Automated Synthesis of Oligosaccharides

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Dedicated to Professor *Duilio Arigoni* on the occasion of his 75th birthday

The chemical synthesis of oligosaccharides with an automated solid-phase synthesizer is described. An octenediol linker served to attach the growing oligosaccharide chain to the solid support, and the desired structures were cleaved from the support *via* olefin metathesis to afford a pentenyl glycoside. The automated syntheses of several important carbohydrates, including a pentarhamnoside, a proteoglycan linkage-region tetrasaccharide, a phytoalexin elicitor dodecasaccharide, and a branched *Leishmania* lipophosphoglycan tetrasaccharide, were accomplished in higher overall yield and *ca.* 20 times faster than with solution-phase methods.

Introduction. – Nonspecialists can routinely access peptides and oligonucleotides for biochemical studies with commercially available machines, while only highly specialized laboratories can synthesize complex carbohydrates. Our effort towards the automated synthesis of oligosaccharides was inspired by automated methods for peptide and DNA synthesis, and the impact these techniques have had on proteomics and genomics. Here, we describe the development and use of the first automated oligosaccharide synthesizer.

The traditional solution-phase chemistry of complex carbohydrates remains a time-consuming art. Although a plethora of new methods for the construction of oligosaccharides, such as enzymatic [1] and one-pot procedures, [2][3] have been described, few are generally applicable. The *OptiMer One-Pot* approach [4] aims to automate synthesis planning by selecting efficient couplings based on a computer program and has been successfully applied to the construction of several complex oligosaccharides [5]. As an alternative to solution-phase assembly, the solid-phase synthesis of oligosaccharides has received increased attention [6]. The elimination of intermediate purification steps has led to the accelerated synthesis of several complex structures. We sought to exploit the benefits of the solid-phase paradigm, without the need for manual manipulations.

Influenced by the peptide-synthesizer platform [7][8], we envisioned a similar machine that could easily accomplish glycosylation and deprotection reactions in an analogous fashion. In this way, the synthesis of complex carbohydrates could be reduced to the acquisition of suitably protected mono- or disaccharide glycosyl donors. The building blocks and reaction cycles required for automated oligosaccharide

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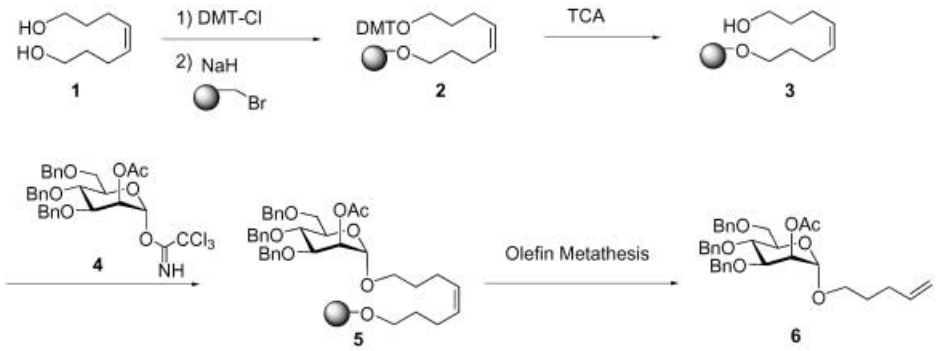
construction were determined through the synthesis of representative structures. The automated synthesis of oligosaccharides must occur in a linear fashion, thereby departing from the traditional goal of convergency. Initial solution-phase syntheses validated the proposed linear synthetic strategy and provided standards for the confirmation of products obtained from the synthesizer. The design of the automated oligosaccharide synthesizer took several key issues into account: appropriate linker systems and polymeric resins; competent glycosylating agents and protecting groups; and efficient cleavage and purification methods.

Results and Discussion. – The solid-phase synthesis of carbohydrates can be accomplished by attaching the first sugar either through the reducing end ('acceptor-bound') or the non-reducing end ('donor-bound'). Several large structures have been synthesized by the donor-bound approach [9], but side reactions involving the resin-bound glycosyl donor result in a direct loss in coupling yield. To increase the overall yield, the acceptor-bound assembly method was chosen for the automated synthesizer. Glycosyl trichloroacetimidates [10][11] and glycosyl phosphates [12] were selected as the building blocks due to their reliability and prior success in solid-phase oligosaccharide synthesis [13].

Oct-4-ene-1,8-diol linker **3** [13], stable to acidolysis and saponification unlike most other linkers [14], provided the basis for our protecting-group concept. Linker **3** is stable under a host of conditions but is cleanly cleaved by olefin metathesis (*Scheme 1*) [15]. Resin loading is determined by detritylation of **2**. Acetate, levulinate, and silyl ether protecting groups are fully compatible with the oct-4-ene-1,8-diol linker, and their removal readily reveals the OH group ready for coupling with the glycosyl donor delivered in solution. The flexibility of this linker system was essential to the success of the automated synthesizer.

Scheme 1. *Functionalization and Use of Oct-4-ene-1,8-diol Linker.* Shaded circle = polystyrene resin.



Initial work on the manual solid-phase synthesis of carbohydrates was accomplished with *Merrifield's* resin (1% crosslinked polystyrene) functionalized with the oct-4-ene-1,8-diol linker. Attempts to functionalize polyethylene glycol (PEG) grafted *Tentagel*® polystyrene support with the linker system were unsuccessful. While *Argogel*®, a similar PEG-grafted resin, and *Argopore*®, a highly crosslinked polystyrene resin, were successfully functionalized, and initial results indicated that *Merrifield*'s

resin was the best choice for the automated synthesis. Resin loadings in the range of 0.1 – 0.3 mmol/g were routinely accomplished with the polystyrene resin.

A commercially available *Applied Biosystems* model *433A* peptide synthesizer was reconfigured to carry out the automated solid-phase oligosaccharide synthesis on a 0.1 – 1.0-mmol scale. Reagent bottles contained the chemicals necessary for oligosaccharide synthesis. Cartridges that stored the glycosyl donor building blocks were advanced in the order of incorporation to a needle that delivered the monomers to the reaction vessel. The computer program *SynthAssist*®, provided by *Applied Biosystems* for peptide construction, was employed to design the automated cycles for oligosaccharide synthesis.

While all the manipulations in peptide chemistry are carried out at room temperature, many transformations in oligosaccharide synthesis occur below ambient temperature. To address the need for variable reaction temperatures, a double-walled glass reaction vessel (8-ml internal volume) was designed with inlet and outlet hose fittings that were connected to an external circulating bath with heating and cooling capabilities. A glass-frit-closed bottom of the reaction vessel, and the resin was loaded and removed from the top of the vessel.

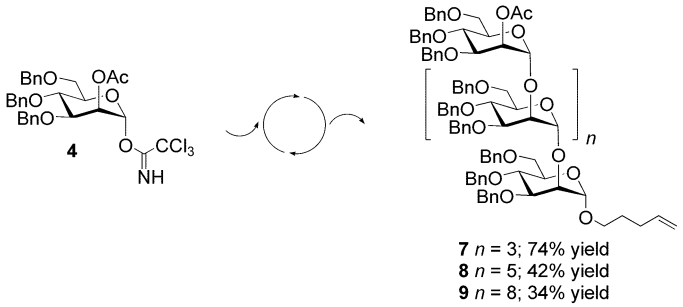
The construction of α-(1 $\rightarrow$ 2)-oligomannosides had been accomplished by manual solid-phase synthesis, and these structures provided a starting point to explore the efficacy of automation [13][16]. Trichloroacetimidate donor **4** utilized a temporary *C(2)*-acetate protecting group that provides anchimeric assistance. The activation of donor **4** was accomplished by the addition of a catalytic amount of trimethylsilyl trifluoromethanesulfonate (TMSOTf) in CH_2Cl_2. Deprotection was achieved through the use of MeONa in a MeOH/CH_2Cl_2 solution. A synthesis cycle consisting of coupling and deprotection steps was developed by varying the concentration and quantity of reagents as well as the reaction times. Repetition of coupling and deprotection in each cycle ensured complete reactivity, and washing cycles effectively removed the excess reagent and solution-phase impurities.

