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Synthesis and Cytotoxicities of Mannose Conjugated *S*-Nitroso-*N*-acetylpenicillamine (SNAP)

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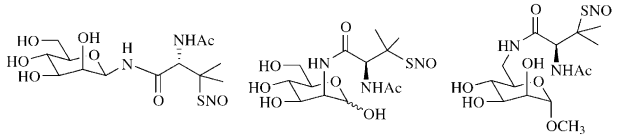
Abstract—A series of mannose conjugated *S*-nitroso-*N*-acetylpenicillamines (SNAPs) has been synthesized, and their cytotoxicities were assessed for DU 145 human prostate cancer cells and Hela R cancer cells.

In the last few years, *S*-nitrosothiols (RSNOs) have been paid special attention because they play important roles in nitric oxide (NO) storage and transport in a wide range of physiological and pathophysiological processes.[1–3] Recently, we have reported that fructose and glucose conjugates of SNAP are more stable than SNAP and they exhibit much stronger effects on killing cancer cell lines.[4–7] For example, Glu-2-SNAP has been shown to be 5000-fold more potent than SNAP in killing A2780S human ovarian cancer cells.[6] The enhanced cytotoxicity can be explained by the expression of GLUT-1 transporter in the cancer cells. The biological activities of glyco-SNAPs are also being investigated in detail by other researchers. Babich et al. have evaluated the cytotoxicities of glucose-1-SNAP, glucose-2-SNAP and fructose-1-SNAP towards the human gingival epithelioid S-G cell line and three human carcinoma cell lines derived from tissues of the oral cavity. They found that glucose-SNAPs are more cytotoxic than SNAP. They also reached a conclusion that the death of S-G cells exposed to glucose-2-SNAP apparently occurred by apoptosis.[8] Incubation of aldose reductase (AR) with glyco-SNAP results in an increase in enzyme activity which is accompanied by the nitrosation of the active-site residue, Cys-298, and the formation of a mixed disulfide with glyco-AP.[9] Treatment of brain-derived cells with glyco-1-SNAP can inhibit the DNA binding activity of transcription factor AP-1 through a NO-dependent mechanism.[10]

Herein, we will report the syntheses and cytotoxicities of three mannose SNAPs: Man-1-SNAP, Man-2-SNAP and Man-6-SNAP (Fig. 1).

Synthesis and Characterization

The synthetic routes toward mannose SNAPs are illustrated in Scheme 1. The 3-acetamido-4, 4-dimethylthietan-2-one (cyclic AP) was synthesized according to our previous method.[4] The β-1-mannosylamine was prepared following known precedure.[11–13] Coupling of β-1-mannosylamine with cyclic AP in pyridine provided Man-1-AP (**2**). Subsequent nitrosation of **2** with $NaNO_2$/HCl gave the Man-1-SNAP in quantitative yield. The Man-2-SNAP was synthesized by nitrosation of Man-2-AP (**4**), which was prepared by coupling the D-mannosamine to cyclic-AP in DMF. The compound **4** is a mixture of α- and β-anomers with a mole ratio of 13:5 as indicated by ^1H and ^{13}C NMR. Finally, the Man-6-AP was synthesized by a relatively long procedure. The 6-OH group of methyl-α-D-mannopyranoside (**5**) was tosylated with tosyl chloride in pyridine. Nucleophilic substitution of the tosyl group with NaN_3



Man-1-SNAP **Man-2-SNAP** **Man-6-SNAP**

Figure 1. Structures of Man-SNAPs.

