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Synthesis and Antiviral Activity of 3-Aminoindole Nucleosides of 2-Acetamido-2-deoxy-D-glucose

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A new method for the construction of 3-aminoindole nucleosides of 2-acetamido-2-deoxy-D-glucose based is presented. Nitration and acetylation of the indole nucleosides by acetic anhydride-nitric acid mixture followed by reduction using silver catalyst (SNSM) impregnated on silica gel, afforded the corresponding amino indole nucleosides. The nucleosides were tested for antiviral activity against hepatitis B virus (HBV) to show different degrees of antiviral activities or inhibitory actions.

Key Words : Nucleosides, Nitroindole, Silver nanoparticles, Antiviral activity

Introduction

Metallic nanoparticles, especially silver nanoparticles, have attracted much attention because they have already shown promises in catalysis[1-4] and SERS (surface enhanced Raman scattering) studies.[5]

Indoles are important in both the biological and material sciences.[6] Among the wide variety of privileged indole scaffold structures, the novel 3-aminoindole core appeared only recently. 3-Aminoindole derivatives, though not easily accessible, have nevertheless emerged as promising agents with potential application against a large number of diseases.[7-13] 3-Aminoindole-based compounds are commonly prepared from the corresponding 3-substituted indoles[7,8,14-17] indoxyls,[9,18] and non-indolic precursors.[10-12,19,20]

As a part of the program aimed at the development of new nucleoside derivatives with potential biological activities,[21] we describes herein the new method for the construction of 3-aminoindole nucleosides of 2-acetamido-2-deoxy-D-glucose and its antiviral activity against hepatitis B virus (HBV).

Experimental Section

Melting points were determined using a Büchi apparatus. All solvents were purified according to the standard procedures. TLC was performed on plastic plates Silica Gel 60 F_{245} (E. Merck, layer thickness 0.2 mm). The detection was achieved by treatment with a solution of 15% H_2SO_4 in methanol, and heating at 150 °C. ^{1}H-NMR spectra were recorded with a Varian Gemini spectrometer at 300 MHz with TMS as a standard. Chemical shifts were reported in δ scale (ppm) relative to TMS as a standard, and the coupling constants (*J* values) are given in Hz. EI-mass spectra were recorded with a HP D5988 A 1000 MHz instrument (Hewlett-Packard, Palo Alto, CA, USA). Elemental analyses (C, H and N) were carried out at the Microanalytical Center of Cairo University, Giza, Egypt. The elemental analyses were found to agree favorably with the calculated values. Silver Nanoparticles were prepared according to the published procedures.[5,22-24] 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (**2**) was prepared according to the published procedure.[25]

1-(2'-Acetamido-3',4',6'-tri-*O*-acetyl-2'-deoxy-β-D-glucopyranosyl)-indoles (3a-c). A mixture of indole derivatives (**1a-c**) (5 mmol) and 50% oil-immersed sodium hydride (0.24 g, 5 mmol) in DMF (30 mL) was stirred at 70-80 °C for 1 h and then cooled to room temperature. α-Chloroacetamido sugar **2**[25] (1.83 g, 5 mmol) was added to the mixture, and stirred at 90 °C for 3 h. The mixture was evaporated till dryness under reduced pressure and the residue was recrystallized from absolute ethanol to afford **3a-c**.

1-(2'-Acetamido-3',4',6'-tri-*O*-acetyl-2'-deoxy-β-D-glucopyranosyl) indole (3a): Yield 84%, mp 210-212 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.75 (s, 3 H, NHCOCH_3), 1.99, 2.05, 2.09 (3s, 9 H, 3 COCH_3), 3.88-3.95 (m, 3 H, H-5', H-6'), 4.50 (q, 1 H, *J* = 9.7 Hz, H-2'), 4.90 (d, 1 H, *J* = 9.0 Hz, H-4'), 5.30 (t, 1 H, *J* = 9.6 Hz, H-3'), 5.66 (d, 1 H, *J* = 9.4 Hz, H-1'), 7.35-7.67 (m, 7 H, N*H*COCH$_3$, Ar-H); MS *m/z* 446 (M^+); Anal. Calcd. for $C_{22}H_{26}N_2O_8$: C, 59.19; H, 5.87; N, 6.27. Found: C, 59.03; H, 5.59; N, 6.07.

