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Oligomerization of a rhamnanic trisaccharide repeating unit of *O*-chain polysaccharides from phytopathogenic bacteria†

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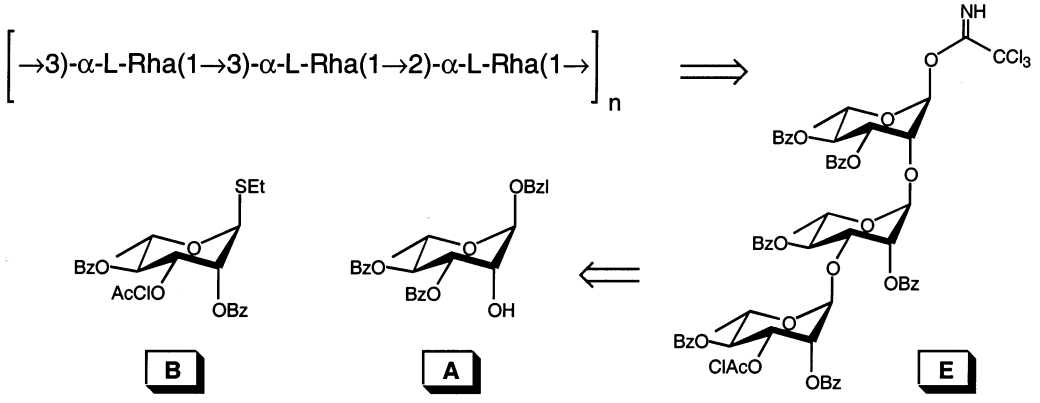
Abstract—An efficient synthesis of a protected trisaccharide building block α-L-Rha(1→3)-α-L-Rha(1→2)-α-L-Rha related to the structure of many lipopolysaccharide *O*-chains from phytopathogenic bacteria has been developed. The protecting group pattern consisting of benzoyl, benzyl and chloroacetyl groups facilitated the use of the trisaccharide building block in the synthesis of two higher oligomers, an oligorhamnosyl hexasaccharide and a nonasaccharide.

It has been recently reviewed[1] that the repeating units of many lipopolysaccharide *O*-chains from phytopathogenic bacteria consist of rhamnan backbones. One of the most frequently found sequences in the rhamnan backbones is the trisaccharide repeating unit $[\rightarrow 3)$-α-L-Rha*p*$(1\rightarrow 3)$-α-L-Rha*p*$(1\rightarrow 2)$-α-L-Rha*p*$(1\rightarrow]_n$ (Scheme 1), which was synthesized some years ago.[2] In order to obtain oligomers of this structural motif for structure–activity relationship studies, a new synthetic strategy was developed. The synthetic approach described in this paper aimed at the synthesis of the trirhamnoside repeating unit **E** as a building block that be easily functionalized as a glycosyl donor and a glycosyl acceptor, thus permitting the stepwise condensation of the repeating units to higher oligosaccharides. In addition this method should be extendable by modifying the building blocks to synthesize the related linear and branched rhamnan repeating units of other lipopolysaccharide *O*-chains of phytopathogenic bacteria.



Scheme 1. Retrosynthesis of the trirhamnoside repeating unit found in the lipopolysaccharide *O*-chains from *Pseudomonas savastanoi* strains ITM 519, ITM 317 and PVF 5, *Pseudomonas tabaci* strain IMV 223, and *Pseudomonas begoniae* strain GSPB 525.

Keywords: glycosylation; oligosaccharide; lipopolysaccharide; repeating unit; phytopathogenic bacteria.

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† This paper is dedicated to Professor Lorenzo Mangoni on the occasion of his 70th birthday.

