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SYNTHESIS AND CHARACTERIZATION OF GLYCOMONOMER ACRYLOYL 2,3:5,6-DI-*O* ISOPROPYLIDENE-α-D-MANNOFURANOSIDE

Ioana Ramona Ciopănoiu[1]*, Geza-Nicolae Bandur[2], Lucian-Mircea Rusnac[2]

1. Faculty of Medicine, Pharmacy and Dental Medicine, "Vasile Goldiș" Western University of Arad, 86 Liviu Rebreanu, 310045, Arad, Romania
2. Faculty of Industrial Chemistry and Environmental Engineering, "Politehnica" University of Timişoara, 6 Carol Telbisz, 300001, Timișoara, Romania

* Correspondence: Ioana Ramona Ciopănoiu, "Vasile Goldiș" Western University of Arad, Faculty of Medicine, Pharmacy and Dental Medicine, Department of Pharmaceutical Sciences, 86 Liviu Rebreanu, 310045, Arad, Romania, Tel. +40729806064, email: salageanioana@yahoo.com

ABSTRACT. Synthetic carbohydrate polymers are being increasingly investigated as biodegradable, biocompatible and biorenewable materials for use as medical devices, water absorbents or chromatographic supports. The aim of this work was to obtain a monosaccharide ester that possesses a double bond in the molecule and can thus function as a monomer. This was accomplished by reacting 2,3:5,6-diisopropylidene-D-mannofuranose with acryloyl chloride in alkaline medium and with methylene chloride as solvent, using triethylamine as capturing agent for the hydrochloric acid. The structure of the glycomonomer thus obtained was confirmed by nuclear magnetic resonance spectroscopy, Fourier transformed infrared spectroscopy and electrospray ionization mass spectrometry.
Keywords: glycomonomer, mannose, chemical synthesis, biorenewable, biocompatible

INTRODUCTION

Glycopolymers can be defined, in a general sense, as synthetic polymers possessing a non-carbohydrate backbone but carrying carbohydrate moieties as pendant or terminal groups (Cerrada et al., 2008). They are of great interest, not only as simplified models of biodegradable polymers bearing oligosaccharides, but also in biological, biochemical and biomedical applications (Wang et al., 2002).

As our fossil raw materials are irrevocably decreasing and the pressure on our environment is building up, the progressive changeover of chemical industry to renewable feedstock for its raw materials, that prevailed before natural gas and oil outpaced all other sources, emerges as an inevitable necessity. Carbohydrates, which represent the most important class of organic compounds in terms of volume produced, account for around 75% of the 200 billion tons of biomass produced annually worldwide (Lichtenthaler et al., 2004). Therefore they offer ideal conditions for industrial development as replacements for raw materials of fossil origin. Specialists estimate that by 2040 the biomass will be a cheaper raw material than petroleum hydrocarbons.

There has been a worldwide realization that nature derived mono-, di-, oligo- and polysaccharides can provide us the raw materials needed for the production of numerous industrial consumer goods (Sălăgean et al., 2008). Unfortunately, in spite of their abundance, the sugar molecules contain multiple hydroxyl groups that allow for non-selective electrophilic acylation. One way to solve this problem is to use specific protection/deprotection steps for some of these groups (Pană et al., 2010).

About 120 years ago Emil Fischer first described the formation of acetals from D-fructose and acetone. Since then, the isopropylidene group has been extensively used as a protecting group in organic synthesis in general, and in drug production in particular (Ladmiral et al., 2004). Acetal migration is also a well-documented phenomenon, and thus di-*O*-isopropylidene sugars are known to exhibit isomers,

each with their own usage and advantages in organic synthesis, making the field of carbohydrate chemistry a complex research area.

Using carbohydrates based materials led to great interest in producing glycopolymers. Because carbohydrates are also important candidates as ligands or signal recognition molecules, efforts have been focused on developing a convenient synthetic route towards amphiphilic glycoconjugate polymers by arranging hydrophobic polystyrene as a main chain bearing pendant hydrophilic oligosaccharides (Wataoka et al., 2005).

Monomers having a sugar moiety as structural unit (glycomonomers) were therefore chosen for this study because of the high biocompatibility and important biological activity which characterize the resulting polymers.

