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Synthesis of the pentasaccharide hapten from the glycopeptidolipid antigen of *Mycobacterium avium* serovar 17

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Abstract—Effective synthesis of the pentasaccharide hapten from the glycopeptidolipid antigen of *Mycobacterium avium* serovar 17 in a *p*-aminophenyl linker-containing form, using 3+2 block synthesis strategy, is described. A 2+3 block synthesis could not be achieved, although different glycosyl donors (1-Br, 1-SPh, 1-*O*-C(NH)CCl$_3$) were used.

Besides *Mycobacterium tuberculosis*[1] and *M. leprae*,[2,3] the pathogenic agents of human tuberculosis and leprosy, respectively, another 'atypical' mycobacteria may also cause infections in humans.[4] Infections with the *M. avium* serocomplex show up in more than 50% of patients with AIDS in some areas of the world.[5–7] This observation initiated a series of investigations, (i) to determine the surface antigens of different human

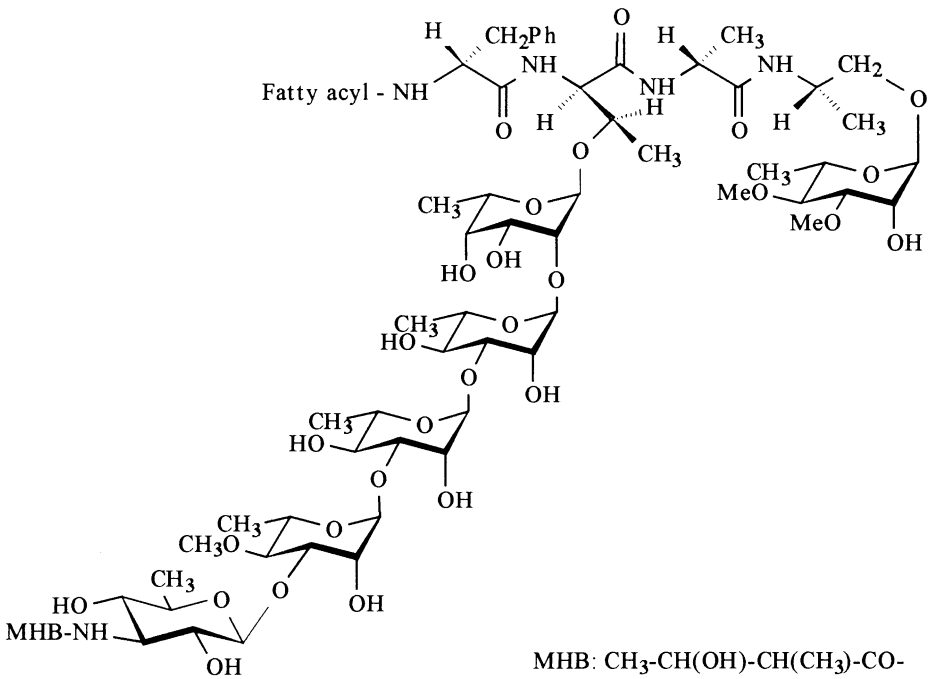

Figure 1.

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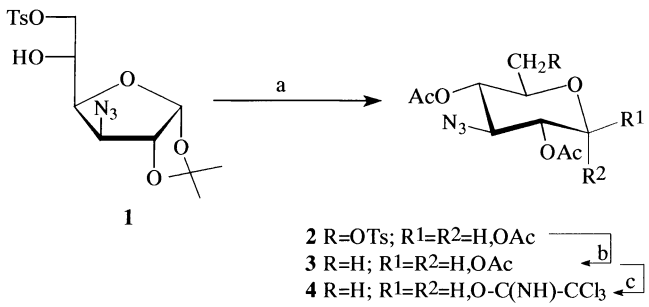
pathogenic mycobacteria, and (ii) to prepare artificial antigens for the serodiagnosis of mycobacterial infections.[8,9]

In this paper we present an effective synthesis of a *p*-aminophenyl linker-containing pentasaccharide which was identified as the GPL type antigen[8,9] of *M. avium* serovar 17 (Fig. 1). The terminal monosaccharide unit in this structure is a 3-amino-3,6-dideoxy-β-D-glucopyranoside[10] acylated with 3-hydroxy-2-methylbutyric acid (nilic acid[11]) at position 3. Nilic acid has two chiral centers and exists in four isomeric forms. We report here the synthesis of the (2*S*,3*S*) isomer containing pentasaccharide hapten.