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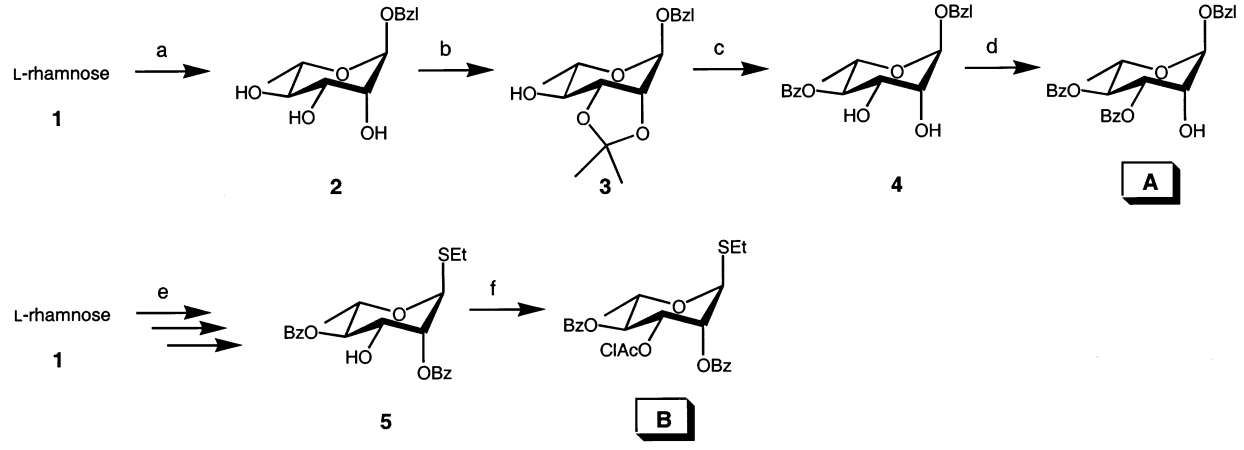
The oligomerization of repeating unit building blocks has been used previously in the synthesis of oligosaccharide chains.[3,4] However, it was not clear from the beginning in which way the envisioned oligorhamnoside sequence could be assembled. Therefore the two rhamnoside building blocks **A** and **B** were deducted allowing maximum flexibility in the sequence of glycosylation.

The glycosyl acceptor **A** was synthesized as outlined in Scheme 2. L-Rhamnose was first protected at the anomeric position by a Fischer glycosylation furnishing benzyl α-L-rhamnopyranoside **2**[5] in 54% yield.

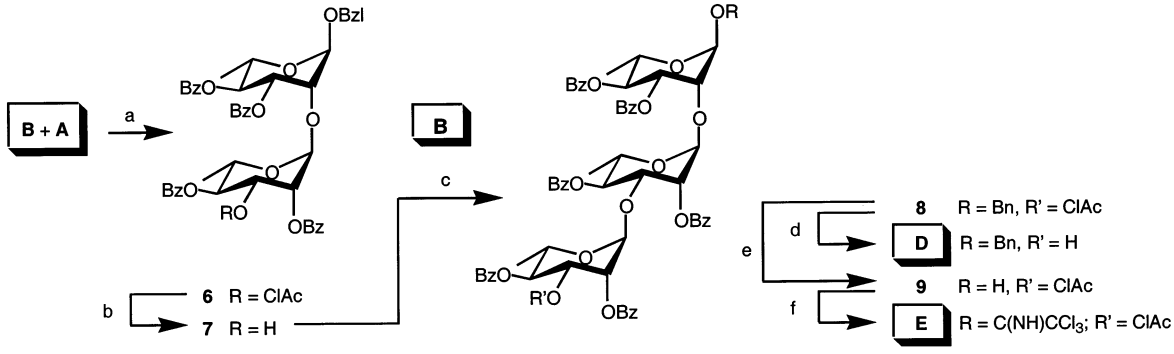
Regioselective isopropylidenation of the triol **2** with 2,2-dimethoxypropane/TsOH gave the alcohol **3** (98%), which was subsequently benzoylated followed by acid hydrolysis of the acetonide. The resulting diol **4** (90% yield) was selectively benzoylated at the more reactive equatorial *OH*-3[6] to afford the glycosyl acceptor **A** (57%). Glycosyl donor **B** was obtained by treating ethyl 2,4-di-*O*-benzoyl-1-thio-α-L-rhamnopyranoside[7] **5** with chloroacetyl chloride in a 1:1 mixture of pyridine and DMF[8] (71%).

