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Synthesis of the pentasaccharide hapten from the glycopeptidolipid antigen of *Mycobacterium avium* serovar 12

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Abstract—An effective synthesis of the pentasaccharide hapten from the glycopeptidolipid antigen of *Mycobacterium avium* serovar 12 in the protected *p*-nitrophenyl glycoside, using a 3+2 block synthesis strategy, is described.

Besides *Mycobacterium tuberculosis*[1] and *M. leprae*,[2,3] the agents of human tuberculosis and leprosy, respectively, another 'atypical' or 'opportunistic' mycobacteria may also cause infections in humans.[4] Infections with the *M. avium* serocomplex are seen in up to 50% of the patients with AIDS in some areas of the world.[5–7] The antigens of various serovars of *M. avium* are glycopeptidolipids (GPLs) located in the outer cell surface of these bacteria, whose structures can be described by a general formula[8] (Fig. 1).

These outer oligosaccharide haptens are responsible for the immunological properties of the bacteria, thus these haptens, after conjugation with suitable proteins, might aid the serodiagnosis of mycobacterial infections. The synthesis of different oligosaccharide haptens and their conjugation to proteins have been recently reviewed.[8,9]

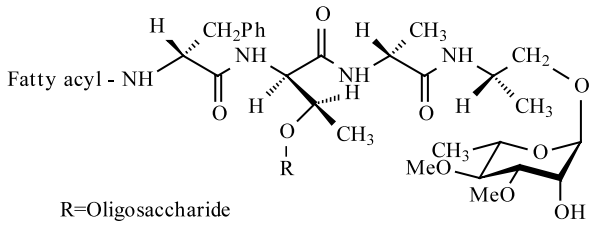

Figure 1.

In this paper we report the synthesis of the protected pentasaccharide hapten of the *M. avium* serovar 12[10] carrying a *p*-nitrophenyl spacer. The structure of the protected hapten **1** is shown in Fig. 2.

The synthesis of the unprotected diastereoisomeric terminal trisaccharides with D- and L-lactamido groups in position 4″ was described by van Boom's group[11] using 3-aminopropyl as the spacer.

One of the most difficult tasks was the synthesis of the 3-*O*-methyl ether of D-viosamin (4-amino-4,6-dideoxy-D-glucose). Regioselective 3-*O*-methylation of ethyl 2-*O*-benzoyl-1-thio-α-D-fucopyranoside by means of the 3,4-*O*-stannylidene acetal protocol was reported,[11] however this procedure did not work in our hands. To overcome these difficulties we started from allyl α-D-fuco-pyranoside[12] **2**, and using the orthoester methodology,[13] allyl 2,4-di-*O*-benzoyl-α-D-fucopyranoside **3** was obtained in a one-pot three-step reaction sequence (Scheme 1). The 3-OH was then methylated with diazomethane in the presence of $BF_3{\cdot}OEt_2$[14] giving **4**.

It is well documented that the isolated 2-*O*-acyl groups of pyranosides are stable under the conditions of the Zemplén transacylation reaction.[15–17] Glaudemans et al. showed that an *O*-acyl group at position 3 was also stable under Zemplén's conditions, when position 2 is deoxygenated and position 4 is blocked.[18] We found that the 2-*O*-benzoate of **4** is more reactive than the 4-OBz, and could be selectively removed under Zemplén's conditions, and the deacylated product **5** proved to be a useful intermediate. The allyl group was isomer-

Keywords: oligosaccharide; glycopeptidolipid; block synthesis; regioselective Zemplén deacylation; *M. avium* serovar 12.

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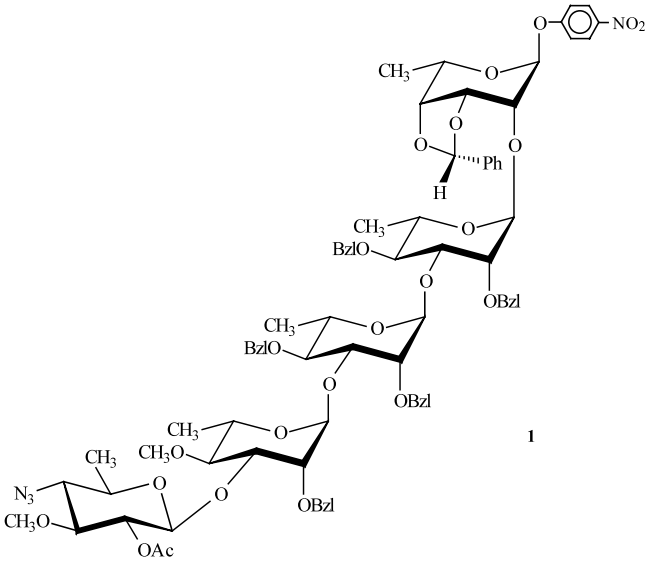


