larger oligomers due to spectral overlap and line broadening. To overcome these difficulties and to allow the conformational analysis of a large, biologically functional DNA or RNA, the introduction of ^{2}H-, ^{13}C, and ^{15}N-labeled oligonucleotide building blocks is paramount for structure elucidation. Therefore, with the help of specific ^{15}N and ^{13}C labeling, key information on local interactions such as hydrogen bonding,[2] protonation,[3] hydration,[4] ligand interactions,[5] and stacking[6] on large oligomers can be provided. A bottleneck in NMR research on nucleic acid structures and dynamics is the availability of these isotopically labeled materials in larger amounts and at reasonable costs. ^{13}C/^{15}N-labeled nucleic acids can be obtained by an enzymatic approach,[7] in a chemical manner, or by a combination of both.[8]

One of the pronounced advantages of the chemical synthesis of labeled nucleosides is the capability of preparing virtually any conceivable isotopomer of a labeled nucleoside.[9] The key step in the chemical preparation of fully labeled nucleosides is the synthesis of protected [$^{13}C_5$]-D-ribose, which starts with the easily available [$^{13}C_6$]-D-glucose. The disadvantage of the published methods is the multiple steps needed to convert [$^{13}C_6$]-D-glucose to [$^{13}C_5$]-D-ribose. The reason for this is that one of the carbon atoms, (C-3) of D-glucose, has to be inverted from configuration while another carbon atom (C-6) has to be removed. An economically much more profitable approach would favor the removal of the carbon with the wrong configuration, i.e., C-3 of D-glucose. Theoretically, this can be achieved by starting with a glucopyranose nucleoside via an oxidative ring-opening−reductive ring-closure procedure removing C-3 of D-glucose.

We have proven the feasibility of this approach by examples in the pyrimidine series, i.e., the synthesis of [1′,2′,3′,4′,5′-$^{13}C_5$]-5′-monomethoxytrityl-[6-^{13}C,1,3-$^{15}N_2$]-uridine [**13**] and [1′,2′,3′,4′,5′-$^{13}C_5$]-5′-monomethoxytrityl-[6-^{13}C,1,3,4-$^{15}N_3$]-*N*^{4}-benzoylcytidine [**14**] in 6 and 7 steps, respectively, starting from [$^{13}C_6$]-D-glucose and labeled nucleic base.

Results and Discussion

Uracil [**1**] was synthesized by hydrogenation of α-cyanoacetylurea in the presence of a nickel catalyst.[10] This three-step synthesis has been adapted to introduce carbon[11] as well as nitrogen labels.[12] The only restriction is that both ring nitrogens are simultaneously introduced so that selective labeling of one of them is not possible. Total synthesis of cytosine [**5**] suffers from the disadvan-

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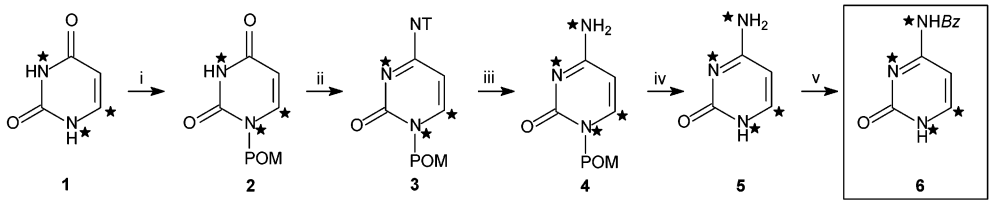
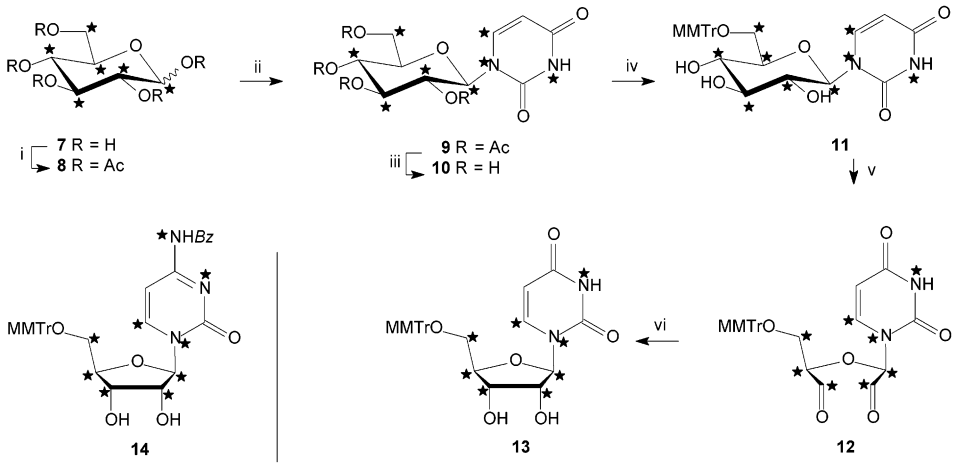
SCHEME 1. Synthesis of Labeled Cytosine [4][a,b]



[a] The labeled positions are indicated with an asterisk. [b]Reagents and conditions: (i) POM-Cl, K_2CO_3, DMF, 54%; (ii) 3NT, TsCl, diphenyl phosphate, pyridine, 83%; (iii) $^*NH_4Cl$, KOH aq, MeCN, THF, 90 %; (iv) 0.5 N KOH, DMF, 82 %; (v) BzCl, pyridine, 76%.