1-(2'-Acetamido-3',4',6'-tri-*O*-acetyl-2'-deoxy-β-D-glucopyranosyl)-6-bromoindole (3b): Yield 82%, mp 264-266 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.75 (s, 3 H, NHCOCH_3), 2.05, 2.07, 2.11 (3s, 9H, 3 COCH_3), 3.81-3.98 (m, 3 H, H-5', H-6'), 4.55 (m, 1 H, H-2'), 4.70-4.81 (m, 2 H, H-3', H-4'), 5.59 (d, 1 H, *J* = 9.4 Hz, H-1'), 7.66-7.88 (m, 4 H, N*H*COCH$_3$, Ar-H), 8.05 (s, 1 H, H-7); MS *m/z* 524/526 (M^+); Anal. Calcd. for $C_{22}H_{25}BrN_2O_8$: C, 50.30; H, 4.80; N, 5.33. Found: C, 50.13; H, 4.64; N, 5.11.

1-(2'-Acetamido-3',4',6'-tri-*O*-acetyl-2'-deoxy-β-D-glucopyranosyl)-6-chloroindole (3c): Yield 80%, mp 240-242

°C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.77 (s, 3 H, NHCOCH_3), 2.04, 2.06, 2.12 (3s, 9 H, 3 COCH_3), 3.78-3.95 (m, 3 H, H-5', H-6'), 4.58 (m, 1 H, H-2'), 4.66-4.78 (m, 2 H, H-3', H-4'), 5.60 (d, 1 H, J = 9.4 Hz, H-1'), 7.65-7.85 (m, 4 H, NHCOCH$_3$, Ar-H), 8.06 (s, 1 H, H-7); MS *m/z* 480/482 (M^+). Anal. Calcd. for $C_{22}H_{25}ClN_2O_8$: C, 54.95; H, 5.24; N, 5.83. Found: C, 54.83; H, 5.03; N, 5.60.

1-(2'-Acetamido-3',4',6'-tri-*O*-acetyl-2'-deoxy-β-D-glucopyranosyl)-3-nitroindoles (4a-c). Acetyl nitrate was generated by the dropwise addition of neat yellow 90% HNO_3 (1.35 mL, 30 mmol) to Ac_2O (20 mL) which cooled at (0 °C) followed by standing at room temperature for 10 min and was used immediately. To a stirred solution of **3a-c** (1 mmol) in Ac_2O (2 mL) cooled at –70 °C was added a solution of the acetyl nitrate dropwise *via* addition funnel over 30 min. The mixture was then allowed to warm to room temperature with stirring overnight. The reaction mixture was poured on crushed ice and then extracted with CH_2C1_2 (100 mL). The solvent was dried over Na_2SO_4, evaporated, and coevaporated with toluene to afford a yellow solid which was purified by recrystallization using absolute ethanol to afford **4a-c**.

1-(2'-Acetamido-3',4',6'-tri-*O*-acetyl-2'-deoxy-β-D-glucopyranosyl)-3-nitroindole (4a): Yield 93%, mp 225-227 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.77 (s, 3 H, NHCOCH_3), 2.06, 2.08, 2.12 (3s, 9H, 3 COCH_3), 3.34-3.49 (m, 2 H, H-6'), 3.69-3.75 (m, 2 H, H-4', H-5'), 3.96-4.12 (m, 1 H, H-3'), 4.18-4.29 (m, 1 H, H-2'), 5.78 (s, 1 H, H-1'), 7.35-7.67 (m, 5 H, NHCOCH$_3$, Ar-H), 7.88 (s, 1 H, H-2); MS *m/z* 491 (M^+). Anal. Calcd. for $C_{22}H_{25}N_3O_{10}$: C, 53.77; H, 5.13; N, 8.55. Found: C, 53.60; H, 5.01; N, 8.32.

1-(2'-Acetamido-3',4',6'-tri-*O*-acetyl-2'-deoxy-β-D-glucopyranosyl)-6-bromo-3-nitroindole (4b): Yield 95%, mp 209-211 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.76 (s, 3 H, NHCOCH_3), 2.06, 2.08, 2.10 (3s, 9H, 3 COCH_3), 3.28-3.39 (m, 2 H, H-6'), 3.54-3.59 (m, 2 H, H-4', H-5'), 3.87 (m, 1 H, H-3'), 4.12 (m, 1 H, H-2'), 5.49 (s, 1 H, H-1'), 7.66-7.88 (m, 3 H, NHCOCH$_3$, Ar-H), 7.92 (s, 1 H, H-2), 8.05 (s, 1 H, H-7); MS *m/z* 569/571 (M^+). Anal. Calcd. for $C_{22}H_{24}BrN_3O_{10}$: C, 46.33; H, 4.24; N, 7.37. Found: C, 46.11; H, 4.09; N, 7.10.