Figure 2.

ized to propenyl and then the enol ether was transformed to the 1,2-*O*-propylidene acetal[19] **6**. Compound **6** was treated with equimolar NaOMe to obtain the 4-OH derivative **7**, which was triflated (→**8**) and a subsequent nucleophilic displacement with LiN_3 resulted in the D-*gluco* epimer **9**.

Acid hydrolysis of the propylidene acetal and direct acetylation of the diol formed gave **10** which was transformed into the trichloroacetimidate **11** using the standard Schmidt procedure.[20]

The next unit of the pentasaccharide was synthesized from phenyl 2,3-*O*-isopropylidene-1-thio-α-L-rhamnopyranoside **12**[21] by methylation of 4-OH (→**13**) followed by the hydrolysis of the isopropylidene group (→**14**) and phase transfer catalyzed selective benzylation[22] of the 2-OH (→**15**). Glycosylation of **15** with the imidate **11** yielded the azido derivative **16** of the terminal disaccharide (Scheme 2).

Our original intent was to use the disaccharide donor **16** for the 2+3 block synthesis of the pentasaccharide. However during the synthesis of an analogous oligosaccharide it was observed[23] that glycosylation of the trisaccharide acceptor **19** with any glycosyl donor failed, but its earlier synthesized[24] disaccharide part **20** reacted readily under various glycosylation conditions (Fig. 3).

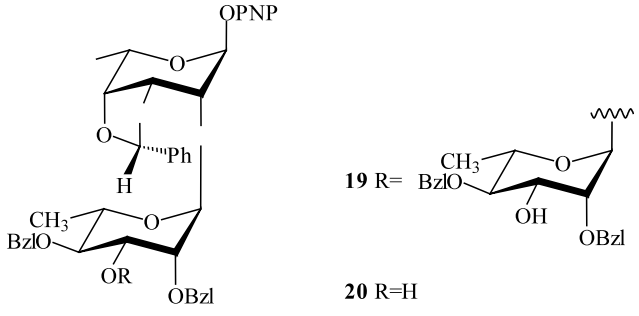
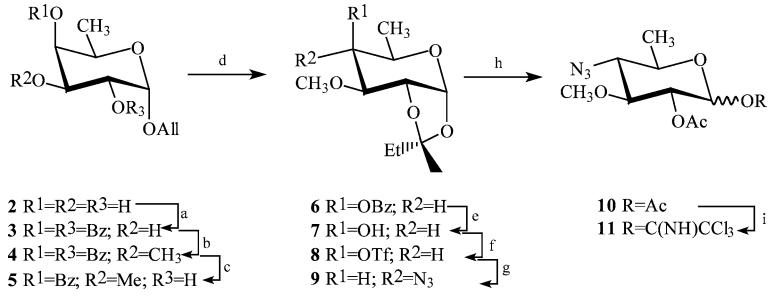
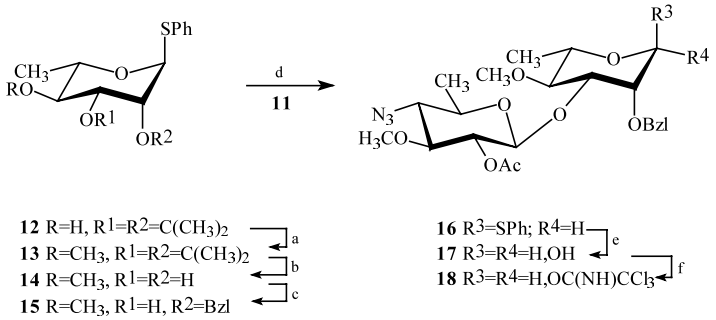


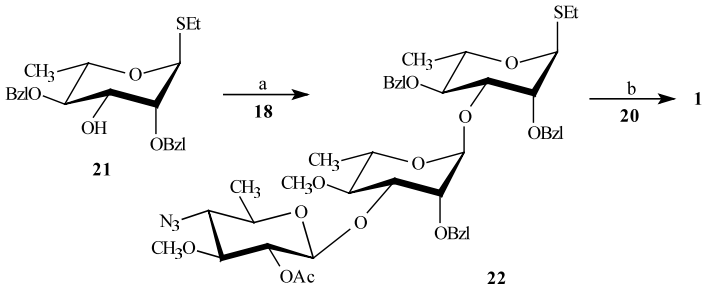
Figure 3.



