Carbohydrate Research 367 (2013) 1–4

Contents lists available at SciVerse ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Gram scale de novo synthesis of 2,4-diacetamido-2,4,6-trideoxy-D-galactose

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ARTICLE INFO

Article history:
Received 5 November 2012
Received in revised form 20 November 2012
Accepted 21 November 2012
Available online 2 December 2012

Keywords:
S. pneumoniae
Lipoteichoic acid
Amino sugar synthesis
Nitro aldol reaction
Deprotection with water

ABSTRACT

In contrast to the infection with Gram-negative bacteria, the precise mechanism of infection with their Gram-positive counterparts is still poorly understood. It has been established, that teichoic and lipoteichoic acids play an important role in host–pathogen interaction. The bacterium *Streptococcus pneumoniae* is one of the severe life-threatening Gram-positive pathogens and elucidation of the structure of its lipoteichoic acid revealed 2,4-diacetamido-2,4,6-trideoxy-D-galactose as a main component of the cell wall glycopolymer. Our approach toward this carbohydrate used an L-threonine derived aldehyde as starting material following the Garner protocol, which was subjected to a nitro aldol reaction with 2-nitroacetaldehyde diethylacetal to generate the *galacto* configurated carbon backbone. Subsequent reduction of the nitro group and peracetylation of the molecule resulted in a fully and orthogonally protected derivative, which was deprotected by a single step in pure deionized water at elevated temperatures.

1. Introduction

The surface of cells is decorated with various carbohydrate polymers.[1] These glycopolymers influence biological functions and effect communication between different cells.[2] Furthermore, host–pathogen interactions and immunological processes are mediated via these molecules and lectins recognize different carbohydrate patterns.[3] The glycopolymers of Gram-negative bacteria, the lipopolysaccharides (LPS), are significantly better characterized than their Gram-positive counterparts the teichoic (TA) and lipoteichoic acids (LTA), and therefore the functions of the TAs and LTAs are still poorly understood.[4] To some extent they are not essential for the survival of the bacteria in vitro and they have been suggested to be mainly involved in defense mechanisms.[5] These biopolymers are important for infection and colonization of the host[6] and for causing immune responses,[7] which makes them interesting targets for medicinal chemistry.[8] Extraction of these complex molecules from natural sources is usually demanding since purification often failed and degradation products were isolated.[4,9] In recent years, the supply of appropriate and chemically pure glycopolymers for biological testing has been provided by chemical synthesis.[10]

The precise structure of the teichoic and lipoteichoic acids differs between species and among strains.[4,5] However, they all consist of different fatty acids and carbohydrates with glucose and galactose as the main constituents.[4] Additionally, amino sugars increase the complexity of the glycopolymers and create further diversity between different bacteria.

The LTA of *S. pneumoniae*, a Gram-positive bacterium which is still a life threatening pathogen, affects the innate immune system. It contains a rather unique 2,4-diamino-2,4,6-trideoxy-D-galactose, which is either mono or di-N-acetylated.[11] In order to detect the receptors involved in the immune response, a chemical synthesis of this glycopolymer was necessary. The synthesis of the galactose derivative led to several publications over the last decades.[12] Glucosamine was the most common starting material and deoxygenation protocols and S_N2 reactions to introduce the nitrogen functionality were applied.[13] Herein, we present a straightforward de novo synthesis starting from L-threonine, which can be easily performed on gram scale.

2. Results and discussion

Our synthetic strategy is outlined in Scheme 1. We started with L-threonine (**1**) and used the Garner protocol[14] to obtain aldehyde **2**,[15] which is the substrate for the following key step. A nitro aldol reaction with 2-nitroacetaldehyde diethylacetal (**7**) under basic conditions generated the carbon backbone of our target molecule. Subsequent reduction of the nitro group with Raney nickel resulted in the amine **4** which was immediately peracetylated. The final global deprotection was achieved using deionized water at high temperatures to yield the di-N-acetylated 2,4-diamino-2,4,6-trideoxy-D-galactose (**6**).

We first investigated the influence of different carbamate protecting groups on the outcome of the nitro aldol reaction (Table 1) with 2-nitroacetaldehyde diethylacetal (**7**).[16] Compound **7** was easily prepared from nitromethane and triethylorthoformate under reflux conditions with Lewis acid catalysis.[17] Simultaneously, an additional oxidation step to generate the aldehyde functionality