For the preparation of the target pentasaccharide using a 2+3 block synthesis, we proceeded as follows. Acetolysis of 3-azido-3-deoxy-1,2-*O*-isopropylidene-6-*O*-(*p*-toluenesulfonyl)-α-D-glucofuranose[12] **1** gave an anomeric mixture of the tri-*O*-acetyl derivative **2**. Deoxygenation of **2** at position 6, leaving the 3-azido group untouched, was performed with the $NaCNBH_3$/NaI reagent in HMPT[13] yielding **3** (73%). Compound **3** itself is a glycosyl donor but with low reactivity, therefore, it was transformed into the glycosyl trichloroacetimidate **4** according to the procedure of Schmidt et al.[14] (Scheme 1).

The TMSOTf-mediated coupling of the trichloroacetimidate **4** with phenyl 2-*O*-benzyl-4-*O*-methyl-1-thio-α-L-rhamnopyranoside **5** as the glycosyl acceptor



Scheme 1. (a) 0.1 M H_2SO_4, 60°C, then Ac_2O/pyridine, 90%; (b) $NaCNBH_3$, NaI, HMPT, 70°C, 73%; (c) H_2NNH_3OAc, DMF, rt, then CCl_3CN, K_2CO_3 CH_2Cl_2, rt, 85%.

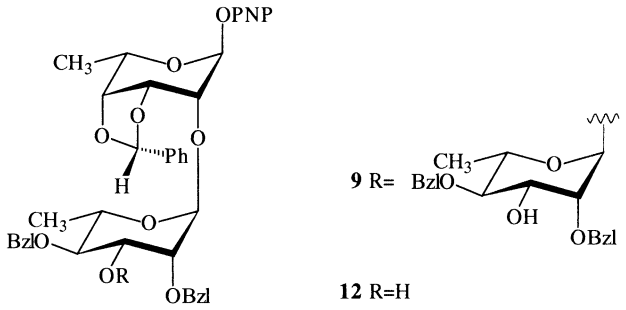


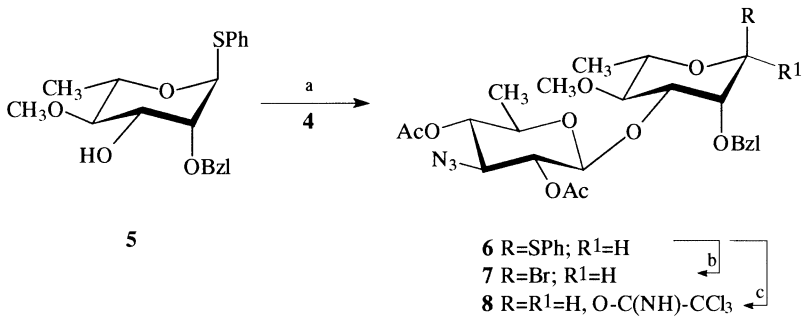
Figure 2.

afforded exclusively the β-disaccharide **6** in 75% yield (Scheme 2).

