Synthesis of Two New Chromogenic Glycosides of 4-nitrocatechol – α-D-N-acetylmannopyranosaminide and α-L-rhamnopyranoside, as Substrates for Exoglycosidases

FLORENTINA DUICA[1], SILVIA STEFANIA GITMAN[1], CORINA LOREDANA HOTOLEANU[1], ALINA NICOLESCU[2], DUMITRU PETRU IGA[1]*

[1] University of Bucharest, Faculty for Biology, 95 Splaiul Independentei, 050095, Bucharest, Romania

[2]"Petru Poni" Institute of Macromolecular Chemistry, Group of Biospectroscopy, 4A Gh. Ghica, 700487, Iasi, Romania

The peracetates of N-acetyl-D-mannosamine and L-rhamnose were reacted with 4-nitrocatechol in a Helferich glycosylation, by using BF_3·OBu_2 as chemical promotor. The synthesized glycosides were purified by column chromatography on silica gel or by crystallization from ethanol and then characterized chemically, chromatographically and by ^1H and ^{13}C NMR spectroscopy. Both synthetic glycosides were hydrolyzed by an enzymatic extract of snail, Helix pomatia.

Key words: N-acetyl-D-mannosamine, L-rhamnose, 4-nitrocatechol, glycoside

Aromatic glycosides are a vast group of natural [1-5] or synthetic [6-9] compounds having important biological and practical implications. Prechromogenic [10-13] chromogenic [14-17], and fluorogenic [18,19] glycosides mimick the structure of natural aromatic glycosides.

A great diversity of aglycons have been used as chemical moiety for mono- or oligosaccharides in the construction of glycosides to be used as substrates for exo-glycosidases. In order to synthesize prechromogenic substrates, phenol [10,13,20-22], 2-naphthol [6,23] and many other derivatized phenols [24,25] were used as aglycons. Instead, for the synthesis of chromogenic substrates, 2-nitrophenol [14,15,22,26], 4-nitrophenol [22,27-29], halogenated indols [30,31] and even 4-nitrocatechol [9,16,17] were aglycons of choice. Many other phenolic derivatives served for the preparation of substrates for exoglycosidases: 4-methyl umbelliferone [18], phenolphthalein [32], cyclo-hexeno-esculetin [33,34], p-naphtholbenzein [35], alizarin [36].

Synthesis of natural (soluble) glycosides constituted the first ample approach in carbohydrate chemistry. β-D-Glucopyranosyl-phenol and helicin were synthesized in methanol by the reaction of tetra-O-acetyl β-D-glucopyranosyl chloride and potassium phenoxide [10]. Other authors kept the idea of halogen-activated sugar reacting with phenoxide but replaced methanol, as chemical environment, by acetone [37]. This method has been extensively used to our days with aromatic aglycons [9, 34, 38]. An important improvement of the glycosylation reaction was the Koenigs and Knorr synthesis (1901) [39]. Alternatively, carbohydrate peracetates were directly linked to phenols in the presence of p-toluenesulfonic acid or anhydrous zinc chloride; the first promotor leads to β-anomers while the second one produces α-glycosides [6]. A modified Koenigs-Knorr alternative recommends BF_3·OEt_2 (or BF_3·OBu_2) as promotor and per-O-acetylated carbohydrates as glycosylation donors [9,23]. A large variety of phenols were glycosylated either by using one of the three metods – Koenigs-Knorr, Michael, Helferich [24] or by reaction with acetobromosugar in the presence of tetrabutylammonium bromide [25].

4-Nitrocatechol is a metabolic intermediate in the degradation of nitrobenzene, being preceded in this pathway by 4-nitrophenol [40,41]. In fact, both products are the result of a hydroxylation reaction and are further metabolized to the corresponding glucuronide and sulfate conjugates[42-44]. A new pathway of biological degradation, by nitrite releasing and 1,2,4-benzenetriol formation was discovered in *Burkholderia cepacia*. Moreover, this metabolic pathway is encoded in plasmid [45].

4-Nitrocatechol sulfate (2-hydroxy 5-nitrophenyl sulfate), a substrate of (aryl)sulfatase [46,47], is a specific and selective inhibitor of *Yersinia* protein tyrosine phosphatase [48,49]. Although a synthetic compound, 4-nitrocatechol bears a widespread natural structural motif, 1,2-dihydroxy-aryl [44]. Moreover, 4-nitrocatechol is an inhibitor of catechol-*O*-methyltransferase and compounds imitating its structure (entacapone, tolcapone, nitecapone) indicated higher inhibitory activity [50].