Using oct-4-ene-1,8-diol functionalized *Merrifield*'s resin **3**, double couplings, and double deprotections (*Table 1*), the synthesis of pent-4-enyl pentamannoside **7** was accomplished in 14 h in 74% isolated yield after cleavage with *Grubbs* catalyst in an atmosphere of ethylene (*Scheme 2*). Heptamannoside **8** and decamannoside **9** were prepared by the same automation cycle in average yields of 90 – 95% per step. The

Table 1. *Automated Cycle for the Synthesis of Oligomannosides*

Step	Function	Reagent	Time [min]
1	Couple	10 equiv. donor and 0.5 equiv. TMSOTf	30
2	Wash	CH_2Cl_2	6
3	Couple	10 equiv. donor and 0.5 equiv. TMSOTf	30
4	Wash	CH_2Cl_2	6
5	Wash	MeOH/CH_2Cl_2 1 : 9	6
6	Deprotection	2 $\times$ 10 equiv. MeONa (MeOH/CH_2Cl_2 1 : 9)	80
7	Wash	MeOH/CH_2Cl_2 1 : 9	4
8	Wash	0.2M AcOH in THF	4
9	Wash	THF	6
10	Wash	CH_2Cl_2	6

Scheme 2. *Automated Synthesis of Polymannosides*



short reaction times – 3 h per monomer unit – allowed for the synthesis of **9** in 20 h. For comparison, heptamannoside **8** was manually synthesized in our laboratory on the solid support in 14 d and 9% overall yield [13]. The cycle developed for the synthesis of polymannosides **7**–**9** provided a basis for general acetate deprotection and trichloroacetimidate donor activation.

Besides glycosyl trichloroacetimidates, which are a staple of many oligosaccharide syntheses, glycosyl phosphates have proven to be readily accessible and versatile glycosylating agents [12]. The use of glycosyl phosphates in an automated protocol demonstrated that the automated machine was compatible with different glycosylation chemistries. The phytoalexin elicitor (PE) family of β-glucans [17] (*Fig. 1*) was selected as the target to evaluate the use of glycosyl phosphates (for a preliminary communication of this work, see [18]).

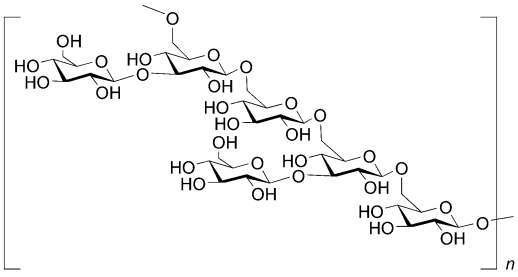
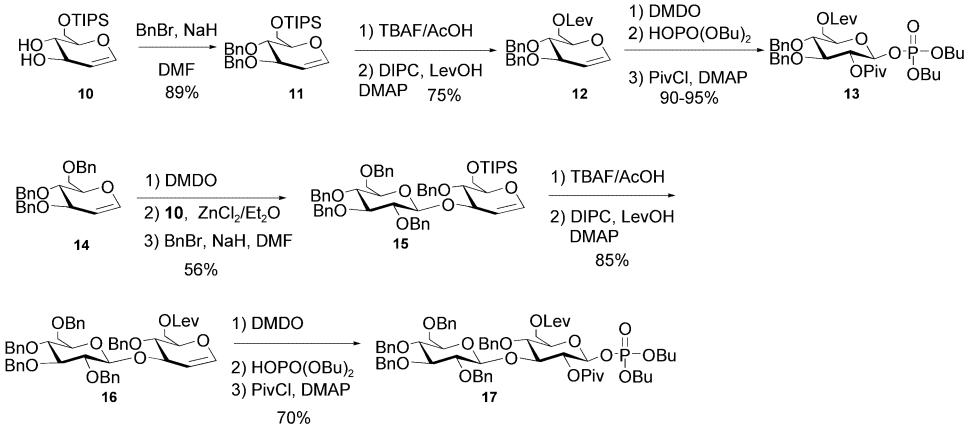


Fig. 1. *Phytoalexin elicitor structure*

Two different glycosyl phosphate donors that were accessible from glycal starting materials were used for the synthesis of the branched β-(1 → 3)/β-(1 → 6) phytoalexin elicitor structure (*Scheme 3*) [19–23]. A levulinoyl (Lev) ester served as the 6-*O*-temporary protecting group while the 2-*O*-pivaloyl (Piv) group ensured β-selectivity in the glycosylation reaction. Monosaccharide glycal **12** was prepared from the corresponding 3,4-di-*O*-benzyl-6-*O*-TIPS (TIPS = (i-Pr)$_3$Si) glucal **11** by treatment with Bu_4NF (TBAF) and subsequent acylation. By our one-pot procedure [24], **12** was converted into phosphate **13** in 95% yield. Disaccharide phosphate **17** was prepared *via*

a similar route. The epoxidation of glucal **14** was followed by regioselective opening with 6-*O*-TIPS glucal **10**, and the crude reaction mixture was benzylated to afford the β-$(1\rightarrow3)$-linked disaccharide glycal **15**. Removal of the *C(6)*-TIPS and subsequent installation of the *C(6)*-levulinoyl protecting group furnished glycal **16**. Glycosyl phosphate **17** was synthesized from **16** again by our one-pot procedure.

Scheme 3. *Synthesis of Phosphate Building Blocks*



The coupling and deprotection conditions for glycosyl phosphates and levulinates were established (*Table 2*) by the synthesis of β-$(1\rightarrow6)$ glucose trisaccharide **18** as a model. Optimal yields with glycosyl phosphate **13** were accomplished with coupling temperatures below ambient temperature. The HPLC traces of the crude reaction products with glycosylations occurring at $+25^\circ$ (run 1) and at -15° (run 2) showed the benefit of utilizing the vessel's temperature-control abilities (*Fig. 2*). The activation of **13** with TMSOTf at -15° was complete within 15 min. The results of these glycosylation experiments mirrored solution-phase oligosaccharide synthesis, where low temperatures are commonly used. The levulinoyl ester was rapidly removed at

Table 2. *Automated Cycle for the Synthesis of Phytoalexin Elicitors* **18**–**20**

Step	Function	Reagent	Time [min]
1	Couple	5 equiv. donor and 5 equiv. TMSOTf	30
2	Wash	CH_2Cl_2	6
3	Couple	5 equiv. donor and 5 equiv. TMSOTf	30
4	Wash	MeOH/CH_2Cl_2 1 : 9	4
5	Wash	THF	4
6	Wash	pyridine/AcOH 3 : 2	3
7	Deprotection	2×20 equiv. Hydrazine (pyridine/AcOH 3 : 2)	80
8	Wash	pyridine/AcOH 3 : 2	3
9	Wash	MeOH/CH_2Cl_2 1 : 9	4
10	Wash	0.2M AcOH in THF	4
11	Wash	THF	4
12	Wash	CH_2Cl_2	6