1-(2'-Acetamido-3',4',6'-tri-*O*-acetyl-2'-deoxy-β-D-glucopyranosyl)-6-chloro-3-nitroindole (4c): Yield 90%, mp 233-235 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.74 (s, 3 H, NHCOCH_3), 2.06, 2.08, 2.13 (3s, 9H, 3 COCH_3), 3.28-3.39 (m, 2 H, H-6'), 3.58-3.63 (m, 2 H, H-4', H-5'), 3.89 (m, 1 H, H-3'), 4.15 (m, 1 H, H-2'), 5.53 (s, 1 H, H-1'), 7.65-7.85 (m, 3 H, NHCOCH$_3$, Ar-H), 7.98 (s, 1 H, H-2), 8.06 (s, 1 H, H-7); MS *m/z* 525/527 (M^+). Anal. Calcd. for $C_{22}H_{24}ClN_3O_{10}$: C, 50.25; H, 4.60; N, 7.99. Found: C, 50.05; H, 4.40; N, 7.77.

Procedure for the Reduction of Nitro-Compounds using Solid Silver as Catalyst, SNSM to Afford 1-(2'-Acetamido-β-D-glucopyranosyl)-3-aminoindoles (5a-c).

Step (i): Compounds **4a-c** (0.5 mmol) in a stirred mixture of methanol (5 mL) and ammonium hydroxide (25%) (5 mL) were stirred at room temperature for 2 h. The resulting solution was evaporated till dryness under reduced pressure.

Step (ii): In a standard quartz cuvette having a 1-cm path length, 2 mL of water and 20 μL of nitrocompounds (resulting from step i) (final concentration in solution: 4.3×10^{-4} M) were taken. To it was added 300 μL of aqueous $NaBH_4$ (0.1 M). It took 5 min for the peak of the colorless product to appear (induction time, IT) in the blue region after the addition of (0.0053 g) solid silica gel impregnated silver catalyst, SNSM.[6] Then the gradual decoloration of the yellow solution (due to nitrocompounds) and the formation of the product were observed through UV–visible spectrophotometry (Fig. 1). After the yellow color was completely discharged, i.e., the completed reaction was, the peak due to nitrocompounds was no longer observed. On the other hand, the appearance of a new peak at 430 nm was noticed 5 min after of the completion of the reaction. The solvent was evaporated under reduced pressure and the residue was dissolved in absolute ethanol and left at room temperature for overnight to afford **5a-c** as a pale yellow crystals.

1-(2'-Acetamido-3',4',6'-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3-aminoindole (5a): Yield 93%, mp 217-219 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.71 (s, 3 H, NHCOCH_3), 3.22-3.38 (m, 2 H, H-6), 3.62-3.79 (m, 2 H, H-4, H-5), 3.85-3.89 (m, 1 H, H-3), 4.12-4.22 (m, 1 H, H-2), 5.37-5.42 (m, 3 H, 3 OH), 5.69 (s, 1 H, H-1), 6.27 (s, 1H, NH_2), 7.12-7.51 (m, 5 H, NHCOCH$_3$, Ar-H); MS *m/z* 335 (M^+). Anal. Calcd. for $C_{16}H_{21}N_3O_5$: C, 57.30; H, 6.31; N, 12.53. Found: C, 57.11; H, 6.16; N, 12.34.

1-(2'-Acetamido-3',4',6'-tri-*O*-acetyl-2'-deoxy-β-D-glucopyranosyl)-3-amino-6-bromoindole (5b): Yield 90%, mp 270-272 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.68 (s, 3 H, NHCOCH_3), 3.22-3.32 (m, 2 H, H-6'), 3.45-3.54 (m, 2 H, H-4', H-5'), 3.80-3.95 (m, 1 H, H-3'), 4.19-4.23 (m, 1 H, H-2'), 5.35-5.45 (m, 3 H, 3 OH), 5.69 (s, 1 H, H-1'), 6.25 (s, 1H, NH_2), 7.19 (s, 1H, H-2), 7.75-7.99 (m, 3 H, NHCOCH$_3$, Ar-H), 8.52 (s, 1 H, H-8); MS *m/z* 413/415 (M^+). Anal. Calcd. for $C_{16}H_{20}BrN_3O_5$: C, 46.39; H, 4.87; N, 10.14. Found: C, 46.22; H, 4.60; N, 10.00.

1-(2'-Acetamido-3',4',6'-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3-amino-6-chloroindole (5c): Yield 90%,

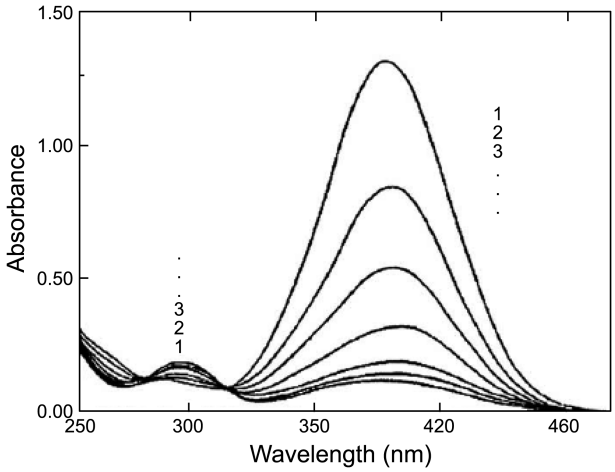


Figure 1. UV-visible spectra for the successive reduction of 3-Aminoindoles by SNSM as catalyst.