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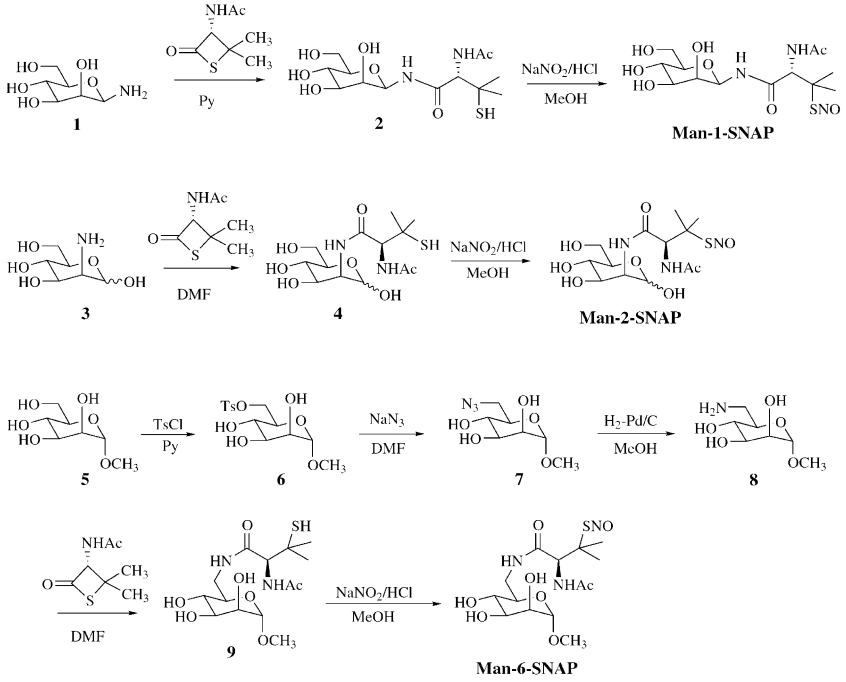

Scheme 1. Synthetic methodology toward Man-1-SNAP, Man-2-SNAP, and Man-6-SNAP.

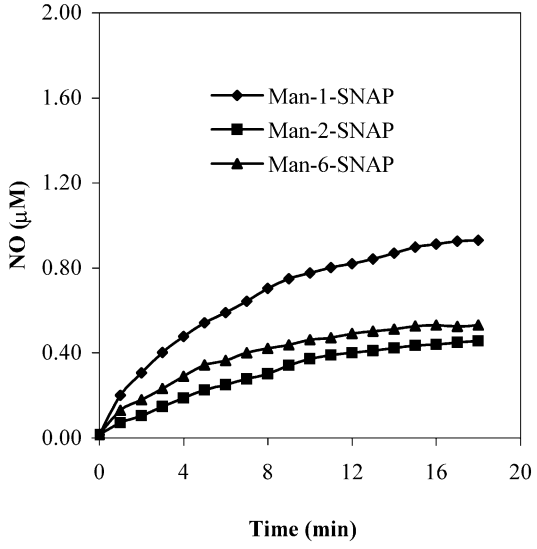

Figure 2. NO generation by a 0.2 mM solution of mannose SNAPs in a phosphate buffer (0.1 M, pH 7.4) and 1.0 mM EDTA.

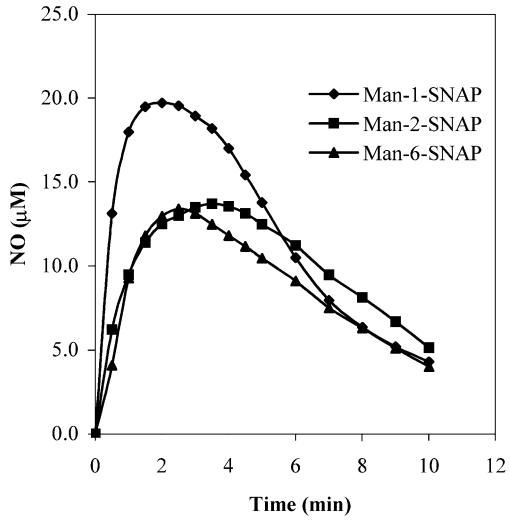

Figure 3. NO generation by a 0.2 mM solution of mannose SNAPs in a phosphate buffer (0.1 M, pH 7.4) and 50 μM Cu^{2+}.

in DMF at 60 C, followed by Pd/C catalyzed hydrogenation, provided 6-amino-α-D-methyl mannoside (**8**). Coupling of **8** with cyclic-AP gave Man-6-AP (**9**), which was further nitrosated to give Man-6-SNAP. The configuration of the anomeric carbon was established by 2-D NOSEY analysis. Man-1-SNAP was assigned as β-anomer, based on the fact that there was strong NOE between H-1 and H-3, as well as between H-1 and H-5.

