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The synthesis and cytotoxicity of fructose-1-SNAP, a novel fructose conjugated *S*-nitroso nitric oxide donor

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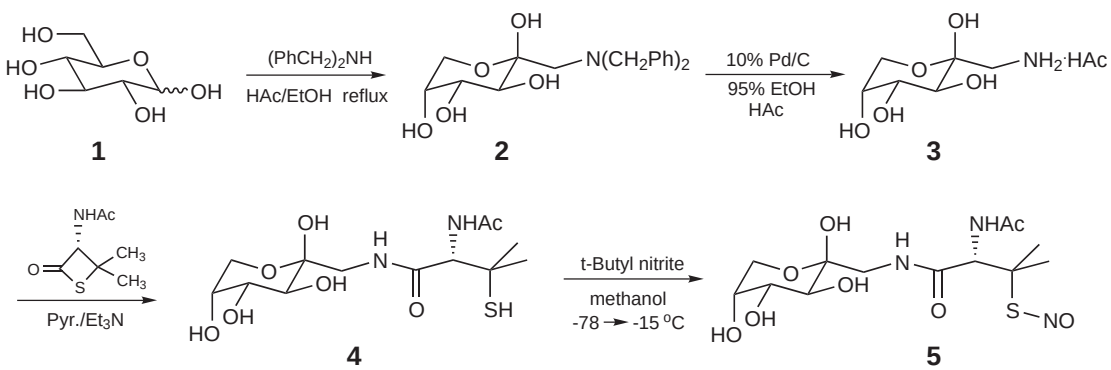
Abstract—An efficient synthetic strategy has been developed for the synthesis of a novel fructose conjugated *S*-nitroso nitric oxide donor, fructose-1-*S*-nitroso-*N*-acetylpenicillamine. This compound showed similar cytotoxicity as the first generation fructose-SNAP, fructose-2-SNAP. A 5- and 26-fold increased cytotoxicity was observed compared to glucose-2-SNAP and SNAP against DU-145 human prostate cancer cells in vitro. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

S-Nitrosothiols (RSNOs) are compounds that play key roles in storing, transporting and releasing nitric oxide (NO) within the mammalian body.[1,2] Numerous biological functions of RSNOs are continuously being discovered. For example, recent reports indicate that the blood flow is regulated by *S*-nitrosohemoglobin via the release of NO.[3] *S*-Nitrosation of specific thiol groups on the calcium release channel may be the regulatory mechanism of the channel.[4] In addition, RSNOs may directly serve as drugs in the treatment of a variety of diseases such as hypertension,[5] atherosclerosis,[6] and congestive heart failure.[7] The design and synthesis of novel *S*-nitrosothiols exhibiting better pharmacokinetic properties has been the current focus in this field.[8] Recently, we have synthesized a series of targeting NO donors, glyco-*S*-nithrosothiols or sugar-*S*-nitroso-*N*-acetylpenicillamines.[9,10] Glyco-*S*-nitrosothiols not only showed improved stability, but an increased cytotoxicity and selectivity against different tumor cells as compared to SNAP. For example, glucose-2-SNAP showed up to 5000-fold more potency than SNAP in killing A2780S human ovarian cancer cells in vitro.[11] The first generation of fructose and SNAP conjugate, fructose-2-SNAP, has been found to be more effective than glucose-2-SNAP against DU-145 human prostate cancers in vitro.[12] However, the difficulties in the synthesis of 2-amino-fructose made it impractical to prepare fructose-2-SNAP on a larger scale. We report here a new and efficient method to synthesize the second generation of fructose-SNAP compounds, fructose-1-SNAP, which showed similar cytotoxicity as fructose-2-SNAP against DU-145 human prostate cancer cells in vitro.



Scheme 1. An efficient synthetic methodology for the synthesis of fructose-1-SNAP.

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2. The chemistry of fructose-1-SNAP

As shown in Scheme 1, we found that 1-amino-1-deoxy fructose **3** could be synthesized from the commercially available anhydrous α-D-glucose **1** via an Amadori rearrangement in nearly quantitative yield.[13] Anhydrous α-D-glucose reacted with dibenzylamine in the presence of acetic acid to afford the product **2**. Compound **2** was the only product formed, as confirmed by NMR analysis. The structure of the Amadori product was also proven by X-ray crystal structure, as shown in Fig. 1. Catalytic hydrogenation of compound **2** using 10% Pd/C produced compound **3**. Compound **4** was obtained by coupling compound **3** to 3-acetamido-4,4-dimethylthietan-2-one (cyclic AP), which was prepared by intramolecular cyclization of *N*-acetylpenicillamine.[9] Fructose-1-SNAP (compound **5**) was obtained as a green powder in nearly quantitative yield by *S*-nitrosation with *t*-butyl nitrite.