In this paper, two new glycosides of 4-nitrocatechol have been synthesized, α-D-N-acetylmannopyranosaminide and α-L-rhamnopyranoside, as potential substrates for exoglycosidases. They were synthesized by a Helferich method and were characterized chemically and by ^1H and ^{13}C NMR spectra.

Experimental part

Materials and methods

N-Acetyl D-mannosamine (D-ManNAc) and L-rhamnose (L-Rha) were from Sigma. All reagents used were either from Fluka or from Merck and they were of analytical grade.

All reactions were followed by thin layer chromatography (TLC) analysis: acetylated glycosides were eluted in the solvent system (SS) 1, chloroform–methanol (19:1), while deacylated glycosides were eluted in chloroform-methanol-water 60:25:4 (v/v) (SS 2). Three types of visualization were used: (a) by dipping the plates in mostain, followed by heating; (b) by exposure to UV light (380 nm); (c) by dipping the plates in a 1 M solution of NaOH in ethanol–water (1:1) [9]. The peracetylated

* *email pdiga49@yahoo.com*

glycosides were separated by column chromatography on silica gel 60 (0.063–0.200 mm, Merck) in a gradient of methanol in chloroform. Pure compounds were submitted to mild alkaline hydrolysis with 0.2 M sodium methoxide and the excess of alkalinity was removed by stirring with Dowex 50 WX2 (H^+). Purification of deacetylated glycosides was accomplished by silica gel column chromatography in a gradient of ethanol in 1,2-dichloroethane. The molar ratio determination of glycosides constituents was preceded by acidic hydrolysis (2 h in 2 M HCl) and partition between water and ethyl ether; in the ether phase 4-nitrocatechol was determined and in the water phase, the sugar, both colorimetrically.

The ^1H and ^{13}C NMR spectra of synthetic intermediates and products were acquired in $CDCl_3$ containing TMS. One-dimensional NMR experiments were performed on a Bruker Avance DRX 400 spectrometer using 400 and 100 MHz for the ^1H and ^{13}C frequencies, respectively. The ^1H-^1H correlation spectroscopy (COSY) and ^1H-^{13}C heteronuclear multiple quantum coherence (HMQC) experiments were carried out with an inverse probe.

T**etra-O-acetyl α-D-N-acetylmannopyranosamine**. 2.5 g (11.31 mmol) ManNAc was stirred overnight at room temperature with an excess of a mixture of pyridine-acetic anhydride (Py-Ac_2O, 2/1, v/v) [51]. The next day, the solvents were removed by rotavapor, the last traces being removed by co-evaporation with dry toluene. The product, tetra-O-acetyl α-D-N-acetylmannopyranosamine (4.18 g; 10.7 mmol 95 %), was used for the next step wthout further purification.

α-D-N-Acetyl-(tri-O-acetyl)mannopyranosaminyl-(2'-O-acetyl)nitrocatechol-1'-yl (1). To a suspension of 1.5 g (3.85 mmol) tetra-O-acetyl α-D-N-acetylmannopyranosamine, 0.7161 g (4.62 mmol) 4-nitrocatechol and 4 g molecular sieves in 15 mL dichloromethane, were added 0.4 mL $BF_3{\cdot}OBu_2$ [9,23]. This suspension was stirred overnight at room temperature. The next day, the solvents were removed by rotavapor under vacuum and the residue was partitioned between chloroform and a saturated solution of sodium bicarbonate. The aqueous layer was separated and extracted two more times with chloroform. The total chloroformic solution was extracted with a small volume of water, dried with magnesium sulfate, filtered, evaporated to dryness and separated by column chromatography on silica gel. The main product, tri-O-acetyl α-D-N-acetylmannopyranosaminyl-nitrocatechol 1-yl (0.7453 g; 1.54 mmol; 40 %), was eluted with a gradient of ethyl acetate in n-hexane, the solution was dried and acetylated with an excess of Py-Ac_2O, 2/1 (v/v). This acetylation was practically quantitative. The final product, tri-O-acetylα-D-N-acetylmannopyranosaminyl-nitrocatechol 1- yl 2-O-acetyl (0.7890 g; 1.50 mmol; 98 %) was analyzed by NMR.

