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A Versatile Synthesis of Pentacosafuranoside Subunit Reminiscent of Mycobacterial Arabinogalactan Employing One Strategic Glycosidation Protocol

Sandip Pasari, Sujit Manmode, Gulab Walke and Srinivas Hotha*

Abstract: Oligosaccharides are involved in myriad of biological phenomena. Many glycobiological experiments can be undertaken if homogenous and well defined oligosaccharides are accessible. Mycobacterial cell wall contains arabinogalactan as one of the major constituents that is challenging for the chemical synthesis. Therefore, the major aim of this investigation is to synthesize a major oligosaccharide portion of the arabinogalactan. The pentacosafuranoside (25mer) synthesis involved installation of several arabinofuranosidic linkages through the neighbouring group participation for 1, 2-*trans* linkages and oxidation-reduction strategy for the 1, 2-*cis* Araf respectively. Strategically placed *n*-pentenyl moiety at the reducing end shall enable ligation of biomolecular probes through celebrated cross metathesis or thiol-ene click reactions. Several linear and branched oligosaccharides were synthesized ranging from trisaccharide to pentadecasaccharide during this endeavor. Synthesis of pentacosasaccharide was accomplished in 77 steps with 0.0012% overall yield. These oligosaccharides are envisioned to be excellent probes for understanding disease biology thereby facilitating discovery of novel anti-tubercular agents, vaccines and/or diagnostics.

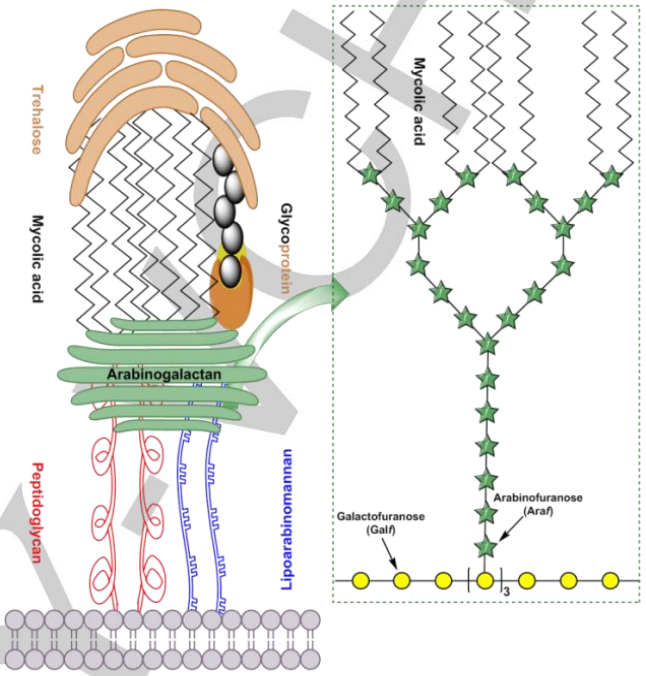

Figure 1. Cartoon representation of the mycobacterial cell wall and the arabinogalactan

Introduction

Tuberculosis (TB) still remains as a global health hazard in spite of it being known for more than 132 years, 122 years since the first vaccine trial and 96 years after the discovery of Bacille Calmette-Guérin (BCG) vaccine.[1-4] Mycobacterial infections have been receiving special attention recently due to the increased incidence and emergence of multidrug-resistant (MDR-TB) and extensively drug-resistant (XDR-TB) strains of *Mycobacterium tuberculosis* (MTb), the organism that causes TB.[5],[6] Usually, treatment of drug-susceptible tuberculosis requires chemotherapy with multiple antibiotics administered over prolonged periods. A number of researchers have attributed such long drawn chemotherapy to the unusually dense, waxy, and unique glycocalyx that acts as a robust permeability barrier to the antibiotics.[7-11] The uniquely dense glycocalyx architecture comprises trehalose, mycolic acid, D-arabinan, D-galactan, D-mannan, linker disaccharide and a peptidoglycan.[12] One of the major structural components of the glycocalyx is the mycolyl arabinogalactan (AG) polysaccharide wherein the mycolic acid (a cyclopropanated fatty acid) is esterified at the non-reducing end of the arabinan; whereas, the reducing end is attached as a pendant to the galactan (Figure 1).[13-16] Finer structure of the mycolyl glycocalyx illustrated that the arabinose and galactose are in the furanosyl form.[13] Most of the glycosidic linkages are in 1,2-*trans* or α-fashion between the Ara*f*-Ara*f* or Gal*f*-Gal*f* or Ara*f*-Gal*f* except the terminal four which are 1,2-*cis* or β-Ara*f* residues which are in turn linked to the C2-position of the penultimate 1,2-*trans* or α-Ara*f* residue. All β-Ara*f* residues are esterified at the C-5 positions with mycolic acid.[13-14] The galactan portion was composed of β-D-Gal*f*-(1→5)-β-D-Gal*f*-(1→6)-β-D-Gal*f*- repeating unit containing pendant arabinan polysaccharide at three sites through the C-5-D-Gal*f*.[13] Access to homogenous synthetic oligosaccharides could significantly augment the discovery of carbohydrate-based vaccines and diagnostics.

Unlike peptides and nucleotides, the synthesis of large oligosaccharides still remains as a significant challenge although many elegant methodologies for chemical glycosidation are developed. Synthesis of oligosaccharides containing more than 20 saccharides in a stereoselective fashion is a herculean task,[17,18] and as a result, fine tuning of the compatible glycosyl donor chemistry is decisive for the success of any multi-step assembly of oligosaccharides. Nevertheless, synthesis of polylactosamine with 25-saccharide residues by Ogawa[19] and *O*-specific polysaccharide containing 24-residues by Pozsgay[20a] were the conspicuous early efforts towards the synthesis of large oligomers. Of mycobacterial origin, mannose-capped LAM fragment by Fraser-Reid,[20b] independent syntheses of docosaarabinofuranoside by Lowary[21] and Ito,[18] heneicosasaccharide (21-residues) of LAM[22] and AG[23]

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syntheses by our group are some of the noticeable efforts in the literature that describe synthesis of large oligosaccharides. Lowary invoked salient features of trichloroacetamidates and thiotolyl donor chemistry whereas Ito utilized thioglycosides for assembling the docosasaccharide.[18,21] Both syntheses accomplished the α-arabinofuranosylation by the neighbouring group participation through the C-2 esters. β-Arabinofuranosylation was accomplished by conformational bias due to the silyl tethering in the Lowary's synthesis whereas an intramolecular aglycon delivery through NAP ether was utilized in the Ito's synthesis of docosasaccharide.[18],[21] Although extensive research has been carried out on mycobacterial arabinogalactan, very few studies exist which have conjugated the arabinan moiety with the β-arabinofuranosides and the galactan moiety until recently. While our studies are in progress, a 92-mer arabinogalactan by Ye[24a] and a 50mer mannan using automated synthesizer by Seeberger[24b] were reported.