Scheme 1. (a) *i*. $PhC(OEt)_3$, CSA, MeCN; *ii*. BzCl, pyridine; *iii*. $AcOH/H_2O$, 72% for three steps; (b) CH_2N_2, $BF_3{\cdot}Et_2O$, 84%; (c) NaOMe, MeOH, 87% (d) *i*. $(Ph_3P)_3RhCl$, DABCO, EtOH-toluene-H_2O; *ii*. CSA, CH_2Cl_2, 52% for two steps; (e) NaOMe, MeOH; quant. (f) Tf_2O, pyridine; (g) LiN_3, DMF, 81% for two steps; (h) *i*. TFA, H_2O, CH_2Cl_2; *ii*. Ac_2O, pyridine, 60% for two steps; (i) *i*. hydrazine acetate, DMF; *ii*. CCl_3CN, K_2CO_3, CH_2Cl_2, 91% for two steps.



Scheme 2. (a) MeI, KOH, DMF, 90%; (b) CF_3COOH, H_2O, CH_2Cl_2, 98%; (c) BzlBr, NaOH, Bu_4NBr, CH_2Cl_2-H_2O, 89%; (d) TMSOTf, CH_2Cl_2, −40°C, 63%; (e) NBS, aq. acetone, 83%; (f) CCl_3CN, K_2CO_3, CH_2Cl_2, quantitative.



Scheme 3. (a) TMSOTf, CH_2Cl_2, −60°C, 74%; (b) NIS/TfOH, CH_2Cl_2-THF, −50°C, 50%.

Based on these experiences the thioglycoside **16** was treated with NBS in aqueous acetone[25] to yield the hemiacetal **17** which was then converted into imidate **18** (Scheme 2).

Glycosylation of ethyl 2,4-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside **21**[26] with **18** afforded the trisaccharide-type glycosyl donor **22**. The dimer **20** was glycosylated with this trisaccharide under NIS/TfOH[27] promotion to give the fully protected pentasaccharide **1** stereoselectively, which was isolated in 50% yield (Scheme 3).

Table 1. Selected NMR data of pentasaccharide **1** (δ, ppm)

Residue		^{1}H	^{13}C
A	1	5.58	98.40
	2	4.17	76.21
	3	4.53	72.02
	4	4.18	71.02
	5	3.95	67.53
	CH_3	1.23	15.47
	$J_{C1,H1}$	176.5 Hz	
B	1	4.97	95.07
	2	3.75	78.17
	3	3.87	78.17
	4	3.52	80.29
	5	3.83	68.63
	CH_3	1.23	17.89
	$J_{C1,H1}$	171.3 Hz	
C	1	4.94	99.20
	2	3.66	78.88
	3	4.01	78.88
	4	3.43	80.29
	5	3.70	68.63
	CH_3	1.07	17.89
	$J_{C1,H1}$	172.7 Hz	
D	1	4.94	100.65
	2	3.72	78.59
	3	3.80	79.89
	4	3.16	81.97
	5	3.62	68.63
	CH_3	1.12	17.89
	OCH_3	3.38	60.75
	$J_{C1,H1}$	173.0 Hz	
E	1	4.47	101.50
	2	4.91	73.25
	3	3.18	83.40
	4	3.00–3.06	66.72
	5	3.00–3.06	70.37
	CH_3	1.05	18.28
	OCH_3	3.48	60.01
	$J_{C1,H1}$	163.9 Hz	

The structures of all intermediates and the end-product were unambiguously confirmed by one and two dimensional ^{1}H and ^{13}C NMR spectroscopic methods. Selected NMR data (CD_3OD, Bruker DRX 500) for **1** is given in Table 1.

In conclusion, we have elaborated a new procedure for the synthesis of the precursor of the terminal 4-amino-4,6-dideoxy-3-*O*-methyl-D-glucose (3-*O*-methyl-D-viosamine); observed and used a new type of the anomalous Zemplén deacylation; verified our earlier observation concerning the very low reactivity of 3″-OH of the **19** trisaccharide acceptor. The 3′-OH of the L-rhamnosyl unit in the disaccharide acceptor **20** showed acceptable reactivity to achieve the synthesis of the fully protected target pentasaccharide **1** using a 3+2 block synthesis.

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