COMMUNICATION

Chemical synthesis of the *N*-glycans of gp63, the major surface glycoprotein from *Leishmania mexicana amazonensis*

Arno Düffels and Steven V. Ley *

Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, UK CB2 1EW

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The chemical synthesis of the conjugated heptasaccharide Man(α1-3)Man(α1-6)[Glc(α1-3)Man(α1-2)Man(α1-2)Man-(α1-3)]Manβ1-O[CH_2]$_8$$CO_2$Me 1 and four closely related biantennary oligomannose type structures derived from the glycoprotein 63 of *Leishmania mexicana amazonensis* is described. Taking advantage of common structural motifs found in these *N*-glycans, a strategy based on the principles of reactivity tuning and orthogonal activation allowed the rapid assembly of a whole class of complex oligosaccharides. Deprotection of all structures was achieved in high yields by hydrogenolysis in one step.

Introduction

Leishmania are digenetic parasites which are the causative agents in a number of serious human diseases affecting populations throughout the tropical and subtropical world. The parasite alternates between the promastigote, a free living flagellate in the gut of the vector sandfly, and the amastigote, the obligatory intracellular form, which resides in phagolysosomes in mammalian macrophages. Essential for the establishment of successful infection are four sequential events: recognition, intracellular entry, survival and replication.[1]

Evidence exists that cell surface oligosaccharides of this parasite play a role in the infection of human macrophages by promastigotes.[2] Investigations into the structure–activity relationship of these carbohydrates, however, are restricted by the poor availability of pure oligosaccharides and the microheterogeneity of the naturally occurring glycoprotein.[3]

The *N*-linked oligosaccharides of gp63, the major surface glycoprotein of *L. mexicana amazonensis*, were characterized in 1990.[3] Interestingly, all the biantennary oligosaccharides isolated from *L. mexicana amazonensis* lack the branched (3,6-linked) α-mannosyl residue on the α-1,6 arm which is usually observed in similar oligomannose structures.[4]

Fig. 1

Here we report the synthesis of the five oligomannose type structures of *L. mexicana amazonensis*. All compounds contain a β-linked methoxycarbonyl octyl chain,[5] replacing the di-*N*-acetylchitobiose (GlcNAc(β1-4)GlcNAc) moiety of the natural occurring *N*-glycans which allows further derivatization of the final glycoconjugates for use in biosynthesis studies of these *N*-glycans.[6]

Results and discussion

Recently we reported the synthesis of a high mannose type nonasaccharide[7,8] as part of our research in the area of protecting group mediated reactivity tuning in oligosaccharide synthesis.[9] In this context seleno- and thioglycosides have proven to be versatile components for the stereoselective construction of complex mannosides.

Based on this result the synthesis of the glycoconjugates **1**–**5** (Fig. 1) was addressed by stepwise addition of the respective mono- and disaccharide precursors **6**–**10** to the central β-mannoside **11**[8] (Fig. 2). This convergent approach allowed the rapid assembly of the complete set of biantennary target