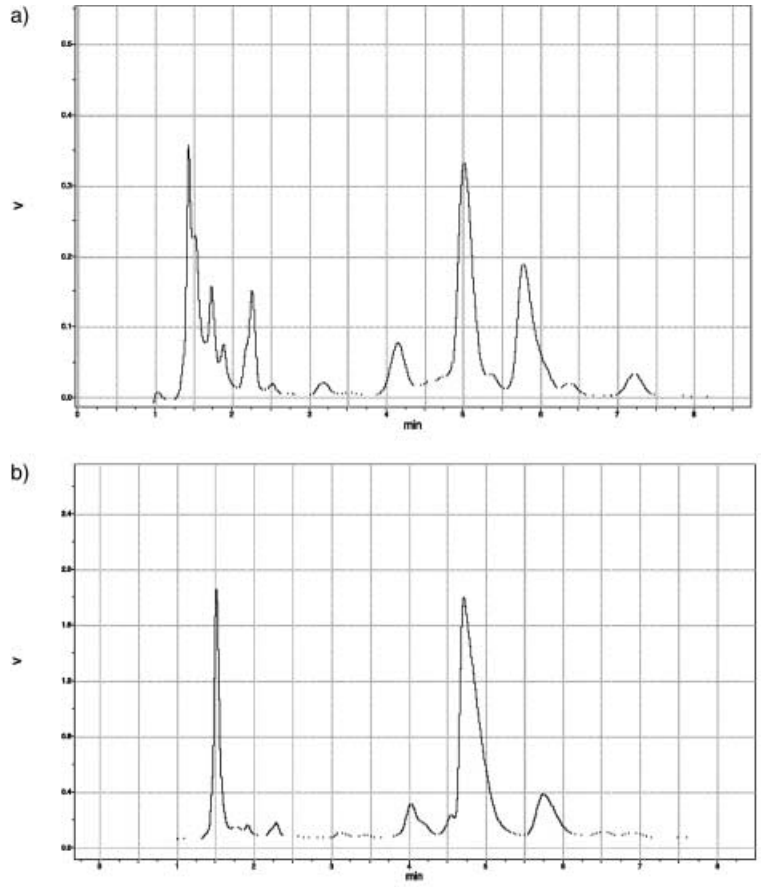
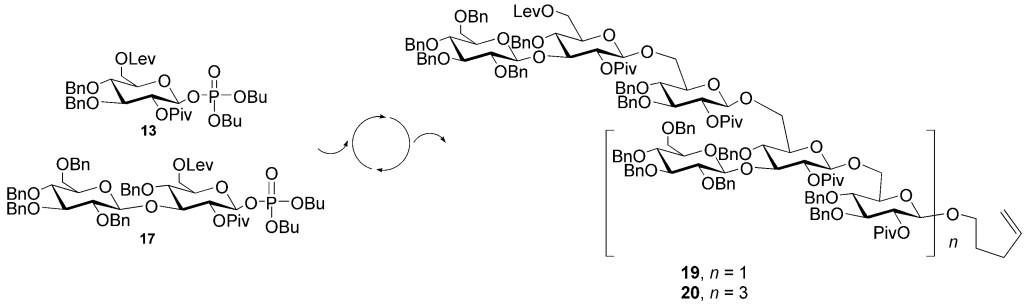


Fig. 2. a) *Analytical HPLC chromatogram of room-temperature triglucoside synthesis following resin cleavage.* b) *Analytical HPLC chromatogram of* $-15°$ *triglucoside synthesis following resin cleavage.* Flow rate: 1 ml/min, 20 → 30% AcOEt/Hexanes (20 min): t_R(**18**) = 5 min.

$+15°$ with hydrazine in a solution of pyridine/AcOH. Double couplings and double deprotections were utilized in analogy to the synthesis of the polymannosides.

The automated cycle developed for glycosyl phosphates and levulinoyl esters was applied to the synthesis of the phytoalexin elicitor oligomers (Scheme 4). Hexasaccharide **19** was constructed by using glycosyl phosphates **13** and **17** in alternating cycles in 10 h. Hexasaccharide **19** was the major product as determined by HPLC analysis. With the same glycosyl donors and automated cycles, we prepared dodecasaccharide **20** in 17 h. Notably, our synthesis of **20** required the solution-phase synthesis of only two glycosyl-phosphate building blocks, greatly reducing the manual labor usually necessary for the assembly of large structures. The coupling cycles for glycosyl phosphates and levulinoyl esters established in the synthesis of the phytoalexin elicitor

Scheme 4. *Automated Synthesis of Phytoalexin Elicitor Structures*



structures may serve as the foundation for other automated syntheses utilizing these groups.

Synthesis of the Proteoglycan Linkage Region. The proteoglycan-linkage region is the attachment point for heparin and related glycosaminoglycan oligomers to the proteoglycan core protein [25][26]. The linkage region is crucial to the biosynthesis of these molecules, and studies to elucidate this biochemical pathway are on-going [27][28]. The tetrasaccharide is attached to the core protein *via* a xylose-threonine/serine bond and consists of xylose, two galactose units, and a terminal glucuronic acid moiety (*Fig. 3*). In the biosynthetic pathway, a galactosamine or glucosamine unit attached to the linkage region tetrasaccharide determines whether the glycosaminoglycan structure will be chondroitin sulfate or heparin/heparan sulfate. Several previous solution-phase syntheses of the tetrasaccharide have been accomplished; however, none were amenable to automation [29–33]. The synthesis of **30** would necessitate unique monosaccharide donors of xylose, galactose, and glucuronic acid, thereby expanding the number of building blocks successfully incorporated in our automated scheme.

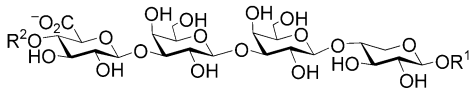
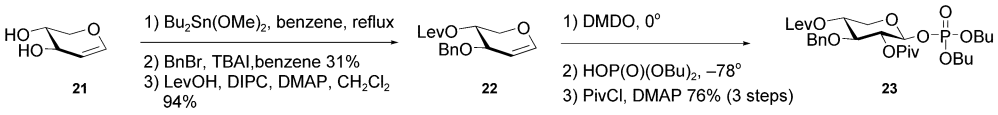


Fig. 3. *Proteoglycan linkage region tetrasaccharide* (R^1 = protein, R^2 = glycosaminoglycan chain)

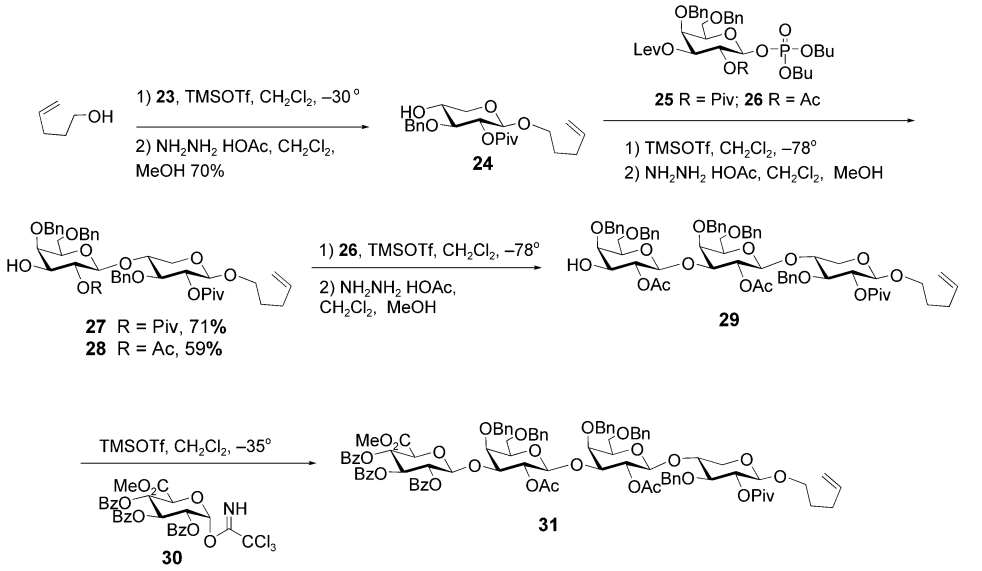
The automated synthesis of the linkage-region tetrasaccharide required an efficient xylose building block. Xylose phosphates had not been explored previously, and synthesis of the glycosyl phosphate from the corresponding glycal starting material was investigated. Starting from xylal **21** [34], the necessary differentiation of the OH groups at C(3) and C(4) proved difficult [35][36]. Unlike monosaccharides containing the *C(5)*-substituent, the two secondary OH groups of xylal are nearly equivalent in reactivity. Utilizing tin-ether chemistry [37], a 3:1 ratio of the 3-*O*-benzyl/4-*O*-benzyl-xylal was obtained in 31% yield (*Scheme 5*). Installation of the 4-*O*-levulinoyl ester, followed by creation of the glycosyl phosphate, afforded xylose donor **23**.

Scheme 5. *Synthesis of Xylose Phosphate* **23**



Solution-phase validation of the proposed synthetic route commenced with the coupling of the xylose phosphate **23** to pent-4-enyl alcohol, a useful model for the functionalized resin. Removal of the temporary levulinoate protecting group afforded monosaccharide acceptor **24**. Glycosylation with galactose phosphate **25** [38], and removal of the levulinoyl protecting group at C(3) gave disaccharide **27** (*Scheme 6*). Attempts to install the second galactose unit with **25** failed, and unreacted acceptor **27** was recovered, thereby necessitating a new route to the Gal-β-(1 → 3)-Gal linkage.