SCHEME 2. Synthesis of [1′,2′,3′,4′,5′-$^{13}C_5$]-5′-*O*-Monomethoxytrityl-[6-^{13}C,1,3-$^{15}N_2$]-uridine [13][a] and the structure of [1′,2′,3′,4′,5′-$^{13}C_5$]-5′-*O*-Monomethoxytrityl-[6-^{13}C,1,3,4-$^{15}N_3$]-*N*^{4}-benzoylcytosine [14]



[a] Reagents and conditions: (i) Ac_2O, pyridine 98 %; (ii) [6-^{13}C,1,3-$^{15}N_2$]-uracil, BSA, TMSOTf, $ClCH_2CH_2Cl$, 87 %; (iii) NH_3/MeOH, 97%; (iv) MMTrCl, pyridine, Et_3N, 90%; (v) $Pb(OAc)_4$, CH_2Cl_2, 90 %; (vi) Bu_3SnH, dioxane, AIBN, 68%.

tage of the formation of isocytosine[13] as byproduct and inferior yields of the desired compound.[14] By condensation of urea with cyanoacetal in the presence of sodium butoxide, cytosine can be obtained in an overall yield of 56%.[15] It is much more efficient, however, to start from the more readily available uracil base [**1**] and convert it into the cytosine base [**5**] (Scheme 1). Starting from [6-^{13}C,1,3-$^{15}N_2$]-uracil [**1**], first N-1 was protected to yield [6-^{13}C,1,3-$^{15}N_2$]-1-pivaloyloxymethyluracil [**2**]. For the introduction of the exocyclic amino group 3-nitro-1,2,4-triazole (NT)[16] is introduced into the C-4 position of the protected uracil [**2**], which is subsequently displaced by $^{15}NH_3$ to obtain 1-[6-^{13}C,1,3,4-$^{15}N_3$]-pivaloyloxymethylcytosine [**4**]. Deprotection, followed by benzoylation of the exocyclic amino group yields [6-^{13}C,1,3,4-$^{15}N_3$]-*N*^{4}-benzoylcytosine [**6**], which is suitable for Vorbruggen condensation.[17]

The published methods to synthesize [$^{13}C_5$]-D-ribose all start from labeled glucose.[18] They differ from each other by (a) first shortening of the side chain of protected glucofuranose by a $NaIO_4-NaBH_4$ reaction followed by inversion of configuration at C-3, using a nucleophilic substitution reaction,[19] (b) first inversion of configuration at C-3 by a PDC−$NaBH_4$ reaction and then oxidative cleavage of the side chain[20] of the protected glucofuranose, and (c) a one-pot procedure of oxidatively cleaving the diol and reducing simultaneously both the 3-keto function and the 5-aldehyde group.[21] A shorter route, which we present here, starts from glucopyranose nucleosides. This method is outlined for the synthesis of [1′,2′,3′,4′,5′-$^{13}C_5$]-5′-monomethoxytrityl-[6-^{13}C,1,3-$^{15}N_2$]-uridine [**13**] and [1′,2′,3′,4′,5′-$^{13}C_5$]-5′-monomethoxytrityl-[6-^{13}C,1,3,4-$^{15}N_3$]-*N*^{4}-benzoylcytidine [**14**]. In this case the C-3 of glucose (i.e., the carbon with the wrong configuration) is removed, which means that the inversion reaction can be omitted. The reaction sequence (Scheme 2) leads directly to the 5'-*O*-monomethoxytrityl protected ribonucleosides (Scheme 2).

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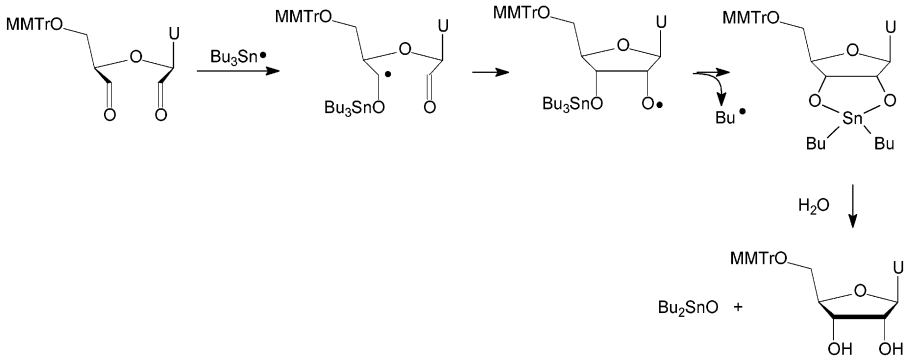

FIGURE 1. Reaction mechanism proposed for the formation of the ribofuranose ring starting from the 1,5-dialdehyde precursor.

