



Glycosylation and functionalization of native amino acids with azido uronic acids



Consulato J. Cara, Danielle Skropeta*

School of Chemistry, Faculty of Science, Medicine and Health, Centre for Medical and Molecular Bioscience, University of Wollongong, NSW 2522, Australia

ARTICLE INFO

Article history:

Received 16 April 2015

Received in revised form 21 September 2015

Accepted 1 October 2015

Available online 22 October 2015

Keywords:

Amino acids

Carbohydrates

Sugar azido acids

Click chemistry

Amides

ABSTRACT

Bifunctional, acetyl-protected, azido glucuronic and galacturonic acid derivatives were coupled via the carboxylic acid moiety to amine- and hydroxyl-containing side chains of the natural amino acids lysine and serine or via the N-terminus of the amino acids leucine, methionine and glutamine. The glycosylated amino acids were then functionalized via the free azido moiety to produce the corresponding anomeric triazole derivatives. This approach enables the dual glycosylation and functionalization of a range of native amino acids in high yields.

Crown Copyright © 2015 Published by Elsevier Ltd. All rights reserved.

1. Introduction

Glycosylation is a key method used to improve the in vivo pharmacokinetic (PK) properties and biodistribution of therapeutic and diagnostic proteins and peptides.^{1–6} This is most often achieved by glycosylation of the N-terminus of peptides via an Amadori reaction,^{2,4} via chemoenzymatic synthesis,⁵ or glycoengineering of proteins.¹ Of particular interest is the incorporation of bifunctional carbohydrates that introduce a site for further functionalization of the peptide/protein of interest. For this purpose, sugars are typically labelled and then coupled with modified substrates such as alkyne- or aminoxy ether functionalised peptides.^{7–14} There is however, only a small number of complementary methods enabling the direct dual glycosylation and functionalization of native amino acids.¹⁵

Sugar amino acids (SAAs) pioneered by Kessler^{16–18} and Fleet,^{19,20} are a class of glycosides of significant interest enabling glycosylation of amino acids via peptide chemistry, while also presenting a free handle for further functionalization. SAAs have been incorporated into a variety of peptides, including cyclic RGD and somatostatin analogues, and linear enkephalin analogues.^{16,19,20} Sugar azido acids such as azido glucuronic and galacturonic acids can be considered as masked SAAs. While a number of anomeric

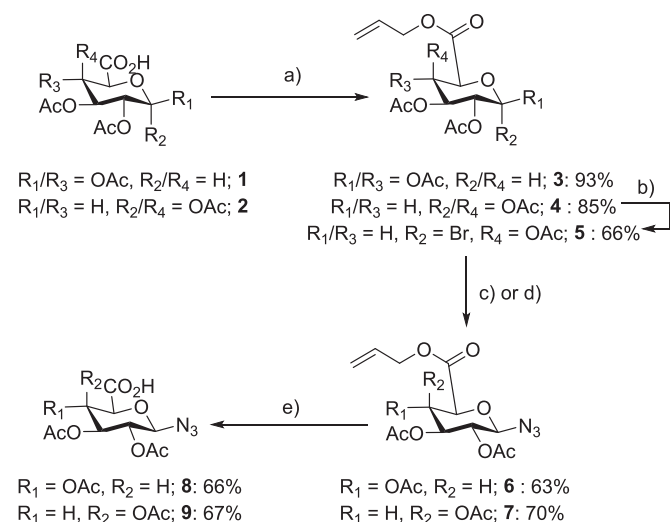
azido sugars have been clicked onto alkyne-modified peptides and proteins,^{9–11} there are relatively few examples of the analogous azido uronic acids where the azido moiety is retained for click chemistry, as it is most often reduced to an amine to produce a SAA, that is, then coupled as an amide.

Herein, we describe an efficient method for functionalization and glycosylation of native amino acids using protected azido glucuronic (**8**) and galacturonic acids (**9**). The uronic acids are readily coupled to amine- and hydroxyl containing side chains of natural amino acids, or via the N-terminus of various amino acids, to produce the corresponding uronamide or uronic acid esters. The nascent glycosylated amino acids retain a free anomeric azido moiety for further functionalization modelled by coupling to an alkynol derivative. This method described herein can be used to introduce both glycosylation to improve the PK profile of therapeutic and diagnostic peptides, as well as an azido labelling site to enable further exploration of glycosylated peptides for a range of purposes including nuclear imaging.