Scheme 6. *Solution-Phase Synthesis of Proteoglycan-Linkage Region Tetrasaccharide* **30**



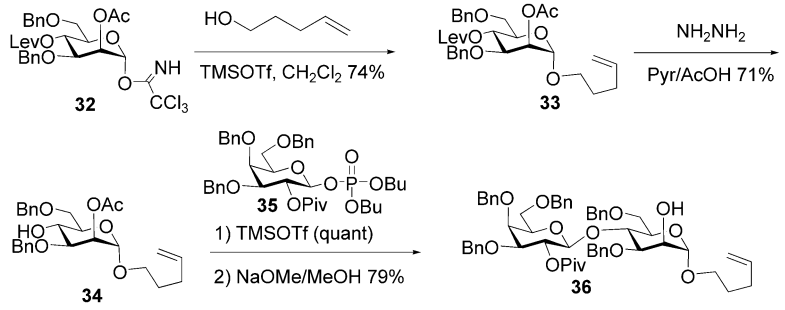
Glycosylation of **24** with *C(2)*-acetate galactose phosphate **26**, followed by deprotection, afforded disaccharide **28** in good yield. Reaction with glycosyl phosphate **26**, in this case, gave good yields of the corresponding trisaccharide. Cleavage of the temporary protecting group and glycosylation with glucuronic acid trichloroacetimidate **30** [39] afforded the final tetrasaccharide **31** in solution.

The automated synthesis of the proteoglycan linkage region was achieved by using glycosyl donors **23**, **26**, and **30**. Double glycosylations and double deprotections were employed to ensure high conversion of all steps. Cleavage from the support with the *Grubbs* catalyst under an atmosphere of ethylene afforded the crude tetrasaccharide **31**. Analysis of the reaction mixture revealed that **31** was obtained as the major product, demonstrating the successful incorporation of xylose, galactose, and glucuronic acid into our automated synthesis scheme.

Synthesis of Branched Structures. Different sections of the *Leishmania* lipophosphoglycan have served as targets for previous synthetic studies [30–42]. During our efforts to identify a potential carbohydrate vaccine candidate for leishmaniasis [43], the automated synthesis of the cap tetrasaccharide **37** was investigated. The automated assembly of **37** presented a synthetic challenge since it required the incorporation of a branching pattern, which had not been attempted previously in an automated context. While acetates and levulinates had been used successfully as temporary protecting groups, we needed to illustrate that these groups, integrated into one monosaccharide, would allow for the synthesis of a branched structure.

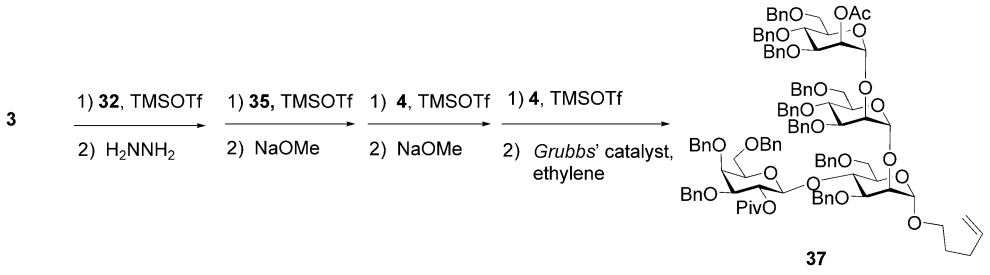
A key challenge of the automated synthesis of **37** was the ability to selectively remove the protecting groups on the central mannose unit. To examine this issue, a solution-phase model study was conducted (*Scheme 7*). Reaction of glycosyl donor **32** with pent-4-enyl alcohol, upon activation with catalytic TMSOTf, generated **33**. Removal of the levulinoyl ester in the presence of the *C(2)*-acetate by treatment with hydrazine furnished acceptor **34** in 71% yield. Installation of the difficult β-(1→4) linkage between galactose and mannose was readily accomplished by reaction with galactose phosphate **35**. Treatment with MeONa provided disaccharide acceptor **36**. These results confirmed the validity of our protecting group strategy *en route* to the automated synthesis of **37**.

Scheme 7. *Solution-Phase Model for Automation of* **37**



With the four monosaccharide building blocks in hand, the automated synthesis of the branched *Leishmania* tetrasaccharide was carried out (*Scheme 8*). Glycosylation of the resin with **32**, followed by levulinate cleavage and glycosylation with **35**, afforded

Scheme 8. *Automation of* Leishmania *Tetrasaccharide* **37**

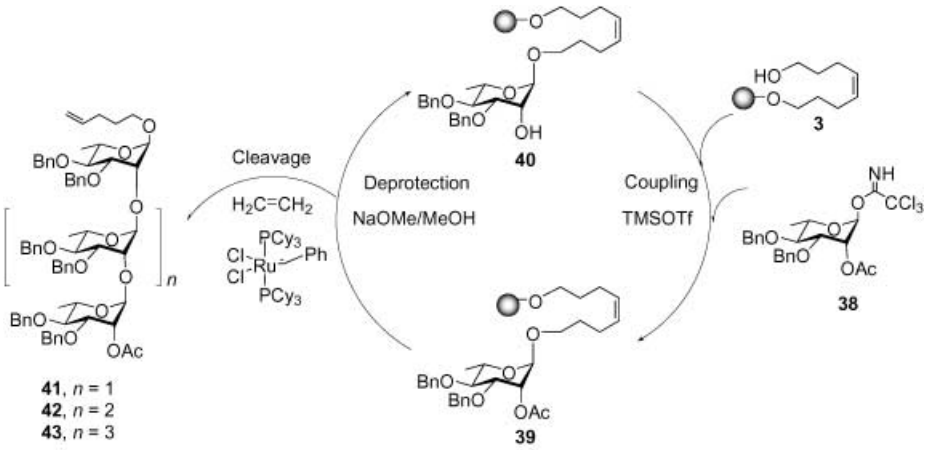


resin-bound disaccharide. Deprotection of the axial acetate and coupling with mannosyl donor **4**, followed by acetate saponification and reaction with **4**, yielded the resin bound *Leishmania* tetrasaccharide. Cleavage of the oct-4-ene-1,8-diol linker using the *Grubbs* catalyst in an atmosphere of ethylene provided crude pent-4-enyl glycoside tetrasaccharide **37**. Analysis of the reaction products showed that the tetrasaccharide was the major product along with the trisaccharide [44].

Synthesis of Difficult Linkages. While mannose donor **4** has been used extensively for the construction of α-(1 → 2)-mannosidic linkages, rhamnose donor **38** had been employed only once previously [45]. Modest yields (53%) were obtained for coupling with the *C(2)*-OH group of glucose under the activation conditions (BF_3OEt_2, CH_2Cl_2/hexanes $-20°$) reported. The synthesis of poly-β-(1 → 2)-rhamnosides would require a significant improvement over previous methods and prompted us to focus attention on this challenge [46].

The initial coupling cycle employed the temperature-control feature of the jacket vessel to synthesize pentarhamnoside **43** (*Scheme 9*). Double glycosylations were performed at $-15°$ for 15 min and the deprotection of the *C(2)*-acetate was carried out

Scheme 9. *Automated Synthesis of Polyrhamnosides*



twice at $+15°$ (*Table 3*). After cleavage from the resin by olefin metathesis, HPLC analysis revealed that the desired pentasaccharide **43** was formed in 35% yield. However, the undesired tetramer **42** (40%) and trimer **41** (18%) were also observed.