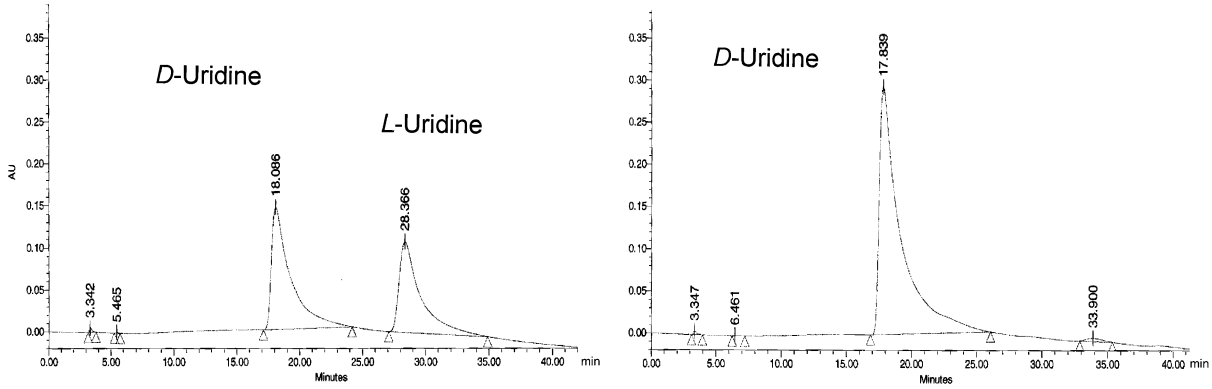

FIGURE 2. Chiral HPLC (solvent system ETOH, *n*-hexane): (left) a 1:1 mixture of D- and L-uridine; (right) a typical run of the uridine obtained by the ring-opening−ring-closure process.

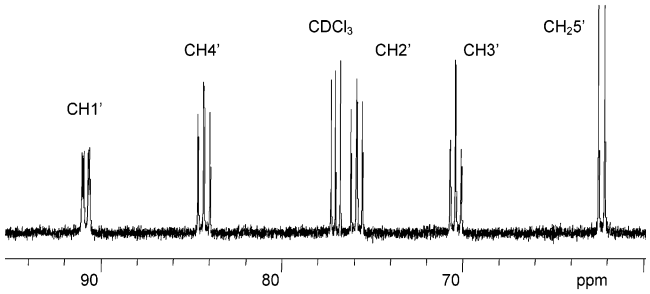

FIGURE 3. [1′,2′,3′,4′,5′-$^{13}C_5$]-5′-*O*-Monomethoxytrityl-[6-^{13}C,1,3-$^{15}N_2$]-uridine **[13]**: ^{13}C NMR sugar region.

After acetylation of [$^{13}C_6$]-D-glucose [**7**] to the corresponding [$^{13}C_6$]-pentaacetyl-D-glucose [**8**], a Vorbruggen condensation with [6-^{13}C,1,3-$^{15}N_2$]-uracil [**1**] is carried out to obtain [1′,2′,3′,4′,5′,6′-$^{13}C_6$]-(2′,3′,4′,6′-tetraacetyl-*β*-D-glucopyranosyl)-[6-^{13}C,1,3-$^{15}N_2$]-uracil [**9**]. After deprotection to the corresponding *β*-D-glucopyranoside [**10**], monomethoxytritylation yielded the 1′,6′-substituted *β*-D-glucopyranoside [**11**], which is the starting compound for the key step of this synthesis—an oxidative ring-opening and reductive ring-closure sequence obtaining [1′,2′,3′,4′,5′-$^{13}C_5$]-5′-*O*-monomethoxytrityl-[6-^{13}C,1,3-$^{15}N_2$]-uridine [**13**] in an overall yield of 43% from [$^{13}C_6$]-D-glucose [**7**]. The synthesis of [1′,2′,3′,4′,5′-$^{13}C_5$]-5′-*O*-monomethoxytrityl-[6-^{13}C,1,3,4-$^{15}N_3$]-N^4-benzoylcytidine [**14**] is carried out in an analogous way, starting from peracetylated [$^{13}C_6$]-D-glucose [**8**] and [6-^{13}C,1,3,4-$^{15}N_3$]-N^4-benzoylcytosine [**6**]. After full deprotection to the corresponding *β*-D-glucopyranoside, the exocyclic amino function was benzoylated. After monomethoxytritylation of the primary alcohol function the formed [1′,2′,3′,4′,5′,6′-$^{13}C_6$]-5′-*O*-monomethoxytrityl-*β*-D-glucopyranosyl-[6-^{13}C,1,3,4-$^{15}N_2$]-N^4-benzoylcytosine is suitable to the oxidative ring-opening and reductive ring-closure sequence obtaining [1′,2′,3′,4′,5′-$^{13}C_5$]-5′-*O*-monomethoxytrityl-[6-^{13}C,1,3,4-$^{15}N_3$]-N^4-benzoylcytidine [**14**] in an overall yield of 31%. The use of periodic acid and its salts or of lead tetraacetate for cleavage of α-glycol groups was described by Malaprade[22] and Criegee.[23] Kinetic studies showed, that *cis* glycols are oxidized more rapidly than *trans* glycols.[24] To obtain nucleoside dialdehydes[25] from the corresponding glucopyranosides, oxidation with 3 equiv of lead tetraacetate turned out to be most efficient.[26]