Nitric Oxide Measurement

NO measurement was carried out with an Electrochemical ISO-NO Mark II Isolated Nitric Oxide Meter, a product of World Precision Instruments, Inc. (Sarasota, FL, USA). The sample was dissolved in small amount of methanol, one portion of the solution was then injected into the sealed O_2-free aqueous buffer solutions. Figure 2 shows the release of NO by a 0.2 mM solution of mannose SNAP in a phosphate buffer solution (0.1 M, pH 7.4) and 1.0 mM EDTA.

Figure 3 illustrates the NO release profiles of mannose SNAPs in a phosphate buffer solution containing Cu^{2+} but without EDTA. The results indicate that the NO release rate could be accelerated about 20- to 30-fold when the Cu^{2+} was presented in the buffer solution. Among the three tested compounds, Man-1-SNAP released NO most efficiently.

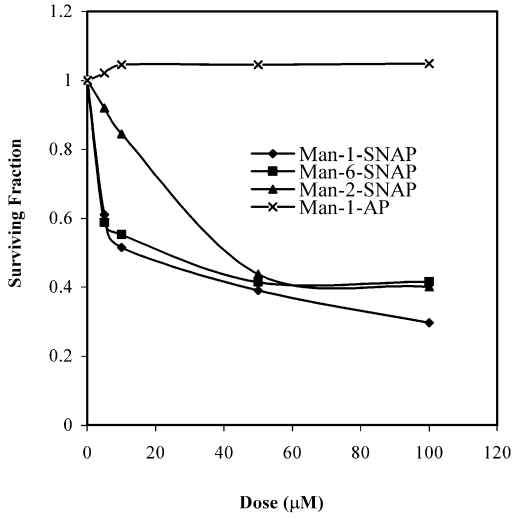

Figure 4. The dose-dependent cytotoxicity of mannose SNAPs against DU-145 human prostate cancer cells.

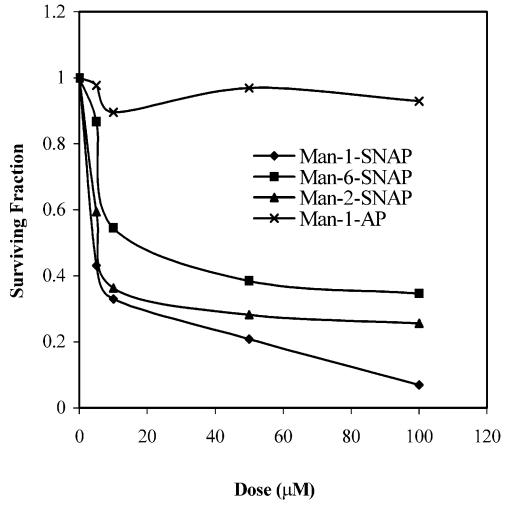

Figure 5. The dose-dependent cytotoxicity of mannose SNAPs against Hela R cancer cells.

Cytotoxicities

The cytotoxicities of mannose SNAPs were assessed by in vitro clonogenic cell survival assays as described previously.[5] Figures 4 and 5 plot the surviving fraction versus dose for DU-145 human prostate cancer cells and Hela R cancer cells.