Since no unreacted *C(2)*-OH compounds were observed in the HPLC chromatogram, it was postulated that the $n-1$ oligomers were due, most likely, to incomplete deprotection of the *C(2)*-acetate and did not stem from incomplete glycosylation. While extending the reaction time to 1 h did improve the overall yield of pentamer **43**, we were interested in developing a shorter coupling cycle. Changing the resin from the swellable *Merrifield*'s resin to the functionalized *Argopore*® support allowed the use of a higher concentration of MeONa (40 equiv.). To ensure full deprotection, the reaction temperature was elevated to $+30°$. This optimized reaction cycle employing the oct-4-

Table 3. *Automated Cycle Utilizing Rhamnosyl trichloracetimidate* **38**

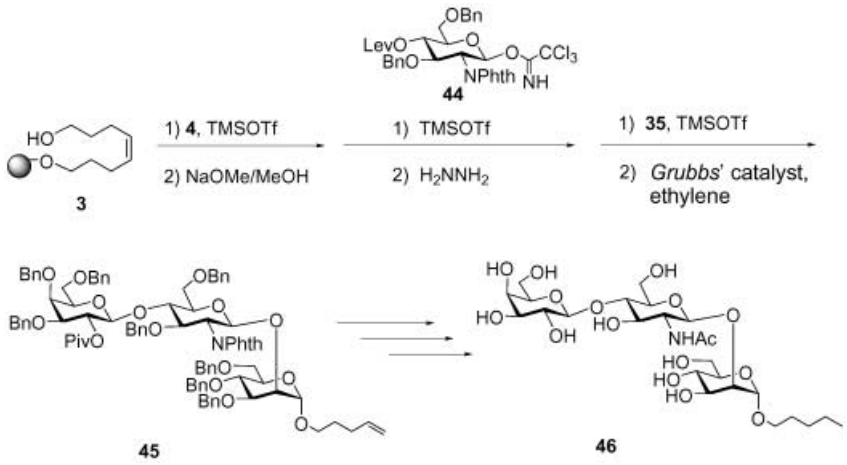
Step	Function	Reagent	Time [min]
1	Couple	5 equiv. donor and 0.2 equiv. TMSOTf (– 15°)	15
2	Wash	CH_2Cl_2	6
3	Couple	2.5 equiv. donor and 0.1 equiv. TMSOTf (15°)	15
4	Wash	CH_2Cl_2	6
5	Wash	MeOH	4
6	Deprotection	2 × 40 equiv. NaOMe, 0.75M in MeOH (+ 30°)	20 each
7	Wash	MeOH	4
8	Wash	0.2M AcOH in THF	4
9	Wash	THF	6
10	Wash	CH_2Cl_2	6

ene-1,8-diol-functionalized *Argopore*® resin resulted in the synthesis of pentarhamnose **43** in 77% yield, a considerable improvement over the previously reported synthesis.

Scale-Up and Deprotection of Automated Syntheses. The ability to scale-up the automated process in order to obtain useful quantities of deprotected oligosaccharides is essential. To address this challenge, we considered the assembly of a Gal-Man-GlcNAc trisaccharide motif found in *N*-linked complex-type glycoproteins [47]. Trisaccharide **45** contains two challenging linkages: glycosylation onto the *C(2)*-OH of mannose with glucosamine donors and glycosylation onto the *C(4)*-OH of glucosamine [48].

The automated synthesis of trisaccharide **45** required the preparation of the monosaccharide building blocks **4**, **44**, and **35**. The reaction scale was increased from 25 μmol in the previous syntheses to 100 μmol, and double couplings and double deprotections were utilized. The desired trisaccharide **45** was isolated in 43% yield, and subsequent removal of all protecting groups was carried out in solution to afford **46** in 62% yield (*Scheme 10*). In comparison, trisaccharide **45** was isolated in 17% yield by

Scheme 10. *Automated Synthesis of the Complex-Type Trisaccharide* **46**



manual solution-phase synthesis. The synthesis and deprotection of trisaccharide **46** demonstrated that automated oligosaccharide synthesis is fully scalable, and the products obtained were readily deprotected by established procedures [49].

Conclusions. – Described is the development of the first automated solid-phase oligosaccharide synthesizer and its application to the assembly of several biologically relevant structures of oligosaccharides. Glycosyl phosphates and glycosyl trichloroacetimidates serve well as building blocks for this automated synthesizer and esters and silyl ethers can be used as temporary protecting groups. Linear and branched structures were prepared along with several structures containing difficult linkages. The automated method is scalable, and the jacketed reaction vessel allows reactions to occur below or above ambient temperature.

The automated synthesizer greatly reduces the necessary labor required to access large oligosaccharide structures. Once the set of building blocks required for the synthesis of a class of oligosaccharides has been established, access to desired structures may be readily achieved by automated synthesis.

This report details the initial efforts directed at the automated synthesis of carbohydrates. Further improvements to the automated method, such as colorimetric determination of coupling efficiencies and alternate methods to cleave from the support not requiring a metal catalyst, are being investigated. Further extension of this technology may allow the automated synthesis of complex glycoproteins, proteoglycans, and glycolipids on one instrument.

Experimental Part

General. All chemicals used were reagent grade and used as supplied except where noted. THF and CH_2Cl_2 used for washing cycles was purchased from *Mallinckrodt* (HPLC-Grade) and used without further purification. CH_2Cl_2 used for reagent preparation was purchased from *J. T. Baker* (*Cycletainer*™) and passed through a neutral alumina column prior to use. THF was purchased from *J. T. Baker* (*Cycletainer*™) and passed through a neutral alumina column prior to use. Pyridine was refluxed over CaH_2 and distilled prior to use. Trimethylsilyl trifluoromethanesulfonate (TMSOTf) was purchased from *Acros Chemicals*. MeONa (25% (*w*/*v*) in MeOH), glacial AcOH, hydrazine acetate, and hydrazine monohydrate (98%) were purchased from *Aldrich Chemicals*. Anal. TLC performed on *E. Merck* silica-gel *60* F_{254} plates (0.25 mm). Compounds were visualized by dipping the plates in a cerium sulfate-ammonium molybdate soln., followed by heating. Liquid column chromatography (CC) was performed by forced flow of the indicated solvent on *Silicycle* 230–400 mesh (60-Å pore diameter) silica gel. HPLC Analysis was performed on a *Waters* model *600E* multisolvent delivery system with anal. (*Nova-Pak*®, 60 Å, 4 μm, 3.6 × 150 mm) and prep. (*Nova-Pak*®, 60 Å, 6 μm, 7.8 × 300 mm) silica columns. ^{1}H-NMR spectra were obtained on a *Varian VXR-500* spectrometer (500 MHz), *Varian VXR-300* (300 MHz), or *Bruker* (400 MHz), and are reported in ppm (δ) relative to $CHCl_3$ (7.27 ppm). Coupling constants (J) are reported in H. ^{13}C-NMR spectra were obtained on a *Varian VXR-500* spectrometer (125 MHz), *Varian VXR-300* (75 MHz), or *Bruker* (100 MHz), and are reported in δ relative to $CDCl_3$ (77.23 ppm). MALDI-TOF mass spectrometry was performed on a *PE Biosystems Voyager System 102* as follows: A 1-μl aliquot of matrix soln. (10-mg/ml 2,5-dihydroxybenzoic acid (DHB) in THF) was spotted on the sample holder and allowed to dry. Addition of a 1-μl aliquot of oligosaccharide soln. (5 mg/ml AcOEt) was co-spotted on the matrix, dried, and analyzed in the positive-ion mode.

Dibutyl 3-O-*Benzyl-4*-O-*levulinoyl-2*-O-*pivaloyl-β*-D-*xylopyranosyl Phosphate* (**23**). *3*-O-*Benzyl-4*-O-*levulinoyl-1,5-anhydro-2-deoxy*-D-threo-*pent-1-enitol* (**22**; 512 mg, 1.67 mmol) was co-evaporated with toluene (3 × 10 ml), dissolved in CH_2Cl_2 (10 ml), and cooled to 0°. Dimethyldioxirane (DMDO; 30 ml, 0.08M in acetone) was added *via* cannula, and the mixture was stirred for 30 min. Solvents were removed *in vacuo*, and the residue was dissolved in CH_2Cl_2 (20 ml). The resulting soln. was cooled to −78°, dibutyl phosphate (320 μl,