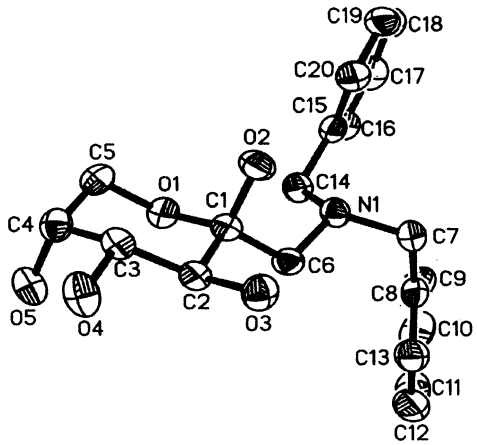

Figure 1. X-Ray structure of 1–dibenzylamino-1–deoxy-D-fructose (compound **2**). Selected bond lengths (Å) and angles (°): O(1)–C(1) 1.434(3), C(1)–O(2) 1.403(3), C(1)–C(6) 1.532(3), C(1)–C(2) 1.540(3); O(2)–C(1)–O(1) 110.6(2), O(2)–C(1)–C(6) 110.1(2), O(2)–C(1)–C(2) 108.2(2), O(1)–C(1)–(2) 108.9(2), O(1)–C(1)–C(6) 107.1(2), C(6)–C(1)–C(2) 111.9(2).

From the NMR analysis it was found that, unlike compound **2**, compounds **3** and **4** each existed as a mixture of up to three tautomeric forms, α- and β-furanose forms and a β-pyranose form. The predominant isomeric form for both compounds **3** and **4** was the β-pyranose form, a thermodynamically favoured isomer. Based on the height of the anomeric carbon in the ^{13}C NMR of fructose-1-AP, a stable precursor of fructose-1-SNAP, we established that the mixture of fructose-1-SNAP consists of a β-pyranose form (72%), a β-furanose form (18%) and an α-furanose form (10%). This agreed with the previous results for similar derivatives of 1-amino-1-deoxy-fructose.[14]

3. NO releasing of fructose-1-SNAP

Experiments to verify and quantify the NO concentration released by a 0.3 mM aqueous solution of fructose-1-SNAP in a HEPES buffer (pH 7.4) with and without the presence of Cu^{2+} (950 μM) were carried out using a NO-specific electrochemical analyzer. As shown in Figs. 2 and 3, the result indicated that in the absence of Cu^{2+}, fructose-1-SNAP released NO mildly and continued at a constant rate when NO reached a maximum concentration, while in the presence of Cu^{2+} (950 μM), NO was generated drastically and reached the maximum concentration in half a minute then decreased quickly.

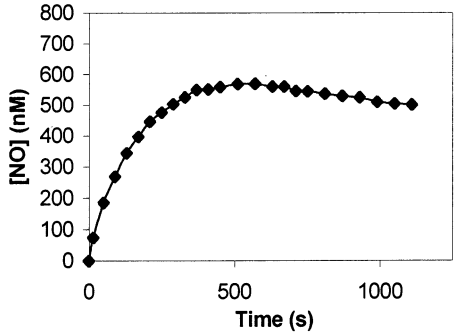

Figure 2. NO releasing by a 0.3 mM solution of fructose-1-SNAP in a HEPES buffer (pH 7.4).

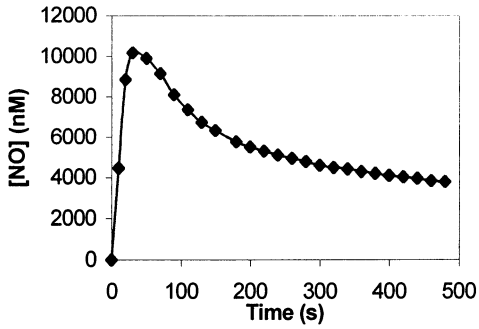

Figure 3. NO releasing by a 0.3 mM solution of fructose-1-SNAP in a HEPES buffer (pH 7.4) in the presence of Cu^{2+} (950 μM).