2. Results and discussion

The key triacetylated *glc*- and *gal*-sugar azido acids were prepared from their respective commercially available uronic acids in five and six steps, in multigram scale and without requiring any chromatographic purification (Scheme 1).²¹ Acetyl protection was chosen to enhance cell permeability and stability of the final

* Corresponding author. Tel.: +61 2 42214360; fax: +61 2 42214287; e-mail address: skropeta@uow.edu.au (D. Skropeta).



Scheme 1. Synthesis of sugar azido acids **8** and **9** from uronic acid building blocks. Reaction conditions: a) i) $(\text{COCl})_2$, DMF (cat.), CH_2Cl_2 , 0°C to RT, 2 h; ii) Allyl-OH, $\text{C}_5\text{H}_5\text{N}$, CH_2Cl_2 , 0°C to RT, 3.5 h; b) 33% HBr/AcOH , 0°C to RT, 4 h; c) TMSN_3 , SnCl_4 , CH_2Cl_2 , RT, 4 h; d) NaN_3 , DMF RT, 3 h; e) $\text{Pd}(\text{PPh}_3)_4$, pyrrolidine, CH_3CN , 0°C , 1 h.

glycopeptides.²² Beginning from the peracetylated *glc*- and *gal*-uronic acid derivatives **1** and **2**, acid chloride formation followed by allylation, resulted in ester derivatives **3** and **4** in 93% and 85% yields, respectively. Conversion of the anomeric acetyl group to a β -azide was initially performed using SnCl_4 -catalyzed conditions,

with neighbouring group participation of the 2-OAc group of **3**, followed by nucleophilic substitution using TMS-N_3 yielding the desired β -azide **5** in 63% yield.

Attempts to replicate this approach in the production of *gal* β -azide **7** proved inefficient, resulting from the differing α -stereochemistry of the anomeric acetyl group of **4**. As a result, the conversion of the α -anomeric acetyl group of **4** to an α -bromide (HBr/AcOH) was performed, yielding the intermediate **5** in 66% yield. Subsequently, $\text{S}_\text{N}2$ nucleophilic substitution of the α -bromo group using NaN_3 in DMF resulted in the production of the desired *gal*- β -azide **7** in 70% yield. Subsequently, Pd-catalyzed de-allylation was utilized to unmask the carboxylic acid of **6** and **7**, resulting in the desired *glc*- and *gal*-azido uronic acids **8** and **9** in 66% and 67% yields, respectively.

Glucuronic acids have previously been coupled to the N-terminus of selected amino acids,^{23–28} but are less often coupled via amino acid side-chains.²⁹ To extend this methodology, herein, clickable glycosyl amino acids **10–13** were produced by coupling the azido uronic acids **8** or **9** to amino acids bearing a free hydroxyl or amino moiety to give ester and amide linked derivatives with the anomeric azido moiety remaining free for further derivatization (Table 1).

Hence, **8** and **9** were coupled to serine using a *tert*-butoxycarbonyl (Boc)/OMe protection strategy and DCC (*N,N'*-dicyclohexylcarbodiimide) as the coupling reagent in the presence of DMAP (4-dimethylaminopyridine), producing the ester-linked *glc*- and *gal*-glycosyl azides **10** and **11** in 80% and 64% yield, respectively (Table 1). In a similar manner, *N*^α-acetyl protected lysine methyl ester was coupled with **8** and **9** using DCC in the presence of HOBT (1-

Table 1
Synthesis of glycosyl amino acids **10–17**

Conditions	SAA	Amino acid	Product	Yield
a)	8 9	Boc.Ser.OMe		<i>glc</i> 10 ; 80% <i>gal</i> 11 ; 64%
b)	8 9	Ac.Lys.OMe·HCl		<i>glc</i> 12 ; 75% <i>gal</i> 13 ; 60%
c)	8 9	H.Leu.OMe·HCl		<i>glc</i> 14 ; 84% <i>gal</i> 15 ; 78%
c)	8	H.Met.OMe·HCl		16 ; 95%
c)	8	H.Gln.OtBu·HCl		17 ; 96%

Reaction conditions: a) DCC, DMAP, CH_2Cl_2 , RT, 24 h; b) DCC, HOBT, DIPEA, DMF, RT, 24 h; c) HBTU, DIPEA, DMF, RT, 24 h.

Download English Version:

<https://daneshyari.com/en/article/5214196>

Download Persian Version:

<https://daneshyari.com/article/5214196>

[Daneshyari.com](https://daneshyari.com)