

Novel D- glucose Based Glycomonomers Synthesis and Characterization

M.S. Mazăre, A. M. Pană, L. M. Ștefan, M. Sillion, M. Bălan, G. Bandur, and L. M. Rusnac

I. INTRODUCTION

Abstract—In the last decade, carbohydrates have attracted great attention as renewable resources for the chemical industry. Carbohydrates are abundantly found in nature in the form of monomers, oligomers and polymers, or as components of biopolymers and other naturally occurring substances. As natural products, they play important roles in conferring certain physical, chemical, and biological properties to their carrier molecules. The synthesis of this particular carbohydrate glycomonomer is part of our work to obtain biodegradable polymers. Our current paper describes the synthesis and characterization of a novel carbohydrate glycomonomer starting from D-glucose, in several synthesis steps, that involve the protection/deprotection of the D-glucose ring via acetylation, tritylation, then selective deprotection of the aromatic-aliphatic protective group, in order to obtain 1,2,3,4-tetra-*O*-acetyl-6-*O*-allyl- β -D-glucopyranose. The glycomonomer was then obtained by the allylation in drastic conditions of 1,2,3,4-tetra-*O*-acetyl-6-*O*-allyl- β -D-glucopyranose with allylic alcohol in the presence of stannic chloride, in methylene chloride, at room temperature. The proposed structure of the glycomonomer, 2,3,4-tri-*O*-acetyl-1,6-di-*O*-allyl- β -D-glucopyranose, was confirmed by FTIR, NMR and HPLC-MS spectrometry. This glycomonomer will be further submitted to copolymerization with certain acrylic or methacrylic monomers in order to obtain competitive plastic materials for applications in the biomedical field.

Keywords—allylation, D-glucose, glycomonomer, trityl chloride

M. S. Mazăre is a PhD student at the “Politehnica” University of Timișoara, Faculty of Industrial Chemistry and Environmental Engineering, C. Telbisz 6, 300001, Timișoara, Romania (corresponding author: phone: +40256404225, fax: +40256403060, email: anghelcumihaelasorina@yahoo.com).

L. M. Ștefan is a PhD student at the “Politehnica” University of Timișoara, Faculty of Industrial Chemistry and Environmental Engineering, C. Telbisz 6, 300001, Timișoara, Romania (email: stefan_marinela@yahoo.com).

A. M. Pană is a postdoctoral research with the “Gheorghe Asachi” Technical University of Iași, Faculty of Chemical Engineering and Environmental Protection (email: ana.chis@chim.upt.ro).

M. Sillion is with the “Petru Poni” Institute of Macromolecular Chemistry, Aleea Gr. Ghica Vodă 41 A, Iași (email: sillion.mihaela@icmpp.ro).

M. Bălan is with the “Petru Poni” Institute of Macromolecular Chemistry, Aleea Gr. Ghica Vodă 41 A, Iași (email: bmihaela16@yahoo.com).

G. Bandur is Professor of Department of Organic Chemical Tehnology of “Politehnica” University of Timișoara, Faculty of Industrial Chemistry and Environmental Engineering (email: geza.bandur@chim.upt.ro).

L. M. Rusnac is the Dean of the Faculty of Industrial Chemistry and Environmental Engineering of “Politehnica” University of Timișoara, and a professor of Department of Organic Chemical Tehnology of Faculty of Industrial Chemistry and Environmental Engineering, email: lucian.rusnac@chim.upt.ro).

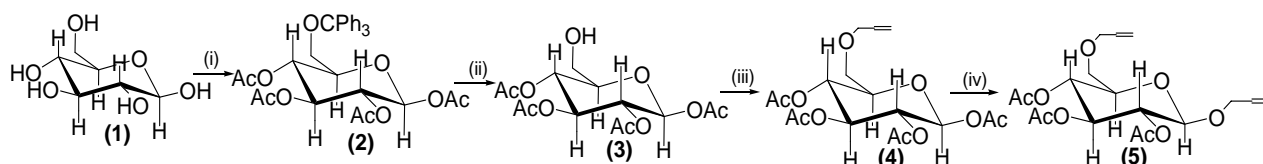
IN Glycopolymers are synthetic polymers with pendent saccharide moieties [2, 3]. It is well known that saccharides decorate the surfaces of all cells (glycocalyx) in the form of polysaccharides, glycoproteins, glycolipids, and other glycoconjugates [4]. The pendent sugar moieties play a significant role in a number of significant biological processes including inflammation, cell-cell contacts, signal transmission and fertilization [5].