Drawing upon these strands of research on the synthesis of mycobacterial arabinogalactan, this study was therefore conducted to synthesize a pentacosafuranoside containing 23-Araf*s* and 2-Gal*f* residues. A careful look at the pioneering work by Brennan group identified five major structural fragments of AG complex (Figure 2A).[13] Later investigations on the biosynthesis of AG showed that 1, 2-*trans* or α-linkages were synthesized by arabinofuranosyl transferases (AraTs) and 1,2-*cis* or β-arabinofuranosides are installed indirectly starting from decaprenylphosphoryl D-ribofuranose as the substrate.[25],[26] Fixing of the 1, 2-*trans* ribofuranoside with DprE1 followed by oxidation-reduction with DprE2 transformed 1,2-*trans* Rib*f* *into* 1,2-*cis* Ara*f* (Figure 2B).[25,26]

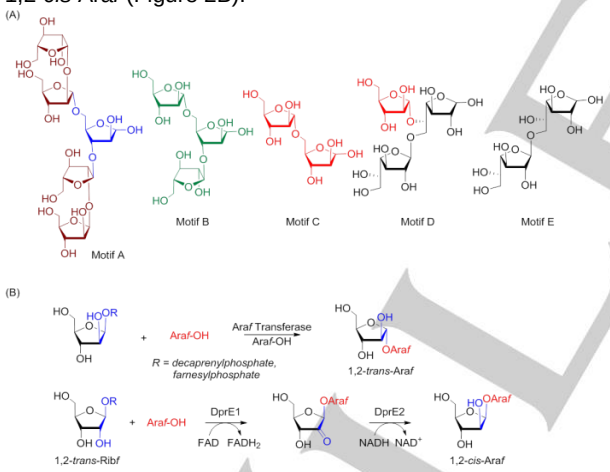
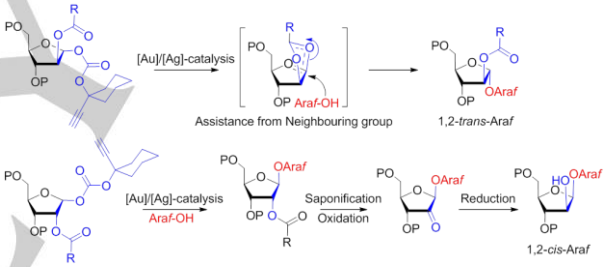


Figure 2. Major glycan motifs (A) of M. tuberculosis cell wall and biosynthesis of 1, 2-*trans* and 1, 2-*cis* arabinofuranosides (B)

Therefore, major challenges in the synthesis of pentacosasaccharide are identified as: (i) stereoselective installation of Ara*f*-(1→2)-Ara*f* linkage; (ii) synthesis of Gal*f*-(1→6)-Gal*f* with free C5-OH for the attachment of arabinan; (iii) a robust furanosylation method that enables preparation of both 1, 2-*trans* and 1, 2-*cis* linkages; (iv) minimum number of different reactions; and (v) final assembly of the pentacosasaccharide from Motifs A-E. In the retrosynthetic analysis, we envisioned that the assembly of pentacosasaccharide (**1**) can be comprehended by a stereoselective glycosidation between a pentadecasaccharide (**2**) and two molar equivalents of a pentasaccharide-carbonate (**3**) in a {2x5+15} fashion. The larger component **2** was envisaged from two disaccharides **4**, **5** and a trisaccharide **6** (Scheme 1).

The carbonate **3** was imagined from the disaccharide-carbonate **7** and a diol **8**. It is interesting to note that the identified advanced building blocks are very similar to motifs A-E as shown in Figure 2A. The highly convergent strategy would enable (i) the impeccable quality control in every stage of the synthesis and (ii) synthesis of enough quantity of the various building blocks *en route* to the final assembly of the target polysaccharide. Installation of the α-(1→x)-Ara*f* linkage by the [Au]/[Ag]-catalysed activation of alkynyl carbonate Ara*f* donor through neighbouring group participation was earlier reported by our group.[27] For the β-(1→2)-Ara*f* linkage, we intended to use the protocol that runs parallel to how mycobacterium biosynthesizes the β-(1→2)-Ara*f* linkage.[28] First, we install the neighbouring group assisted 1,2-*trans* linkage on a Rib*f*-derivative and subsequent saponification and oxidation-reduction enables us to achieve desired 1,2-*cis* or β(1→2)Ara*f* linkage (Scheme 2).

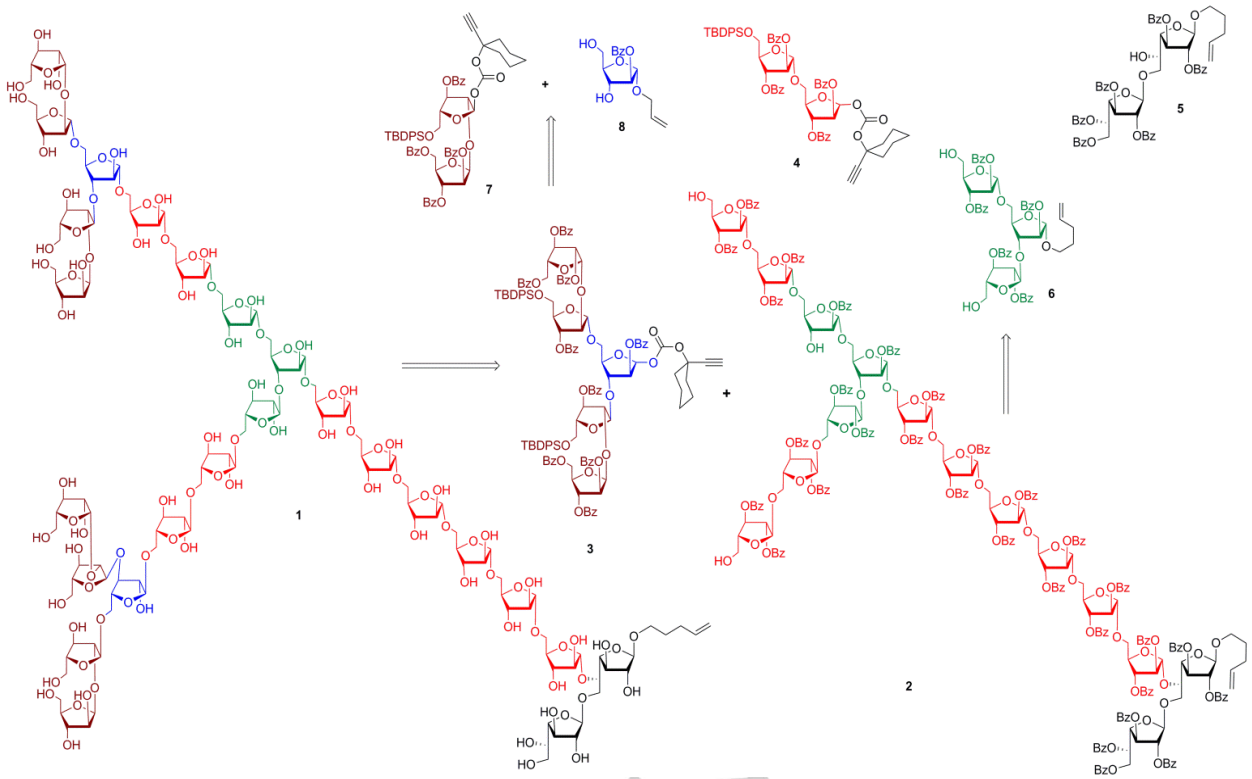


Scheme 2. Synthesis protocols for 1, 2-*trans* and 1, 2-*cis* Araf*s*

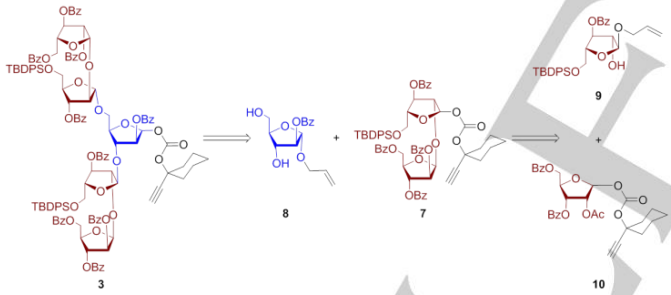
One furanosylation method, minimal number of complex reactions and protecting groups are the key features of this endeavour. With the identification of major motifs and generally stereoselective arabinofuranosylation protocols, our exploration towards the synthesis of pentacosasaccharide **1** started with the synthesis of major structural motifs with appropriate protecting groups in sufficient quantities.