^1H NMR. ($CDCl_3$; δ ppm; *J* Hz) (fig. 1): 5.55 (s, 1H) (H-1â); 4.81 (1H) (H-2); 5.37 (dd, 4.4 Hz, 6 Hz, 1H) (H-3); 5.19 (1H) (H-4); 4.03 (1H) (H-5); 4.27 (1H) (H-6a); 3.97 (1H) (H-6b); 6.29 (d, 8.8 Hz) (NH of NH–Ac); 1.98 (Me of NH–Ac); 7.25 (d, 8.8 Hz, 1H) (H-3'); 8.01 (d, 2.8 Hz, 1H) (H-5'); 8.14 (d, 2.8 Hz, 1H) (H-6'); 2.03, 2.08, 2.09, 2.46 (s) (Me groups of –OAc linked to sugar and 4-nitrocatechol).

^{13}C NMR. ($CDCl_3$; δ ppm; *J* Hz) (fig. 1): 97.57 (C-1); 50.12 (C-2); 68.47 (C-3); 65.30 (C-4); 69.58 (C-5); 62.23 (C-6); 23.09 (Me of NH–Ac); 152.10 (C-1'); 142.61 (C-2'); 114.56 (C-3'); 140.04 (C-4'); 119.23 (C-5'); 122.64 (C-6'); 20.27, 20.48, 20.62, 20.69 (Me groups of –OAc linked to sugar and 4-nitrocatechol); 169.87, 169.96, 169.99, 170.57, 170.61 (>C=O groups of Ac).

Tetra-O-acetyl α-L-rhamnopyranose. L-Rhamnose (3 g; 18.29 mmol) was stirred overnight with an excess of Py-Ac_2O (2/1, v/v) [52,53]. The next day, the excess reagents were removed by rotavapor, by adding small amounts of dry toluene. The residue, tetra-O-acetyl α-L-rhamnopyranose (5.83 g; 17.56 mmol; 96 %) was crystallized once from ethanol.

α-L-Tri-O-acetyl)rhamnopyranosyl-(2'-O-acetyl)nitrocatechol-1'-yl (2). To a suspension of 2 g (6.02 mmol) tetra-O-acetyl α-L-rhamnopyranose, 4 g molecular sieves and 0.1119 g (7.22 mmol) 4-nitrocatechol in 10 mL dichloromethane, 0.4 mL $BF_3{\cdot}OBu_2$ was added. This suspension was stirred overnight at room temperature in the absence of moisture. The next day the suspension was diluted with chloroform and filtered by Celite. The solvents of the filtrate were removed and the residue dried in a dessicator, acetylated with an excess of Py-Ac_2O and crystallized from ethanol (1.0693 g; 2.28 mmol; 38 %); the product was analyzed by NMR, submitted to mild alkaline hydrolysis and analyzed chemically and chromatographically.

^1H NMR. ($CDCl_3$; β ppm; *J* Hz) (fig. 1): 5.51 (d, 2.4 Hz, 1H) (H-1); 5.60 (dd, 1.6 Hz; 8.8 Hz, 1H) (H-2); 5.47 (1H) (H-3); 5.160 (1H) (H-4); 3.96 (1H) (H-5); 1.20 (d, 6.4 Hz, 3H) (CH_3 group); 7.25 (d, 6.0 Hz, 1H) (H-3'); 7.95 (dd, 2.8 Hz, 6.4 Hz, 1H) (H-5'); 8.05 (d, 2.4 Hz, 1H) (H-6'); 1.99, 2.07, 2.20, 2.45 (s), 12H (Me groups of Ac linked to sugar and 4-nitrocatechol).

^{13}C NMR. ($CDCl_3$; β ppm; *J* Hz) (fig. 1): 96.40 (C-1); 68.17 (C-2); 69.40 (C-3); 68.69 (C-4); 70.37 (C-5); 17.42 (C-6); 151.08 (C-1'); 145.49 (C-2'); 112.99 (C-3'); 143.04 (C-4'); 116.17 (C-5'); 119.53 (C-6'); 20.68, 20.74, 20.87 (Me groups of Ac linked to sugar and 4-nitrocatechol); 169.38, 169.45, 170.08, 170.11 (>C=O groups of Ac).