mp 248-250 ºC; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.75 (s, 3 H, NHCOCH_3), 3.28-3.39 (m, 2 H, H-6'), 3.60-3.77 (m, 2 H, H-4', H-5'), 3.84 (m, 1 H, H-3'), 4.18 (m, 1 H, H-2'), 5.35-5.39 (m, 3 H, 3 OH), 5.69 (s, 1 H, H-1'), 6.29 (s, 1H, NH_2), 7.17 (s, 1 H, H-2), 7.38-7.69 (m, 4 H, N*H*COCH$_3$, Ar-H); MS *m/z* 369/371 (M^+). Anal. Calcd. for $C_{16}H_{20}ClN_3O_5$: C, 51.97; H, 5.45; N, 11.36. Found: C, 51.82; H, 5.31; N, 11.22.

1-(2'-Acetamido-β-D-glucopyranosyl)-3-nitro-6-substitutes amino-indoles (6-11). A solution of **4b** (0.570 g, 1 mmoL) and an excess of the appropriate amine was stirred under reflux for 2 h. The solvent was removed *in vacuo* and the residue was recrystallized from ethanol to give **6-11**.

1-(2'-Acetamido-β-D-glucopyranosyl)-6-methylamino-3-nitroindole (6): Yield 78%, mp 280-282 ºC; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.76 (s, 3 H, NHCOCH_3), 2.65 (s, 3 H, NHCH_3), 3.30-3.43 (m, 2 H, H-6'), 3.55-3.65 (m, 2 H, H-4', H-5'), 3.89-3.99 (m, 1 H, H-3'), 4.16 (m, 1 H, H-2'), 5.19-5.27 (m, 3 H, 3 OH), 5.51 (s, 1 H, H-1'), 6.09-6.26 (m, 2 H, H-5, H-7), 7.05 (m, 1 H, N*H*CH$_3$), 7.80-7.88 (m, 3 H, N*H*COCH$_3$, H-2, H-4); MS *m/z* 394 (M^+). Anal. Calcd. for $C_{17}H_{22}N_4O_7$: C, 51.77; H, 5.62; N, 14.21. Found: C, 51.60; H, 5.39; N, 14.07.

1-(2'-Acetamido-β-D-glucopyranosyl)-6-ethylamino-3-nitroindole (7): Yield 80%, mp 243-245 ºC; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.11 (t, 3 H, J = 7.1 Hz, CH$_3$), 1.89 (s, 3 H, NHCOCH_3), 3.35-3.45 (m, 4 H, H-6', NHCH_2), 3.64-3.75 (m, 2 H, H-4', H-5'), 3.99 (m, 1 H, H-3'), 4.23 (m, 1 H, H-2'), 5.31-5.37 (m, 3 H, 3 OH), 5.73 (s, 1 H, H-1'), 6.06-6.25 (m, 2 H, H-5, H-7), 6.98 (m, 1 H, N*H*CH$_2$), 7.78-7.89 (m, 3 H, N*H*COCH$_3$, H-2, H-4); MS *m/z* 408 (M^+). Anal. Calcd. for $C_{18}H_{24}N_4O_7$: C, 52.94; H, 5.92; N, 13.72. Found: C, 52.66; H, 5.78; N, 13.66.

1-(2'-Acetamido-β-D-glucopyranosyl)-3-nitro-6-propyl-aminoindole (8): Yield 79%, mp 270-272 ºC; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 0.89 (t, 3 H, J = 7.1 Hz, CH$_3$), 1.36 (m, 2 H, CH$_2$), 1.75 (s, 3 H, NHCOCH_3), 3.22-3.40 (m, 4 H, H-6', NHCH_2), 3.62-3.78 (m, 2 H, H-4', H-5'), 3.85-3.93 (m, 1 H, H-3'), 4.12-4.28 (m, 1 H, H-2'), 5.37-5.48 (m, 3 H, 3 OH), 5.71 (s, 1 H, H-1'), 6.07-6.18 (m, 2 H, H-5, H-7), 7.21 (m, 1 H, N*H*CH$_2$), 7.75-7.88 (m, 3 H, N*H*COCH$_3$, H-2, H-4); MS *m/z* 422 (M^+). Anal. Calcd. for $C_{19}H_{26}N_4O_7$: C, 54.02; H, 6.20; N, 13.26. Found: C, 53.90; H, 6.10; N, 13.10.