4. The cytotoxicity studies of fructose-1-SNAP

The cytotoxicity of fructose-1-SNAP was tested and compared with fructose-2-SNAP, glucose-2-SNAP and SNAP using DU-145 human prostate cancer cells in vitro.[12] Fig. 4 shows a median effect dose (MED) plot for the cytotoxicity of fructose-1-SNAP, fructose-2-SNAP, glucose-2-SNAP and SNAP toward DU-145 human prostate cancer cells in vitro. The data indicate that the MED is approximately 120, 25.6, 8.7 and 4.5 μM for SNAP, glucose-2-SNAP, fructose-2-SNAP, and fructose-1-SNAP, respectively. Fig. 5 plots the surviving fraction versus dose (semi log format). The data suggest that our new generation fructose-1-SNAP is approximately as potent as our first generation fructose-2-SNAP. In addition, fructose-1-SNAP is found to be approximately 5- and 26-fold more potent than glucose-2-SNAP and SNAP, respectively.

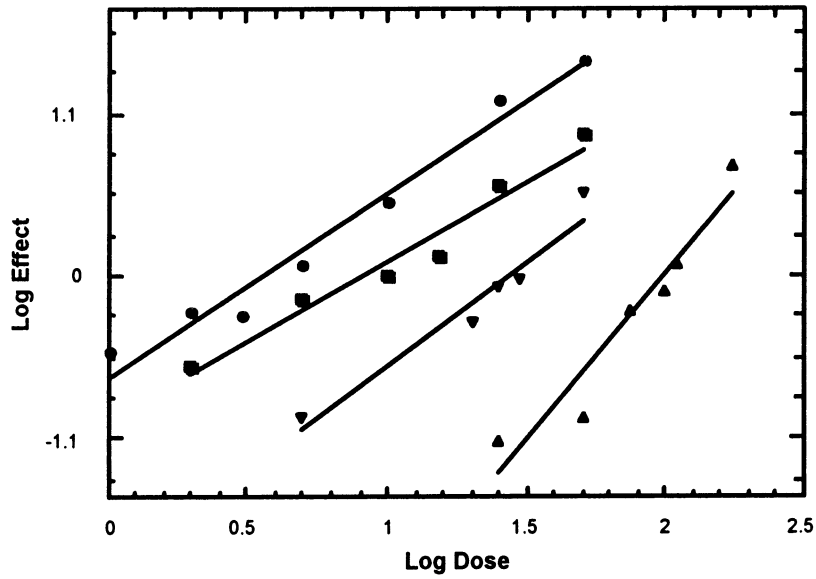

Figure 4. Cytotoxicity toward DU-145 human prostate cancer cells. Fructose-1-SNAP (●), fructose-2-SNAP (■), glucose-2-SNAP (▼), and SNAP (▲).

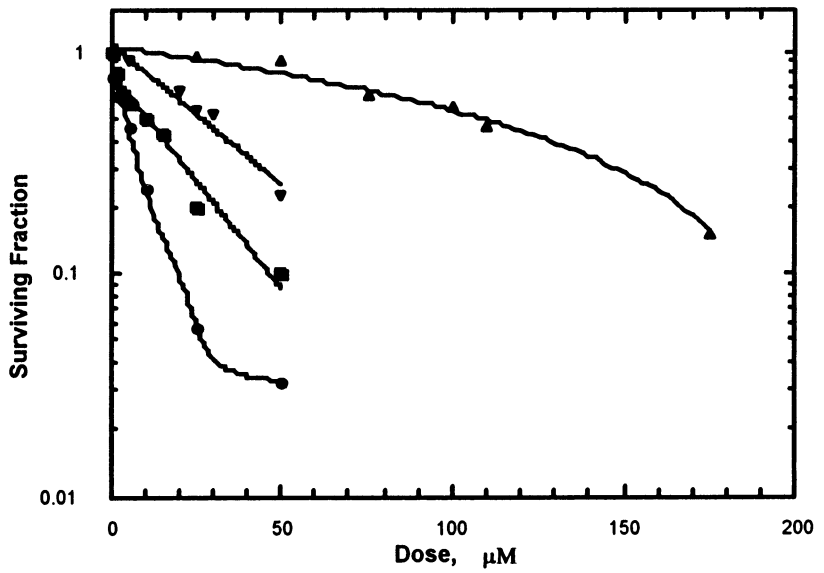

Figure 5. Surviving fractions versus doses toward DU-145 human prostate cancer cells. Fructose-1-SNAP (●), fructose-2-SNAP (■), glucose-2-SNAP (▼), and SNAP (▲).

In summary, fructose-2-SNAP and fructose-1-SNAP were found to be a novel class of NO donor compounds. These types of compounds exhibit potent, selective cytotoxicity against human prostate cancer cells. Moreover, fructose-1-SNAP is much easier to prepare and purify than fructose-2-SNAP.