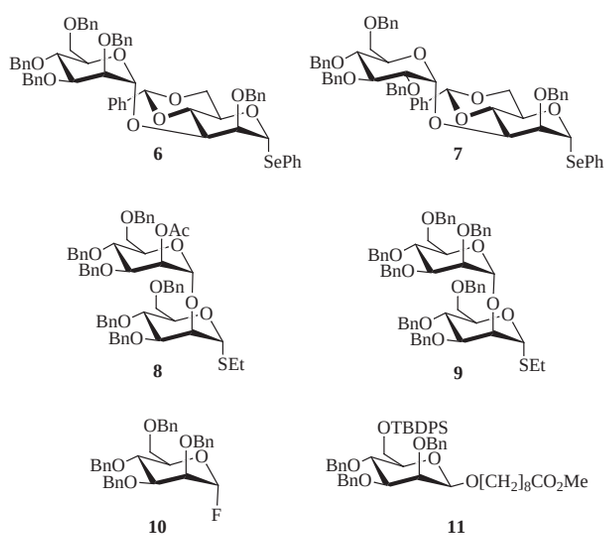
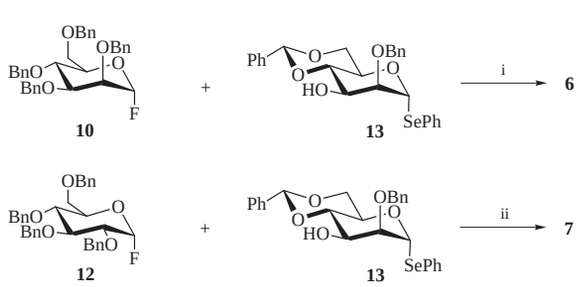
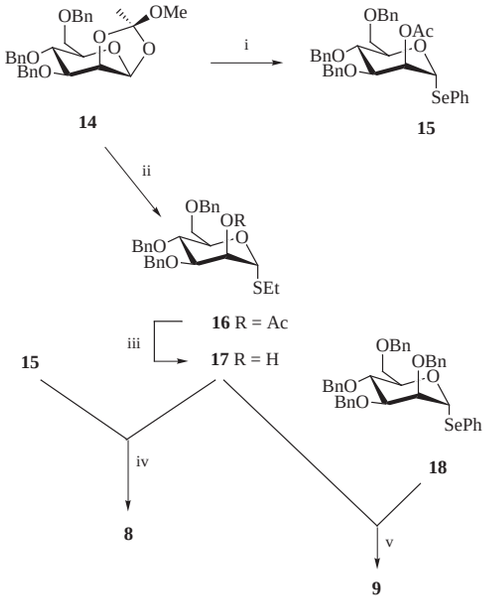


Fig. 2



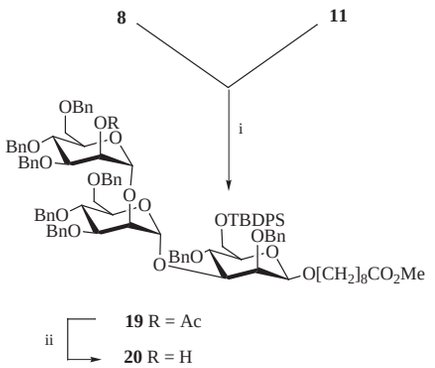
Scheme 1 *Reagents and conditions*: i, $AgClO_4$, $HfCp_2Cl_2$, 4 Å molecular sieves, Et_2O, 74%; ii, $AgClO_4$, $HfCp_2Cl_2$, 4 Å molecular sieves, Et_2O, 72%.