1-(2-Acetamido-β-D-glucopyranosyl)-6-benzylamino-3-nitroindole (9): Yield 85%, mp 223-225 ºC; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.68 (s, 3 H, NHCOCH_3), 3.22-3.36 (m, 2 H, H-6'), 3.45-3.59 (m, 2 H, H-4', H-5'), 3.80-3.97 (m, 1 H, H-3'), 4.19-4.25 (m, 1 H, H-2'), 4.49 (s, 2 H, CH$_2$), 5.35-5.48 (m, 3 H, 3 OH), 5.74 (s, 1 H, H-1'), 7.19-7.35 (m, 8 H, N*H*CH$_2$, Ar-H), 7.74-7.81 (m, 3 H, N*H*COCH$_3$, H-2, H-4); MS *m/z* 470 (M^+). Anal. Calcd. for $C_{23}H_{26}N_4O_7$: C, 58.72; H, 5.57; N, 11.91. Found: C, 58.60; H, 5.31; N, 11.67.

1-(2'-Acetamido-β-D-glucopyranosyl)-3-nitro-6-pyrro-lidinoindole (10): Yield 83%, mp 209-211 ºC; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.26-1.38 (m, 4 H, 2 x CH$_2$), 1.76 (s, 3 H, NHCOCH_3), 3.28-3.41 (m, 6 H, H-6', 2 x NCH$_2$), 3.60-3.79 (m, 2 H, H-4', H-5'), 3.85 (m, 1 H, H-3'), 4.19 (m, 1 H, H-2'), 5.33-5.41 (m, 3 H, 3 OH), 5.66 (s, 1 H, H-1'), 6.09-6.28 (m, 2 H, H-5, H-7), 7.72-7.83 (m, 3 H, N*H*COCH$_3$, H-2, H-4); MS *m/z* 434 (M^+). Anal. Calcd. for $C_{20}H_{26}N_4O_7$: C, 55.29; H, 6.03; N, 12.90. Found: C, 55.20; H, 5.90; N, 12.78.

1-(2'-Acetamido-β-D-glucopyranosyl)-6-morpholino-3-nitroindole (11): Yield 84%, mp 221-223 ºC; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.74 (s, 3 H, NHCOCH_3), 3.24-3.36 (m, 6 H, H-6', 2 x NCH$_2$), 3.53-3.68 (m, 6 H, H-4', H-5', 2 x OCH$_2$), 4.15-4.27 (m, 1 H, H-3'), 4.30-4.43 (m, 1 H, H-2'), 5.25-5.36 (m, 3 H, 3 OH), 5.64 (s, 1 H, H-1'), 6.04-6.23 (m, 2 H, H-5, H-7), 7.75-7.80 (m, 3 H, N*H*COCH$_3$, H-2, H-4); MS *m/z* 450 (M^+). Anal. Calcd. for $C_{20}H_{26}N_4O_8$: C, 53.33; H, 5.82; N, 12.44. Found: C, 53.22; H, 5.70; N, 12.22.

Procedure for the Reduction of Nitro-Compounds using Solid Silver as Catalyst, SNSM to Afford 1-(2'-Acetamido-β-D-glucopyranosyl)-3-amino-6-substituted aminoindoles (12-17). Compounds **12-17** were prepared according to **step (ii)** as mentioned in the preparation of **5a-c**.

1-(2-Acetamido-β-D-glucopyranosyl)-3-amino-6-methyl-aminoindole (12): Yield 93%, mp 247-249 ºC; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.74 (s, 3 H, NHCOCH_3), 2.68 (s, 3 H, NHCH_3), 3.34-3.45 (m, 2 H, H-6'), 3.56-3.68 (m, 2 H, H-4', H-5'), 3.85-3.93 (m, 1 H, H-3'), 4.17 (m, 1 H, H-2'), 5.15-5.29 (m, 3 H, 3 OH), 5.53 (s, 1 H, H-1'), 6.15-6.40 (m, 4H, H-5, H-7, NH_2) 7.05-7.19 (m, 2 H, N*H*CH$_3$, H-2), 7.80-7.83 (m, 2 H, N*H*COCH$_3$, H-4); MS *m/z* 364 (M^+). Anal. Calcd. for $C_{17}H_{24}N_4O_5$: C, 56.03; H, 6.64; N, 15.38. Found: C, 55.86; H, 6.52; N, 15.22.