Therefore, polymers with sugar moieties have been of interest due to their biomimetic properties and significance in biological applications [6].

The design and synthesis of polymer materials with ability of molecular recognition can be regarded as the finest exhibit of this biomimetic approach [7]. Stimuli-responsive polymers have received much attention in the last years, because they undergo abrupt physical or chemical change in response to change of environmental conditions such as pH, temperature, light, magnetic field, and glucose [8].

Different approaches to synthesize glycopolymers using conventional and controlled radical polymerization, living anionic polymerization, cyanoxyl mediated polymerization, ring opening polymerization and post-polymerization modification have been described. In these procedures glycomonomers with protected and unprotected carbohydrate groups have been utilized. That is dependent on the simplicity of saccharide stereospecific functionalization, the solubility of the monomer and polymer, the potential incompleteness of the protective group removal, and the purification easiness [9, 10].

In accordance with our continuing interest to obtain biodegradable polymers, in this study we report the results obtained in the synthesis and characterization of 2,3,4-tri-*O*-acetyl-1,6-di-*O*-allyl- β -D-glucopyranose. The infrared and NMR analyses confirmed the structure of the synthesized compound, and additionally their molecular weight was confirmed by HPLC-MS analysis. The synthesis of this derivative based on D-glucose (**1**) was achieved according to Scheme 1, and involves several steps of protection/deprotection of the D-glucose in the pyranosic form.



Scheme 1. Preparation of 2,3,4-tri-*O*-acetyl-1,6-di-*O*-allyl- β -D-glucopyranose: i) $(C_6H_5)_3CCl$, Py, Ac_2O , 12h, r.t., AcOH, H_2O , 2h, $10^\circ C$; ii) AcOH, $60^\circ C$; HBr (AcOH 33%), 45s, $10^\circ C$; iii) $AlCl_3$, NaH, DMF, MeOH, 24h., r.t.; iv) CH_2Cl_2 anhydrous, molecular sieves 4 Å, $SnCl_4$, AlOH, 18 h, r.t.

II. EXPERIMENTAL

The key intermediate, 1,2,3,4-tetra-*O*-acetyl-6-trityl- β -D-glucopyranose (TrTAG) was obtained according to the literature [11], [12]. The TrTAG derivative (2) was deprotected of the aromatic-aliphatic protective group with glacial acetic acid solution of hydrogen bromide (33%). The intermediary (3) (TAG) was allylated with allyl chloride ($AlCl_3$) in strong basic catalysis (NaH) in anhydrous DMF. The excess catalyst was neutralized with excess MeOH [12], [13]. After purification by silicagel column chromatography (hexane:AcOEt 6:1), 1,2,3,4-tetra-*O*-acetyl-6-*O*-allyl- β -D-glucopyranose, product (4) was isolated. Next step consisted in the forced allylation of the anomeric centre of D-glucose, using allylic alcohol in methylene chloride, in the presence of $SnCl_4$ (Scheme 1).

The synthesis of (5) was achieved using allylic alcohol at room temperature: 1 g of (4) was dissolved in 17.25 mL methylene chloride anhydrous in presence of molecular sieves 4 Å and the reaction mass was stirred vigorously at room temperature. After 30 minutes, 0.33 ml stannic chloride and 0.12 ml allylic alcohol were added at once. The mixture was additionally stirred for 18 hours, then filtered and concentrated in vacuo. 2,3,4-tri-*O*-acetyl-1,6-di-*O*-allyl- β -D-glucopyranose was purified by silicagel column chromatography (hexane:AcOEt 6:1), (yield 86.1%), then characterized using FTIR and NMR spectroscopy, and HPLC-MS.

III. MATERIALS AND METHODS

D-(+)-glucose, trityl chloride, pyridine, allyl alcohol, ethyl acetate, hexane, allyl chloride, DMF, sodium hydride, diethyl ether, dichloromethane and methanol were purchased from Merck. Sodium bicarbonate was purchased from ChimoPar, and sodium sulfate from Acros Organics. All this materials were used without further purification.

All syntheses were monitored using thin-layer chromatography (TLC) performed on silica gel plates, Merck, DC-Autofolien Kiesegel 60 F 254, using different eluants.