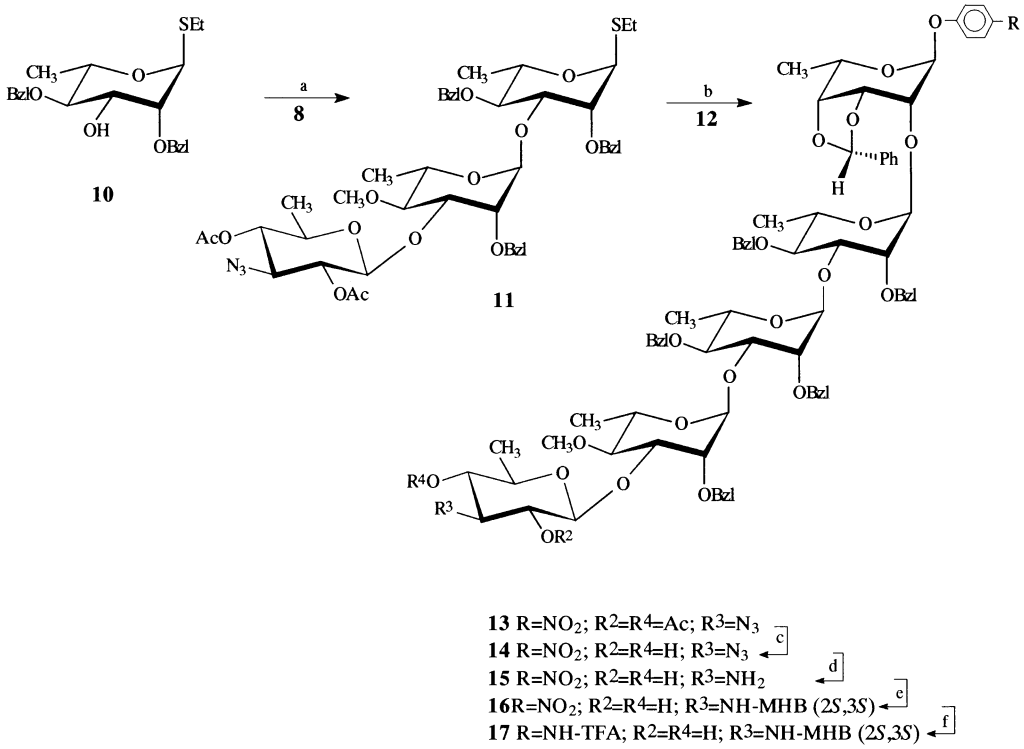
However, in contrast to our expectation, the disaccharide donor **6** did not react with the trisaccharide acceptor **9** (Fig. 2), and thus to enhance its reactivity, thioglycoside **6** was transformed into the bromosugar **7** and the trichloroacetimidoyl derivative **8** (Scheme 2). However, neither **7** nor **8** reacted sufficiently, probably due to the extremely low nucleophilicity of the trisaccharide acceptor.

The unusual behavior of **9** forced us to change the original synthetic route to a 3+2 approach. Thus, the disaccharide donor **8** was reacted with ethyl 2,4-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside **10** in the presence of TMSOTf to give the trisaccharide block **11** in a yield of 55%. The sterically 'mismatched' situation in this glycosylation led to a relatively low yield of coupling. The thioglycoside donor **11** was then allowed to react with the disaccharide acceptor **12** in the presence of NIS/TfOH to furnish the desired pentasaccharide **13** in a 69% yield (Scheme 3). Although glycosylations were performed with donors having non-participating group at C-2, in both reactions the formation of an α-1,2-*trans* linkage was observed exclusively.

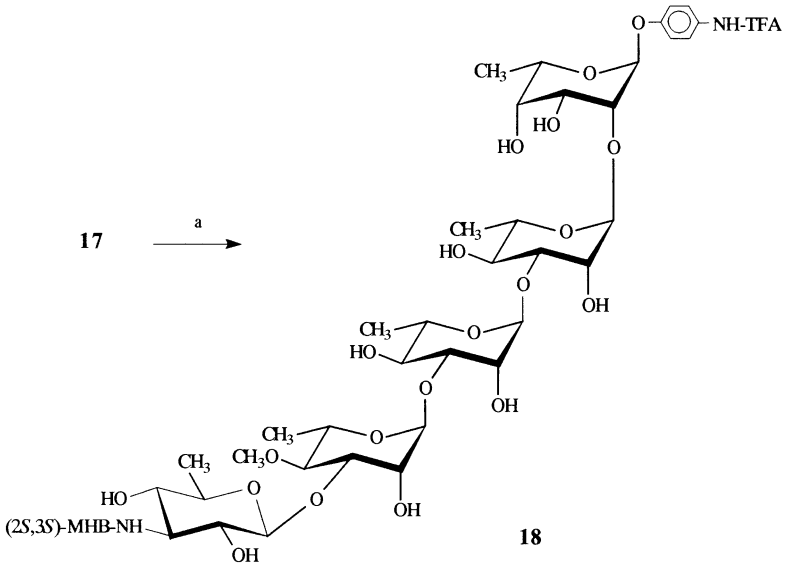
To avoid a possible *O*→*N* acetyl migration, the two isolated *O*-acetyl groups were removed with KO^tBu,[15] prior to the reduction of the azide, to obtain the diol **14**. It is to be noted, that in accord with other literature examples, removal of the hindered or isolated *O*-acetyl protecting groups by the classical Zemplén procedure