5. Experimental

5.1. General procedures

All the reagents were purchased from commercial suppliers and used without further purification. ^{1}H and ^{13}C NMR spectra were recorded on a GE Gemini 300 MHz, a Varian Mercury 400 MHz or a Varian Unity 500 MHz NMR spectrometer. All chemical shifts were reported by using the following abbreviations: s, singlet; d, doublet; b, broad; m, multiple; *J*, coupling constants (Hz). Silica gel plates (Merck F254) and silica gel 60 (Merck, 70–230 mesh) were used in analytical thin-layer chromatography (TLC) and column chromatography, respectively. NO measurements were carried out in an Electrochemical ISO-NO Mark-II Isolated Nitric Oxide Meter, a product of World Precision Instruments, Inc (Sarasota, Florida). The samples dissolved in small amounts of methanol were injected into the sealed O_2-free (argon atmosphere) aqueous buffer solutions. $CuSO_4 \cdot 5H_2O$ was used as the source of Cu^{2+}.

3-Acetamido-4,4-dimethylthietan-2-one (cyclic AP) and glucose-2-SNAP were synthesized according to previous methods.[10] SNAP was synthesized following a procedure by Field et al.[15]

5.2. Synthesis of 1-amino-1-deoxy fructose acetic acid salt (3)

Anhydrous α-D-glucose (10.00 g, 53.3 mmol) and dibenzylamine (10.57 mL, 53.3 mmol) were suspended in absolute ethanol (70 mL), then glacial acetic acid (3.05 mL) was added. The reaction mixture was refluxed for 3 h then cooled to room temperature. The precipitate was collected by filtration, washed till colorless with ethanol and then dried to give Amadori product (18.14 g, 51.0 mmol) in a 96% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ_{ppm} 2.60–2.70 (m, 2H), 3.42 (dd, 1H, J=12 Hz, 1.6 Hz), 3.55–3.65 (m, 5H), 3.68–3.80 (m, 3H), 4.18 (d, 1H, J=5.6 Hz), 4.41 (m, 2H), 5.25 (s, 1H), 7.19–7.34 (m, 10H). MS (FAB) 360.1 (M+H$^+$). This compound (3.00 g, 8.4 mmol) was dissolved in 95% ethanol (40 mL) and glacial acetic acid (20 mL) was added. Using 10% Pd/C as the catalyst, it was hydrogenated at 40 Psi for 3 h then filtered. The filtrate was concentrated to half of the original volume, then ether (20 mL) was added to precipitate the product, which was washed with EtOH:Et_2O (3:1) and then dried to give 1-amino-1-deoxy fructose acetic acid salt (1.84 g, 7.7 mmol) in a 92% yield.^{1}H NMR (300 MHz, CD_3OD) δ_{ppm} 1.75 (s, 3H, Ac), 3.00–3.10 (m, 2H), 3.54–3.60 (m, 2H), 3.71 (d, 1H), 3.80–3.88 (m, 2H).

5.3. Synthesis of fructose-1-AP (4)

1-Amino-1-deoxy fructose acetic acid salt (1.0 g, 4.2 mmol) dissolved in 20 mL of pyridine was neutralized with a mixture of 1.8 mL of triethylamine (Et_3N) in 20 mL of pyridine at 0°C. The solution was stirred for 20 min followed by the addition of cyclic-AP (1.1 g, 6.3 mmol). After 20 h the reaction was complete and the solvent was removed in vacuo. Purification was accomplished by column chromatography using dichloromethane and methanol (7:1, 5.5:1, 4.5:1, in volume). The conjugate, fructose-1-AP, was obtained as a white powder (1.2 g, 3.41mmol, yield 81%). ^{1}H NMR (500 MHz, CD_3OD) δ_{ppm} 1.40 (s, 3H, CH_3), 1.46 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 3.18–4.02 (m, 5H), 3.35 (s, 2H, CH_2), 4.53–4.56 (m, 1H, CH). ^{13}C NMR (125 MHz, CD_3OD) δ_{ppm} 98.14 (anomeric carbon of β-pyranose form), 101.82 (anomeric carbon of β-furanose form), 105.28 (anomeric carbon of α-furanose form). MS (FAB) 375.1 (M+Na$^+$).