Results and Discussion

Synthesis of pentaarabinofuranosyl donor 3: One of the most complex portions of the pentacosasaccharide (**1**) is the terminal pentasaccharide which requires installation of two β-Ara*f*-(1→2)-Ara*f* linkages that are notorious for their difficulty. In the retrosynthetic plan of this motif, a {2x2+1} furanosylation between the glycosyl donor **7** and the diol acceptor **8** was recognized to be the most convenient. The glycosyl donor was set out easily from two starting substrates **9** and **10** (Scheme 3). The synthetic effort started with a known arabinofuranoside **11**[29] whose isopropylidene group was opened with allyl alcohol



Scheme 1. Retrosynthetic analysis of pentacosasaccharide motif of arabinogalactan of *Mycobacterium tuberculosis*

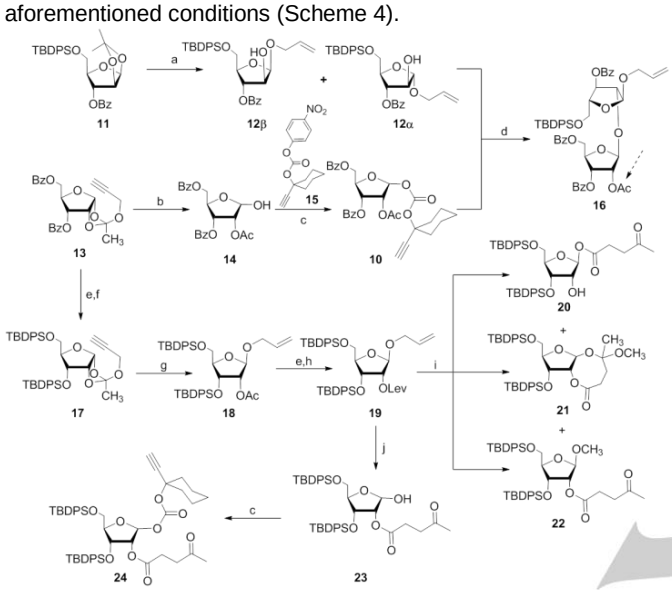


Scheme 3. Retrosynthetic analysis of pentasaccharide

under acidic conditions to afford a separable mixture of allyl arabinofuranosides **12α** and **12β** in 1:1 ratio which are utilized as glycosyl acceptors later.[30] 1,2-*cis* Arabinofuranosylation was envisioned through oxidation-reduction strategy, hence, the 1, 2-*trans* furanosyl donor of Rib*f* was required; in addition, the protecting group at the *C*-2 position of the Rib*f*-donor needs to be orthogonal to the remaining appendages present in the molecule. Unmasking of acetates in the presence of benzoate esters was reported and the installation of the acetate at the *C*-2 position is relatively easier if we start with an orthoacetate **13**.[31-33] Accordingly, the orthoacetate **13** was synthesized by invoking a protocol developed in our group and transformed to hemiacetal **14** by a gold-catalyzed glycosidation using $AuCl_3/H_2O/CH_3CN$ at 25 ^{0}C and subsequently converted to the arabinofuranosyl donor **10** by reacting the hemiacetal **14** with the reagent **15** in very high yield (Scheme 4).

Earlier studies from our group showed that the anomeric ratio in the donor does not significantly affect the overall outcome of the glycosidation reaction.[27] Hence, the carbonate **10** was not separated into individual isomers. In continuation, the [Au]/[Ag]-catalyzed glycosidation between the donor **10** and **12α** underwent smoothly giving the desired diarabinofuranoside **16** in 67% yield as pale yellow syrup. At this stage, chemoselective saponification of acetates in the presence of benzoates became a herculean task. Both chemical and lipase mediated hydrolysis of acetate ester failed to give required alcohol in sufficient quantities that has prompted us take a detour and continue our synthetic effort. Without much difficulty, orthoacetate **13** was transformed to di-*O*-silyl ether **17** that was treated with allyl alcohol and $AuBr_3$ conditions to obtain allyl arabinoside **18** with acetate at the C-2 position. In continuation, acetate was saponified easily under Zemplén conditions[34] and protected again with the levulinic acid under DIC/DMAP conditions to obtain levulinoate **19**. The next step in the sequence is to convert the allyl glycoside into the hemiacetal **23**; however, under normal conditions, the Pd-catalyzed allyl deprotection resulted in the formation of compounds **20**-**22**. The formation of the compound **20** can be envisaged from the migration of levulinoyl group from C-2 position to the more reactive hemiacetal position where the formation of bicyclic compound **21** was more intriguing.[36] Departure of the allyl glycoside can result in the formation of an oxocarbenium ion intermediate that

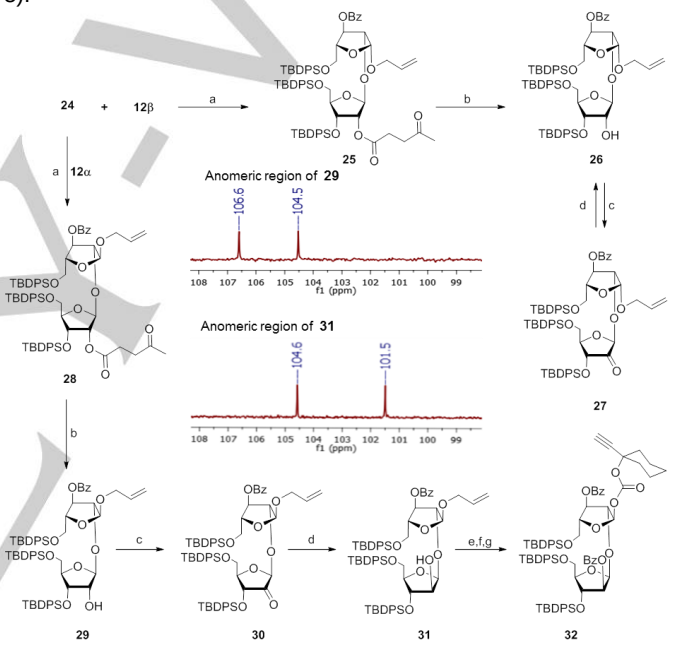
could be trapped by the ketone intramolecularly to give bicyclic **21** or be trapped by the methanol to afford the compound **22**. Nevertheless, the problem was circumvented by inviting a method that was reported through a ternary solvent system. Accordingly, compound **19** was hydrolyzed successfully to hemiacetal **23** by employing 1,2-DME-CH_2Cl_2-MeOH (3:1:1)/25 ^{0}C in 83% yield.[40] Subsequently, the hemiacetal **23** was conveniently converted into the desired carbonate donor **24** by aforementioned conditions (Scheme 4).