1.67 mmol) was added dropwise. After 15 min, the mixture was warmed to amb. temp., and pivaloyl chloride (410 µl, 3.34 mmol) and 4-(dimethylamino)pyridine (DMAP; 815 mg, 6.68 mmol) were added. The resulting mixture was stirred for 2 h, hexanes/AcOEt 3:1 (100 ml) were added, and the white precipitate was filtered off through a silica-gel plug. The filtrate solvents were removed *in vacuo* and by CC (hexanes/AcOEt 3:1) afforded **23** (593 mg, 58%). $[\alpha]_D^{24} = -2.6°$ ($c = 3.84$, CH_2Cl_2); IR (thin film, NaCl): 2962, 2934, 1742, 1151. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.34–7.27 (*m*, 5 H); 5.42 (*dd*, $J = 4.4$, 6.2, 1 H); 5.08 (app. *t*, $J = 6.2$, 1 H); 4.95–4.93 (*m*, 1 H); 4.74 (*d*, $J = 11.6$, 1 H); 4.67 (*d*, $J = 11.6$, 1 H); 4.26 (*dd*, $J = 3.6$, 12.4, 1 H); 4.09–3.99 (*m*, 4 H); 3.72 (app. *t*, $J = 6.0$, 1 H); 3.58 (*dd*, $J = 5.5$, 12.4, 1 H); 2.77–2.72 (*m*, 2 H); 2.58–2.53 (*m*, 2 H); 2.20 (*s*, 3 H); 1.68–1.60 (*m*, 4 H); 1.44–1.26 (*m*, 4 H); 1.25 (*s*, 9 H); 0.98–0.90 (*m*, 6 H); ^{13}C-NMR (125 MHz, $CDCl_3$): 206.4; 177.1; 172.1; 137.8. 128.6; 128.5; 128.5; 127.9; 127.7; 127.6; 127.6; 96.2; 96.2; 75.9; 75.0; 73.3; 69.4; 69.3; 69.3; 68.1; 68.1; 68.0; 68.0; 61.3; 39.0; 38.0; 37.9; 37.9; 32.4; 32.4; 32.3; 32.3; 32.3; 30.0; 30.0; 28.0; 27.9; 27.3; 27.3; 27.3; 18.8; 18.8; 18.8; 13.8; 13.8; 13.8. ^{31}P-NMR (120 MHz, $CDCl_3$): −2.5; ESI-MS: 637.2752 ($[M + Na]^+$; calc. 637.1856).

*Dibutyl 2-*O*-Acetyl-4,6-di-*O*-benzyl-3-*O*-levulinoyl-β-*D*-galactopyranosyl Phosphate* (**26**). 4,6-Di-*O*-benzyl-3-*O*-levulinoyl-D-galactal (430 mg, 1.01 mmol) was co-evaporated with toluene (3×10 ml), dissolved in CH_2Cl_2, and the soln. was cooled to 0°. DMDO (40 ml, 0.08M in acetone) was added, and the resulting mixture was stirred for 30 min. The solvents were removed *in vacuo*, the residue was dissolved in CH_2Cl_2 (20 ml), and the soln. was cooled to −78°. Dibutyl phosphate (200 µl, 1.05 mmol) was added dropwise. After 30 min, the soln. was warmed to amb. temp., and Ac_2O (190 mg, 2.0 mmol) and DMAP (493 mg, 4.0 mmol) were added, and the soln. was stirred for 1 h. Hexanes/AcOEt 3:1 (100 ml) and the mixture were passed through a silica-gel plug, and the solvents were removed *in vacuo*. CC (hexanes/AcOEt 3:2 → 1:1) afforded **26** (375 mg, 65%). Clear oil. $[\alpha]_D^{24} = +18.3°$ ($c = 1.15$, CH_2Cl_2). IR (thin film, NaCl): 2961, 2934, 1750, 1028. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.30–7.26 (*m*, 10 H); 5.43 (*dd*, $J = 8.0$, 10.2, 1 H); 5.21 (*app. t*, $J = 7.4$, 1 H); 4.98 (*dd*, $J = 3.0$, 10.2, 1 H); 4.72 (*d*, $J = 11.7$, 1 H); 4.55 (*d*, $J = 11.7$, 1 H); 4.15–3.96 (*m*, 5 H); 3.83 (*app. t*, $J = 6.6$, 1 H); 3.63–3.58 (*m*, 2 H); 2.69–2.65 (*m*, 2 H); 2.46–2.65 (*m*, 2 H); 2.15 (*s*, 3 H); 2.09 (*s*, 3 H); 1.67–1.57 (*m*, 4 H); 1.41–1.27 (*m*, 4 H); 0.94–0.86 (*m*, 6 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 206.3; 172.0; 169.8; 138.2; 137.7; 128.6; 128.5; 128.2; 128.0; 128.0; 127.9; 97.0; 96.9; 75.3; 74.0; 73.9; 73.6; 69.8; 69.7; 68.2; 68.1; 68.1; 68.1; 67.6; 37.7; 32.3; 32.2; 32.2; 29.9; 28.0; 21.0; 18.8; 13.8; 13.7. ^{31}P-NMR (120 MHz, $CDCl_3$): −2.4. ESI-MS: 715.2861 ($[M + Na]^+$; calc. 715.1962).

*Pent-4-enyl (Methyl 3,4-di-*O*-benzyl-2-*O*-pivaloyl-β-*D*-glucopyranosiduronate)-(1 → 3)-2-*O*-acetyl-4-6-di-*O*-benzyl-β-*D*-galactopyranosyl-(1 → 3)-2-*O*-acetyl-4-6-di-*O*-benzyl-β-*D*-galactopyranosyl-(1 → 4)-3-*O*-benzyl-2-*O*-pivaloyl-β-*D*-xylopyranoside* (**31**). Oct-4-ene-1,8-diol-functionalized resin **3** (25 µmol, 61 mg; 0.40 mmol/g loading) was manually loaded into a reaction vessel, and the vessel was inserted into a modified *ABI-433A* peptide synthesizer. The coupling, deprotection, and washing steps were controlled by the synthesizer. The resin was glycosylated with donor **23** (5 equiv., 77mg) delivered in CH_2Cl_2 (4 ml) and TMSOTf (5 equiv., 1 ml; 0.125M in CH_2Cl_2) at −20°. Mixing of the suspension was performed (10 s vortex, 50 s rest) for 15 min. The resin was then washed with CH_2Cl_2 (4×4 ml each) and glycosylated a second time. Removal of any soluble impurities was accomplished by washing the resin with CH_2Cl_2 (4×4 ml each) and THF (4×4 ml each). Deprotection of the levulinoyl ester was carried out by treating the resin with hydrazine acetate (40 equiv., 4 ml, 0.25M $N_2H_4 \cdot H_2O$ in pyridine/AcOH 3:2) for 15 min at 15°. The resin was subjected to the deprotection conditions a second time for 15 min. Removal of any soluble impurities was accomplished by washing the resin with DMF (4×4 ml), 0.2M AcOH in THF (4×4 ml each), THF (4×4 ml each), and CH_2Cl_2 (4×4 ml each). Identical glycosylation/deprotection cycles were used with glycosyl donors **26** ($2 \times$, 5 equiv., 86 mg, 5 equiv. TMSOTf) and **30** (5 equiv., 83 mg, 0.5 equiv. TMSOTf). The glycosylated resin (25 µmol) was dried in vacuo over P_2O_5 for 12 h and transferred to a 10 ml flask. The flask was purged with ethylene and *Grubbs*' catalyst (bis(tricyclohexylphosphine)benzylideneruthenium(IV) dichloride; 4.1 mg, 20 mol%) was added. The mixture was diluted with CH_2Cl_2 (3 ml) and stirred under 1 atm ethylene for 36 h. The mixture was diluted with CH_2Cl_2 (5 ml) and washed with H_2O (3×5 ml) and saturated aqueous $NaHCO_3$ (3×5 ml). The aq. phase was extracted with CH_2Cl_2 (3×5 ml), and the combined org. extracts were dried (Na_2SO_4), filtered through a plug of silica gel, and the solvents were removed to afford crude **31** (6.1 mg). The resulting crude mixture was analyzed by HPLC (*Fig. 4*). Mass spectrometry also confirmed the presence of **31** in the crude mixture. ESI-MS: 1682.0 ($[M + NH_4]^+$; calc. 1681.4). $[\alpha]_D^{24} = +26.9°$ ($c = 0.16$, CH_2Cl_2). ^{1}H-NMR (500 MHz, $CDCl_3$): 7.95 (*d*, $J = 7.2$, 2 H); 7.92 (*d*, $J = 7.3$, 2 H); 7.83 (*d*, $J = 7.2$, 2 H); 7.56–7.13 (*m*, 34 H); 5.94 (app. *t*, $J = 9.6$, 1 H); 5.81–5.73 (*m*, 1 J); 5.68 (app. *t*, $J = 9.8$, 1 H); 5.53 (*dd*, $J = 7.5$, 9.3, 1 H); 5.31 (*dd*, $J = 8.1$, 10.1, 1 H); 5.20 (*dd*, $J = 7.8$, 10.2, 1 H); 5.16 (*d*, $J = 11.9$, 1 H); 5.01–4.85 (*m*, 7 H); 4.71 (*d*, $J = 11.8$, 1 H); 4.55–4.14 (*m*, 10 H); 3.91–3.53 (*m*, 18 H); 3.17–3.14 (*m*, 2 H); 2.11–2.03 (*m*, 2 H); 2.02 (*s*, 3 H); 1.48 (*s*, 3 H); 1.16 (*s*, 9 H). ESI-MS 1685.6492 ($[M + Na]^+$; calc. 1685.6506).