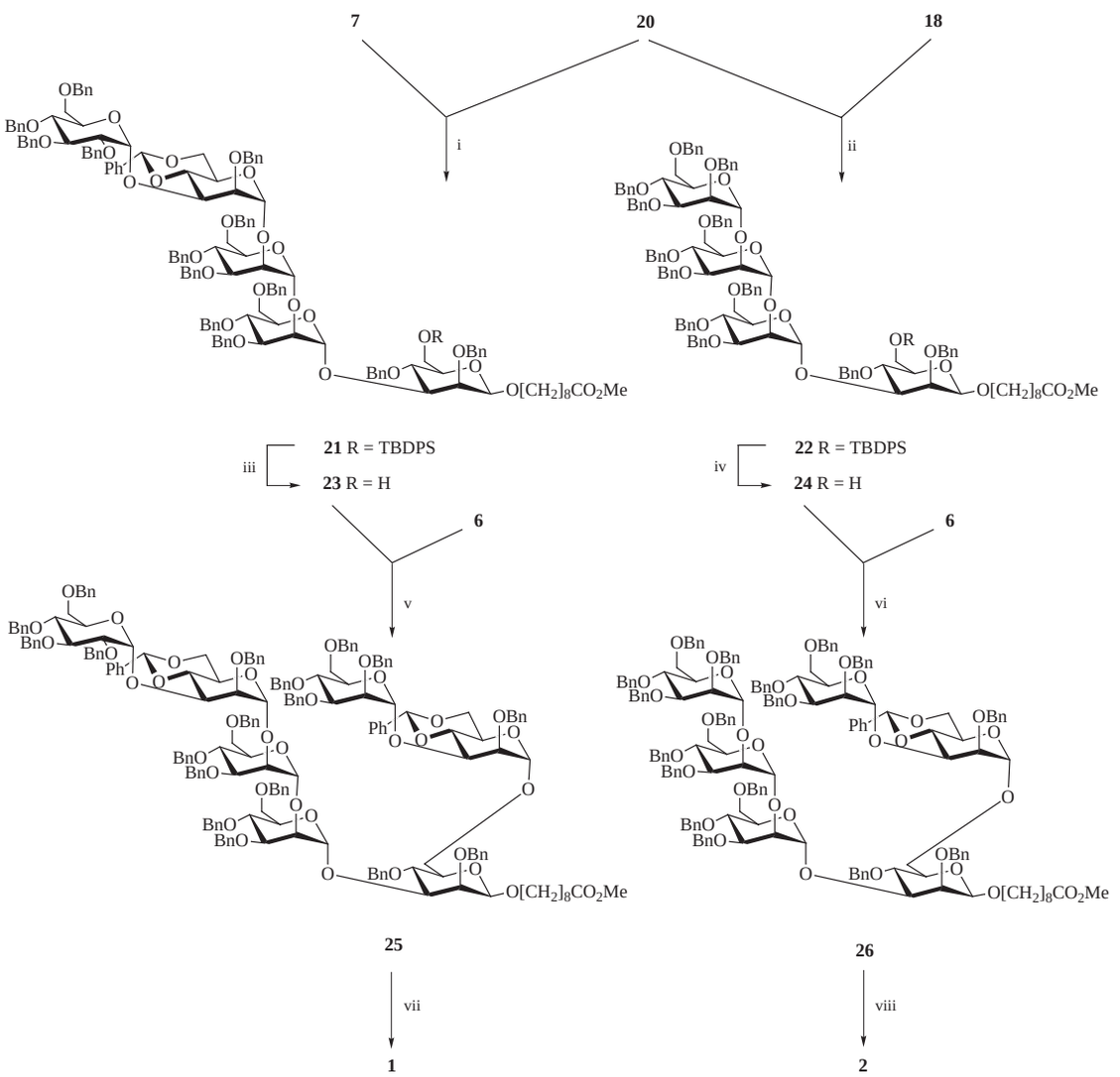
Scheme 2 *Reagents and conditions*: i, $HgBr_2$ (cat.), PhSeH, MeCN, 60 °C, 96%; ii, $HgBr_2$ (cat.), EtSH, MeCN, 60 °C, 94%; iii, K_2CO_3, MeOH, 85%; iv, NIS, TfOH (cat.), 4 Å molecular sieves, DCM–Et_2O 1:1, 75%; v, NIS, TfOH (cat.), 4 Å molecular sieves, DCM–Et_2O 1:1, 78%.

molecules **1**–**5** in a minimum number of steps from a common set of building blocks.

The synthesis of the building blocks **6**–**11** began with the preparation of the α-1,3 linked disaccharides **6** and **7**. Starting from the readily available benzylidene protected selenomannoside **13**[10] (Scheme 1), orthogonal activation of 2,3,4,6-*O*-benzyl-α-D-mannopyranosyl fluoride **10** and 2,3,4,6-*O*-benzyl-α-D-glucopyranosyl fluoride **12** using $HfCp_2Cl_2$–$AgClO_4$[11] as the activation system in the presence of **13** gave the desired disaccharides **6** (74%) and **7** (72%), respectively, as single



Scheme 3 *Reagents and conditions*: i, NIS, TfOH (cat.), 4 Å molecular sieves, DCM–Et_2O 1:1, 81%; ii, K_2CO_3, MeOH, 88%.