5.4. Synthesis of fructose-1-SNAP (5)

Fructose-1-AP (148 mg, 0.42 mmol) was dissolved in 2.5 mL of methanol and cooled to −78°C, then t-butyl nitrite (3 mL) cooled to −78°C was added in a single portion. The reaction mixture was stirred and allowed to gradually warm up to −15°C within 2–3 h. The solvent, excess t-butyl nitrite and the formed t-butyl alcohol were evaporated in vacuo. The residue was dissolved in methanol and extracted with hexane several times to remove t-butyl nitrite/t-butyl alcohol. Any trace amount of t-butyl nitrite/t-butyl alcohol was removed by repeated washing with chloroform. The final product, fructose-1-SNAP, was obtained as a green powder (152 mg, 0.40 mmol, yield 95%). ^{1}H NMR (400 MHz, CD_3OD), δ_{ppm} 1.95–1.96 (m, 3H, CH_3), 1.99–2.03 (m, 3H, CH_3), 2.08–2.09 (m, 3H, CH_3), 3.28–4.02 (m, 5H), 3.34 (s, 2H, CH_2), 5.30–5.32 (m, 1H, CH).

5.5. Biological testing

The cytotoxicity of fructose-1-SNAP, fructose-2-SNAP and glucose-2-SNAP was evaluated and compared to SNAP using DU-145 human prostate cancer cells. Cancer cells were plated in T25 flasks at a density of 2×10^4 cell/cm^2. After 4 h, the cells were washed twice and 4 mL of fresh serum free media was added. NO donor solutions were prepared immediately before each experiment by dissolving fructose-1-SNAP, fructose-2-SNAP, glucose-2-SNAP and SNAP in sterile saline, filtered through a 0.2 micron filter, diluted, and added to the cells. Cancer cells were exposed to NO donors for 30 min at 37°C. The cells were then washed twice with serum free media or phosphate buffered saline, trypsinized from the flask, washed, counted, diluted, and then plated out at several dilutions in fresh tissue culture media containing 10% serum. The cultures were then incubated for 10–14 days at which time the colonies were fixed, stained (methylene blue) and counted.

Acknowledgements

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References

1. Stamler, J. S. In *S-Nitrosothiols and the Bioregulatory Actions of Nitric Oxides Through Reactions with Thiol Groups, The Role of Nitric Oxide in Physiology and Pathophysiology*; Koprowski, H.; Maeda, H., Eds.; Springer-Verlag, 1993; pp. 19–36.
2. Williams, D. L. H. *Chem. Soc. Rev.* **1985**, 171–196.
3. Stamler, J. S.; Jia, L.; Eu, J. P.; McMahon, T. J.; Demchenko, I. T.; Bonaventura, J.; Gernert, K.; Piantadosi, C. A. *Science* **1997**, *276*, 2034–2037.
4. Xu, L.; Eu., J. P.; Meissner, G.; Stamler, J. S. *Science* **1998**, *279*, 234–237.
5. Luscher, T. F.; Raij, L.; Vanhoutte, P. M. *Hypertension* **1987**, *9*, 157–163.
6. Lefer, A. M.; Sedar, A. W. *Pharmacol. Res.* **1991**, *23*, 1–12.
7. Drexler, H.; Hayoz, D.; Munzel, T.; Hornig, B.; Just, H.; Brunner, H.; Zelis, R. *Am. J. Cardiol.* **1992**, *69*, 1596–1601.

8. Wang, K.; Zhang, W.; Xian, M.; Hou, Y.-C.; Chen, J.-P.; Wang, P. G. *Curr. Med. Chem.* **2000**, *7*, 821–834.
9. Ramirez, J.; Yu, L.-B.; Li, J.; Brauschweiger, P. G.; Wang, P. G. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2575–2580.
10. Hou, Y.-C.; Wang, J.-Q.; Ramirez, J.; Wang, P. G. *Methods Enzymol.* **1999**, *301*, 242–249.
11. Cantuaria, G.; Magalhaes, A.; Angioli, R.; Mendez, L.; Mirhashemi, R.; Wang, J.-Q.; Wang, P. G.; Penalver, M.; Averette, H.; Braunschweiger, P. *Cancer* **2000**, *88*, 381–388.
12. Hou, Y.-C.; Wang, J.; Andreana, P. R.; Cantauria, G.; Tarasia, S.; Sharp, L.; Braunschweiger, P. G.; Wang, P. G. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2255–2258.
13. Hodge, J. E.; Fisher, B. E. In *Methods in Carbohydrate Chemistry*, Whistler, R. L.; Wolfrom, M. L.; Bemiller, J. N., Ed.; Academic Press: New York, 1963; Vol. II, pp. 99–103.
14. Gómez-Sánchez, A.; Martin, M. G. G.; Pascual, C. *Carbohydr. Res.* **1986**, *149*, 329–345.
15. Field, L.; Dilts, R. V.; Ravichandran, R.; Lenhert, P. G.; Carnahan, G. *J. Chem. Soc., Chem. Commun.* **1978**, 249–250.