Scheme 2. (a) TMSOTf, CH_2Cl_2, −50°C, 75%; (b) Br_2, CH_2Cl_2, 0°C; (c) NBS, acetone–H_2O, 0°C, then CCl_3CN, K_2CO_3 CH_2Cl_2, rt, 75%.



Scheme 3. (a) TMSOTf, CH_2Cl_2, −60°C, 55%; (b) NIS/TfOH, CH_2Cl_2–THF, −50°C, 69%; (c) KO^{t}Bu, dioxane–MeOH, rt, quant.; (d) Ph_3P, THF, 87%; (e) (2*S*,3*S*)-nilic acid, BOP/DIPEA, 72%; (f) H_2/Pt, EtOAc, rt, then Py, TFA-anhydride 0°C, 78%.



Scheme 4. (a) H_2/Pd–C, MeOH–H_2O (5:1), 85%.

failed in this case.[16] For the effective, selective reduction of the azido group in the presence of the *p*-nitrophenyl aglycone, Ph_3P[17] was used. The resulting amine **15** was acylated with (*S*)-3-hydroxy-(*S*)-2-methylbutyric acid (prepared from ethyl (*S*)-3-hydroxybutyrate[11,18]) using BOP/DIPEA as the coupling reagents. Pentasaccharide **16** was deprotected as follows. The aromatic nitro group was reduced with Adam's catalyst and the product was converted into the trifluoroacetamido derivative **17**. Finally, the benzyl and benzylidene functions were cleaved by hydrogenolysis in the presence of Pd–C catalyst to furnish the target pentasaccharide **18**[†] (Scheme 4). Complete NMR data (CD_3OD, BRUKER DRX 500) of **18** are given in Table 1.

The conjugation of the spacer-armed pentasaccharide hapten to protein, and the biological testing of the neoglycoconjugate are in progress and will be reported elsewhere.

[†] MALDI-TOF for pentasaccharide $C_{44}H_{67}F_3N_2O_{23}$+Na^+: 1072.54.

Table 1. NMR data of pentasaccharide **18** (δ, ppm)

Residue		^{1}H	^{13}C
A	1	5.64	98.3
	2	4.03	77.4
	3	4.10	70.6
	4	3.63	72.6
	5	3.89	68.1
	CH_3	1.23	15.8
	p-subst. aromatic	7.13, 7.60	
B	1	5.04	103.4
	2	4.09	66.4
	3	3.81	77.6
	4	3.59	72.1
	5	3.83	69.5
	CH_3	1.31	17.1
C	1	5.07	102.7
	2	4.07	70.8
	3	3.88	78.9
	4	3.56	72.2
	5	3.88	70.1
	CH_3	1.29	17.1
D	1	5.02	102.6
	2	4.17	71.2
	3	4.06	79.2
	4	3.24	82.6
	5	3.88	68.1
	CH_3	1.28	16.9
	OCH_3	3.58	60.5
E	1	4.63	104.6
	2	3.37	72.6
	3	3.78	58.1
	4	3.12	74.3
	5	3.39	73.7
	CH_3	1.30	17.0
Nilic acid	C_2-*H*	2.33	49.0
	C_3-*H*	3.32	48.3
	C_2-CH_3	1.14	13.6
	C_3-CH_3	1.22	19.8

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