In the DU-145 human prostate cancer cells, the MEDs (median effect doses) are approximately 13, 42 and 22 μM for Man-1-SNAP, Man-2-SNAP and Man-6-SNAP, respectively. Compared with our previous data of other sugar SNAPs, the mannose conjugated SNAPs are approximately as potent as Glu-2-SNAP or Fru-2-SNAP.

In the Hela R cancer cells, the MEDs are approximately 4, 6 and 15 μM for Man-1-SNAP, Man-2-SNAP and Man-6-SNAP, respectively. For both cancer cells, Man-1-SNAP appears to be the most potent agent. It is believed that the potency of the compound is correlated to both NO releasing efficacy and steric configuration, which may affect its interaction with sugar transporters.

Experimental

General

All reagents were purchased from commercial suppliers and were used without further purification. ^{1}H and ^{13}C NMR were recorded on a Varian Gemini-300, a Mercury-400, or a Varian Unity-500 spectrometer. Mass spectra were recorded on a Kratos MS 80RFT spectrometer. Silica gel F_{254} plates (Merck) and Silica Gel 60 (70–230 mesh, Merck) were used in analytical thin-layer chromatography (TLC) and flash column chromatography, respectively.

Man-1-AP (2). A mixture of 1-β-D-mannosylamine (3.27 g) and cyclic-AP (1.25 g, 7.22 mmol) was dissolved in 25 mL of pyridine. The reaction mixture was stirred vigorously at room temperature for 16 h, it was then evaporated to give an oily residue, which was purified by column chromatography eluted with CH_2Cl_2/CH_3OH (10:1–7:1). The product **2** was obtained as a white foam (710 mg, 2.02 mmol, yield 28.0%). ^{1}H NMR (400 MHz, CD_3OD) δ_{ppm} 5.17 (s, 1H, H-1), 4.62 (s, 1H, H-2′), 3.83 (dd, 1H, $J=11.2$, 2.4 Hz, H-6), 3.76 (d, 1H, $J=1.6$ Hz, H-2), 3.67 (dd, 1H, 11.2, 5.6 Hz, H-6), 3.56 (t, 1H, $J=9.6$ Hz, H-4), 3.52 (dd, 1H, $J=9.6$, 2.4 Hz, H-3), 3.29–3.25 (m, 1H, H-5), 2.03 (s, CH_3 of Ac), 1.45 (s, CH_3-4′), 1.39 (s, CH_3-5′); ^{13}C NMR (100 MHz, CD_3OD) δ_{ppm} 171.99 (C=O), 170.27 (C=O), 78.90 (C-5), 78.02 (C-1), 74.46 (C-3), 71.14 (C-2), 66.89 (C-4), 61.61 (C-6 and C-2′), 45.24 (C-3), 29.61 (CH_3-4′), 28.15 (CH_3-5), 21.26 (CH_3 of Ac). MS (ESI) m/z found 353 ($M+H^+$), 375 ($M+Na^+$), 391 ($M+K^+$).

Man-2-AP (4). D-Mannosamine hydrochloride (3.24 g, 15.0 mmol) was stirred with sodium bicarbonate (1.26 g, 15.0 mmol) in 30 mL of dry DMF for 1.5 h, to which was added cyclic-AP (2.60 g, 15.0 mmol). After TLC indicated the disappearance of D-mannosamine the solvent was removed under vacuum and the crude was purified by silica gel chromatography eluted with CH_2Cl_2/CH_3OH (9:1–7:1). The compound **4** was obtained as a white powder (1.74 g, 4.94 mmol, yield 32.9%). NMR indicated that the product was a mixture of α-, β-anomers with a molar ratio of 13:5. α-Anomer: ^{1}H NMR (CD_3OD, 400 MHz) δ_{ppm} 5.03 (d, 1H, $J=1.6$ Hz, H-1), 4.64 (s, 1H, H-2′), 4.30 (dd, 1H, $J=4.8$ Hz, 1.6 Hz, H-2), 4.02 (dd, 1H, $J=9.4$, 4.6 Hz, H-3), 3.84 (dd, 1H, $J=14.4$, 4.4 Hz, H-6), 3.78–3.73 (m, 2H, H-5 and H-6), 3.60 (t, 1H, $J=9.6$ Hz, H-4), 2.03 (s, 3H, CH_3 of Ac), 1.46 (s, 3H, CH_3-4′), 1.41 (s, 3H, CH_3-5′); ^{13}C NMR (CD_3OD, 100 MHz) δ_{ppm} 171.89 (C=O), 170.82 (C=O), 93.53 (C-1), 72.25 (C-5), 69.61 (C-3), 67.26 (C-4), 61.77 (C-2), 60.95 (C-6), 53.91 (C-2), 45.58 (C-3′), 29.20 (CH_3 of Ac), 28.56 (CH_3-4′), 21.42 (CH_3-5). MS (ESI) m/z found 375 ($M+Na^+$), 391 ($M+K^+$).