1-(2'-Acetamido-β-D-glucopyranosyl)-3-amino-6-ethyl-aminoindole (13): Yield 92%, mp 217-219 ºC; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.13 (t, 3 H, J = 7.1 Hz, CH$_3$), 1.84 (s, 3 H, NHCOCH_3), 3.35-3.42 (m, 4 H, H-6', NHCH_2), 3.60-3.71 (m, 2 H, H-4', H-5'), 3.90 (m, 1 H, H-3'), 4.20 (m, 1 H, H-2'), 5.31-5.39 (m, 3 H, 3 OH), 5.78 (s, 1 H, H-1'), 6.11-6.37 (m, 4H, H-5, H-7, NH_2) 7.08-7.22 (m, 2 H, N*H*CH$_3$, H-2), 7.78-7.89 (m, 2 H, N*H*COCH$_3$, H-4); MS *m/z* 378 (M^+). Anal. Calcd. for $C_{18}H_{26}N_4O_5$: C, 57.13; H, 6.93; N, 14.81. Found: C, 57.00; H, 6.82; N, 14.62.

1-(2'-Acetamido-β-D-glucopyranosyl)-3-amino-6-prop-ylaminoindole (14): Yield 91%, mp 233-235 ºC; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 0.87 (t, 3 H, J = 7.1 Hz, CH$_3$), 1.34 (m, 2 H, CH$_2$), 1.71 (s, 3 H, NHCOCH_3), 3.25-3.41 (m, 4 H, H-6', NHCH_2), 3.60-3.83 (m, 2 H, H-4', H-5'), 3.88-3.99 (m, 1 H, H-3'), 4.10-4.25 (m, 1 H, H-2'), 5.34-5.45 (m, 3 H, 3 OH), 5.73 (s, 1 H, H-1'), 6.15-6.40 (m, 4H, H-5, H-7, NH_2) 7.05-7.23 (m, 2 H, N*H*CH$_2$, H-2), 7.80-7.83 (m, 2 H, N*H*COCH$_3$, H-4); MS *m/z* 392 (M^+). Anal. Calcd. for $C_{19}H_{28}N_4O_5$: C, 58.15; H, 7.19; N, 14.28. Found: C, 58.00; H, 7.04; N, 14.11.

1-(2'-Acetamido-β-D-glucopyranosyl)-3-amino-6-benz-ylaminoindole (15): Yield 94%, mp 212-214 ºC; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.71 (s, 3 H, NHCOCH_3), 3.24-3.38 (m, 2 H, H-6'), 3.43-3.58 (m, 2 H, H-4', H-5'), 3.79-3.99 (m, 1 H, H-3'), 4.17-4.24 (m, 1 H, H-2'), 4.45 (s, 2 H, CH$_2$), 5.35-5.45 (m, 3 H, 3 OH), 5.74 (s, 1 H, H-1'), 6.15-6.40 (m, 4H, H-5, H-7, NH_2) 7.11-7.26 (m, 7 H, N*H*CH$_2$, H-2, Ar-H), 7.80-7.83 (m, 2 H, N*H*COCH$_3$, H-4); MS *m/z* 440 (M^+). Anal. Calcd. for $C_{23}H_{28}N_4O_5$: C, 62.71; H, 6.41; N, 12.72.

Found: C, 62.50; H, 6.30; N, 12.60.

1-(2'-Acetamido-β-D-glucopyranosyl)-3-amino-6-pyrrolidinoindole (16): Yield 93%, mp 244-246 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.26-1.35 (m, 4 H, 2 x CH_2), 1.75 (s, 3 H, NHCOCH_3), 3.28-3.45 (m, 6 H, H-6', 2 x NCH_2), 3.55-3.75 (m, 2 H, H-4', H-5'), 3.83 (m, 1 H, H-3'), 4.21 (m, 1 H, H-2'), 5.33-5.44 (m, 3 H, 3 OH), 5.65 (s, 1 H, H-1'), 6.15-6.37 (m, 4H, H-5, H-7, NH_2), 7.05-7.15 (m, 1 H, H-2), 7.77-7.87 (m, 2 H, N*H*COCH_3, H-4); MS *m/z* 404 (M^+). Anal. Calcd. for $C_{20}H_{28}N_4O_5$: C, 59.39; H, 6.98; N, 13.85. Found: C, 59.12; H, 6.72; N, 13.66.

1-(2'-Acetamido-β-D-glucopyranosyl)-3-amino-6-morpholinoindole (17): Yield 94%, mp 210-212 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.70 (s, 3 H, NHCOCH_3), 3.24-3.30 (m, 6 H, H-6', 2 x NCH_2), 3.50-3.65 (m, 6 H, H-4', H-5', 2 x OCH_2), 4.15-4.25 (m, 1 H, H-3'), 4.30-4.45 (m, 1 H, H-2'), 5.25-5.35 (m, 3 H, 3 OH), 5.65 (s, 1 H, H-1'), 6.11-6.33 (m, 4H, H-5, H-7, NH_2) 7.03-7.18 (m, 2 H, N*H*CH_3, H-2), 7.75-7.87 (m, 2 H, N*H*COCH_3, H-4); MS *m/z* 420 (M^+). Anal. Calcd. for $C_{20}H_{28}N_4O_6$: C, 57.13; H, 6.71; N, 13.33. Found: C, 57.02; H, 6.60; N, 13.21.