Ring closure to obtain the monomethoxytrityl protected ribonucleosides was achieved *via* an intramolecular pinacol-coupling reaction. This reduction with either SmI_2[27] or tributyltin hydride shows a high *cis*-selectivity. For tributyltin hydride, the reaction is proposed to occur *via* an addition of a tin ketyl to a carbonyl group followed by an intramolecular homolytic substitution step (S_H2).[28]

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This distinguishes this pinacol cyclization from other reductive cyclizations of tin ketals and causes the high *cis*-selectivity in the cyclization of 1,5-dicarbonyl compounds (Figure 1).

In the case of the furanose sugars, the reaction may also proceed via a di-enol intermediate. This mechanism would give rise to a mixture of D- and L-nucleosides. However, this possibility was ruled out by the fact that L-nucleosides were not detected during the reaction (Figure 2).

Figure 3 shows the ^{13}C spectrum of the sugar region of the obtained MMT-uridine [**13**]. The isotopic purity was verified by exact mass spectrometry.

Conclusion

In summary, the synthesis of fully [$^{13}C/^{15}N$]-labeled pyrimidine nucleosides has been achieved from ^{13}C-glucose and labeled nucleobases. The reaction scheme leads directly to the monomethoxytrityl-protected nucleosides without the need for the inversion of configuration of C-3 of ^{13}C-glucose. This was achieved by an oxidative ring-opening reaction removing the carbon with the wrong configuration. Due to its shortness and high yield, this reaction scheme can be considered as a considerable improvement for existing methods, making labeled nucleotide material more easily available.

Experimental Section

Materials and General Methods. ^{13}C and ^{1}H is referred to TMS, ^{15}N to liquid NH_3. Exact mass measurements were performed on a quadrupole time-of-flight mass spectrometer equipped with a standard electrospray ionization (ESI) interface. TLC was performed with TLC aluminum sheets and silica (200−425 mesh) was used for column chromatography. For all reactions dry (molecular sieve) analytical grade solvents were used. Solvents for column chromatography were distilled before use. Isotopically labeled materials were obtained from Campro Scientific, [6-^{13}C,1,3-$^{15}N_2$]-Uracil **[4]** was synthesized according to literature procedures.[11,12]

Preparation of [6-^{13}C,1,3-$^{15}N_2$]-1-Pivaloyloxymethyluracil [**2**]. To a suspension of [6-^{13}C,1,3-$^{15}N_2$]-uracil [**1**] (0.66 g, 5.86 mmol) and anhydrous K_2CO_3 (0.68 g, 6.45 mmol) in anhydrous DMF (80 mL) was added pivaloyloxymethyl chloride (972 mg, 6.45 mmol). The reaction was stirred at room temperature for 3 days, protected from atmospheric moisture by a $CaCl_2$ drying tube. The reaction mixture was concentrated in vacuo and the resulting residue was extracted continuously in a Soxhlet extractor with CH_2Cl_2 (175 mL) for 18 h. The extract was concentrated and purified by column chromatography (silica, CH_2Cl_2:MeOH 95:5). Besides the desired product (0.71 g, 56%), 1,3-bispivaloyl derivative (0.06 g, 3%) and unreacted starting material (0.25 g, 36%) could be isolated. R_f (CH_2Cl_2/MeOH 95:5) 0.33; mp 127−128 °C (CH_2Cl_2); ^{13}C NMR (50 MHz, DMSO-d_6) δ 26.5 (C(CH_3)$_3$), 38.2 (C(CH$_3$)$_3$), 70.6 (d, J = 54.6 Hz, OCH_2N), 101.7 (d, J = 291.4, 5-C), 145.3 (J = 242.4, 6-C), 155.4 ((t), J = 11 Hz, 2-C), 163.5 (d, J = 11 Hz, 4-C), 177.2 (C=O); ^{15}N NMR (50 MHz, DMSO-d_6) δ 137.5 (d, J = 11.8 Hz, N1), 156.1 (s, N3); exact mass calcd for C_9*CH_{14}*N_2O_4Na 252.0825, found 252.0823.