This paper presents the synthesis of an unsaturated monosaccharide ester, obtained by reacting 2,3:5,6-diisopropylidene-D-mannofuranose with acryloyl chloride. The presence of a C=C double bond in the structure of such an ester allows its subsequent copolymerization with other unsaturated monomers. To ensure the stability of the acetal, the reactions were performed in organic alkaline media, using triethylamine as capture agent for the hydrochloric acid formed as byproduct. Triethylamine is a very useful organic base which, alongside pyridine and 4-dimethylaminopyridine (DMAP), finds multiple applications in organic synthesis as a nucleophilic solvent and catalyst. However, the advantages of using triethylamine over pyridine and DMAP consist in its lower toxicity and decreased boiling point (which allows for easy removal). Also, in contrast with a previous study in which sodium hydroxide aqueous solution was used to assure the necessary alkaline conditions for esterification (Sălăgean et al., 2009), triethylamine allows instead a completely anhydrous environment, being readily miscible with other organic solvents while at the same time assuring the complete solubility of the nonpolar reagents used and the stability of the acetal protecting groups.

EXPERIMENTAL PART

Materials

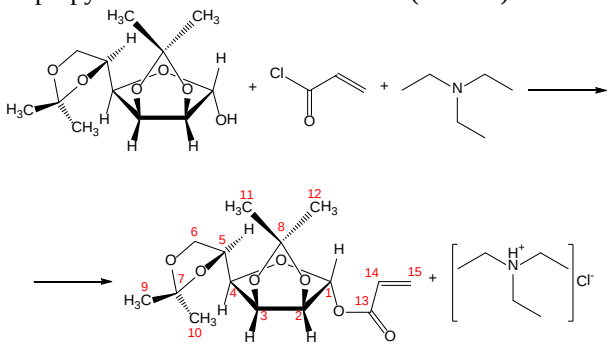
The reagents were used as purchased. Along with their acronyms and their supplier, are given in Table 1.

Table 1. The reagents used and their acronyms

Reagent	Used acronyms	Manufacturer
Triethylamine	TEA	Merck
Methylene chloride	DCM	Chimopar
Acryloyl chloride	AC	Aldrich

Glycomonomer Synthesis

2,3:5,6-Di-O-isopropylidene-D-mannofuranose (also known by the improper name of "diacetone mannose" and abbreviated as DAM) was obtained according to literature (Rauter et al., 1995; Rajput et al., 2006) and further reacted with AC according to Scheme 1, giving acryloyl 2,3:5,6-di-O-isopropylidene-α-D-mannofuranoside (ADAM):



Scheme 1. The synthesis of ADAM and its atom numbering

Methylene chloride (CH_2Cl_2) was used as a solvent for the synthesis of the glycomonomer, because it provides good solubility for both reagents (DAM and AC). To ensure the stability of the cyclic acetals, the reaction was carried out in alkaline conditions, using TEA as quenching agent of hydrochloric acid (HCl).

The glycomonomer was synthesized according to the protocol described below.

A mixture of 25.28 g TEA (0.25 mol) and 26.00 g DAM (0.10 mol) in DCM (120 mL) was cooled to 0˚C in an ice bath and maintained under stirring. 18.10 g AC (0.20 mol), previously dissolved in methylene chloride (100 mL), was added dropwise (2.5-3.0 h). All this time the temperature was maintained at 0˚C. Afterwards, the mixture was stirred for 3 hours in an inert atmosphere, until the thin layer chromatography plate (hexane/ethyl acetate 3:1 as eluent) showed the formation of the desired product and the disappearance of the starting material spot. The reaction mixture was washed with 2M sulfuric acid (2x10 mL), saturated aqueous sodium bicarbonate (3x20 mL) and saturated aqueous sodium chloride solution (2x20 mL), and then dried over sodium sulfate. The remaining solvent was evaporated under reduced pressure, after which the product was purified by flash column chromatography on silica gel (hexane/ethyl acetate 3:1 as mobile phase). The final product is a colorless viscous syrup (75% yield), soluble in acetone, benzene, dichloromethane, chloroform, DMF, DMSO, alcohols and petroleum ether.

Analytical instruments and methods

The syntheses were monitored using thin-layer chromatography performed on silica gel plates.

The FTIR spectra were recorded on a Bruker Vector 22 FT-IR spectrometer. The analyses were performed using KBr pellets for solid samples and also with the help of an ATR DIAMANT cell. Absorption band values are given as wavenumbers (cm^{-1}). The spectra were recorded over the range 4000-400 cm^{-1}.