Scheme 4 *Reagents and conditions*: i, NIS, TfOH (cat.), 4 Å molecular sieves, DCM–Et_2O 1:1, 81%; ii, NIS, TfOH (cat.), 4 Å molecular sieves, DCM–Et_2O 1:1, 83%; iii, TBAF–AcOH (3%), THF, 88%; iv, TBAF–AcOH (3%), 92%; v, NIS, TfOH (cat.), 4 Å molecular sieves, DCM–Et_2O 1:1, 51%; vi, NIS, TfOH (cat.), 4 Å molecular sieves, DCM–Et_2O 1:1, 53%; vii, $Pd(OH)_2$/C, H_2 1 atm, DCM–MeOH–H_2O 3:3:1, 84%; viii, $Pd(OH)_2$/C, H_2 1 atm, DCM–MeOH–H_2O 3:3:1, 98%.

isomers. High α-selectivity for the glycosidation of anomeric halides in the presence of $AgClO_4$ has been reported.[12]

The α-1,2 linked disaccharides **8** and **9** were prepared using the selectively acetylated seleno- and thiomannosides **15** and **17**, which in turn were derived from the known orthoester precursor **14**[13] (Scheme 2). Following a protocol introduced by Sinäy and co-workers[14] orthoester **14** was reacted with benzeneselenol or ethanethiol in the presence of catalytic amounts of $HgBr_2$ to yield the seleno- and thiomannosides **15** and **16** in excellent 96 and 94% yields respectively. Deacetylation of **16** under standard conditions furnished the glycosyl acceptor **17** which was subsequently reacted with the selenoglycosyl donors **15** and **18** using van Boom's NIS–TfOH activation conditions.[15] Due to their inherent higher reactivity the selenoglycosyl donors **15** and **18** were selectively activated in the presence of the thioglycosyl donor **17** resulting in the stereoselective formation of the disaccharides **8** and **9** in 75 and 78% yield respectively.

With building blocks **6**–**11** in hand the target glycoconjugates **1**–**5** were prepared in four to six steps.

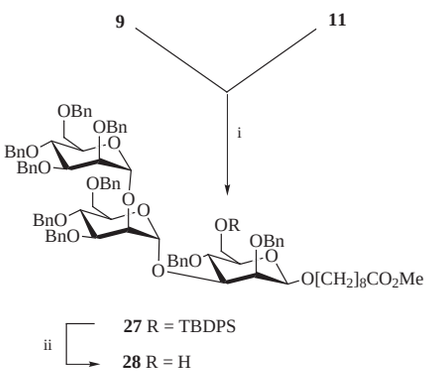
Synthesis of heptasaccharide **1** and hexasaccharide **2** was accomplished *via* the common intermediary trisaccharide **20**. According to Scheme 3 the dimannose-thioglycoside **8** was reacted with the selectively protected central β-mannoside **11** under NIS–TfOH activation conditions to give the fully protected product trisaccharide **19** in 81% yield on a gram scale. Standard deacetylation of **19** followed by glycosidation of the resulting acceptor saccharide **20** with the selenophenyl glycoside **18** and **7** under NIS–TfOH conditions yielded the desired tetra- and pentasaccharides **21** and **22** in 81 and 83% yields respectively and completed the construction of the α-1,3-arm of the target compounds **1** and **2** (Scheme 4).

Desilylation of the fully protected glycoconjugates **21** and **22** with tetrabutylammonium fluoride (TBAF) in THF proceeded smoothly and was followed by the coupling of the resulting glycosyl acceptors **23** and **24** with the seleno glycoside **6** to complete the synthesis of the fully protected target molecules **25** and **26**. The moderate yields for the last glycosidations (51 and 53%) are acceptable considering the complexity of the product molecules. The saccharides **25** and **26** were deprotected by hydrogenation in a homogeneous mixture of dichloromethane, methanol and water (3 : 3 : 1) using $Pd(OH)_2/C$ as a catalyst. The solvent system was chosen to keep both the starting material and the product in solution. The deprotections were carried out on a 150 mg scale and the resulting saccharides **1** and **2** were isolated as colourless amorphous solids after size exclusion chromatography on Sephadex® G-15 (eluent: H_2O–^{n}PrOH, 95 : 5) in 84 and 98% yield respectively.