Scheme 4. Synthesis of building blocks for the terminal pentasaccharide motif: Reagents: (a) PTSA (0.2 eq), allylOH, CH_2Cl_2, 55 ^{0}C, 3 h, 43% for **12β** and 46% for **12α**; (b) 8mol% $AuCl_3$, CH_3CN, H_2O, 25 °C, 2 h, 88%; (c) **15**, CH_2Cl_2, DMAP, 25 °C, 4 h, 83% for **10** and 94% for **24** (α:β = 1:3); (d) 8mol% each of chloro[tris(2,4-di-*tert*-butylphenyl)phosphite]gold and AgOTf, CH_2Cl_2, 4Å MS powder, 25 °C, 30 min, 67%; (e) NaOMe (0.3 eq), CH_2Cl_2-MeOH (1:1), 25 °C, 2 h; (f) TBDPSCl, Im., DMF, 0-25 °C, 4 h, 68% over two steps; (g) 10mol% $AuBr_3$, allylOH, CH_2Cl_2, 4Å MS powder, 25 °C, 3 h, 89%; (h) Levulinic acid, DIC, DMAP, CH_2Cl_2, 0-25 °C, 2 h, 82% over two steps; (i) $PdCl_2$ (0.2 eq), CH_2Cl_2-MeOH (1:4), 25 °C, 4 h, 31% for **21**, 18% for **22**, 36% for **23**; (j) $PdCl_2$ (0.2 eq), 1,2-DME/CH_2Cl_2/MeOH (3:1:1), 25 °C, 4 h, 83%.

Synthesis of desired building blocks set the stage for the preparation of β-Ara*f*-(1→2)-Ara*f* disaccharide. Earlier investigations from our group showed that the stereoelectronic factors around the glycosyl acceptor govern the stereoselectivity of β-arabinofuranosylation.[37] So, initially, donor **24** was treated with excess amount of sterically hindered glycosyl acceptor **12β** under standard [Au]/[Ag]-catalyzed glycosidation conditions. Gratifyingly, the disaccharide **25** was isolated in very high yield whose structural homogeneity was confirmed by the spectroscopic studies. For example, the two anomeric protons of compound **25** were noticed at δ 5.51 (d, *J* = 4.6 Hz, 1H), 5.33 (s, 1H) ppm and δ 106.1, 100.9 ppm in the ^{1}H and ^{13}C NMR spectra respectively (Scheme 5).[36]

An uneventful deprotection of levulinoate using 70% aqueous solution of hydrazine hydrate in the presence of 1:1 mixture of pyridine-acetic acid buffer in CH_2Cl_2 at 25 ^{0}C afforded the alcohol **26** that was oxidized smoothly to its 2-ulose derivative **27**;[28] however, the reduction of the ketone by various reducing agents gave back the original starting alcohol **26**. Hence, the glycosidation between glycosyl donor **24** and the acceptor **12α** was carried out to obtain the disaccharide **28** under gold/silver-catalyzed glycosidation conditions. The levulinoate deprotection of **28** to obtain alcohol **29** followed by oxidation afforded 2-ulose derivative **30** that underwent smooth reduction with $NaBH_4$ in EtOAc-EtOH (2:1) converted β-Rib*f*-(1→2)-Ara*f* into a much desired β-Ara*f*-(1→2)-Ara*f* **31**. In the ^{1}H NMR spectrum of compound **31**, the two anomeric protons were noticed at δ 5.22 and 5.33 ppm as two singlets. Additionally, the ^{13}C NMR spectrum confirmed the presence of 1,2-*cis* and 1,2-*trans* linkages by displaying resonances at δ 101.5 and 104.6 ppm respectively. Successfully synthesized disaccharide **31** was transformed into the carbonate donor **32** in three steps (Scheme 5).[36]



Scheme 5. Synthesis of the key β-Ara*f*-(1→2)-Ara*f* donor: Reagents: (a) 8mol% each of chloro[tris(2,4-di-*tert*-butylphenyl) phosphite]gold and AgOTf, CH_2Cl_2, 4Å MS powder, 25 °C, 40 min, 83% for **25**, 81% for **28**; (b) 70% $N_2H_4.H_2O$ (4.5 eq), CH_2Cl_2, AcOH (22 eq), Py (27 eq), 25 °C, 2 h, 89% for **26**, 92% for **29**; (c) PDC (0.6 eq), Ac_2O (3.3 eq), CH_2Cl_2, 25 °C, 5 h; (d) $NaBH_4$ (1.2 eq), EtOH/EtOAc (2:1), 0 °C, 2 h, 78% for **31** over two steps; (e) Py, PhCOCl, CH_2Cl_2, 0-25 °C, 2 h, 93%; (f) $PdCl_2$ (0.2 eq), CH_2Cl_2-MeOH (1:4), 25 °C, 4 h; (g) **15**, DMAP, CH_2Cl_2, 25 °C, 5 h, 79% over two steps.

Having achieved the key milestone encouraged us to move forward to synthesize the pentasaccharide donor **3**. The synthesis started with the conversion of easily accessible propargyl orthoester **33** to the di-*O*-TBDPS compound **34** that was subjected to the gold-catalyzed glycosidation to obtain desired allyl arabinofuranoside whose silyl ethers were cleaved off under HF·py condition to afford the acceptor-diol **8** in 54% yield over four steps (Scheme 6).

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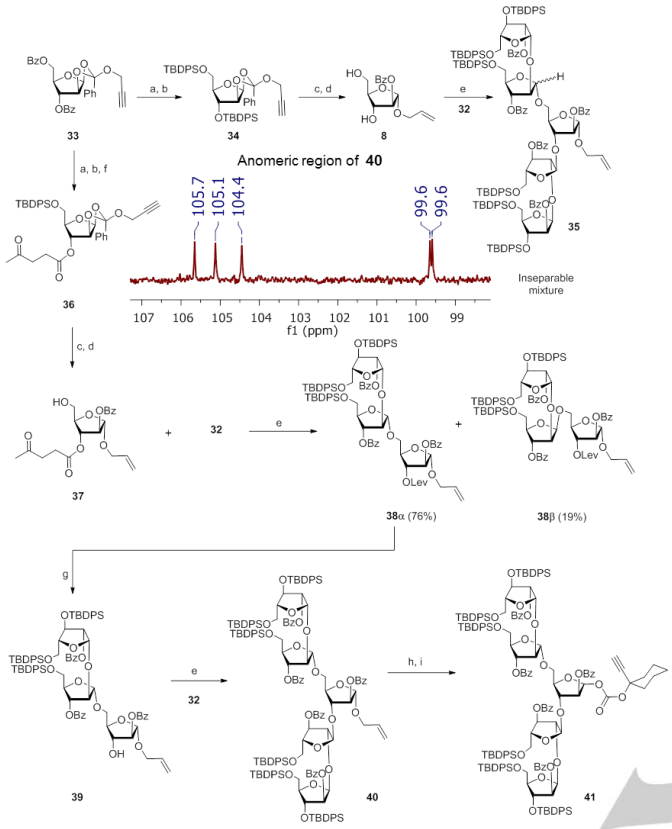


Scheme 6. Synthesis of pentaarabinofuranosyl carbonate donor **40**: Reagents: (a) NaOMe (0.3 eq), CH_2Cl_2-MeOH (1:1), 25 °C, 2 h; (b) TBDPSCl, Im., DMF, 0-25 °C, 4 h, 68% over two steps for **34**; (c) 10mol% $AuBr_3$, allylOH, CH_2Cl_2, 4Å MS powder, 25 °C, 3 h, 86% towards **8** and 85% towards **37**; (d) Pyridine, HF.py, 0-25 °C, 5 h; 92% for **8** and 87% for **37**; (e) 8mol% each of chloro[tris(2,4-di-*tert*-butylphenyl)phosphite]gold and AgOTf, CH_2Cl_2, 4Å MS powder, 25 °C, 40 min, 84% for **35**, 76% for **38α**, 19% for **38β** and 84% for **40**; (f) Levulinic acid, DIC, DMAP, CH_2Cl_2, 0-25 °C, 2 h, 61% over three steps for **36**; (g) 70% $N_2H_4.H_2O$ (4.5 eq), CH_2Cl_2, AcOH (22 eq), Py (27 eq), 25 °C, 2 h, 93%; (h) $PdCl_2$ (0.2 eq), CH_2Cl_2-MeOH (1:4), 25 °C, 4 h; (i) **15**, DMAP, CH_2Cl_2, 25 °C, 5 h, 77% over two steps.