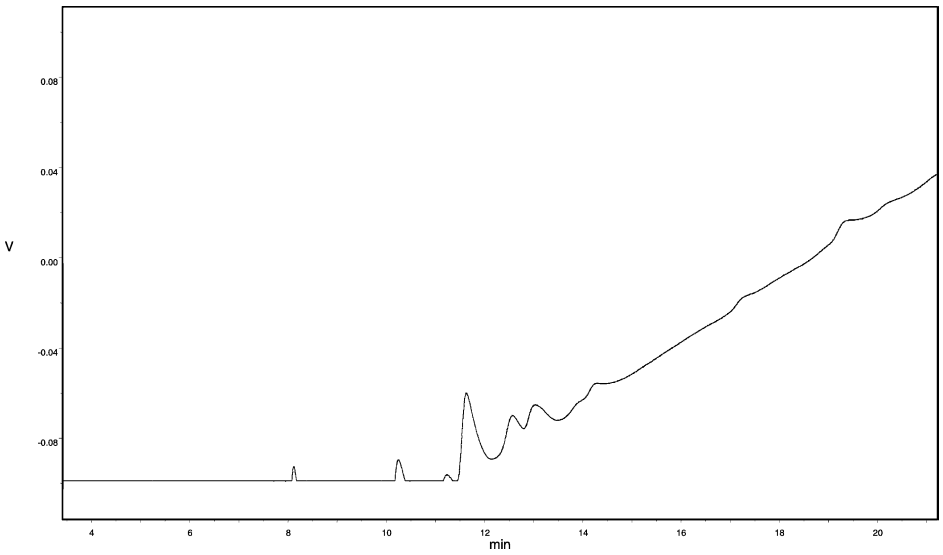


Fig. 4. *Analytical HPLC chromatogram of crude* **31**. Flow rate: 1 ml/min, 25 → 40% AcOEt/Hexanes (30 min); t_R(**31**) 11.5 min.

*Automated Synthesis of Poly-α-(1 → 2)-*L*-rhamnosides.* Oct-4-ene-1,8-diol-functionalized *Argopore*® resin **3** (0.100 μmol, 256 mg, 0.39 mmol/g) was manually loaded into a reaction vessel and inserted into a modified *ABI-433A* peptide synthesizer. The coupling, deprotection, and washing steps were controlled by the synthesizer. Double glycosylations were performed for 15 min each at −15°, and double deprotections (40 equiv. MeONa, 0.75M MeONa/MeOH) were carried out at 30° for 20 min each. Elongation to the pentamer stage was followed by cleavage from the resin. HPLC Analysis gave pentamer **43** (77%), tetramer **42** (18%), and trimer **41** (4%).

*Pent-4-enyl 2-*O*-Acetyl-3,4-di-*O*-benzyl-α-*L*-rhamnopyranosyl-(1 → 2)-3,4-di-*O*-benzyl-α-*L*-rhamnopyranosyl-(1 → 2)-3,4-di-*O*-benzyl-α-*L*-rhamnopyranoside* (**41**). $[\alpha]_D^{24} = -10.5°$ ($c = 0.81$, CH_2Cl_2). IR (thin film): 2927, 1714, 1365, 1236, 1088. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.52–7.12 (*m*, 30 H); 5.81–5.76 (*m*, 1 H); 5.56–5.54 (*m*, 1 H); 5.07 (*app. s*, 1 H); 5.02–4.95 (*m*, 3 H); 4.90 (*d*, *J* = 10.7, 1 H); 4.89 (*d*, *J* = 11.0, 1 H); 4.86 (*d*, *J* = 11.3, 1 H); 4.72–4.58 (*m*, 11 H); 4.54 (*d*, *J* = 11.0, 1 H); 4.11 (*app. s*, 1 H); 3.98 (*dd*, *J* = 3.4, 9.5, 1 H); 3.92 (*app. s*, 1 H); 3.89 (*dd*, *J* = 3.2, 9.5, 1 H); 3.84–3.81 (*m*, 2 H); 3.77–3.74 (*m*, 1 H); 3.65–3.59 (*m*, 3 H); 3.47–3.31 (*m*, 5 H); 2.14 (*s*, 3 H); 2.11, 2.07 (*m*, 2 H); 1.65–1.59 (*m*, 2 H); 1.28 (*d*, *J* = 6.7, 3 H); 1.27 (*d*, *J* = 7.0, 3 H); 1.22 (*d*, *J* = 6.4, 3 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 170.3; 138.7; 138.6; 138.5; 138.3; 138.2; 128.6; 128.5; 128.4; 128.3; 128.2; 128.0; 127.9; 127.8; 127.7; 115.1; 100.5; 99.3; 99.0; 80.5; 80.3; 79.8; 78.0; 75.6; 75.5; 74.8; 74.7; 72.3; 72.0; 69.1; 68.6; 68.5; 68.0; 66.9; 30.5; 28.8; 21.4; 18.2; 18.1; ESI-MS: 1129.5231 ($[M + Na]^+$; calc. 1129.5284).

*Pent-4-enyl 2-*O*-Acetyl-3,4-di-*O*-benzyl-α-*L*-rhamnopyranosyl-(1 → 2)-3,4-di-*O*-benzyl-α-*L*-rhamnopyranosyl-(1 → 2)-3,4-di-*O*-benzyl-α-*L*-rhamnopyranosyl-(1 → 2)-3,4-di-*O*-benzyl-α-*L*-rhamnopyranoside* (**42**). $[\alpha]_D^{24} = -9.0°$ ($c = 0.20$, CH_2Cl_2). IR (thin film): 2927, 1714, 1366, 1234, 1088. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.38–7.20 (*m*, 40 H); 5.89–5.77 (*m*, 1 H); 5.61 (*dd*, *J* = 1.8, 3.1, 1 H); 5.15 (*app. s*, 1 H); 5.07–4.88 (*m*, 8 H); 4.80 (*d*, *J* = 11.0, 1 H); 4.76–4.58 (*m*, 12 H); 4.16 (*d*, *J* = 2.0, 1 H); 4.10 (*d*, *J* = 2.0, 1 H); 4.04 (*dd*, *J* = 3.0, 9.4, 1 H); 3.96–3.74 (*m*, 7 H); 3.68–3.59 (*m*, 2 H); 3.51–3.33 (*m*, 6 H); 2.19 (*s*, 3 H); 2.16–2.08 (*m*, 2 H); 1.68–1.62 (*m*, 2 H); 1.32–1.23 (*m*, 12 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 170.5; 138.9; 138.7; 138.6; 138.5; 128.8; 128.7; 128.6; 128.5; 128.4; 128.3; 128.1; 115.3; 101.0; 100.7; 99.5; 99.2; 79.6; 79.3; 78.2; 77.8; 75.9; 75.8; 75.2; 75.1; 75.0; 72.7; 72.5; 72.4; 72.2; 69.3; 68.9; 68.7; 68.2; 67.2; 30.7; 29.0; 21.6; 18.4; 18.3. ESI MS: 1455.6810 ($[M + Na]^+$; calc. 1455.6802).