Preparation of [6-^{13}C,1,3-$^{15}N_2$]-1-Pivaloyloxymethyl-4-(3-nitrotriazol-1-yl)-2-pyrimidone [**3**]. A solution of [6-^{13}C,1,3-$^{15}N_2$]-1-pivaloyloxymethyluracil [**2**] (0.93 g, 4.12 mmol) in pyridine (10 mL) was evaporated to an oil that was treated with 3-nitrotriazole (0.94 g, 8.25 mmol) in pyridine (10 mL) to form a solution which was again evaporated to an oil. This was treated with diphenyl phosphate (1.2 g, 4.95 mmol) and pyridine (10 mL), and the solution was again evaporated to an oil. To this residual oil was added pyridine (10 mL) and *p*-toluenesulfonyl chloride (1.57 g, 8.25 mmol). The solution was stirred at room temperature for 30 h and then quenched by the addition of H_2O (1 mL). The reaction mixture was extracted with CH_2Cl_2 (100 mL) and saturated Na_2CO_3 (100 mL). After drying over Na_2SO_4 the solvent was removed in vacuo to obtain a dark-brown solid (1.10 g, 83%), which was used without further purification in the preparation of [6-^{13}C,1,3,4-$^{15}N_3$]-1-pivaloylmethylcytosine [**4**]. R_f (CH_2Cl_2/MeOH 95:5) 0.55; mp 195−197 °C (CH_2Cl_2); ^{13}C NMR (50 MHz, $CDCl_3$) δ 26.7 (C(CH_3)$_3$), 38.9 (C(CH$_3$)$_3$), 71.7 (d, J = 55 Hz, OCH_2N), 96.7 (d, J = 291 Hz, 5-C), 143.1 ((t), J = 11 Hz, 2-C), 144.8 (3-C, NT), 147.4 (5-CH NT), 155.4 (J = 48.6, 6-C), 164.7 (d, J = 11 Hz, 4-C), 177.8 (C=O); ^{15}N NMR (50 MHz, DMSO-d_6) δ 165.6 (N1), 241.0 (N3); exact mass calcd for C_{11}*$CH_{15}N_4$*N_2O_5 326.1078, found 326.1103.

Preparation of [6-^{13}C,1,3,4-$^{15}N_3$]-1-Pivaloylmethylcytosine [**4**]**.** A mixture of NH_4Cl (0.19 g, 3.3 mmol) and KOH (0.25 g, 3.4 mmol) in CH_3CN (8 mL) was cooled in an ice bath. Through a septum was added H_2O (5 mL), followed by triethylamine (0.32 g, 3.12 mmol). To the resulting clear, colorless solution was added a solution of [6-^{13}C,1,3-$^{15}N_2$]-1-pivaloyloxymethyl-4-(3'-nitrotriazol-1'-yl)-2-pyrimidone [**3**] (1.04 g, 3.2 mmol) in CH_3CN (8 mL) and H_2O (5 mL). The reaction was allowed to warm to room temperature. After 48 h, the formation of a precipitate (KCl) was noted and the reaction mixture was diluted with MeOH to form a solution that was adsorbed onto 10 g of silica gel and purified by column chromatography (50 g, silica, 1% MeOH in CH_2Cl_2). Yield 0.69 g (90%); R_f (CH_2Cl_2/MeOH 9:1) 0.73; mp 265−266 °C (CH_2Cl_2); ^{13}C NMR (50 MHz, DMSO-d_6) δ 26.6 (C(CH_3)$_3$), 38.1 (C(CH$_3$)$_3$), 71.8 (d, J = 54.6 Hz, OCH_2N), 94.4 (J = 267 Hz, 5-C), 145.9 (J = 48.4, 6-C), 156.8 ((t), J = 11 Hz, 2-C), 166.4 (d, J = 55 Hz, 4-C), 177.4 (C=O).