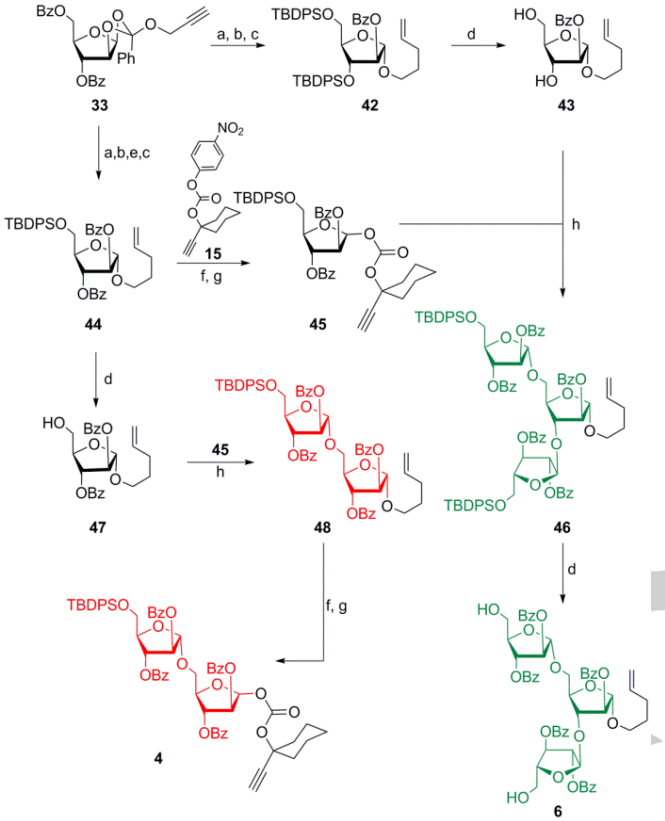
Now, the stage was set for the gold/silver-catalyzed glycosidation in a {2x2+1} fashion. The glycosidation between diol **8** and the acceptor **32** was performed affording an inseparable mixture of pentasaccharides **35**. Earlier reports on the synthesis of pentasaccharide motifs indicated that the diastereomeric ratio would likely be resulting from the C-5 position of the aglycon due to its inherently more reactive nature compared to the C-3-OH.[18],[21-23] Temperature controlled glycosidations and reversing the addition sequence of glycosyl donor and acceptor did not improve the diastereoselectivity and more frustratingly, the isomers could not be separated. Hence, sequential addition of the disaccharide was investigated and installation at the C-5 position prior to the C-3 position would be beneficial because the mixture of compounds, if any, shall be easy to purify. With this idea, orthoester **33** was saponified, monosilylated using 1.1 equivalent of TBDPS-Cl at 0 ^{0}C and installation of the levulinoate protection was carried-out at the C-3 position afforded the compound **36** in 61% over three steps. Gold-catalyzed glycosidation with allyl alcohol followed by the cleavage of the silyl ether using HF·py afforded the glycosyl acceptor **37** for further exploration. Much to our satisfaction, the gold-phosphite and AgOTf catalyzed glycosidation between donor **32** and acceptor **37** resulted in a 4:1 diastereomeric mixture of trisaccharides **38**.[36] Furthermore, the two trisaccharides could be easily separated by flash silica gel chromatography and enabled thorough characterization. The three anomeric carbons of trisaccharide **38α** were noticed at δ105.5, 104.8, 100.0 ppm for two 1,2-*trans* and lone 1,2-*cis* linkage respectively (Scheme 6).[36]

The major isomer of the trisaccharide **38α** was treated with hydrazine hydrate to afford alcohol **39** and further treated with the glycosyl donor **32** under 8mol% each of Au-phosphite catalyst and AgOTf to obtain structurally homogenous pentasaccharide **40** in very high yield. In the ^{13}C NMR spectrum of pentasaccharide **40**, resonances of the five anomeric carbons were noticed at δ 105.7, 105.1, 104.4, 99.6 (2C) ppm along with very good matching of molecular ion in the mass spectrum (found: 2692.0864; calcd: 2692.0874 for $C_{159}H_{174}NaO_{26}Si_6$).[36] The successfully synthesized pentasaccharide **40** was converted easily into the corresponding carbonate donor **41** in two easy steps (Scheme 6).

Synthesis of disaccharide donor 4 and trisaccharide acceptor 6: Easily accessible propargyl orthoester **33** was saponified under Zemplén conditions to obtain a diol which was quickly split into two halves; one half was treated with 2.2 equivalents of TBDPS-Cl and imidazole in DMF at 25 ^{0}C to obtain a di-*O*-TBDPS orthoester and subsequently subjected to gold-catalyzed glycosidation conditions to afford monosaccharide **42** in 55% over three steps (Scheme 7). The second half was treated with 1.1 equivalent of TBDPS-Cl, the remaining *C*-3 hydroxyl group was protected as its benzoate under BzCl/py./25 ^{0}C and treated with pent-4-en-1-ol/$AuBr_3$/CH_2Cl_2/4Å MS powder/25 ^{0}C to afford the required *n*-pentenyl furanoside **44** in 46% overall yield. Hydrolysis of the *n*-pentenyl glycoside **44** to the hemiacetal and subsequent reaction with freshly prepared reagent **15** afforded the arabinofuranosyl donor **45** as a fluffy solid.[38] Cleavage of the silyl ether of compound **42** gave the diol **43** which will serve as the glycosyl acceptor. Next, [Au]/[Ag]-catalyzed reaction between donor **45** and acceptor **43** underwent glycosidation resulting in a trisaccharide **46** which upon treatment with HF·py afforded the desired motif B analogue **6** that is ready for further explorations (Scheme 7).

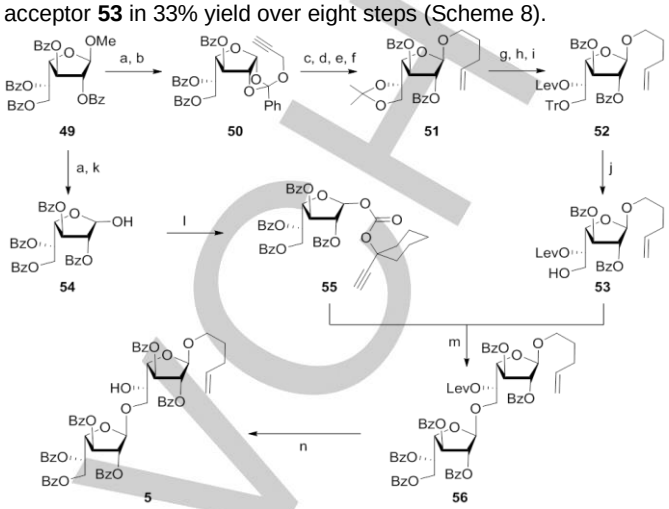
The ^{1}H NMR spectrum of compound **6** confirmed the presence of the three anomeric protons by displaying resonances at δ 5.24, 5.21 and 5.10 ppm and the presence of olefin was evident from the resonances δ 4.96-4.86 (m, 2H) and 5.78-5.66 (m, 1H) ppm along with all other protons in the aromatic and aliphatic regions. In the ^{13}C NMR spectrum of trisaccharide **6**, resonances due to the three anomeric carbons were noticed at δ106.0, 105.4, 105.3 ppm and the terminal olefin carbon was observed at δ 115.1 ppm and resonances from five carbonyls were identified at δ 166.4-165.1 ppm. In addition, MS studies further confirmed the compound (found: 1025.3214; calcd: 1025.3207 for $C_{55}H_{54}NaO_{18}$).[36] In continuation, F$^-$

assisted cleavage of the silyl ether of monosaccharide **44** resulted in alcohol **47** that was glycosylated with the donor **45** prepared *vide supra* to obtain the disaccharide **48** in high yield. Conversion of the *n*-pentenyl glycoside **48** to the carbonate **4** was carried out by aforementioned two-step sequence (Scheme 7). Presence of the carbonate of disaccharide **4** was confirmed by its transmittance at 1726 cm^{-1} in the IR spectrum.