^{1}H-NMR and ^{13}C-NMR data were recorded on a Bruker Avance 300 spectrometer, at a frequency of 300.133 MHz for ^{1}H and 75.464 MHz for ^{13}C, using deuterochloroform as solvent (about 50 mg of sample dissolved in 0.5 mL $CDCl_3$). Chemical shifts are given in ppm (parts per million), relative to tetramethylsilane (TMS) as internal standard.

Mass spectrometry was conducted on a Bruker Daltonics mass spectrometer. A completely automated nanoESI chip (NanoMate robot which incorporates ESI 400 Chip technology, Advion BioScience) in conjunction with a high-capacity ion trap (HCT Ultra PTM Discovery) were used. Electrospray ionization was conducted in positive ion mode (1.0 – 1.7 kV; capillary exit 20 V) using nitrogen as nebulizer gas (dry gas temperature: 100°C). The data were acquired using Compass v.1.2 software while Bruker Daltonics DataAnalysis v.3.4 software was used for the storage and processing of mass spectra. The mass spectrum of the investigated compound was acquired in the *m*/*z* range 50–900. The glycomonomer was solubilized in methanol at a maximum concentration of 0.01 mg/mL.

Atom numbering is based on IUPAC carbohydrate nomenclature and includes both the carbon atoms and the protons directly attached to them.

RESULTS AND DISCUSSIONS

The glycomonomer ADAM was characterized using Fourier transformed infrared spectroscopy (FTIR), nuclear magnetic resonance spectroscopy (^{1}H-NMR and ^{13}C-NMR) and electrospray ionization mass spectrometry (ESI-MS).

FTIR spectra of DAM and ADAM are presented overlapped in Fig. 1.

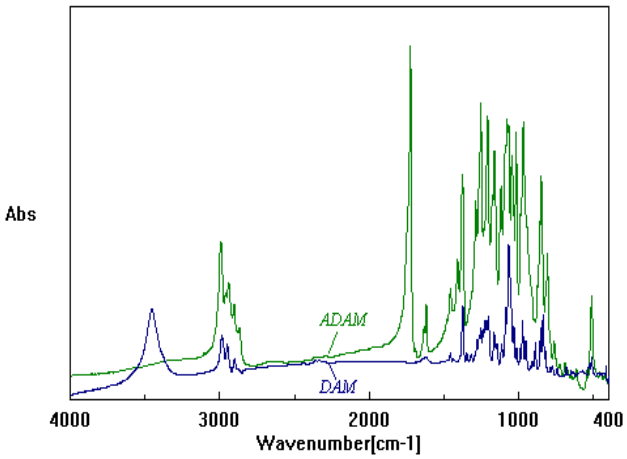


Fig. 1. FTIR spectra for DAM and ADAM

When comparing the two spectra, the disappearance of the absorption band specific to the valence vibration of the hydroxyl group at 3436 cm^{-1}, simultaneously with the appearance of the bands characteristic to the ester group at 1725 cm^{-1} in the glycomonomer spectrum, can readily be noticed (Sălăgean et al., 2009).

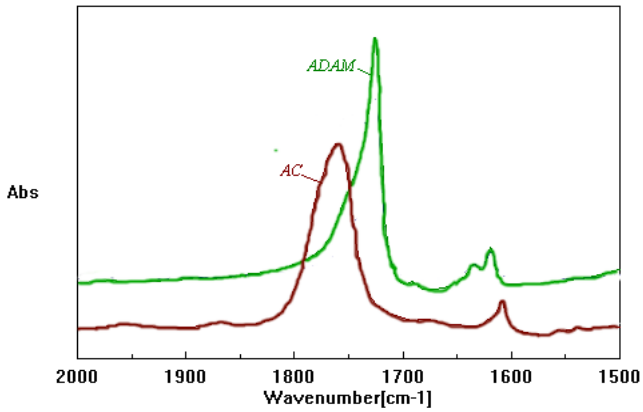


Fig. 2. FTIR spectra for ADAM and AC in the 1500-2000 cm^{-1} domain

By comparing the FTIR spectra of ADAM glycomonomer with the FTIR spectra of AC (Fig. 2) it can be easily seen that the band from 1761 cm^{-1}, which confirms the C=O double bond in the AC spectrum, does not appear in the glycomonomer spectrum, being replaced by the band at about 1725 cm^{-1}, specific for the esteric linkage. The C=C group absorption band present in the acryloyl chloride spectrum at 1610 cm^{-1} can be found in the glycomonomer spectrum at 1619 cm^{-1}.