Antiviral Testing

The HepG2.2.2.15 cell line, supplied by State Serum Institute, Denmark, was maintained in RPMI-1640 Glutamax, Gibco BRL Life technologies.[26,27] The standard drug Lamivudine was from GlaxoSmithKline. The cell line was maintained in RPMI-1640 (Glutamax) culture medium containing 100 IU/mL nystatin, 380 μg/mL G418 (genetecin) and 10% fetal calf serum (FCS) (Gibco BRL Life Technologies). The transferred HEPG2.2.2.15 cells were kept in a tissue culture flask at 37 °C and 5% CO_2. Subcultures were set up after a week by trypsination (10% versin/trypsin (Biochrome KG) and transferred to a 96-well tissue culture plate. 5-Fold serial dilutions of tested compounds with final concentrations ranging from 100 to 0.03 μM were added to the cell suspension and incubated for 6 d at 37 °C and 5% CO_2. Each compound was tested in triplicate. Cells with no compounds added to their culture were used for comprison (blank cells).

DNA Extraction. DNA extraction was done by incubating 10 μL of diluted supernatant with 10 μL of 0.2 M NaOH at 37 °C for 1 h, then carefully adding 9.6 μL of 0.2M HCl followed by addition of 90 μL of Tris–EDTA buffer [(2-amino-2-(hydroxymethyl)-1,3-propanediol–EDTA) (Gibco BRL Life Technologies)].

PCR-ELISA Detection of HBV DNA. The DNA content in the cell culture supernatant was determined by polymerase chain reaction amplification of the HBV DNA using 1 μmol/L of each of the following primers: HCID-1 primer (5'-GGAAAGAAGTCAGAAGGCA-3') and HCID-2 primer (5'-TTGGGGGAGGAGATTAGGTT-3'), in a reaction mixture containing 14 μL extracted supernatant, 4 mmol/L $MgCl_2$, 10 μmol/L DIG-11-dUTP (Roche, Germany), 190 μmol/L dTTP, 200 μmol/L dATP, dGTP, dCTP (Roche) 1.5 U Taq polymerase (Roche), in a total volume 50 μL. PCR reaction conditions were: 32 cycles of 10 min at 94 °C, 30 s at 58 °C, and 30 s at 72 °C with a 3 s increment for each cycle in a Perkin Elmer 480 thermal cycler (Perkin Elmer, USA). The PCR product was detected by DIG-ELISA assay (Roche). The optical density from DNA of the test compound was compared to that of the blank culture.[28,29]

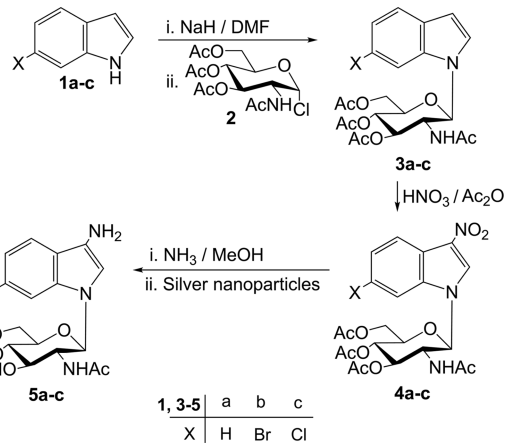
Cytotoxicity Assay. 3-(3,5-Dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (Sigma, USA) is a colorless substrate that is transformed to a colored product by living cells, but not by dead cells. The assay utilizes this compound to test for the viability of the cells with the test compound added compared to the viability of the blank cells.[30]

Results and Discussion

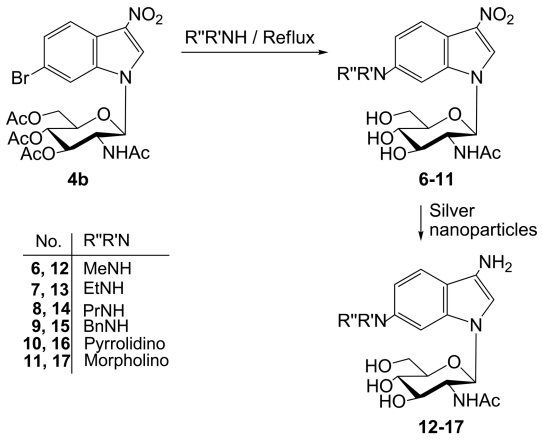
The ease of accessibility and the biological significance of 2-acetamido-2-deoxy-D-glucose[31-33] have prompted us to use this aminosugar as a starting material in Sasaki glycosylation reaction.[34] Thus, the sodium salt of indole derivatives **1a-c** was condensed with 2-acetamido-1-chloro-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucose (**2**)[25] in dry DMF. The reaction was proceeded at 90 °C to give the desired 1-(2'-acetamido-3',4',6'-tri-*O*-acetyl-2'-deoxy-β-D-glucopyranosyl)indoles (**3a-c**) (Scheme 1).