FT-IR Analysis. The FTIR spectra were recorded on a Jasco FT/IR-410 spectrometer. The IR analyses were done using KBr pellets.

NMR-Spectroscopy. The NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer using $CDCl_3$ as reference.

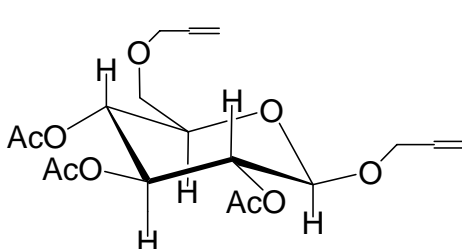
HPLC-MS Analysis. Mass spectrometry experiments were performed using a quadrupole time-of-flight mass spectrometer, equipped with an electrospray ion source (Agilent 6520 Accurate Mass Q-ToF LC/MS). The samples were dissolved in a chloroform/methanol 6:4 (v/v) mixture. The solutions were introduced into the ESI source via a syringe pump at a flow-rate of 0.2 mL/min. The electrospray interface was set in positive ionization mode with the capillary voltage at 4000 V and a heat source of $325^\circ C$, in full scan spectra (m/z 100–1000). Nitrogen was used as a drying (7 L/min) and nebulizing gas (35 psi). Data were collected and processed using a MassHunter Workstation software.

IV. RESULTS AND DISCUSSION

A. FT-IR Results

Table 1 presents the IR spectra of 2,3,4-tri-*O*-acetyl-1,6-di-*O*-allyl- β -D-glucopyranose (5). The signals assigned to the pyranosic D-glucose ring, mainly the aliphatic C-H stretching, are placed between 2850 and 3000 cm^{-1} , also the acetic skeleton exhibits the esteric C=O bond at about 1750 cm^{-1} , while the C-O bond is traceable at about 1250 cm^{-1} . The double C=C bond from the allylic residue expresses asymmetric stretching at about 3100 cm^{-1} , while its bond stretching is placed in the spectrum at about 1650 cm^{-1} , proving that the given structure is accurate [13].

TABLE I
THE FTIR SPECTRA OF THE D-GLUCOSE DERIVATIVE

Compound	Band frequency (cm ⁻¹)	Assignment
 2,3,4-tri-O-acetyl-1,6-di-O-allyl-β-D-glucopyranose	3082.65; 1648.84	=CH ₂ asymmetric stretching; C=C bond stretching
	2955.38; 2926.45; 2854.13	aliphatic C-H stretching
	1750.08; 1440.56; 1369.21; 1230.36	C-O stretching
	768.49; 691.35; 601.68	out of plane γ vibrations for aliphatic C-H stretching

B. NMR-Spectroscopy

The NMR spectroscopy also confirmed the D-glucose derivatives structure. The ¹H-NMR spectrum of **5** is shown in fig. 2. The spectrum shows the characteristic signals for the acetic CH₃ protons at about 2 ppm (parts per million), thus proving that this protective group has not been lost during the allylation in strong oxidative conditions. The protons from the sugar ring display signals between 4.4 and 5.0 ppm. The signals of the protons in the allylic group, involved into the double bond, are the most shifted to the left of the ¹H-NMR spectrum, displaying signals between 5.4 and 5.6 ppm. The methylene attached to the double bond express signals around 4.5 ppm [14, 15].

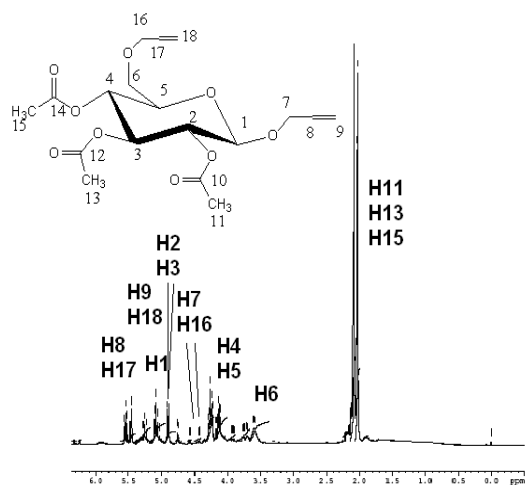


Fig. 1 The ¹H-NMR spectrum of **5** in CDCl₃

The ¹³C-NMR spectrum of **5** is shown in fig. 2 and confirms its structure as well. The acetic protective group expresses its signals at about 20 ppm for CH₃ and around 170 ppm for the C=O esteric bond. The signals characteristic to the C=C bond are placed from about 127 to about 132 ppm, Whereas the CH₂ attached to the double bond expresses

signals at about 60 and respectively 73 ppm, the one linked to the anomeric O belonging to the D-glucose ring being more shifted to the right.