6-*O*-Toluenesulfonyl-methyl-α-D-mannopyranoside (6). Methyl-α-D-mannopyranoside (20 g, 103.0 mmol) was dissolved in 200 mL of pyridine and cooled to 0 °C, to which was added toluenesulfonyl chloride (24 g, 125.9 mmol) in portions. The reaction was monitored with TLC. After the removal of solvent, the crude product was purified by silica gel column chromatography eluted with CH_2Cl_2/CH_3OH (16:1). The product **6** was obtained as a colorless syrup (33.38 g, 95.92 mmol, yield 93.1%). ^1H NMR ($CDCl_3$, 400 MHz) δ_{ppm} 7.79 (d, 2H, $J=8.8$ Hz), 7.31 (d, 2H, $J=8.8$ Hz), 4.76 (s, 1H), 4.65 (s, 1H), 4.82 (s, 1H), 4.31 (s, 3H), 3.91 (s, 1H), 3.75 (s, 1H), 3.71 (s, 1H), 3.26 (s, 3H, OCH_3), 2.40 (s, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ_{ppm} 145.22, 132.83, 130.15, 128.25, 101.17 (C-1), 71.85 (CH), 70.70 (CH), 70.32 (CH), 70.05 (CH_2), 55.21 (OCH_3), 21.86 (CH_3). MS (ESI) m/z found 371 ($M+Na^+$), 719 ($2M+Na^+$).

6-Azido-6-dexoxy-methyl-α-D-mannopyranoside (7). The compound **6** (5.15 g, 14.8 mmol) was dissolved in 45 mL of dry DMF, and the solution was heated to 60 C. Then sodium azide (10.64 g, 163.7 mmol) was added in portions. When TLC indicated that all of the starting material disappeared, the white precipitate was removed by vacuum filtration and the solvent was evaporated under vacuum. Column chromatography purification of the crude provided **7** (3.04 g, 13.9 mmol, yield 93.9%) as a colorless syrup. ^1H NMR (CD_3OD, 500 MHz) δ_{ppm} 4.64 (d, 1H, $J=1.5$ Hz, H-1), 3.80 (dd, 1H, $J=3.0$, 1.5 Hz, H-2), 3.65–3.60 (m, 2H, H-3 and H-5), 3.58 (t, 1H, $J=9.1$ Hz, H-4), 3.48 (dd, 1H, $J=13.2$, 2.5 Hz, H-6), 3.43 (dd, 1H, $J=13.2$, 7.1 Hz, H-6); ^{13}C NMR (CD_3OD, 125 MHz) δ_{ppm} 101.64 (C-1), 72.50 (C-5), 71.18 (C-3), 70.75 (C-2), 68.26 (C-4), 54.19 (OCH_3), 51.71 (C-6). MS (ESI) m/z found 242 ($M+Na^+$).