Preparation of [6-^{13}C,1,3,4-$^{15}N_3$]-Cytosine [**5**]**.** [6-^{13}C,1,3,4-$^{15}N_3$]-1-Pivaloylmethylcytosine [**4**] (0.75 g, 3.30 mmol) was treated with DMF (25 mL) and 0.5 N KOH (25 mL). The suspension was stirred at room temperature for 5 days, during which time all initially insoluble material dissolved. The reaction mixture was concentrated in vacuo, dissolved in MeOH, and adsorbed onto 5 g of silica gel and chromatographed from 55 g of silica gel with use of a gradient ranging from 3% to 12.5% MeOH in CH_2Cl_2. Yield 0.31 g (82.3%); R_f (CH_2Cl_2/MeOH 9:1) 0.80; mp >300 °C; ^{13}C NMR (50 MHz, DMSO-d_6) δ 92.6 (J = 267 Hz, 5-C), 142.8 (d, J = 48.4, 6-C), 156.8 ((t), J = 11 Hz, 2-C), 166.7 (d, J = 55 Hz, 4-C); ^{15}N NMR (50 MHz, DMSO-d_6) δ 91.3 (d, J = 5.8 Hz, 4-NH_2), 139.7 (d, J = 11.1 Hz, N1), 209.9 (d, J = 5.8 Hz, N3); exact mass calcd for C_3*CH_6*N_3O 116.0455, found 116.0472.

Preparation of [6-^{13}C,1,3,4-$^{15}N_3$]-*N*^{4}-Benzoylcytosine [**6**]**.** [6-^{13}C,1,3,4-$^{15}N_3$]-Cytosine [**5**] (0.5 g, 4.5 mmol) was suspended in pyridine (25 mL). Under cooling benzoyl chloride (2.5 g, 17 mmol) was added dropwise. After being stirred at room temperature for 6 h, the reaction mixture was cooled in an ice bath and first treated with EtOH (5 mL), then after 20 min H_2O (5 mL) was added. The reaction mixture was allowed to warm to room temperature with stirring for 18 h. After filtration and washing with cold H_2O (2 × 3 mL) the precipitate was dried over P_2O_5. Because of the bad solubility the product only was verified by MS. Yield 0.65 g (76%); R_f (CH_2Cl_2/MeOH 9:1) 0.15; mp >300 °C; exact mass calcd for C_{10}*CH_{13}*N_3O_2 220.0717, found 220.0700.

Preparation of [1,2,3,4,5,6-$^{13}C_6$]-Glucosepenta-*O*-acetate [**8**]**.** After pyridine (20 mL) was cooled to 0 °C, acetic anhydride (15 g, 14 mL, 0.15 mmol) was added. The fully labeled glucose [**7**] (3.0 g, 0.016 mmol) was added in small portions at 0 °C. The reaction temperature was slowly allowed to come to room temperature and stirred overnight. After

(28) (a) Hays, D. S.; Fu, G. C. *J. Org. Chem*. **1998**, *63*, 6375−6381. (b) Bebbington, D.; Bentley, J.; Nilsson, P. A.; Parsons, A. F. *Tetrahedron Lett.* **2000**, *41*, 8941−8945.

addition of ice−water (60 mL) an oil was formed, which turned solid with scrapping. The resulting precipitate was filtered off and recrystallized from water. The α:β enantiomer ratio was ∼1:1 (^{13}C NMR). Yield 6.20 g (97%); R_f (CH_2Cl_2/MeOH 95:5) 0.63; mp 110−112 °C (H_2O); ^{13}C NMR (50 MHz, $CDCl_3$) δ 20.2−20.5 (5 × CH_3 Ac), 169.5−170.7 (5 × C═O Ac), 60.9, 61.8 (d, J = 44 Hz, CH_2-6′), 67.2−71.0 (m, 3 CH, CH-4′,2′,3′), 72.7 (t, J = 44 Hz, CH-5′), 88.0, 88,6 (d, J = 44 Hz, CH-1′α), 91.4, 92.0 (d, J = 44 Hz, CH-1′β); MS (LSIMS; Thgly; *m*/*z* (%)) 337 (73.3) $[M + H]^+$.

Synthesis of [1′,2′,3′,4′,5′,6′-$^{13}C_6$]-(2′,3′,4′,6′-Tetraacetyl-β-D-glucopyranosyl)-[6-^{13}C,1,3-$^{15}N_2$]-uracil [9]. To the suspension of [6-^{13}C,1,3-$^{15}N_2$]-uracil [**1**] (0.96 g, 8.32 mmol) and [$^{13}C_6$]-glucosepenta-*O*-acetate [**8**] (3.3 g, 8.32 mmol) in dichloroethane (40 mL) was added BSA (4.8 mL, 20 mmol). The mixture was stirred under nitrogen at ambient temperature for 20 min. A clear, colorless solution was obtained. After addition of TMSOTf (3.60 mL, 20 mmol) under nitrogen the reaction mixture was heated under reflux for 2 h. After cooling to ambient temperature the resulting brown mixture was evaporated in vacuo. The resulting oil was diluted in ethyl acetate (200 mL) and washed with $NaHCO_3$ (150 mL) and brine (2 × 100 mL). After the solution was dried over Na_2SO_4 and the solvent evaporated, the resulted oil was purified by column chromatography (silica, 24 × 3 cm, ethyl acetate/*n*-hexane 2:1). Yield 3.29 g (87.5%); R_f (ethyl acetate/*n*-hexane 2:1) 0.34; mp 153−155 °C (EtOH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 20.2, 20.3, 20.4, 20.6 (4 × CH_3 Ac), 61.5 (d, J = 43 Hz, CH6′), 67.8 (t, J = 43 Hz, CH4′), 69.3 (t, J = 43 Hz, CH2′), 72.7 (t, J = 43 Hz, CH3′), 75.0 (t, J = 43 Hz, CH5′), 80.3 (d × d, J^1 = 14.6 Hz, J^2 = 45 Hz, CH1′), 103.7 (d × d, J^1= 14.6 Hz, J^2= 66 Hz, 5-CH), 139.2 (d, J = 14.6 Hz, 6-CH), 150.5 ((t), J = 11 Hz, 2-C), 162.9 (d, J = 11 Hz, 4-C), 169.5, 169.6, 169.8, 170.5 (4 × C═O Ac); ^{15}N NMR (50 MHz, $CDCl_3$) δ 130.6 (N1), 152.5 (N3); exact mass calcd for C_{11}*C_7H_{23}*N_2O_{11} 452.1473, found 452.1510.

