Synthesis and applications of alkylated C-sugars as peptide bioconjugates

Florence M. Brunel, Anne-Marie Leduc, Mark S. Mashuta, K. Grant Taylor & Arno F. Spatola*
Department of Chemistry, University of Louisville, Louisville, KY, U.S.A.
(* Author for correspondence, e-mail: spatola@louisville.edu, Fax: 502 852 3899)

Received 20 October 2002; Accepted 6 December 2002

Key words: bioavailability, bioconjugate, C-sugar, NR box analog, X-ray

Summary

Permethylated C-sugars affect the stability and solubility of their carbohydrate precursors and may represent an important group of bioconjugates. When properly functionalized, these units can be appended to the N- and C-termini or to the side chains of peptides or other therapeutic candidates. In this report, we describe the synthesis of an amine-functionalized alkylated mannose derivative and confirm the configuration by determining the X-ray crystal structure of its nitrile precursor. An acid functionalized counterpart, when attached to the N-terminus of a NR box peptide analog, improved binding to estrogen receptor β (ERβ) but not to ERα.

Introduction

Posttranslational modifications such as phosphorylation, hydroxylation, sulfation, nucleotidylation, and especially glycosylation can have profound effects on numerous complex biochemical functions, especially in mammals. In an effort to understand these events, much attention has been devoted to the synthesis of glycopeptides and glycoproteins as vital mediators of intracellular targeting, intercellular recognition and of the immune response, among other cell-based processes [1].

C-glycosides [2], stabilized derivatives of O-glycosides, have proven to be popular synthetic targets, much as pseudopeptides have been useful analogs as stabilized peptide mimics [3].

We have reported the synthesis of a new class of compounds known as alkylated C-sugars [4]. The structure of our first functionalized analog, a permethylated carboxylic acid derivative of mannose 1, is shown in Figure 1. These compounds may retain some of the properties of their hydroxylated precursors, but we view them primarily as a potentially powerful new class of bioconjugates. Their polyethereal structures, if appropriately modified to permit facile attachment to peptides or other therapeutically important molecules, could provide PEG-like (polyethyleneglycol) [5] benefits in terms of solubility improvement and half-life extensions, necessary attributes of compounds with enhanced bioavailability profiles [6].

In this report, we describe the synthesis of an amine functionalized alkylated C-sugar and document the biological consequences of incorporating a carboxy-functionalized version onto a previously reported new class of protein-protein inhibitory peptides based on the LXXLL nuclear receptor (NR box) motif (Table 4) [7].

Materials and methods

Boc protected amino acids were purchased from Bachem and Chem-Impex International. MBHA
resin was a gift from Peptides International. BOP was purchased from Advanced ChemTech, HOBt from Quantum Technologies, TFFH from PerSeptive Biosystems, and sodium hydride, methyl iodide, DIEA, TEA, TMSOTf, TMSCN, oxalyl chloride, LiAlH₄, anhydrous solvents and deuterated solvents were obtained from Aldrich Chemical Co. The RP-HPLC was performed on a Vydac 218TP54C18 column (4.6 × 250 mm) on a Hitachi 655A system equipped with a Hitachi L-5000 controller, D-2000 integrator, and Hitachi 655A detector. HPLC analysis was performed using the following gradient: 5–90% CH₃CN/0.05% TFA in 30 min, flow 1 ml min⁻¹.

Purification was carried out on a pre-packed C18 column (Varian, Mega Bond Elut C18, 1.4 × 3 cm) using a gradient of CH₃CN/0.05% TFA between 0 and 50%.

Molecular weight determinations were made on a Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) Mass Spectrometer, DE-Pro, made by Applied Biosystems. The in vitro biological activities were performed at Lilly Research Laboratories in Indianapolis, Indiana, by T. Burris and K. Bramlett.

**Synthesis of 5b**

β-D-(2,3,4,6-tetra-O-methyl-mannosyl) methanamine (Scheme 1)

The permethylated methyl α-D-mannopyranoside, 3, (0.581 g; 2.31 mmol) (prepared from 2 as described previously [4]) and trimethylsilyl cyanide (0.62 ml; 4.62 mmol) were dissolved in freshly distilled CH₃CN at 0 °C. Trimethylsilyl triflate (0.42 ml; 2.31 mmol) was slowly added and the reaction was stirred for 6 h at 0 °C and gradually warmed to room temperature. After stirring overnight, the reaction mixture was quenched by water and, the separated organic phases were washed with saturated NaHCO₃ solution, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure to afford a light brown oil. Column chromatography (EtOAc hexane, 1:4 to 1:2 v:v) provided 23% of the β isomer, (4a) 7% of the α isomer (4b) and 48% of a mixture. The compounds were characterized by ¹H and ¹³C NMR spectroscopy. (We notice the 2 CN peaks at 117.6 ppm for the β isomer and 116.9 ppm for the α isomer in DMSO). The structure of the β isomer was confirmed by X-ray crystallography as described below.

To a solution of the β-D-(2,3,4,6-tetra-O-methyl-mannosyl) cyanide isomer (4b) (212 mg; 8.7 mmol) in freshly distilled THF was added LiAlH₄ (35 mg, 0.66 mmol). The mixture was stirred overnight at room temperature, and then it was carefully quenched with cold water, followed by excess 5% H₂SO₄ solution. The mixture was extracted with EtOAc (2 × 15 ml) to remove unreacted organic compounds. The water extracts were combined and treated with 5.0 N NaOH solution and extracted with EtOAc twice. The combined organic extracts were washed with brine, dried over Na₂SO₄, and concentrated to give a pale yellow oil (90%) 5b. The α isomer (4a) was reduced using the same procedure to provide 5a. A Kaiser test [8] was performed to confirm the presence of an amine; then the compounds were characterized by ¹H and ¹³C NMR spectroscopy, and the molecular weights were confirmed (Mass calc.: 244.267; Mass found (M + 1): 245.2).

**Crystallographic studies**

A colorless needle (0.424 × 0.070 × 0.045 mm) crystal of 4b was obtained by diffusion of hexane into an ethyl acetate solution at room temperature. The crystal was mounted on a 0.05 mm CryoLoop with Paratone oil for collection of X-ray data using a Bruker SMART APEX CCD diffractometer. The SMART [9] software package (v 5.625) was used to acquire a total of 1868 thirty-second frame exposures of data at 100 K using monochromated Mo Kα radiation (0.71073 Å) from a sealed tube and a monocapillary. Frame data were processed using SAINT [10] (v 6.22) to produce the raw hkl data that were then corrected for absorption using SADABS [11] (v 2.02). The structure was solved by direct methods using SHELXS-90 [12] and refined by least squares methods on F² using SHELXL-97 [13] incorporated into the SHELXTL [14] (v 6.12) suite of programs. There are 4 molecules in the unit cell, 3 of which are independent. All non-hydrogen atoms except for O15B and C28B were refined anisotropically. The methoxymethyl group bonded to ring atom C27 was disordered and modeled with O15A and C28A for the major component and O15B and C28B as the minor isotropic group with occupancies of 60 and 40%, respectively. Methine and methylene hydrogen atoms were placed in their geometrically generated positions and refined as a riding model. For all 8575 unique reflections (R(int) = 0.030) the final anisotropic full matrix least-squares refinement on F² for 533 variables converged at R1 = 0.063 and wR2 =