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Preliminary Communication

POLYMER-SUPPORTED SOLUTION SYNTHESIS OF A HEPTAGLUCOSIDE HAVING PHYTOALEXIN ELICITOR ACTIVITY

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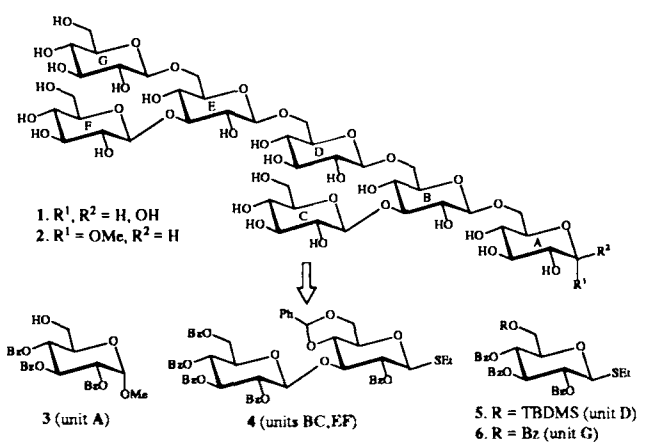
Abstract. An efficient synthesis of methyl heptaglucoside **2** using poly(ethyleneglycol)monomethyl ether as a polymer support is described. Elongation of the acceptor **11**, anchored *via* a succinoyl linkage to PEG, with the appropriate glycosyl donors **4-6** and intermittent protective group manipulations gave fully protected heptamer **18**. Deprotection of **18** furnished, after purification as its peracetate, title compound **2** in a good overall yield.

It is now well established[1] that the branched and β-linked glucoheptaose **1** triggers the production of phytoalexins by binding to a receptor[2] tethered in soybean membranes. With the objective to gain a detailed insight into the structural requirements[3] for optimal interaction of the methyl analogue of **1** (*i.e.* compound **2**)[4] with the plant receptor, the availability[5] of a wide range of modified heptasaccharides would be desirable. In order to meet this requirement, we developed[6] an efficient and high yielding route to the heptaglucoside **2** based on the retrosynthetic scheme depicted below. However, the recurrence of work up and purification steps renders the synthesis of **2** and analogues thereof rather laborious and time-consuming.



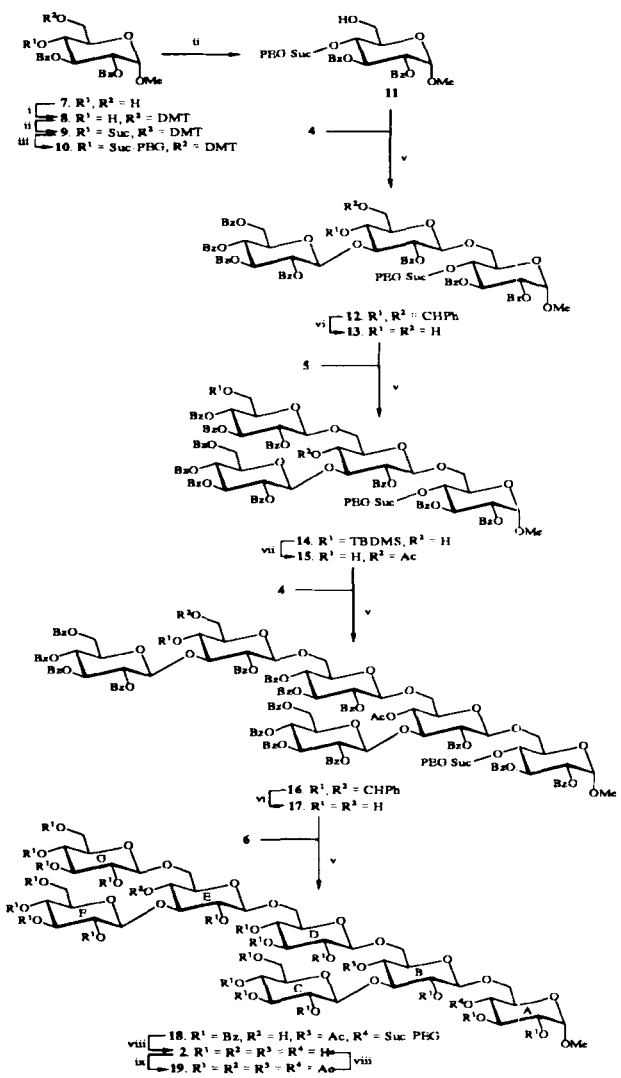
Recently, *Krepinsky et al.*[7] reported the preparation of disaccharides using poly(ethyleneglycol)monomethyl ether[8] (PEG) as a polymer support. A characteristic feature of the PEG-methodology, recently applied by *Bonora et al.*[9] for the rapid and large-scale synthesis of oligonucleotides, is the fact that the coupling product resulting after each elongation step can be collected by simple precipitation, thus nullifying the earlier mentioned disadvantages of a solution methodology of oligosaccharide synthesis. In order to achieve our goal, we adopted the following crucial elements of the in our laboratory devised[6] successful synthetic route to the target molecule **2**. Thus, the same terminal (*i.e.* monomer **6**) and non-terminal (*i.e.* dimer **4** and monomer **5**) synthons, all of which contain a participating benzoyl group at C-2 to secure the stereocontrolled construction of β-glycosidic linkages, will be used. In addition, the reliable regioselective glycosylation of the primary hydroxyl of the 4,6-diol function, generated after deblocking of the benzylidene protecting group from adducts derived from the iodonium-promoted elongation of dimer **4** at the anomeric centre, plays a pivotal role in the PEG-supported synthesis of **2**.