Deprotection: Typical Procedure. A solution of the tetra-*O*-acetyl-protected nucleoside [**9**] (10 mmol) in NH_3/MeOH (20 mL) was stirred overnight at ambient temperature. After removal of the solvent in vacuo the precipitate was recrystalized from dichloromethane. [1′,2′,3′,4′,5′,6′-$^{13}C_6$]-(β-D-Glucopyranosyl)-[6-^{13}C,1,3-$^{15}N_2$]-uracil [**10**]: Yield 2.77 (98%); R_f (EE) 0.1; mp 202 °C (MeOH); ^{13}C NMR (50 MHz, DMSO-d_6) δ 60.9 (d, J = 43 Hz, CH6′), 69.6 (t, J = 43 Hz, CH4′), 70.8 (t, J = 43 Hz, CH2′), 76.9 (t, J = 43 Hz, CH3′), 79.9 (t, J = 43 Hz, CH5′), 82.5 (d × d, J^1 = 14.6 Hz, J^2 = 45 Hz, CH1′), 101.3 (d × d, J^1 = 14.6 Hz, J^2 = 66 Hz, 5-CH), 141.0 (d, J = 14.6 Hz, 6-CH), 151.0 ((t), J = 11 Hz, 2-C), 163.3 (d, J = 11 Hz, 4-C); ^{15}N NMR (50 MHz, DMSO-d_6) δ 141.2 (N1), 155.4 (N3); exact mass calcd for C_3*C_7H_{15}*N_2O_7 284.1055, found 284.1061.

Monomethoxytritylation in the 6′ Position: Typical Procedure. A mixture of glucopyranosylnucleoside (i.e.[**10**]) (3.5 mmol) and MMTCl (2.16 g, 7.0 mmol) in pyridine (20 mL) and triethylamine (5 mL) was stirred in an inert atmosphere at ambient temperature for 18 h. After the solvent was removed in vacuo the resulted oil was coevaporated with toluene (4 × 2 mL). The resulting foam was purified by column chromatography (silica, 15 × 3 cm, CH_2Cl_2/MeOH 9:1). [1′,2′,3′,4′,5′,6′-$^{13}C_6$]-6′-*O*-Monomethoxytrityl-(β-D-glucopyranosyl)-[6-^{13}C,1,3-$^{15}N_2$]-uracil [**11**]: Yield 1.8 g (92%); R_f (CH_2Cl_2/MeOH 9:1) 0.31; mp 168−170 °C; ^{13}C NMR (50 MHz, DMSO-d_6) δ 55.1 (s, CH_3O), 63.7 (d, J = 43 Hz, CH6′), 69.6 (t, J = 43 Hz, CH4′), 70.6 (t, J = 43 Hz, CH2′), 76.6 (t, J = 43 Hz, CH3′), 77.9 (t, J = 43 Hz, CH5′), 82.4 (d × d, J^1 = 14.6 Hz, J^2 = 45 Hz, CH1′), 85.8 (C, MMT), 101.3 (d × d, J^1 = 14.6 Hz, J^2 = 66 Hz, 5-CH), 113.3 (s, 2CH, AA′BB′, MMT), 126.9−130.3 (CAr, MMT), 135.4 (s, 1C, AA′BB′, MMT), 141.4 (d, J = 14.6 Hz, 6-CH), 144.7 (s, 2 × 1C, MMT), 151.0 ((t), J = 11 Hz, 2-C), 158.3 (s, 4C, AA′BB′, MMT), 163.3 (d, J = 11 Hz, 4-C); ^{15}N NMR (50 MHz, DMSO-d_6) δ 145.7 (N1), 159.9 (N3); exact mass calcd for C_{23}*C_7H_{29}*$N_2O_8Na_2$ 600.1895, found 600.1864.