The assembly of the trisaccharide was initiated by coupling of the glycosyl acceptor **A** and the donor **B** (Scheme 3). Following the typical conditions for the activation of the disarmed thioglycosides with *N*-iodosuccinimide and triflic acid (NIS/TfOH),[9] the disaccharide **6** was obtained in high yield (83%). The α-configuration of the newly formed glycosidic linkage in **6** was ascertained by the heteronuclear C-1′,H-1′ coupling constant of 173 Hz measured in a coupled HMQC-COSY experiment.[10] Selective removal of the chloroacetyl moiety was achieved by treating the disaccharide **6** with thiourea in ethanol.[11] The disaccharide acceptor **7** was obtained in 80% yield without any detectable benzoyl migration. Subsequent coupling with glycosyl donor **B** using the NIS/TfOH activation method afforded the α-configurated trisaccharide **8** in high yield (96%, $J_{C\text{-}1'',H\text{-}1''}=174$ Hz). Trisaccharide **8** was readily converted to the acceptor trisaccharide **D** (83%) by dechloroacetylation with thiourea in ethanol.

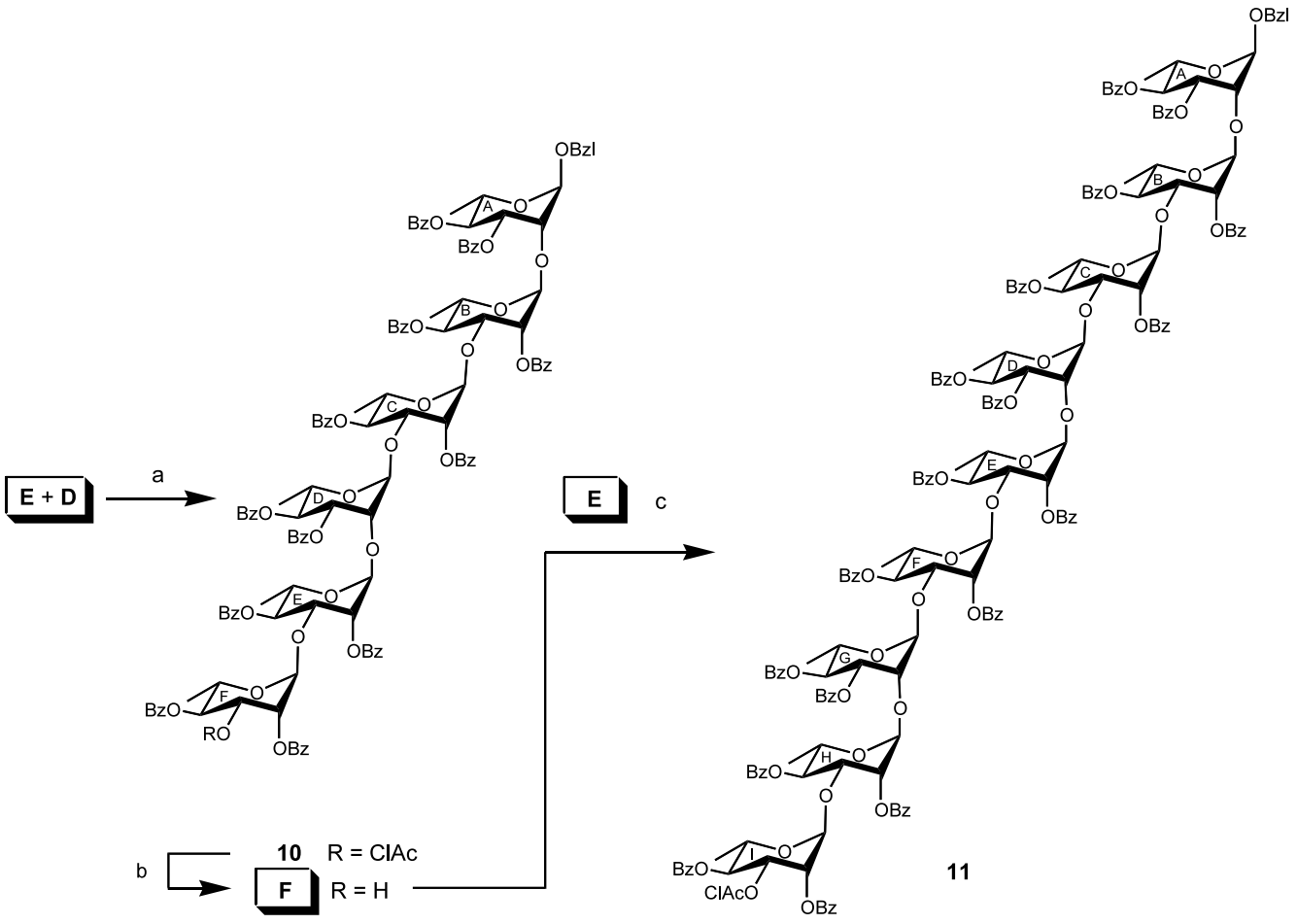
In order to obtain a glycosyl donor from trisaccharide **8**, the deprotection the benzylated anomeric position was required, thus allowing further activation of the