6-Amino-6-dexoxy-methyl-α-D-mannopyranoside (8). The compound **7** (0.56 g, 2.56 mmol) was dissolved in 50 mL of methanol, to which was added 50 mg of Pd/C as catalyst. The reaction vessel was shaken at room temperature for 17 h under 52 psi of hydrogen atmosphere. The mixture was filtered through Celite, and the filtrate was concentrated to a very small volume. Flash column chromatography of the residue with CH_2Cl_2/CH_3OH (2:1–1:1) as eluting solvent provided **8** (356 mg, 1.84 mmol) as a colorless solid. ^1H NMR (CD_3OD, 300 MHz) δ_{ppm} 4.64 (d, 1H, $J=1.2$ Hz, H-1), 3.79 (dd, 2H, $J=3.0$, 1.8 Hz, H-2), 3.65 (dd, 1H, $J=9.0$ Hz, 3.0 Hz, H-3), 3.52 (t, 1H, $J=9.0$ Hz, H-4), 3.47–3.38 (m, 1H, H-5), 3.37 (s, 3H, OCH_3), 2.98 (dd, 1H, $J=13.2$, 2.4 Hz, H-6), 2.79 (dd, 1H, $J=13.2$, 6.6 Hz, H-6); ^{13}C NMR (CD_3OD, 75 MHz) δ_{ppm} 101.63 (C-1), 72.98 (C-5), 71.30 (C-3), 70.87 (C-2), 68.63 (C-4), 54.17 (OCH_3), 42.43 (C-6). MS (ESI) m/z found 194 ($M+H^+$), 216 ($M+Na^+$).

Man-6-AP (9). The compound **8** (210 mg, 1.09 mmol) was dissolved in 15 mL of dry DMF, to which was added cyclic AP (226 mg, 1.31 mmol). The reaction mixture was stirred for one day, then the solvent was removed under vacuum. The crude was purified by silica gel column chromatography eluted with CH_2Cl_2/CH_3OH (14:1–12:1). The compound **9** was obtained as a white powder (346.2 mg, 0.95 mmol, yield: 86.8%). ^1H NMR (D_2O, 400 MHz) δ_{ppm} 4.67 (s, 1H, H-1), 4.40 (s, 1H, H-2′), 3.87 (d, 1H, $J=1.6$ Hz, H-2), 3.68 (dd, 1H, $J=9.6$, 3.2 Hz, H-3), 3.63 (d, 1H, $J=14.4$, H-6), 3.56 (dd, 1H, $J=8.8$, 7.6 Hz, H-5), 3.51 (dd, 1H, $J=9.6$, 8.8 Hz, H-4), 3.42 (dd, 1H, $J=14.4$, 7.2 Hz, H-6), 3.31 (s, 3H, OCH_3), 2.03 (s, 3H, Ac) 1.43 (s, 3H, CH_3-4′), 1.36 (s, 3H, CH_3-5′); ^{13}C NMR (D_2O, 100 MHz) δ_{ppm} 173.93 (C=O), 171.31 (C=O), 100.94 (C-1), 71.02 (C-5), 70.34 (C-3), 69.91 (C-2), 68.10 (C-4), 62.38 (C-2′), 54.91 (OCH_3), 45.16 (C-3′), 40.01 (C-6), 29.69 (CH_3-4′), 29.02 (CH_3-5′), 21.86 (CH_3 of Ac). MS (ESI) m/z found 389 ($M+Na^+$), 755 ($2M+Na^+$).

Nitrosation of glyco-*N*-acetyl-penicilliamines. The mannose AP was dissolved in methanol and cooled to −20 °C using a dry ice/acetone/water bath, to which was added 1.0 M HCl. Then a aqueous solution of sodium nitrite was added dropwise. After stirring for several hours under dark, the solvent was removed under vacuum. Cold methanol was added to precipitate the salt. After filtration and solvent removal the mannose SNAP was obtained as a green powder in quantitative yield.