Oxidative Ring Opening: Typical Procedure. To a solution of the 1′,6′-disubstituated β-D-glucopyranoside [**11**] (1 mmol) in dry CH_2Cl_2 (10 mL) was added $Pb(OAc)_4$ (1.33 g, 3 mmol) in dry CH_2Cl_2 (10 mL) under nitrogen at ambient temperature. The solution became warm and turned yellow. After 2 h a colorless precipitate of $Pb(OAc)_2$ was formed. Stirring was continued for another 5 h. After the solvent was reduced on a rotavapor the residue was purified by column chromatography (silica, 10 × 2 cm, CH_2Cl_2/MeOH 95:5). The formation of the dialdehyde has been verified with HR-MS, but because of the complicated equilibrium observed in the NMR it is not possible to interpret the NMR spectra in a straightforward way.[25a] [1′,2′,3′,4′,5′-$^{13}C_5$]-5′-*O*-Monomethoxytrityl-[6-^{13}C,1,3-$^{15}N_2$]-uridine dialdehyde [**12**]: Yield 0.49 g (90%); R_f (CH_2Cl_2/MeOH 9:1) 0.44; exact mass calcd for C_{23}*C_6H_{26}*N_2O_8Na 545.1774, found 545.1766.

Reductive Ring Closure: Typical Procedure. Dialdehyde [**12**] (1 mmol) was dissolved in absolute dioxane (8 mL) under nitrogen atmosphere. Tri-*n*-butyltinhydride (0.43 g, 0.4 mL, 1.25 mmol) was added via a septum. The reaction mixture was heated to 90 °C. A solution of AIBN (0.02 g, 0.12 mmol) in dioxane (2 mL) was added via the septum. After being stirred for 30 min at 90 °C the reaction mixture was checked with TLC (CH_2Cl_2/MeOH 95:5). If unreacted aldehyde was monitored on TLC, more AIBN (0.01 g, 0.06 mmol) and tri-*n*-butyltinhydride (0.11 g, 0.1 mL, 0.31 mmol) were added and stirring at 90 °C was continued for another 30 min. After the solvent was evaporated under reduced pressure the resulting foam was dissolved in CH_2Cl_2 (15 mL) and water (5 mL), and after vigorous stirring for another 3 h KF·$x$$H_2O$ (0.2 g) was added to remove all organo-tin compounds, stirring was continued for another 60 min, a white voluminous precipitate was formed, after filtration the organic layer was separated, the filter was washed with CH_2Cl_2, and the collected organic layers were dried and purified by column chromatography (silica, 20 × 3 cm, CH_2Cl_2 (100 mL) → CH_2Cl_2/MeOH 95:5). Final purification to obtain enantiomerically pure nucleosides [**13**] and [**14**], respectively, was carried out with reverse phase HPLC.

[1′,2′,3′,4′,5′-$^{13}C_5$]-5′-Monomethoxytrityl-[6-^{13}C, 1,3-$^{15}N_2$]-uridine [**13**]: Yield 0.32 g, (60%); R_f (CH_2CL_2:MeOH 95:5) 0.41; mp 100−105 °C (CH_2Cl_2); ^{13}C NMR (50 MHz, DMSO-d_6) δ 55.2 (s, CH_3O), 63.0 (d, J = 43 Hz, CH5′), 69.6 (t, J = 43 Hz, CH3′), 73.6 (d × d, J ≈ 43 Hz, CH2′), 82.5 (d × d, J ≈ 43 Hz, CH4′), 89.0 (d × d, J^1 = 12 Hz, J^2 = 45 Hz, CH1′), 86.3 (C, MMT), 101.6 (d × d, J^1= 14.6 Hz, J^2= 66 Hz, 5-CH), 113.5 (s, 2CH, AA′BB′, MMT), 124.1−130.3 (CAr, MMT), 134.8 (s, 1C, AA′BB′, MMT), 140.8 (d, J = 14.6 Hz, 6-CH), 144.1, 144.4 (s, 2 × 1C, MMT), 150.7 ((t), J = 11 Hz, 2-C), 158.5 (s, 4C, AA′BB′, MMT), 163.3 (d, J = 11 Hz, 4-C); ^{15}N NMR (50 MHz, DMSO-d_6) δ 143.5 (N1), 155.2 (N3); exact mass calcd for C_{23}*C_6H_{28}*N_2O_7-Na 547.1930, found 547.1957.

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Supporting Information Available: Spectroscopic data concerning the synthesis of 4-benzoyl-5′-monomethoxytrityl-cytosine [**14**]. This material is available free of charge via the Internet at http://pubs.acs.org.

Note Added after ASAP Posting. The C′2 amd C′3 NMR assignment were incorrect in Figure 3 and the NMR data fo **13** in the version posted January 29, 2003; the correct version was posted February 14, 2003.

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