Scheme 2. *Reagents and conditions*: (a) Amberlyst 15-H^+, BnOH, 45°C, 16 h, 54%; (b) pTsOH, 2,2-dimethoxypropane, rt, 1 h, 98%; (c) i. BzCl, pyridine, 0°C, 1 h; ii. TFA/water 4:1, rt, 45 min, 90%; (d) BzCl, pyridine/CH_2Cl_2 1:2, –25°C, 100 min, 57%; (e) lit.[6]; (f) $ClCH_2COCl$, pyridine/DMF 1:1, –45°C, 3 h, 71%.



Scheme 3. *Reagents and conditions*: (a) NIS, TfOH, molecular sieves 4 Å, CH_2Cl_2, –20°C, 90 min, 82%; (b) NH_2CSNH_2, EtOH, rt, 16 h, 80%; (c) NIS, TfOH, molecular sieves 4 Å, –20°C, 15 min, 96%; (d) NH_2CSNH_2, EtOH, rt, 2 days, 83%; (e) anhydrous $FeCl_3$, CH_2Cl_2, rt, 45 min, 58%; (f) Cl_3CCN, DBU, CH_2Cl_2, 0°C, 80 min, 76%.



Scheme 4. *Reagents and conditions*: (a) $BF_3{\cdot}OEt_2$, molecular sieves 4 Å, CH_2Cl_2, −30°C, 1 h, 71%; (b) NH_2CSNH_2, EtOH/DMF 1:1, rt, 2 days, 78%; (c) $BF_3{\cdot}OEt_2$, molecular sieves 4 Å, CH_2Cl_2, −30°C, 90 min, 53%.

resulting hemiacetal as a glycosyl donor of the trichloroacetimidate type.[12] At first trisaccharide **8** was treated with H_2/Pd–C in 10:1 MeOH/AcOH to remove the benzyl group by catalytic hydrogenation. This however, resulted in a 1:1 mixture of the desired product **9** and a homologous hemiacetal where the chloroacetyl group had been reduced to an acetate. To avoid reduction of the chloroacetyl group, the benzyl group was alternatively replaced by an acetate using acetolysis conditions (acetic anhydride with 1% H_2SO_4, 84% yield). The following cleavage of the anomeric acetate by hydrazinium acetate afforded, both in DMF and ethyl acetate, the simultaneous loss of the chloroacetate at *OH*-3″. This reduced chemoselectivity at the anomeric center can be explained considering the relative rates for alkaline hydrolysis of acetates and chloroacetates (1:760)[3,13] and the generally high reactivity of the equatorial position 3 of rhamnose as shown before.

Finally, a method for the conversion of **8** to hemiacetal **9** in a single step was found in a procedure employing anhydrous $FeCl_3$ in CH_2Cl_2.[14] It is noteworthy that the stability of the chloroacetyl protective group under these reaction conditions was not reported previously. The hemiacetal **9** could be converted to the trichloroacetimidate building block **E** in an overall yield of 44% over two steps.