Scheme 7. Synthesis of disaccharide **4** and trisaccharide **6**: Reagents: (a) NaOMe (0.3 eq), CH_2Cl_2-MeOH (1:1), 25 °C, 2 h; (b) TBDPSCl, Im., DMF, 0-25 °C, 4 h; (c) 10mol% $AuBr_3$, 4-pentene-1-ol, CH_2Cl_2, 4Å MS powder, 25 °C, 3 h, 55% for **42** and 75% for **44** over three steps; (d) Pyridine, HF.py, 0-25 °C, 5 h; 86% for **43**, 82% for **47** and 87% for **6**; (e) BzCl, py., 25 ^{0}C; (f) NIS (2.2 eq), TfOH (0.2 eq) $CH_2Cl_2/CH_3CN/H_2O$ (5:3:0.5), -10 °C to 0 °C, 1 h, 81% towards **45**, and 78% towards **4**; (g) **15**, DMAP, CH_2Cl_2, 25 °C, 4 h, 85% for **45** and 82% for **4**; (h) 8 mol% each of chloro[tris(2,4-di-*tert*-butylphenyl)phosphite]gold and AgOTf, CH_2Cl_2, 4Å MS powder, 25 °C, 40 min, 95% for **46** and 92% for **48**.

Synthesis of α-Gal*f*-(1→6)-Gal*f* 5: Synthesis of disaccharide **5** started with the easily accessible methyl 2,3,5,6-tetra-*O*-benzoyl galactofuranoside **49**[39] which was converted to the orthoester **50** in 76% yield over two steps. Characteristic resonances due to the orthoester moiety of **50** are noticed at δ 123.4 ppm for the quaternary carbon of the orthoester. Global saponification under Zemplén conditions (NaOMe/MeOH), isopropylidenation and esterification of the remaining two hydroxyl moieties under BzCl/py resulted in the formation pentenyl galactoside **51**. Hydrolysis of the isopropylidene of compound **51**, regioselective protection of *C*-6 position as its trityl ether and further treatment with levulinic acid/DIC/DMAP led to the synthesis of compound **52**. Unblocking of the *O*-trityl ether under acidic conditions afforded the galactofuranosyl acceptor **53** in 33% yield over eight steps (Scheme 8).

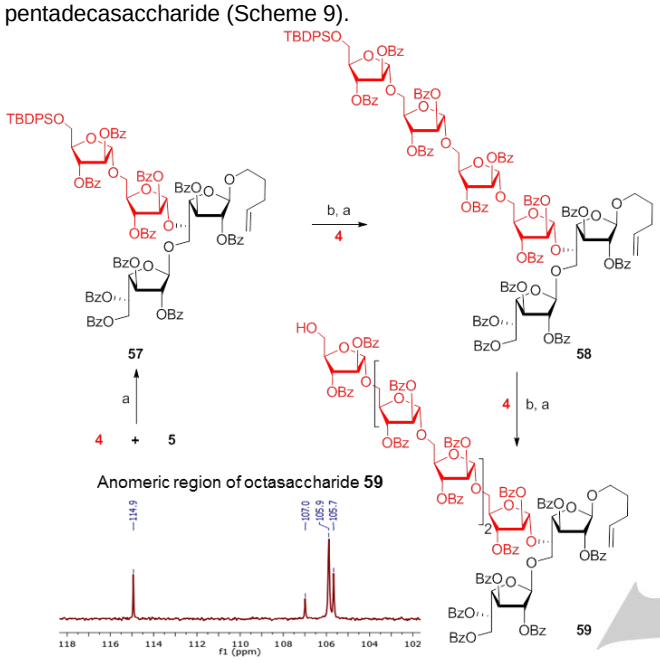


Scheme 8. Synthesis of Motif E: Reagents: (a) CH_2Cl_2, CH_3COBr (6.5 eq), MeOH (4.5 eq), 0 °C, 3 h; (b) CH_2Cl_2, propargyl alcohol, 2,6-lutidine (2.2 eq), TBAI (0.5 eq), 25 °C, 10 h, 76% over two steps for **50**; (c) 10mol% $AuBr_3$, CH_2Cl_2, pent-4-ene-1-ol, 4Å MS powder, 25 °C, 3 h, 82%; (d) NaOMe (0.3 eq), CH_2Cl_2-MeOH (1:1), 25 °C, 2 h; (e) PTSA (0.2 eq.), 2,2-DMP, Acetone, 8 h; (f) Py, PhCOCl, CH_2Cl_2, 0-25 °C, 2 h, 64% over three steps for **51**; (g) 80% AcOH, aq. THF, 60 °C, 3 h, 85%; (h) TrCl, py, CH_2Cl_2, 60 °C, 24 h; (i) Levulinic acid, DIC, DMAP, CH_2Cl_2, 25 °C, 3 h, 74% over two steps for **52**; (j) Et_3SiH, TFA, CH_2Cl_2, 0 °C, 5 min, 96%; (k) CH_3CN, H_2O, 25 °C, 6 h; (l) **15**, DMAP, CH_2Cl_2, 25 °C, 4 h, 58% over three steps (a,k,l) for **55**; (m) 8mol% each of chloro[tris(2,4-di-*tert*-butylphenyl)phosphite]gold and AgOTf, CH_2Cl_2, 4Å MS powder, 25 °C, 40 min, 89%; (n) 70% $N_2H_4.H_2O$ (4.5 eq), CH_2Cl_2, AcOH (22 eq), Py (27 eq), 25 °C, 2 h, 80%.

In parallel, methyl furanoside **49** was converted into the hemiacetal **54** under strongly acidic conditions (excess AcBr/MeOH) and subsequently treated with reagent **15**/DMAP/CH_2Cl_2 to obtain the galactofuranosyl donor **55** in 58% yield over two steps. Galactofuranosyl donor **55** and the acceptor **53** were conveniently glycosylated under aforementioned [Au]/[Ag]-catalyzed glycosidation conditions to afford the digalactofuranoside **56** which was transformed to compound **5** by unmasking the levulinoate employing 70% hydrazine hydrate solution (Scheme 8). In the ^{1}H NMR spectrum of disaccharide **5**, characteristic resonances of the two anomeric protons are identified as singlets at δ 5.12, 5.29 ppm and the ^{13}C NMR spectral studies further confirmed presence of the two 1,2-*trans* linkages by displaying C-1 carbons at δ 105.9 and 106.4 ppm. In addition, disaccharide **5** gave good matching of molecular weight to that of calculated molecular weight (found: 1057.3266; calcd: 1057.3259 for $C_{59}H_{54}NaO_{17}$).[36]

Synthesis of octasaccharide. Accomplishing sufficient quantities of all major structural motifs encouraged us to move ahead towards the total synthesis of pentacosasaccharide **1**. The next major milestone will be the synthesis of an octasaccharide and a heptasaccharide. The glycosidation between arabinofuranosyl donor **4** and digalactoside **5** enabled us to conveniently synthesize the arabinogalactan **57** in 92% yield. Unmasking of the silyl ether using HF·py at 25 ^{0}C

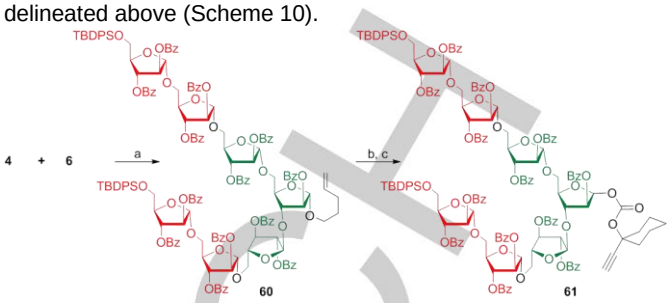
followed by the repetition of the gold-phosphite and AgOTf assisted glycosidation afforded the hexasaccharide **58** and one more repetition of this two-step sequence gave us an octasaccharide with two Gal*f*- and six Ara*f*- residues.[36] Fluoride ion mediated cleavage of silyl ether afforded the required acceptor **59** that will be utilized *in futuro* for the synthesis of pentadecasaccharide (Scheme 9).