Results and discussions

As indicated above, TLC chromatograms were visualized by three methods (Experimental part). TLC chromatograms of the reaction mixture disclosed two nuances in UV: one was blue-violet and the other one, red-brown. The blue-violet bands released 4-nitrocatechol and sugar by acidic hydrolysis, so it corresponded to glycosylated nitrocatechol. The red-brown bands were less investigated but some data indicated that they corresponded to free and (partially) acetylated nitro-catechol. By exposure to sodium hydroxide, the blue-violet bands were coloured to yellow, while the red-brown ones turned red [16,17]. The acetylated glycoside of 4-nitrocatechol could be separated by column chromato-graphy on silica gel or by crystallization from ethanol. Both α-D-N-acetylmanno-pyranosaminyl-4-nitrocatechol 1-yl and α-L-rhamno-pyranosyl-4-nitrocatechol 1-yl presented a molar ratio of

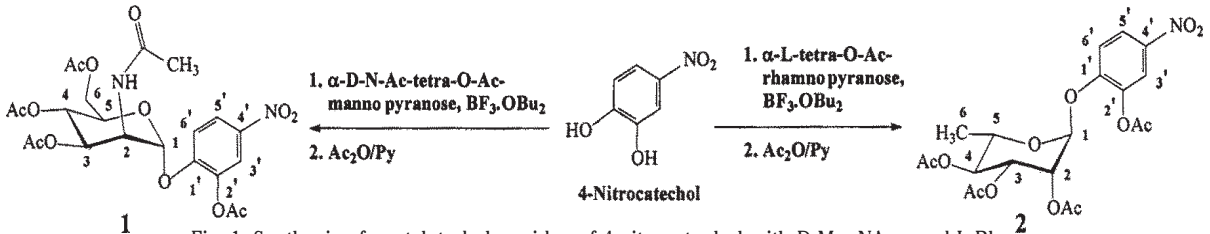

Fig. 1. Synthesis of acetylated glycosides of 4-nitrocatechol with D-ManNAcp and L-Rhap

1:1 between the sugar and the aglycon. The two glycosides presented one band by TLC, before and after mild alkaline hydrolysis, as well as by the three visualization systems.

There was a good agreement between our results and the results of others concerning the NMR spectral data on α-D-ManNAcp glycoside [54,55] of 4-nitrocatechol as well as of the glycoside of α-L-Rhap [56, 57] with the same phenol. Also, our present results agreed with preceding data concerning the glycosides of 4-nitrocatechol [9,16,17].

A comparison of ^{1}H NMR signals of ester methyl groups of peracetylated 17α-estradiol 17-β-D-glucopyranoside, disclosed a *downfield* shift for phenolic acetate in contrast with aliphatic ones [58]. We have encountered the same phenomenon in case of peracetates of the following compounds: 4-nitrocatechol, DOPA, estrone, DL-α-tocopherol (D. P. Iga, unpublished data). This feature of acetate aromatic ester could serve to distinguish between diglycosylated 4-nitrocatechol [25] and monoglycosylated one [9, 16, 17]. The characteristic signal for α-D-N-acetyl-(tri-O-acetyl)mannopyranosaminyl-(2'-O-acetyl) nitrocatechol-1'-yl was 2.46 ppm (s) and for α-L-(tri-O-acetyl)rhamnopyranosyl-(2'-O-acetyl)nitrocatechol-1'-yl 2.45 ppm (s) (see above). A smilar *downfield* shift (2.29 ppm) was noticed in case of peracetylated mono-glucoside of hydroquinone [59].

An enzymatic extract of digestive tract of snail, *Helix pomatia*, hydrolyzed both substrates in a slightly acidic buffer (D. P. Iga, unpublished data).

Conclusions

Peracetylation of N-acetyl D-mannosamine and L-rhamnose at low temperature and use of the protected sugar in Helferich glycosylation of 4-nitrocatechol, with dibutyletherated boron trifluoride as promotor, produced α-D-N-acetyl-(tri-O-acetyl)mannopyranosaminyl-nitrocatechol-1'-yl and α-L-(tri-O-acetyl)rhamnopyranosyl-nitrocatechol-1'-yl, respectively.

The two major products of glycosylation were separated, acetylated, and characterized by ^{1}H and ^{13}C NMR spectroscopy.

When the two acetylated glycosides were submitted to mild alkaline hydrolysis they gave α-D-N-acetyl-mannopyranosaminyl-nitrocatechol-1'-yl and for α-L-rhamnopyranosyl-nitrocatechol-1'-yl, respectively; both contained sugar and 4-nitrocatechol in a molar ratio 1:1.

Both synthetic glycosides were cleaved by an enzymatic extract of digestive tract of snail, *Helix pomatia*.