The signals assigned to the pyranosic D-glucose ring are placed between 62 and 70 ppm, the anomeric C though is shifted much more to the left to about 90 ppm

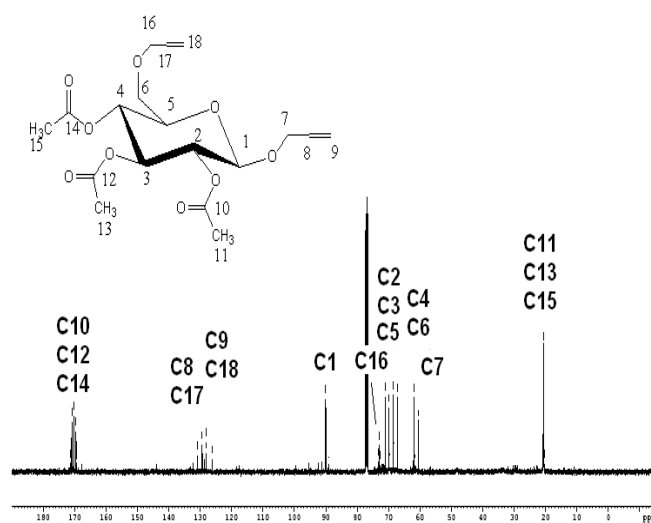


Fig. 2 The ¹³C-NMR spectrum of **5** in CDCl₃

C. HPLC-MS Analysis Additionally

The HPLC-MS analysis confirms the molecular weight of product **5**. Fig. 3 displays the mass spectrum for compound **3**. It shows that the most abundant ion corresponds to the [M+Na]⁺ single-charge sodium adduct at m/z = 373.65. The peak observed at m/z = 720.37 is associated with the presence of the [2*M+Na+H]⁺ adduct, corresponding to the dimer of the molecule associated with Na⁺ and H⁺.

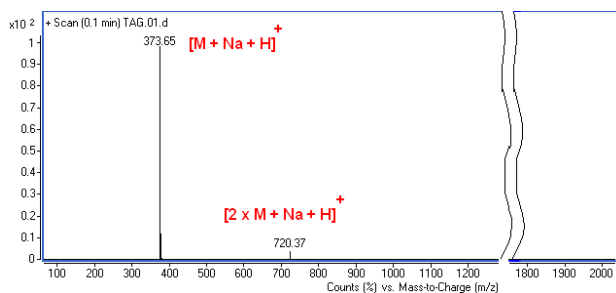


Fig. 3 The HPLC-MS spectrum of **3**

Fig. 4 shows the collected mass spectrum of **4**, showing the molecular peak as an adduct with K^+ at $m/z = 439.02$ and as an adduct with Na^+ and H^+ at $m/z = 424.83$, thus reassuring the proposed structure for the new glycomonomer. The accompanying peak at $m/z = 373.21$ can be assigned to the single charged peak of the molecule which has lost one double bond from the two protective allylic groups.

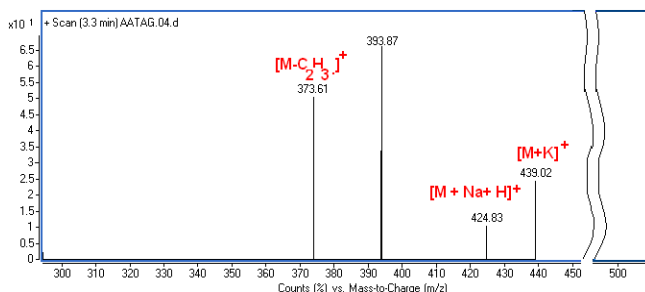


Fig. 4 The HPLC-MS spectrum of **5**

V. CONCLUSION

One novel glycomonomer deriving from D-glucose was successfully synthesized and analyzed using FTIR and NMR (1H-NMR and 13C-NMR) spectroscopy and HPLC-MS. The FTIR and NMR spectroscopy results confirmed the structure of this compound. The HPLC-MS analysis confirmed the molecular weights of **5**. The synthesis of this D-glucose derivative (in high yields, 86% for each step) is part of our work to obtain biodegradable polymers. Polymer materials derived from carbohydrates are potentially biodegradable and they lead to minimum environmental pollution.