Both ^{1}H-NMR and ^{13}C-NMR spectra (Fig. 3 and 4) confirmed the presence of the double bond in the glycomonomer, showing the characteristic signals for C=C group at 127.9 ppm (C_{14}) and 132.2 ppm (C_{15}), and also the proton signals in the range 5.88 - 6.50 ppm (H_{14}, H_{15}). In addition, the ^{13}C-NMR spectra shows the O-C=O acrylic ester signal at 164.4 ppm (C_{13}). ^{1}H-NMR shows four signals at 1.26, 1.35, 1.46 and 1.50 ppm corresponding to the methyl groups, confirming that the product has kept both its isopropylidene protecting groups. This was also established using ^{13}C-NMR, which clearly shows the corresponding four signals: 24.7, 25.1, 26.0 and 27.0 ppm (Sălăgean et al., 2009).

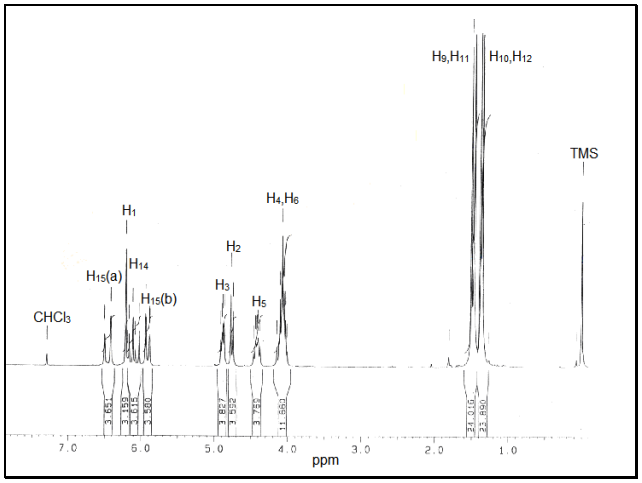


Fig. 3. ^{1}H-NMR spectrum for ADAM (atom numbering in Scheme 1)

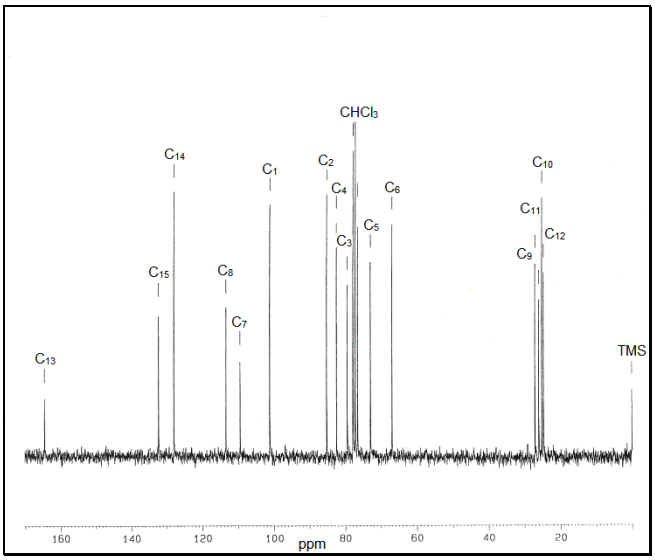


Fig. 4. ^{13}C-NMR spectrum for ADAM (atom numbering in Scheme 1)

The mass spectrum for ADAM is presented in Fig. 5. The peak at *m*/*z* 337.1, which has almost the same intensity as the base peak (*m*/*z* 208.6), is associates with the presence of the single charge sodium adduct of the glycomonomer, $[ADAM+Na]^{+}$, and confirms the calculated molecular mass for ADAM (314.1 atomic mass units). The potassium adduct, $[ADAM+K]^{+}$, can also be seen at *m*/*z* 353.1.