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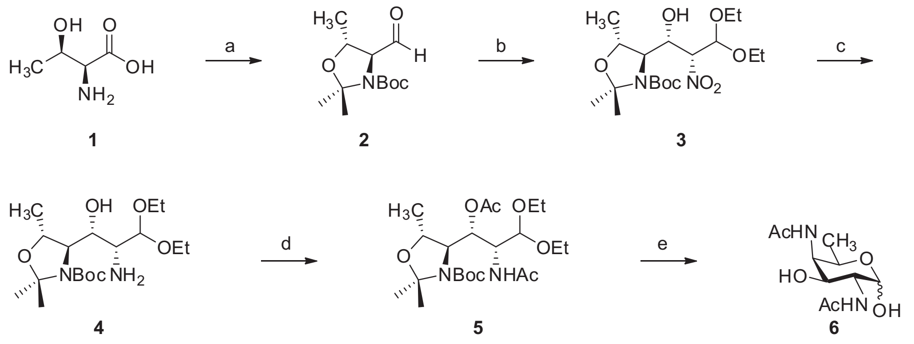
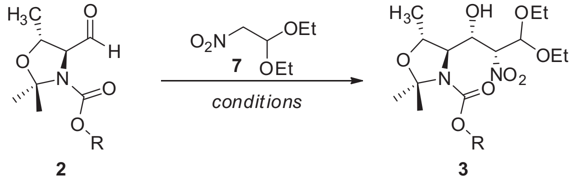
0008-6215/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carres.2012.11.019



Scheme 1. Synthesis of target compound **6**. (a) see Ref. 15; (b) 2-nitroacetaldehyde diethylacetal (**7**), LiOH, THF–H_2O, 0 °C (*74%*); (c) Raney nickel, H_2, MeOH (*98%*); (d) Ac_2O, DMAP, pyridine, 0 °C (*92%*); (e) H_2O, 100 °C (*37%*).

Table 1
Reaction conditions for the nitro aldol reaction[a]



Entry	R	Reaction conditions[b]	Base[c]	Yield **3**[d] (%)
1	Benzyl	THF (rt)	TBAF/NEt_3/BuLi/LiOH	nd
2	Phenyl	THF (rt)	TBAF/NEt_3/BuLi/LiOH	nd
3	*tert*-Butyl	THF (rt)	TBAF/CsF/KF + crown ether	Traces
4	*tert*-Butyl	THF (rt)	LiOH	43 (*gal*:*talo* 2:1)
5	*tert*-Butyl	CH_2Cl_2 (0 °C)	LiOH	60 (*gal*:*talo* 3:1)
6	*tert*-Butyl	THF (0 °C)	LiOH	77 (*gal*:*talo* 3:2)
7	*tert*-Butyl	THF:H_2O 3:2 (0 °C)	LiOH	74 (*gal*:*talo* 5:1)
8	*tert*-Butyl	THF:H_2O 1:4 (0 °C)	LiOH	41 (*gal*:*talo* 4:3)

[a] Reactions were performed on a 50 mg scale of **2** and 7 equiv of **7** in 10 mL solvent for 16 h.
[b] rt = room temperature.
[c] When various bases are stated, they were used in different experiments.
[d] Isolated yields; nd = not detected; *gal* = *galacto*.

of the target molecule was avoided, compared to the more common reaction with nitroethanol for C2-elongation.[18]

To our surprise we could not observe any aldol product formation when we used the benzyloxycarbonyl or the corresponding phenyl derivative (entries 1 and 2). We used different bases and solvent systems but only the reaction with the *tert*-butyloxycarbonyl (Boc) protected compound **2** gave the desired product **3**. The use of amine bases or lithiating reagents resulted in decomposition, whereas bases like fluoride ions gave very low yields throughout (entry 3). We could raise the yield up to 77% using lithium hydroxide in tetrahydrofuran at 0 °C for 16 h (entry 6). Prolonged reaction times did not further improve the yield and when higher temperatures were applied, elimination products were detected. The diastereoselectivity of the reaction turned out to strongly depend on the solvent composition. In anhydrous tetrahydrofuran we could observe a diastereomeric ratio of 3:2 in favor of the *galacto* configurated derivative over its *talo* diastereomer (entry 6). We also detected only two out of the four possible diastereomeric products, which is in agreement with results obtained using compound **7** in nitro aldol reactions.[16] The stereochemistry of the diastereomers was determined unambiguously by NMR spectroscopy of the deprotected final carbohydrates. Analysis of the coupling constants of the major product showed typical values for *galacto* configuration.[19] The chemical shifts and ratios of the furanose and pyranose forms of the minor isomer are fully consistent with published data for the *talo* configurated product.[20]

The addition of water to the reaction medium increased the selectivity up to 5:1 (entry 7). The optimized solvent mixture was tetrahydrofuran/water = 3:2 and further addition of water led to decreasing yields and selectivities (entry 8). The *syn* configuration between the two new stereocenters C-2 and C-3 is

Table 2
Reaction conditions for the deprotection[a]

Entry	*c* (**5**) (mg/mL)	Time (h)	Temperature (°C)	**8** (%)	**9** (%)	**6** (%)
1	10	12	80	68	5	nd
2	10	12	100	47	5	10
3	1	12	100	3	12	37
4	1	48	100	nd	7	22

[a] Reactions were performed with 50 mg of **5** in deionized water. Yields are isolated yields. nd = not detected.