Scheme 9. Synthesis of octasaccharide: Reagents: (a) 8mol% each of chloro[tris(2,4-di-*tert*-butylphenyl)phosphite]gold and AgOTf, CH_2Cl_2, 4Å MS powder, 25 °C, 40 min, 92% for **57**, 87% for **58**, 83% for **59** ; (b) Pyridine, HF.py, 0-25 °C, 5 h, 85% towards **58**, 81% towards **59**.

Structural homogeneity of octasaccharide **59** was confirmed by spectroscopic techniques. The ^{1}H NMR spectrum of octasaccharide **59** showed the presence of the eight anomeric protons from δ 5.42 to 5.15 ppm and the presence of olefin was evident from the resonances δ 4.87-4.78 (m, 2H) and 5.95 (m, 1H) ppm along with all other protons in the aromatic and aliphatic regions. Further, ^{13}C NMR spectrum confirmed the formation of α-linkages of octasaccharide **59** by showing resonances due to the anomeric carbons of Gal*f*- at δ 107.0 and 105.7 ppm whereas the remaining six anomeric carbons of Ara*f*-residues were noticed between δ 105.9 and 105.7 ppm; the terminal olefinic carbon was identified at δ 114.9 ppm and resonances from eighteen carbonyls were identified from δ166.1 to 165.1 ppm. In addition, MS studies further confirmed formation of the octasaccharide **59**.[36]

Synthesis of heptasaccharide. In continuation, synthesis of the pentacosasaccharide **1** required access to the middle heptasaccharide. In view of this, the glycosidation between the glycosyl donor **4** and the acceptor **6** was carried-out using [Au]/[Ag]-catalyzed glycosidation procedure under Argon atmosphere at 25 ^{0}C (Scheme 10). Furthermore, heptasaccharide **60** was transformed into the ethynylcyclohexyl carbonate **61** in 77% yield reinvoking a protocol that was delineated above (Scheme 10).



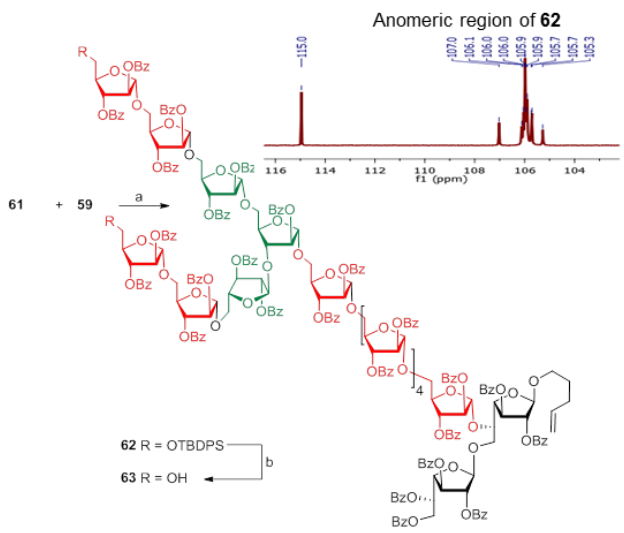
Scheme 10. Synthesis of heptasaccharide: Reagents: (a) 8mol% each of chloro[tris(2,4-di-*tert*-butylphenyl)phosphite]gold and AgOTf, CH_2Cl_2, 4Å MS powder, 25 °C, 40 min, 91%; (b) NIS (2.2 eq), TfOH (0.2 eq) $CH_2Cl_2/CH_3CN/H_2O$ (5:3:0.5), -10 °C to 0 °C, 1 h; (c) **15**, DMAP, CH_2Cl_2, 25 °C, 4 h, 77% over two steps.

Structural authenticity of the heptasaccharide **60** was evident from the ^{1}H NMR spectrum wherein the presence of the seven anomeric protons were identified at δ 5.62, 5.50, 5.46, 5.45, 5.41, 5.38 and 5.26 ppm and the presence of olefin was confirmed by the characteristic resonances at δ 5.11-5.01 (m, 2H) and 5.87 (m, 1H) ppm. The ^{13}C NMR spectrum of heptasaccharide **60** confirmed the formation of α-linkages by displaying resonances due to the anomeric carbons at δ 106.1(2C), 106.0 (4C), 105.3 ppm; the terminal olefinic $\underline{C}H_2$- was identified at δ 115.0 ppm.[36]

Assembly of the pentadecasaccharide. Stapling of the heptasaccharyl donor **61** and the octasaccharyl acceptor **59** was accomplished successfully by re-inviting the gold-catalyzed, silver assisted glycosidation reaction in the presence of 8mol% each of Au-phosphite and AgOTf catalysts in CH_2Cl_2 at 25 ^{0}C for 40 min. The two silyl ethers at the non-reducing end of the pentasaccharide **62** were unmasked by the treatment of HF·py to afford the aglycon **63** in 79% yield (Scheme 11).

The ^{1}H NMR spectrum of pentadecasaccharide **63** showed the presence of *n*-pentenyl moiety by displaying characteristic resonances at δ 6.02 (m, 1H), 4.91 (m, 2H), 3.48 (m, 2H), 2.06 (m, 2H) and 1.59 (m, 2H). No resonances were observed from the TBDPS- moiety and many overlapping signals were noticed in the anomeric and aromatic regions. Additionally, the ^{13}C NMR confirmed the presence of 15-saccharide residues by displaying fifteen signals at δ 107.0, 106.0 (8C), 105.9 (3C), 105.8, 105.7 and 105.5 ppm. ESI-MS spectrum of compound **63** revealed *m/z*= 5377.5426 for the corresponding sodium adduct (calcd. 5377.5407 for $C_{299}H_{258}NaO_{94}$).[36]

Synthesis of pentacosasaccharide 1. The glycosidation by {2x5+15} fashion was envisioned to accomplish the targeted pentacosasaccharide **1**. Accordingly, pentadecasaccharide **63** and the pentafuranosyl donor **41** were called upon to complete the assembly of pentacosasaccharide **1**. The glycosidation was carried out by the catalytic protocol containing 8mol% each of Au-phosphite and AgOTf at 25 ^{0}C for 40 min.