The structure of the nucleosides **3a-c** was determined on the basis of its respective ^{1}H-NMR, mass spectra and microanalyses, which was found to be consistent with the assigned structure by comparison with the structures of glycopyranoside analogues.[35-37] The ^{1}H-NMR spectrum of **3a-c** showed the anomeric proton peaks at δ 5.59-5.66 ppm as a doublet with $J_{1',2'}$ coupling constant of 9.4 Hz. Coupling constant of 9.4 Hz in sugar moieties normally results from the diaxial orientation of H-1' and H-2' protons, which is clearly indicative of the β-configuration of the products.[35-37] The methyl protons of the acetamido group (NHCOCH_3) appeared as singlet at δ 1.75-1.77 ppm, while the other three acetyl groups appeared as singlet at δ 1.99-2.12, respectively.

Nitration of **3a-c** using acetic anhydride-nitric acid mixture afforded the corresponding 3-nitroindole nucleoside derivatives **4a-c**. The ^{1}H-NMR spectra showed a singlet at δ 7.88-7.98 ppm corresponding to H-2. Treatment of **4a-c** with ammonium hydroxide (25%) in methanol at room temper-



Scheme 1. Synthesis of nucleosides **3-5**.



Scheme 2. Synthesis of compounds **6-17**.

ature resulted in the aminolysis of three acetyl groups and the nitro group of the crude intermediates were reduced directly by solid silica gel impregnated silver catalyst (SNSM) to afford the corresponding amino indole nucleosides **5a-c**.

Treatement of **4b** with different primary and secondary amines at reflux temperature afforded the corresponding 3-nitro-6-substituted aminoindoles **6-11**, respectively. Reduction of the 3-nitro group again by solid silica gel impregnated silver catalyst (SNSM) provided the new amino indole nucleosides **12-17**.

The compounds were tested for their antiviral activity and cytotoxicity against HBV using the HepG2.2.2.15 cell line, a human hepatoma cell line producing HBV viral particles.[26,27] The drug Lamivudine (4-amino-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2(1*H*)-one), a potent selective inhibitor of HBV replication,[28] has been used as a standard positive control. The 50% inhibitory concentration (IC_{50}) of an antiviral drug was determined by plotting the DNA content in the cell culture supernatant *versus* the concentration of the test compound. The 50% cytotoxic effect (CC_{50}) was calculated from the average viability of the cells in proportion to the concentration of the drug; for all the tested compounds its value was 100 μM. The selectivity

Table 1. Cytotoxic effect (CC_{50})[a] and inhibitory concentration (IC_{50}) of newly synthesized compounds

Compd	HBV DNA Hep2.2.2.15 IC_{50} [μM]	CC_{50} [μM]	Compd	HBV DNA Hep2.2.2.15 IC_{50} [μM]	CC_{50} [μM]
Lamivudine	0.1	1000.0	**7**	0.9	111.1
3a	1.3	76.9	**8**	0.5	200.0
3b	1.4	71.4	**9**	0.8	156.2
3c	0.3	333.3	**10**	0.5	200.0
4a	0.5	200.0	**11**	0.4	250.0
4b	0.5	200.0	**12**	0.9	111.1
4c	0.4	250.0	**13**	0.8	156.2
5a	0.3	333.3	**14**	0.4	250.0
5b	0.4	250.0	**15**	1.4	71.4
5c	0.3	333.3	**16**	0.2	500.0
6	0.2	500.0	**17**	0.3	333.3

[a]Cytotoxic effect (CC_{50}) of all tested compounds is 100 μM.

index (SI) was calculated as CC_{50}/IC_{50}.[29,30] The results of the antiviral activity measurements against HBV are shown in the Table 1. Preliminary screening indicated that compound **6** and **16** showed the highest inhibitory activity against HBV among this series of tested compounds with low cytotoxicity and a selectivity index of 2500.0 followed by compounds **3c**, **5a**, **5c** and **17**. Compounds **4a-c**, **5b**, **8**, **10**, **11** and **14** showed moderate inhibition with moderate cytotoxicity while the other tested compounds exhibited less activity against HBV.

Conclusions

In conclusion, a new and versatile approach to the synthesis of 3-aminoindole nucleosides of 2-acetamido-2-deoxy-D-glucose was established. The use of modified heterocyclic base in nucleoside synthesis as well as the convenient experimental conditions merits the efficiency of this approach.

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