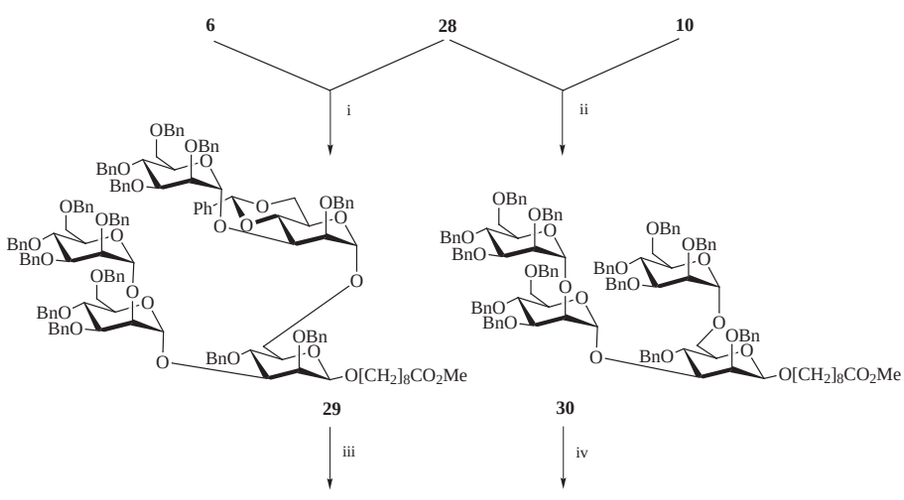
As for the preparation of the hexa- and heptasaccharide **1** and **2**, a common trisaccharide intermediate was chosen in the synthesis of the high mannose type saccharides **3** and **4**, taking advantage of the common structural motifs in both target molecules (Scheme 5). Thus stereoselective glycosidation of the central β-mannoside **11** with the α-1,2 linked selenoglycoside **9** under NIS–TfOH conditions gave the trimannoside **27** in 68% yield. Subsequent desilylation with TBAF in THF gave the key glycosyl acceptor **28** which underwent glycosidation reactions with fluoro mannoside **6** and the seleno dimannoside **10** (Scheme 6). The resulting high mannosides **29** and **30** were isolated as single anomers in 64 and 67% yield respectively. When the perbenzylated selenophenyl mannoside **18** was reacted with the primary alcohol of trisaccharide **28** an anomeric mixture at the newly formed glycosidic linkage was observed (82%; α : β = 3 : 1). Cleavage of the benzyl and benzylidene protecting groups was achieved under the previously described conditions and completed the synthesis of the glycoconjugates **3** and **4**. The final products were isolated as colourless amorphous solids in 99 and 98% yield after size exclusion chromatography on Sephadex® G-15 (eluent: H_2O–^{n}PrOH, 95 : 5).

Synthesis of the *N*-glycans of *L. mexicana amazonensis* was completed with the four step preparation of tetramannoside **5** (Scheme 7). Thus NIS–TfOH mediated coupling of the perbenzylated selenomannoside **18** with the free secondary alcohol of the central β-mannoside **11** resulted in the α-stereoselective formation of dimannoside **31** in 78% yield. Standard desilylation followed by glycosidation of the resulting primary alcohol with the α-1,3 linked dimannoside **6** furnished the fully protected tetrasaccharide **33** as its α-anomer in 64% yield, which was deprotected in one step by hydrogenation using the conditions described above. The desired tetrasaccharide **5** was isolated in 96% yield as a colourless amorphous solid after size exclusion chromatography on Sephadex® G-15 (eluent: H_2O–^{n}PrOH, 95 : 5).