ACKNOWLEDGMENT

This work was partially supported by the strategic project POSDRU 107/1.5/S/77265 (fellowship of M.S. Mazăre), inside POSDRU Romania 2007-2013 co-financed by the European Social Fund – Investing in People. This paper was supported by the project PERFORM-ERA "Postdoctoral Performance for Integration in the European Research Area" (ID-57649) (fellowship of A. M. PANĂ), financed by the European Social Fund and the Romanian Government. This work was partially supported

REFERENCES

- [1] D. Teng, W. Yin, X. Zhang, Z. Wang, C. Li, "New glycoconjugate polyacrylamide with water-solubility and additional activated groups: synthesis and characterization", *Journal of Polymer Research*, 16, 2009, pp. 311-316.
- [2] V. Ladmiraal, E. Melia, D. M. Haddleton, "Synthetic glycopolymers: An overview", *European Polymer Journal*, 40, 2004, pp. 431-449.
- [3] S. Thadke, M. Kar, S.S. Gupta, S. Hortha, "Gold catalyzed glycosidations for the synthesis of sugar acrylate/acrylamide hybrids and their utility", *Carbohydrate Research*, 346, 2011, pp. 1511-1518.
- [4] M.X. Hu, Z.K. Xu, "Carbohydrate decoration of microporous polypropylene membranes for lectin affinity adsorption: comparison of mono- and disaccharides", *Colloids and Surfaces B: Biointerfaces*, 85, 2011, pp. 19-25.
- [5] K. Jain, P. Kercharwani, U. Gupta, N. Jain, "A review of glycosylated carriers for drug delivery", *Biomaterials*, 33, 2012, pp. 4166-4186.
- [6] Z. Yu, M.M. Cui, J. Yan, Y. You, "One-pot synthesis of hyperbranched poly(amido amine) clicked with a sugar shell via Michael addition polymerization and thiol click reaction", *Science China Chemistry*, 53:8, 2010, pp. 1663-1668.
- [7] N. Peppas, Y. Huang, "Polymers and gels as molecular recognition agents", *Pharmaceutical Research*, 19:5, 2002, pp. 578-587.
- [8] Y. Wang, X. Zhang, Y. Han, C. Cheng, C. Li, "pH- and glucose-sensitive glycopolymer nanoparticles based on phenylboronic acid for triggered release of insulin", *Carbohydrate Polymers*, 89, 2012, pp. 124-131.
- [9] A. Munoz-Bonilla, V. Bordege, O. Leon, R. Cuervo-Rodriguez, M. Sanchez-Chaves, M. Fernandez-Garcia, "Influence of glycopolymers structure on the copolymerization reaction and on their binding behavior with lectins", *European Polymer Journal*, 48, 2012, pp. 963-973.
- [10] J. Nicolas, Y. Guillaneuf, C. Lefay, D. Bertin, D. Gigmes, B. Charleux, "Nitroxide-Mediated polymerization", *Progress in Polymer Science*, 2012, accepted manuscript, doi:10.1016/j.progpolymsci.2012.06.002.
- [11] M. I. Osborn, *Academic Press*, 2002, pp. 50.
- [12] D. D. Reynolds, W. Lloyd Evans, *Organic Syntheses*, 3, 1955, pp.432.R. W. Lucky, "Automatic equalization for digital communication," *Bell Syst. Tech. J.*, vol. 44, no. 4, pp. 547-588, Apr. 1965.
- [13] L.M. Ștefan, A.M. Pană, C. Pascariu, E. Șișu, G. Bandur, L.M. Rusnac, "Synthesis and characterization of a new methacrylic glycomonomer", *Turk J Chem*, 35, 2011, pp. 657-667.
- [14] A.M. Pană, L.Rusnac, G.Bandur, E. Șișu, V.Badea, M. Silion, "Synthesis and Characterization of New Glycopolymers Based on Monosachharides and Maleic Anhydride I. Glucose derivatives", *Materiale Plastice, București*, 2010, 47(1), pp.28-34.
- [15] Pană A.M., Rusnac L.M., Bandur G., Silion M., Deleanu C., and Bălan M., "Novel D-glucose and D-mannose based oligomers. Synthesis and characterization", *e-Polymers*, 2011, no.004, pp. 1-13.