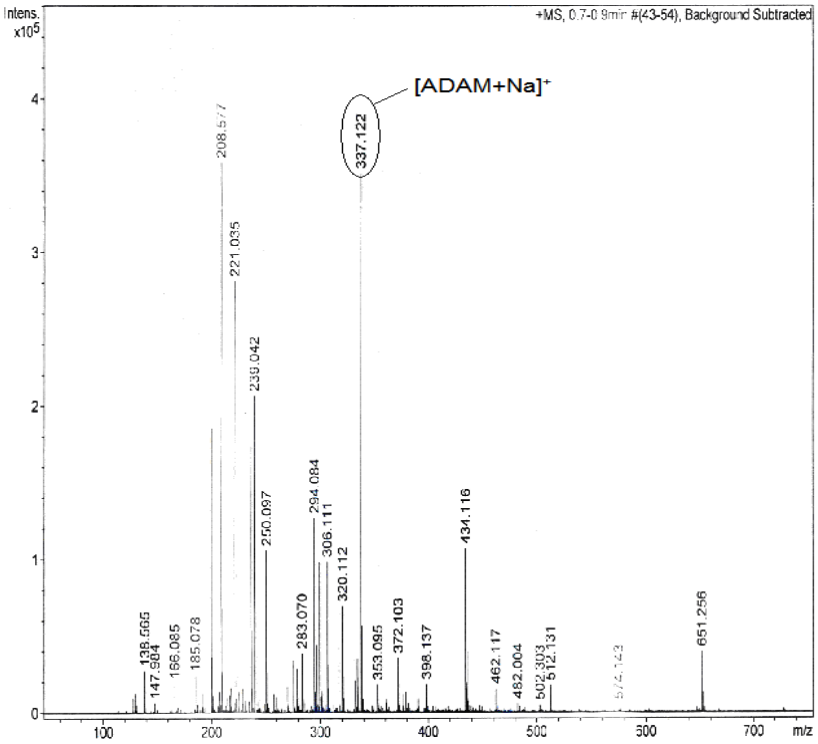
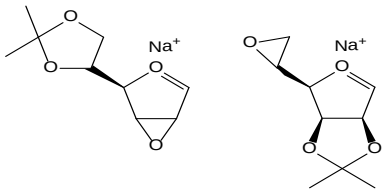


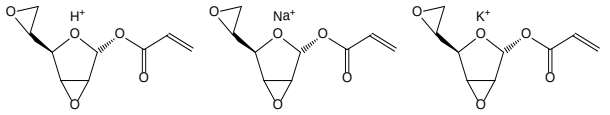
Fig. 5. Mass spectra of ADAM

The peak of lower intensity at *m*/*z* 651.3 corresponds to glycomonomer dimer as sodium adduct, $[2ADAM+Na]^+$. The base peak at *m*/*z* 208.6 could result from the complete loss of the lateral chain and one of the isopropylidene protecting units (in the form of acetone, 58.0 atomic mass units), as illustrated in Scheme 2. Some proposed structures for other peaks observed in the ESI-MS spectra (*m*/*z* 199.1, 221.0 and 237.0), which would be produced by the loss of both isopropylidene protecting groups in the form of acetone, are given in Scheme 3.



Chemical Formula: $C_9H_{13}NaO_4^+$
Exact Mass: **208.1**
Molecular Weight: **208.2**

Scheme 2. Possible fragmentation cations that could explain the base peak



Chemical Formula: $C_9H_{11}O_5^+$
Exact Mass: **199.1**
Molecular Weight: **199.2**

Chemical Formula: $C_9H_{10}NaO_5^+$
Exact Mass: **221.0**
Molecular Weight: **221.2**

Chemical Formula: $C_9H_{10}KO_5^+$
Exact Mass: **237.0**
Molecular Weight: **237.3**

Scheme 3. Possible fragmentation cations that could explain the observed peaks at *m/z* 199.1, 221.0 and 237.0

CONCLUSIONS

Glycopolymers are being increasingly investigated as biodegradable, biocompatible and biorenewable materials. This paper therefore focused on the synthesis and preparation of a glycomonomer which could easily lead to such a glycopolymer after copolymerization with different suitable monomers, like acrylic or methacrylic monomers.

The glycomonomer synthesis took place with an overall yield of about 75%, loses arising from the separation processes.

Its structure was confirmed by FTIR spectrometry, the complete acylation reaction being marked by the disappearance of OH band, together with the simultaneous appearance of the C=O esteric band and the C=C vinyl band.

The NMR spectrometry was also used to verify the structure of the glycomonomer obtained, proving that the acylation reaction took place without the loss of the isopropylidene protecting groups and that the product was perfectly isolated from the starting compounds and any byproducts that could have formed during the synthesis.

Lastly, mass spectrometry allowed confirmation of the molar weight for the glycomonomer (determined as sodium and potassium adducts) while also showing its fragmentation pattern, which seem to be based on the loss of isopropylidene protecting groups in the form of acetone. Possible fragmentation cation structures, which could account for some of the peaks found in the ESI-MS spectrum, have been proposed.

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