*Pent-4-enyl 2-*O*-Acetyl-3,4-di-*O*-benzyl-α-*L*-rhamnopyranosyl-(1 → 2)-3,4-di-*O*-benzyl-α-*L*-rhamnopyranosyl-(1 → 2)-3,4-di-*O*-benzyl-α-*L*-rhamnopyranosyl-(1 → 2)-3,4-di-*O*-benzyl-α-*L*-rhamnopyranosyl-(1 → 2)-3,4-di-*O*-benzyl-α-*L*-rhamnopyranoside* (**43**). $[\alpha]_D^{24} = -11.7°$ ($c = 0.41$, CH_2Cl_2). IR (thin film): 2931, 1744, 1453, 1364, 1087. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.36–7.23 (*m*, 46 H); 7.21–7.15 (*m*, 4 H); 5.86–5.75 (*m*, 1 H); 5.61 (*dd*,

$J = 1.8, 3.1, 1$ H); 5.16 (*d*, $J = 1.3, 1$ H); 5.09 (*d*, $J = 1.3, 1$ H); 5.06–5.04 (*m*, 2 H); 5.01–4.86 (*m*, 7 H); 4.81–4.58 (*m*, 16 H); 4.17 (*t*, $J = 2.3, 1$ H); 4.11 (*t*, $J = 2.1, 1$ H); 4.06–4.02 (*m*, 2 H); 3.96 (*dd*, $J = 2.8, 9.3, 1$ H); 3.93–3.72 (*m*, 9 H); 3.66–3.59 (*m*, 2 H); 3.51–3.30 (*m*, 6 H); 2.18 (*s*, 3 H); 2.17–2.06 (*m*, 2 H); 1.67–1.60 (*m*, 2 H); 1.34–1.21 (*m*, 15 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 170.5; 138.9; 138.7; 138.5; 128.9; 128.8; 128.6; 128.5; 128.4; 128.3; 128.1; 128.0; 127.9; 115.3; 101.0; 100.9; 100.7; 99.5; 99.2; 80.9; 80.8; 80.7; 80.5; 79.8; 79.7; 79.4; 79.2; 78.2; 75.9; 75.8; 75.3; 75.2; 75.1; 75.0; 72.7; 72.5; 72.4; 72.2; 69.3; 68.9; 68.8; 68.7; 68.2; 67.1; 30.7; 29.0; 21.6; 18.4. ESI MS: 1781.8241 ($[M + Na]^+$; calc. 1781.8320).

*Pent-4-enyl 3,4,6-Tri-*O*-benzyl-β-*D*-galactopyranosyl-(1 → 4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl-(1 → 2)-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranoside* (**45**). Oct-4-ene-1,8-diol functionalized resin **3** (100 μmol, 333 mg, 0.30 mmol/g loading) was manually loaded into a reaction vessel and inserted into a modified *ABI-433A* peptide synthesizer. The coupling, deprotection, and washing steps were controlled by the synthesizer. The resin was glycosylated with donor **4** (4 equiv.) delivered in CH_2Cl_2 (4 ml) and TMSOTf (0.4 equiv., in CH_2Cl_2). Mixing of the suspension was performed (10 s vortex, 50 s rest) for 30 min. The resin was then washed with CH_2Cl_2 (6 × 4 ml each) and glycosylated a second time. Upon completion of the double glycosylation, the resin was washed with CH_2Cl_2 (6 × 4 ml each) MeOH/CH_2Cl_2 1:9 (4 × 4 ml each). Deprotection of the Ac ester was carried out by treatment of the resin with MeONa (5 equiv.; 0.5M MeONa in MeOH) in CH_2Cl_2 (5 ml) for 30 min. The resin was then washed with MeOH/CH_2Cl_2 1:9 (1 × 4 ml) and subjected to the deprotection conditions a second time for 30 min. Removal of any soluble impurities was accomplished by washing the resin with MeOH/CH_2Cl_2 1:9 (4 × 4 ml each), 0.2M AcOH in THF (4 × 4 ml each), THF (4 × 4 ml each), and CH_2Cl_2 (6 × 4 ml each). The resulting resin-bound *C(2)*-OH was glycosylated with donor **44** (4 equiv.) delivered in CH_2Cl_2 (4 ml) and TMSOTf (0.4 equiv., in CH_2Cl_2) at −15°. Mixing of the suspension was performed (10 s vortex, 50 s rest) for 30 min. The resin was then washed with CH_2Cl_2 (6 × 4 ml each) and glycosylated a second time. Deprotection of the levulinoyl ester was carried out by treating the resin with hydrazine acetate (40 equiv., 4 ml; 0.25M $N_2H_4 \cdot$ HOAc in pyridine/AcOH 3:2) for 15 min at 15°. The resin was subjected to the deprotection conditions a second time for 15 min. Removal of any soluble impurities was accomplished by washing the resin with pyridine/AcOH 3:2 (3 × 4 ml), 0.2M AcOH in THF (4 × 4 ml each), THF (4 × 4 ml each), and CH_2Cl_2 (6 × 4 ml each). The *C(4)*-OH was glycosylated using donor **35** (4 equiv.) delivered in CH_2Cl_2 (4 ml) and TMSOTf (4 equiv., in CH_2Cl_2) at −15°. Mixing of the suspension was perfomed (10 s vortex, 50 s rest) for 15 min. The resin was then washed with CH_2Cl_2 (6 × 4 ml each) and glycosylated a second time. Upon completion of the double glycosylation the resin was washed with CH_2Cl_2 (4 × 4 ml each) and THF (4 × 4 ml). The glycosylated resin was dried *in vacuo* over P_2O_5 for 12 h and transferred to a 10-ml flask. The flask was purged with ethylene and the *Grubbs* catalyst (bis(tricyclohexylphosphine)benzylidene ruthenium(IV) dichloride; 16.4 mg, 20 mol-%) was added. The mixture was diluted with CH_2Cl_2 (6 ml) and stirred under 1 atm ethylene for 36 h. Et_3N (440 ml, 3.20 mmol, 80 equiv.) and tris(hydroxymethyl)phosphine (200 mg, 1.60 mmol, 80 equiv.) were added, and the resulting soln. was stirred at r.t. for 1 h. The pale yellow mixture was diluted with CH_2Cl_2 (25 ml) and washed with H_2O (3 × 25 ml), sat. aq. $NaHCO_3$ soln. (3 × 25 ml), and brine (3 × 25 ml). The aq. phase was extracted with CH_2Cl_2 (3 × 25 ml), and the combined org. extracts were dried (Na_2SO_4), filtered, and concentrated. Purification by FC (20% AcOEt/hexanes) gave **13** (65 mg, 43% isolated yield). IR (thin film, NaCl): 2918, 2869, 1776, 1739, 1714, 1087. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.65 (br. *s*, 1 H); 7.57–7.54 (*m*, 1 H); 7.37–7.10 (*m*, 40 H); 6.94 (*d*, $J = 7.8$, 1 H); 6.86 (*t*, $J = 7.3$, 1 H); 6.78 (*t*, $J = 6.9$, 1 H); 5.78–5.63 (*m*, 1 H); 4.97–4.64 (*m*, 6 H); 4.54–4.23 (*m*, 8 H); 4.13–3.93 (*m*, 4 H); 3.84–3.75 (*m*, 3 H); 3.57–3.39 (*m*, 6 H); 3.21 (app. *dd*, $J = 7.0, 16.3, 1$ H); 2.96 (*dd*, $J = 6.4, 11.3, 1$ H); 2.01–1.93 (*m*, 2 H); 1.56–1.50 (*m*, 2 H); 1.19 (*s*, 9 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 176.9; 139.0; 138.9; 138.7; 138.7; 138.3; 138.0; 133.7; 132.0; 128.7; 128.6; 128.6; 128.4; 128.4; 128.4; 128.3; 128.3; 128.2; 128.1; 128.0; 127.9; 127.8; 127.6; 127.6; 127.5; 127.5; 127.3; 126.9; 123.3; 114.9; 100.1; 97.2; 97.1; 81.1; 77.9; 77.5; 76.7; 76.6; 75.4; 75.0; 74.9; 74.5; 73.9; 73.7; 73.6; 73.5; 73.1; 72.8; 72.1; 71.9; 71.9; 70.6; 70.2; 69.0; 68.5; 67.2; 55.8; 39.0; 30.4; 28.8; 27.5. ESI-MS: 1528.6715 ($[M + Na]^+$; calc. 1529.7562).

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