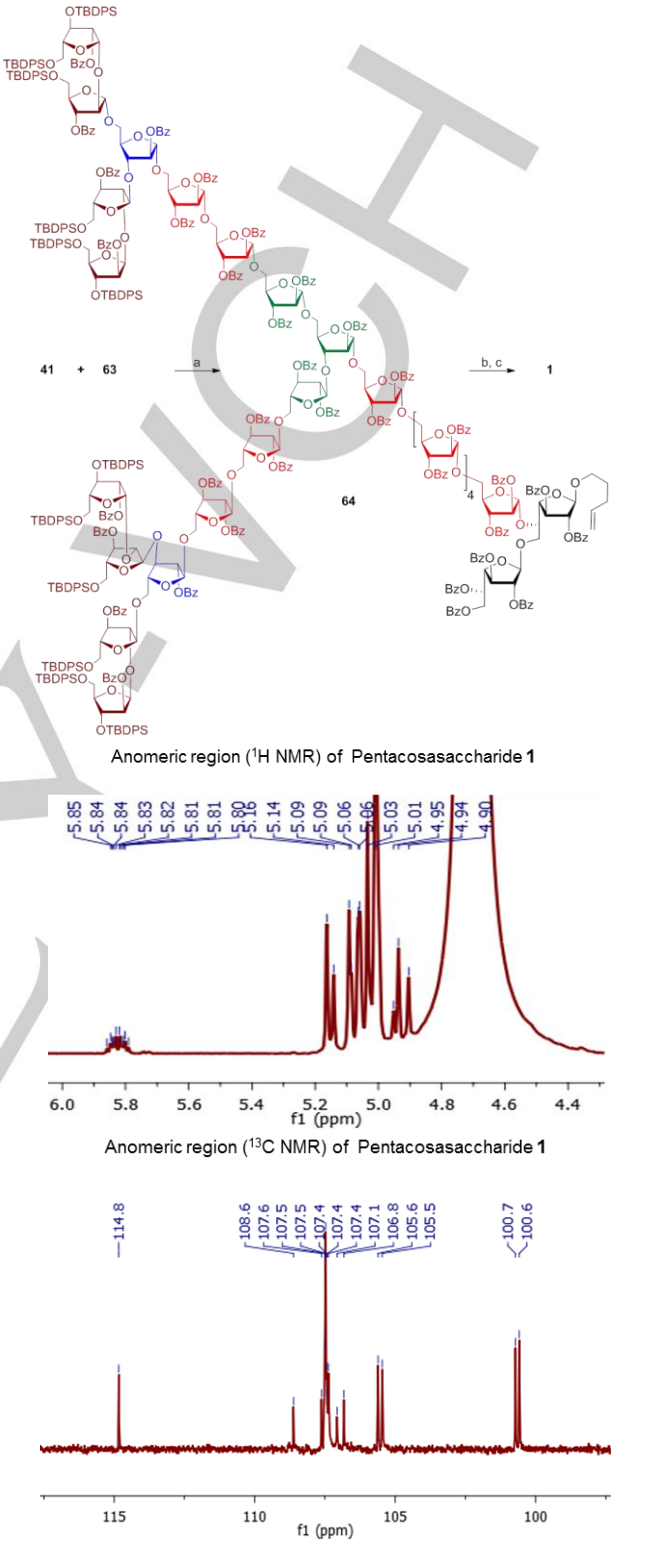


Scheme 11. Synthesis of pentadecasaccharide: Reagents: (a) 8mol% each of chloro[tris(2,4-di-tert-butylphenyl)phosphite]gold and AgOTf, CH_2Cl_2, 4Å MS powder, 25 °C, 40 min, 88%; (b) Pyridine, HF.py, 0-25 °C, 5 h, 79%.

The pentacosasaccharide **64** was carefully isolated by the flash silica gel column chromatography. The presence of large number of benzoates and furanose ring protons have obscured anomeric protons in the ^{1}H NMR spectrum; however, evidence of pentenyl moiety at δ 6.04 (m, 1H), 4.96-4.88 (m, 2H), 3.34 (m, 2H), 2.07 (m, 2H) and 1.60 (m, 2H) ppm and *tert*-butyl of TBDPS-moieties at 0.99, 0.96, 0.91, 0.88, 0.80 and 0.79 ppm as six pairs of singlets supported the successful formation of the pentacosasaccharide **64** (Scheme 12). Full confirmation and the structural integrity of polysaccharide **64** were obtained from the high field ^{13}C NMR spectrum (150.97 MHz). All the twenty five anomeric carbons were identified between δ 107.0, 106.2 (3C), 106.0-105.9 (13C), 105.7 (2C), 105.3, 105.0, 99.9 (2C) and 99.8 (2C) ppm. The anomeric carbon at the reducing end of the arabinogalactan **64** was confirmed at the δ 99.9 and 99.8 ppm as anticipated. Besides, the pentacosasaccharide showed satisfactory matching of sodium adduct of the molecular ion.[36] Subsequently, cleavage of the silyl ether under standard HF·py conditions followed by Zemplén saponification conditions afforded the free pentacosasaccharide as a pentenyl glycoside **1** (Scheme 12). The free pentacosasaccharide **1** was purified by gel filtration chromatography using Bio-Rad P4 gel followed by lyophilization to afford polysaccharide **1** as a fluffy white powder (13 mg).

Conclusions

In summary, the synthesis of pentacosasaccharide with twenty four interglycosidic linkages achieved by invoking salient features of alkynyl carbonate glycosyl donor strategy involving [Au]/[Ag]-catalysis. Major features of this endeavor are: (i) installation of all 1,2-*trans* linkages by the neighbouring group assistance from the *C*-2 acyl group, (ii) conversion of β- or 1,2-*trans* Rib*f* to β- or 1,2-*cis* Ara*f* through an oxidation-reduction



Scheme 12. Synthesis of pentacosasaccharide motif of Arabinogalactan: Reagents: (a) 8mol% each of chloro[tris(2,4-di-*tert*-butylphenyl)phosphite]gold and AgOTf, CH_2Cl_2, 4Å MS powder, 25 °C, 40 min, 87%; (b) Pyridine, HF.py, 0-25 °C, 5 h; (c) 0.5M NaOMe (2 mL), CH_2Cl_2-MeOH (1:1), 25 °C, 2 h; 67% over two steps.

strategy, (iii) single glycosylation method for the stapling of glycans, (iv) effective utilization of gold-silver catalyzed glycosidations, and (v) highly modular and convergent assembly of the pentacosasaccharide as a *n*-pentenyl glycosides. Synthesis of pentacosasaccharide was achieved in 0.0012% overall yield. This route enabled us to synthesize 13mg of the final pentacosasaccharide **1** which be useful for investigating the mycobacterial arabinogalactan biosynthesis. The presence of *n*-pentenyl moiety can be exploited for conjugating immunogenic proteins, biomarkers, and chemical probes for further biological explorations.

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Keywords: Tuberculosis • Arabinogalactan • Oligosaccharide • Synthesis • Gold-catalysis

Dedicated to Dr. Mukund K Gurjar on his 65th Birthday

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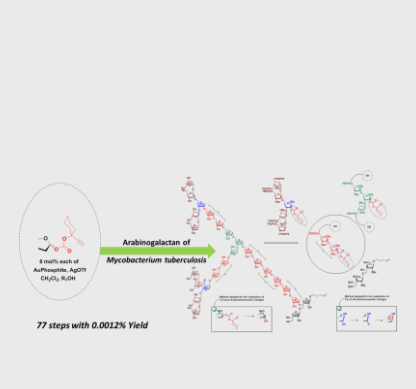
Layout 1:

FULL PAPER

A 77 step synthesis of a pentacosafuranoside of *Mycobacterium tuberculosis* glycocalyx employing single glycosidation protocol is accomplished in 0.0012% yield.



*Sandip Pasari, Sujit Manmode, Gulab Walke and Srinivas Hotha**

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A Versatile Synthesis of Pentacosafuranoside Subunit Reminiscent of Mycobacterial Arabinogalactan Employing One Strategic Glycosidation Protocol