The structures of the final glycoconjugates **1**–**5** and their



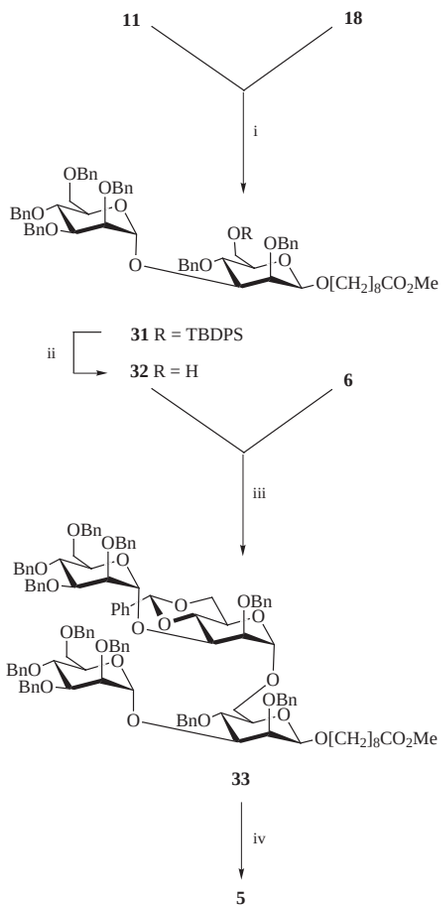
Scheme 5 *Reagents and conditions*: i, NIS, TfOH (cat.), 4 Å molecular sieves, DCM–Et_2O 1 : 1, 68%; ii, TBAF–AcOH (3%), THF, 92%.



Scheme 6 *Reagents and conditions*: i, NIS, TfOH (cat.), 4 Å molecular sieves, DCM–Et_2O 1 : 1, 64%; ii, $AgClO_4$, $HfCp_2Cl_2$, 4 Å molecular sieves, Et_2O, 67%; iii, $Pd(OH)_2/C$, H_2 1 atm, DCM–MeOH–H_2O 3 : 3 : 1, 99%; iv, $Pd(OH)_2/C$, H_2 1 atm, DCM–MeOH–H_2O 3 : 3 : 1, 98%.

Table 1 Selected ^{1}H NMR and ^{13}C NMR data (δ_H, δ_C in ppm) for the deprotected oligosaccharide derivatives **1**–**5** (in D_2O)

Residue	Atom	**1**	**2**	**3**	**4**	**5**
Man[a]	H-1	4.58	4.47	4.49	4.47	4.49
	C-1	99.7	100.2	100.3	100.2	100.2
Man[b]	H-1	5.28	5.38	5.48	5.37	5.07
	C-1	100.6	100.6	100.8	100.8	102.5
Man[c]	H-1	5.23	5.28	5.00	4.99	
	C-1	101.0	101.0	102.7	102.8	
Man[d]	H-1	4.97	4.98			
	C-1	102.7	102.7			
Glo[e]	H-1	5.19				
	C-1	102.2				
Man[b′]	H-1	4.83	4.83	4.89	4.83	4.83
	C-1	100.2	100.2	100.2	100.0	100.1
Man[c′]	H-1	5.08	5.08	5.10		5.08
	C-1	102.3	102.3	102.3		102.3



Scheme 7 *Reagents and conditions*: i, NIS, TfOH (cat.), 4 Å molecular sieves, DCM–Et_2O 1:1, 78%; ii, TBAF–AcOH (3%), THF, 95%; iii, NIS–TfOH (cat.), 4 Å molecular sieves, DCM–Et_2O 1:1, 64%; iv, $Pd(OH)_2$/C, H_2 1 atm, DCM–MeOH–H_2O 3:3:1, 96%.

precursors were confirmed by ^{1}H and ^{13}C NMR spectroscopy (DQF–COSY, TOCSY, HMQC and HMBC; see Table 1), and by mass spectrometry (high resolution, ESMS).[16]

To summarize, the first chemical synthesis of the *N*-glycans of gp63 of *L. mexicana amazonensis* has been achieved using a strategy based on the principles of reactivity tuning and orthogonal activation.

Acknowledgements

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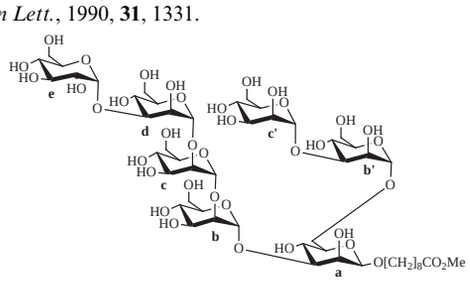

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