Man-1-SNAP. ^1H NMR (CD_3OD, 500 MHz) δ_{ppm} 5.37 (s, 1H, H-2), 5.16 (d, 1H, $J=1.0$ Hz, H-1), 3.83 (dd, 1H, $J=11.6$, 2.5 Hz, H-6), 3.73 (dd, 1H, $J=3.0$, 1.0 Hz, H-2), 3.69 (d, 1H, $J=11.6$, 5.6 Hz, H-6), 3.58 (t, 1H, $J=9.6$ Hz, H-4), 3.52 (dd, 1H, $J=9.6$, 3.0 Hz, H-3), 3.32–3.28 (m, 1H, H-5), 2.06 (s, 3H, CH_3-4′), 2.00 (s, 3H, CH_3-5′), 1.96 (s, 3H, CH_3 of Ac); ^{13}C NMR (CD_3OD, 125 MHz) δ_{ppm} 172.03 (C=O), 169.49 (C=O), 78.84 (C-5), 78.09 (C-1), 74.39 (C-3), 71.06 (C-2), 66.81 (C-4), 61.40 (C-6), 60.09 (C-2′) 58.25 (C-3′), 26.01 (CH_3-4′), 24.46 (CH_3-5′), 21.19 (CH_3 of Ac). UV λ_{max}: 229 nm, 350 nm. MS (ESI) m/z found 404 ($M+Na^+$), 785 ($2M+Na^+$).

Man-2-SNAP. ^1H NMR (CD_3OD, 500 MHz) δ_{ppm} 5.38 (s, 1H, H-2′), 5.03 (s, 1H, H-1), 4.32 (d, 1H, $J=4.1$ Hz, H-2), 4.04 (dd, 1H, $J=9.4$, 4.8 Hz, H-3); 3.86 (dd, 1H, $J=10.9$, 3.8 Hz, H-6), 3.81–3.73 (m, 2H, H-5 and H-6), 3.61 (t, 1H, $J=9.6$ Hz, H-4), 2.16 (s, 3H, CH_3-4′), 2.02 (s, 3H, CH_3-5′), 1.92 (s, 3H, CH_3 of Ac); ^{13}C NMR (CD_3OD, 125 MHz) δ_{ppm} 171.74 (C=O), 169.95 (C=O), 93.54 (C-1), 72.21 (C-5), 69.52 (C-3), 67.25 (C-4), 60.88 (C-6), 60.20 (C-2′), 58.99 (C-3′), 54.04 (C-2), 26.95 (CH_3-4′), 23.96 (CH_3-5′), 21.16 (CH_3 of Ac). UV λ_{max} 229 nm, 350 nm. MS (ESI) m/z found 404 ($M+Na^+$), 785 ($2M+Na^+$).

Man-6-SNAP. ^1H NMR (CD_3OD, 400 MHz) δ_{ppm} 5.32 (s, 1H, H-2′), 4.61 (s, 1H, H-1), 3.78 (d, 1H, $J=1.6$ Hz, H-2), 3.69–3.56 (m, 2H, H-3 and H-6), 3.56–3.44 (m, 3H, H-5, H-4 and H-6), 3.34 (s, 3H, OCH_3), 2.07 (s, 3H, CH_3 of Ac), 2.00 (s, 3H, CH_3-4′), 1.96 (s, 3H, CH_3-5′); ^{13}C NMR (CD_3OD, 100 MHz) δ_{ppm} 174.35 (C=O), 171.90 (C=O), 101.63 (C-1), 71.32 (C-5), 70.94 (C-3), 70.82 (C-2), 68.32 (C-4), 60.30 (C-2′), 58.33 (C-3′), 54.35 (OCH_3), 40.23 (C-6), 26.28 (C-4′), 22.38 (C-5′), 21.24 (CH_3 of Ac). UV λ_{max} 228 nm, 346 nm. MS (ESI) m/z found 418 ($M+Na^+$), 753 ($2M+Na^+$).

Acknowledgement

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