References

1.ANDERSEN, F. M., MARKHAM, K. R., (eds.), FLAVONOIDS: Chemistry, Biochemistry, and Applications, Taylor & Francis Group, Boca Raton London New York, 2006.
2.DAY, A. J., DUPONT, M. S., RIDLEY, S., RHODES, M., RHODES, M. J. C., MORGAN, M. R. A., WILLIAMSON, G., FEBS Letters **436**, 1998, p. 71
3.HIRANO, R., SASAMOTO, W., MATSUMOTO, A., ITAKURA, H., IGARASHI O., KONDO, K., J. Nutr. Sci. Vitaminol., **47**, 2001, p. 357
4.LEONARD, E., YAN, Y., LIM, K. H., KOFFAS, M. A. G., Appl. Environ. Microbiol. **71**, 2005, p. 8241
5.DEEB, D., GAO, X., JIANG, H., ARBAB, A. S., DULCHAVSKY, S. A., GAUTAM, S. C., Anticancer Res. **30**, 2010, p. 3333
6.HELFERICH, B., SCHMITZ-HILLEBRECHT, E., Chem. Ber. **66**, 1933, p. 378
7.HOPPE, H.-G. In Handbook of Methods in Aquatic Microbial Ecology; Kemp, P. P.; Sherr, B. F.; Sherr, E. B.; Cole, J. J., Eds.; Lewis Publishers, Boca Raton, Fla., 1993.
8.MANAFI, M., KNEIFEL, W., BASCOMB, S., Microbiol. Rev. **55**, 1991, p. 335
9.IGA, D. P., SCHMIDT, R. R., IGA, S., HOTOLEANU, C. L., DUICA, F., NICOLESCU, A., GITMAN, S. S., Turk. J. Chem. **37**, 2013, p. 299
10.MICHAEL, A., Am. Chem. Soc., **I**, 1879, p. 305
11.FISCHER, E., ARMSTRONG, E. F., Ber. Dtsch. Chem. Ges., **34**, 1901, p. 2885
12.FISCHER, E., RASKE, K., Ber. deut. Chem. Ges. **42**, 1909, p. 1465
13.FISCHER, E., v. MECHEL, L., Chem. Ber. **49**, 1916, p. 2813
14.SEIDMAN, M., LINK, K. P., J. Am. Chem. Soc. **72**, 1950, p. 4324
15.LEDERBERG, J., J. Bacteriol. **60**, 1950, p. 381
16.IGA, D. P., IGA, S., NICOLESCU, A., CHIRA, N.-A., Rev. Roum. Chim. **55**, 2010, p. 357
17.IGA, D. P., IGA, S., VELICU, N., PETRUT, T., CONDUR, N.-D., Roum. Biotechnol. Lett. **15**, 2010, p. 5096
18.ROBINSON, D., Biochem. J. **63**, 1956, p. 39
19.COLOMBO, V., VIEIRA, A. A. H., MORAES, G., Brazilian J. Microbiol. **35**, 2004, p. 110
20.MONTGOMERY, E. M., RICHTMYER, N. K., C. S. HUDSON, J. Am. Chem. Soc. **64**, 1942, p. 690
21.MONTGOMERY, E. M., RICHTMYER, N. K., HUDSON, C. S., J. Org. Chem., **10**, 1945, p. 194
22.CONCHIE, J., FINDLAY J., LEVVY, G. A., Biochem. J. **71**, 1959, p. 318
23.SMITS, E., ENGBERTS, J. B. F. N., KELLOGG, R. M., VAN DOREN, H. A., J. Chem. Soc., Perkin Trans. **1**, 1996, p. 2873
24.DE BRUYNE, C. K. WOUTERS-LEYSEN, J., Carbohydr. Res. **18**, 1971. p. 124
25.KRÖGER, L., THIEM, J., Carbohydr. Res. **342**, 2007, p. 467
26.BOROOAH, J., LEABACK, D. H., WALKER, P. G., Biochem. J. **78**, 1961, p. 106
27.AIZAWA, K., J. Biochem. (Tokyo), **30**, 1939, p. 89
28.JERMYN, M. A., Aust. J. Chem. **8**, 1955, 403
29.FINDLAY, J., LEWVY G. A., MARSH, C. A., Biochem. J., **69**, 1958, p. 467
30.BURTON, M. EP 1 436 411 B1/2005.
31.ROTH, N. G., ROTH, N. J. US Patent 7,273,719 B2/2007.
32.TALALAY, P., FISHMAN, W. H., HUGGINS, C., J. Biol. Chem. **166**, 1946, p. 757
33.JAMES, A. L., PERRY, J. D., FORD, M., ARMSTRONG, L., GOULD, F. K., Appl. Environ. Microbiol. **62**, 1996, p. 3868
34.JAMES, A. L., PERRY, J. D., FORD, M., ARMSTRONG, L., GOULD, F. K., J. Appl. Microbiol. **82**, 1997, p. 532
35.JAMES, A. L., CHILVERS, K. F., PERRY, J. D., AMSTRONG, L., GOULD, F. K., Appl. Environ. Microbiol. **66**, 2000, p. 5521
36.JAMES, A. L., PERRY, J. D., CHILVERS, K., ROBSON, I. S., ARMSTRONG L., ORR, K. E., Lett. Appl. Microbiol. **30**, 2000, p. 336
37.MANNICH, C., Ann., **394**, 1912, p. 223
38.MOTABAR, O., SHI, Z.-D., GOLDIN, E., LIU, K., SOUTHALL, N., SIDRANSKY, E., AUSTIN, C. P., GRIFFITHS, G. L., ZHENG, W., Anal Biochem. **390**, 2009, p. 79
39.KOENIGS, W., KNORR, E., Chem. Ber. **34**, 1901, p. 957
40.ROBINSON, D., SMITH, J. N., WILLIAMS, R. T., Biochem. J. **50**, 1951, p. 221
41.CHRASTIL, J., WILSON, T. J., J. Pharmacol. Exp. Ther. **193**, 1975, p. 631
42.LUUKANEN, L., KILPELÄINEN, I., KANGAS, H., OTTOILA, P., ELOVAARA, E., TASKINEN, J., Bioconjugate Chem. **10**, 1999, p. 150
43.ANTONIO, L., GRILLASCA, J.-P., TASKINEN, J., ELOVAARA, E., BURCHELL, B., PIET, M.-H., ETHELL, B., OUZZINE, M., FOURNEL-GIGLEUX, S., MAGDALOU, J., Drug Metab. Dispos. **30**, 2002, p. 199
44.ELOVAARA, E., MIKKOLA, J., LUUKKANEN, L., ANTONIO, L., FOURNEL-GIGLEUX, S., BURCHELL, B., MAGDALOU, J., TASKINEN, J., Drug Metab. Dispos. **32**, 2004, p. 1426
45.CHAUHAN, A., SAMANTA, S. K., JAIN, R. K., J. Appl. Microbiol. **88**, 2000, p. 764
46.FAROOQUI, A. A., Clin. Chim. Acta **100**, 1980, p. 285
47.RIP, J. W., GORDON, B. A., Clin. Biochem. **31**, 1998, p. 29
48.SUN, J.-P., WU, L. FEDOROV, A. A., ALMO, S. C. , ZHANG, Z.-Y., J. Biol. Chem. **278**, 2003, p. 33392
49.HUANG, Z., WONG, C. F., Biophys. J. **93**, 2007, p. 4141