The assembly of **2** commences, as delineated in Scheme 1, with the stepwise immobilization[10] of methyl 2,3-di-*O*-benzoyl-α-D-glucopyranoside[11] (**7**) to PEG. Thus, tritylation of **7** with 4,4'-dimethoxytrityl (DMT) chloride, and subsequent treatment of **8** with succinic (Suc) anhydride in the presence of 4-(dimethylamino)pyridine (DMAP) gave the Suc derivative **9**. The anchorage of **9** to the hydroxyl function of PEG was readily effected with *N,N'*-dicyclohexylcarbodiimide in the presence of DMAP to give, after capping of unreacted PEG followed by precipitation and washing of the precipitate with diethyl ether, the PEG-Suc-bound derivative **10** having a loading capacity of 148 μmol/g PEG as gauged spectrophotometrically[12] by the released trityl cation. Acidolysis of the DMT group in **10** provided the immobilized acceptor **11**, which was elongated[13] with dimer **4** using *N*-iodosuccinimide and catalytic

trific acid (NIS/TfOH)[14] as the promoter. Precipitation, after 10 min at 20°C, yielded[15] the PEG-bound trimer **12**, the benzylidene group of which was removed by acid, resulting in the isolation of the diol derivative **13**. Regioselective NIS/TfOH-assisted glycosylation of immobilized acceptor **13** with donor **5** followed by acetylation and subsequent removal of the *tert*-butyldimethylsilyl group, led to the isolation of the PEG-Suc-bound tetrameric fragment **15**. Finally, regioselective extension of the hexameric fragment **17**, obtained (*cf.* conversion of **4** → **13**) by elongation of **15** with donor **4** and subsequent debenzylidenation of **16**, gave the fully protected and immobilized heptameric fragment **18**. Deprotection of **18** under Zemplén conditions with concomitant release from the support led to crude **2**, which was purified[16] as its peracetate **19**. Saponification of **19** gave homogeneous **2** (overall yield 18%, based on **11**) having the same biological activity as the methyl glucoside prepared earlier *via* solution methodology[6]. Furthermore, the homogeneity and identity of **2**, and its peracetylated derivative **19**, was unambiguously ascertained by NMR, FAB-MS and HPLC analysis.

In conclusion, the successful assembly of **2** *via* a PEG-supported liquid synthesis[17] indicates that this methodology may be adapted for the future synthesis of biologically interesting analogues of **2**.

Scheme 1



Reagents and conditions: i. DMTCl, pyridine (86%); ii. Suc_2O, DMAP, pyridine (71%) iii. DCC, CH_2Cl_2 then PEG, DMAP; iv. $PhSO_3H$, $MeOH/CH_2Cl_2$; v. NIS/cat.TfOH, MS(0.4 nm), $ClCH_2CH_2Cl/Et_2O$, 0°C; vi. HCl, $MeOH/CH_2Cl_2$; vii. Ac_2O, pyridine then HCl, $MeOH/CH_2Cl_2$; viii. NaOMe, MeOH; ix. Ac_2O, pyridine.

References and notes

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4. In an independent study it was found that the methyl analogue **2** exhibited the same biological activity as its natural congener **1**.
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8. The supporting polymer poly(ethyleneglycol)-monomethyl ether ($H(OCH_2CH_2)_nOCH_3$, n = 80-160; PEG, average MW 5000) is commercially available from Aldrich Chemie, Bornem, Belgium. See also: a) Technical Bulletin: Polyglycols Hoechst. Polyethylene Glycols: Properties and Applications; Hoechst Frankfurt, 1983; b) Dale, J., *Isr. J. Chem.*, **1980**, *20*, 3.
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13. General procedure - A 0.1 M stock-solution of NIS/cat.TfOH was prepared by adding trifluoromethanesulfonic acid (20 µL, 226 µmol) to a solution of *N*-iodosuccinimide (460 mg, 2.04 mmol) in DCE/Et_2O (1/1, v/v, 20 mL).
A solution of NIS/cat.TfOH in DCE/Et_2O (0.1 M, 1 equiv. rel. to donor) was added to a mixture of acceptor and donor (2 equiv.) in DCE (2.0 mL) at 0°C under an atmosphere of nitrogen. After 10 min at 0°C, the reaction mixture was neutralized with Et_3N and added dropwise to Et_2O (150 mL) under vigorous stirring. The precipitate was collected by filtration, thoroughly washed with Et_2O and then dried.
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15. Monitoring of the reaction course, as advocated by *Krepinsky et al.* (see ref. 7a), by NMR spectroscopy using the methyl group of PEG as internal standard was not very satisfactory in our case.
16. The acetylated heptasaccharide **19** was purified by silica gel column chromatography using the eluent light petroleum/EtOAc, 2/3 → 1/4, v/v.
17. Preliminary experiments indicated that heptasaccharide **2** could also be prepared on the PEG-based solid support TentaGel™ (see ref. 18) by the same sequence of reactions depicted in Scheme 1.
18. TentaGel™ was purchased from Rapp Polymere, Tübingen, Germany. See also: Bayer, E., *Angew. Chem. Int. Ed. Engl.*, **1991**, *30*, 1991.

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