With the two key building blocks **D** and **E** in hands a sequence of oligomerization reactions was investigated. When activated with $BF_3{\cdot}OEt_2$ the two trisaccharides **D** and **E** gave the fully protected hexasaccharide **10** (71%) (Scheme 4). The α-configuration of the newly established glycosidic bond was confirmed by the C-1^D,H-1^D coupling constant of 174 Hz. The hexasaccharide **10** was converted to glycosyl acceptor **F** (78%) by cleavage of the chloroacetyl group with thiourea in a 1:1 mixture of EtOH–DMF to allow the complete solubility of **10**. Finally, hexasaccharide acceptor **F** was elongated with the trisaccharide imidate **E** to afford the nonasaccharide **11**[15] (53%). Deprotection of the hexasaccharide **10** using basic conditions (NaOMe in MeOH at $T=40$°C) assured the complete removal of all protective functions except of the anomeric benzyl group.

In conclusion we have developed an efficient strategy for the preparation of oligomeric repeating units of the rhamnan type found on many *O*-chain polysaccharides from phytopathogenic bacteria thus providing sufficient quantities for future structure–activity studies of the deprotected oligomers.

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15. Compound **11**: $[\alpha]_D^{22} = +156$ (*c* 0.5, dichloromethane); ESI-MS $C_{189}H_{171}O_{56}Cl$ M_r (calcd) 3371.02, M_r (found) 3394.24 (M+Na);$^+$ ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.10–7.23 (90H, H-Ar), 5.77 (d, $J_{3,4} = 9.4$ Hz, 1H, H-3^A), 5.60 (d, 1H, H-2^B), 5.43 (m, 4H, H-1^C, H-4^A, H-2^E, H-2^H), 5.37 (m, 2H, H-1^F, H-4^B), 5.28 (m, 4H, H-1^B, H-1^I, H-3^D, H-3^G), 5.21 (m, 8H, H-3^I, H-4^C, H-4^D, H-4^E, H-4^F, H-4^G, H-4^H, H-4^I), 5.12 (d, 1H, H-2^C), 5.09 (d, 1H, H-2^F), 5.05 (m, 2H, H-1^A, H-2^I), 4.99 (m, 2H, H-1^D, H-1^G), 4.80 (m, 3H, H-1^E, H-1^H, $OCH_2\Phi$), 4.65 (m, 2H, H-3^B, $OCH_2\Phi$), 4.55 (m, 2H, H-3^E, H-3^H), 4.39 (m, 1H, H-2^A), 4.26 (m, 2H, H-3^C, H-3^F), 4.12 (m, 3H, H-5^A, H-5^B, H-5^I), 4.01 (m, 4H, H-5^C, H-5^F, OCH_2Cl), 3.90 (m, 4H, H-2^D, H-2^G, H-5^E, H-5^H), 3.76 (m, 2H, H-5^D, H-5^G), 1.28–1.01 (m, 15H, H-6^A, H-6^B, H-6^C, H-6^F, H-6^I), 0.74 (m, 6H, H-6^E, H-6^H), 0.67 (m, 6H, H-6^D, H-6^G); ^{13}C NMR (125 MHz, DMSO-d_6): δ 166.3–164.0 (19 C=O), 136.9 (C_i Bn), 134.0–133.2 (18 C_i Bz), 129.7–127.9 (C-Ar), 99.7 (C-1^D, C-1^G), 98.4 (C-1^F, C-1^C), 97.9 (C-1^B, C-1^E, C-1^H, C-1^I), 97.0 (C-1^A), 76.3 (C-2^A), 75.5 (C-2^D, C-2^G), 74.5 (C-3^B), 74.1–73.3 (C-3^C, C-3^E, C-3^F, C-3^H), 72.7 (C-4^B, C-4^E, C-4^H), 71.9–70.7 (C-2^B, C-4^A, C-2^E, C-2^H, C-2^C, C-2^F, C-4^I, C-4^D, C-4^G, C-3^I), 70.4 (C-3^A), 69.9–69.6 (C-4^C, C-4^F, C-3^D, C-3^G), 69.2 (C-2^I), 68.7 ($OCH_2\Phi$), 66.9–66.2 (C-5^A, C-5^B, C-5^C, C-5^D, C-5^E, C-5^F, C-5^G, C-5^H, C-5^I), 40.5 (OCH_2Cl), 17.5 (C-6^A), 17.3–16.9 (C-6^B, C-6^C, C-6^E, C-6^F, C-6^H, C-6^I), d = 16.5 (C-6^D, C-6^G).