50.MÄNNISTÖ, P. T.; KAAKKOLA, S., Pharmacol. Rev. **51**, 1999, p. 593
51.KISO, M., ANDERSON, L., Carbohydr. Res., **72**, 1979, c12-c14.
52.ASNANI, A., AUZANNEAU, F.-I., Carbohydr. Res. **338**, 2003, p. 1045
53.COMEGNA, D., Synthesis of rhamnanic oligosaccharides, PhD dissertation, Universita Degli Studi Di Napoli Federico II, 2006-2007, p 49.
54.VAN DER KAADEN, A., GERWIG, G. J., KAMERLING, J. P., VLIEGENTHART, J. F. G., TIESJEMA, R. H., Eur. J. Biochem. **152**, 1985, p. 663
55.REZANKA, T., SIRISTOVA, L., MELZOCH, K., SIGLER, K., Lipids **46**, 2011, p. 249
56.YATES, E. A., VALDOR, J.-F., HASLAM, S. M., MORRIS, H. R., DELL, A., MACKIE W., KNOX, J. P., Glycobiology **6**, 1996, p. 131
57.GRANJA-PEREZ, P. E., GAMBOA-ANGULO, M. M., ESCALANTE-EROSA, F., PENA-RODRIGUEZ, L. M., Rev. Soc. Quim. Mex. **43**, 1999, p. 188

Manuscript received: 16.12.2014