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High-resolution mass spectra of biotinylated, HEG-spacered molecular probes with oligosaccharide fragments of the capsular polysaccharides from *Streptococcus pneumoniae*

Alexander O. Chizhov, Elena V. Sukhova, Elena A. Khatuntseva, Yury E. Tsvetkov and Nikolay E. Nifantiev*

N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 119991 Moscow, Russian Federation. Fax: +7 499 135 5328; e-mail: nen@ioc.ac.ru

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High-resolution electrospray mass spectra (MS and MS/MS) of biotinylated, hexaethylene glycol (HEG) spacered molecular probes bearing immunogenic oligosaccharides, fragments of capsular polysaccharides of *Streptococcus pneumoniae*, were interpreted (four examples). Characteristic fragmentation of the aglycone at the end of the HEG chain distant from the biotin fragment has been revealed.

Carbohydrate determinants play an important role in various life processes, for example, in cell–cell or host–pathogen interactions.[1] To study specificity of carbohydrate–protein interactions, molecular probes bearing a carbohydrate determinant and a chemical label ('tag') are used, where the tag makes the probe specifically distinguishable. Biotin fragment is generally used as a label which is able to bind strongly to such proteins as avidin and streptavidin.[2] To reduce undesirable hydrophobicity and to optimize orientation of carbohydrate fragment in specific interactions, a flexible hydrophilic spacer is placed between the carbohydrate fragment and the tag. Such molecular probes of the general structure carbohydrate–C2/C3-spacer–hexaethylene glycol–biotin, carrying biologically valuable carbohydrate determinants, have been previously synthesized in our lab and used to reveal and quantitate protein–carbohydrate interactions by surface plasmon resonance.[3] These glycoconjugates are multifunctional and can be employed for the preparation of hybrid systems,[4] *e.g.*, glycochips and glycoarrays of different types (for example, see ref. 5), where oligosaccharide ligands are immobilized on a solid surface and do not undergo unspecific and thus not regulated sorption on microtiter plates as it is in the case of polymer-bound coating antigens.[6]

Molecular probes **6**–**9** (Scheme 1) carrying immunogenic fragments of capsular polysaccharides of dangerous pathogens *Streptococcus pneumoniae*[7] of types 3 and 14 were prepared from 3-aminopropyl glycoside **1**[8(a)] (represents the repeating unit of the polysaccharide from *S. pneumoniae* type 3) and 2-aminoethyl glycosides **2**–**4**[8(*b*)] (correspond to fragments of the polysaccharide from *S. pneumoniae* type 14).

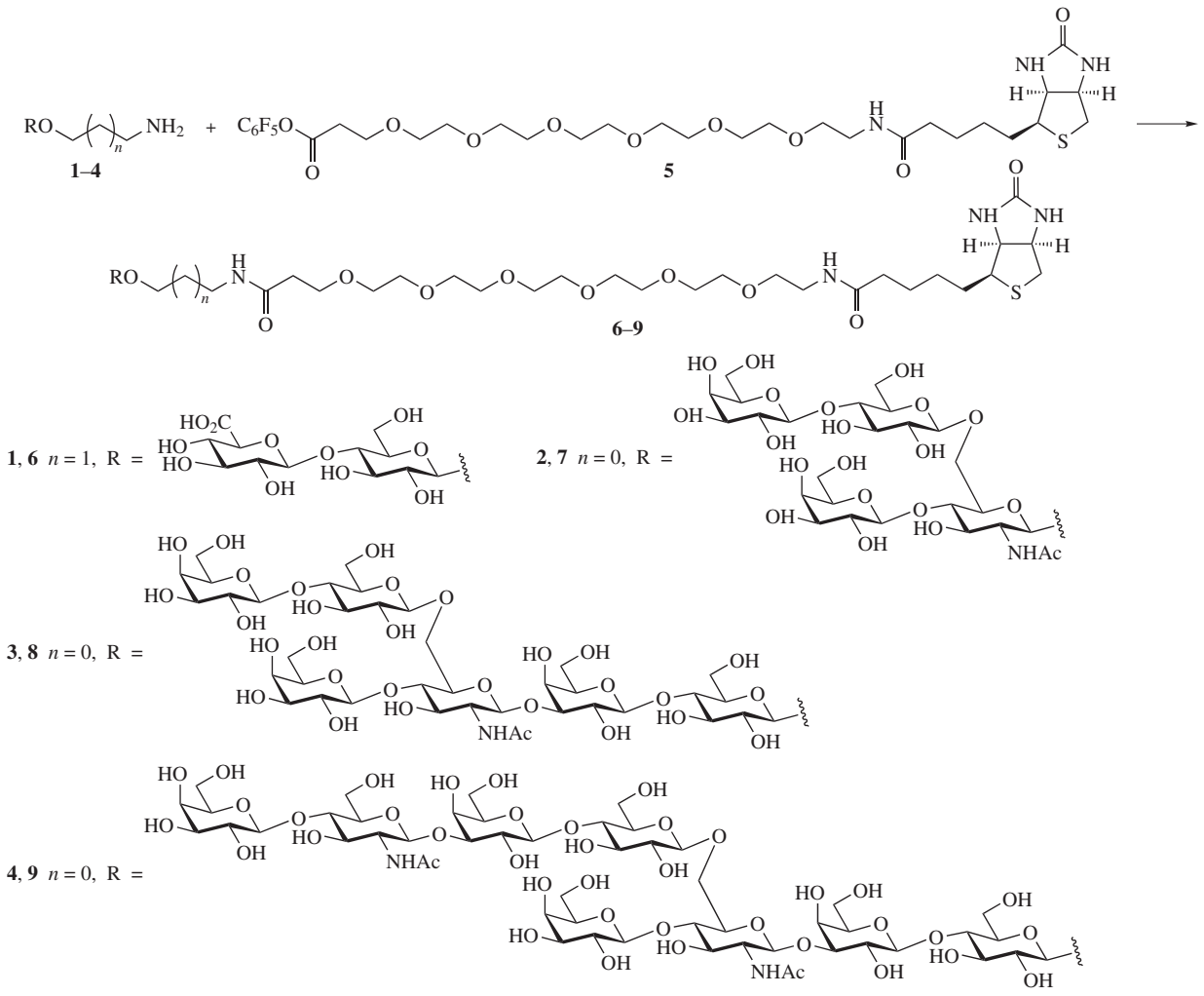
Preparation of biotinylated conjugates **6**–**9**, which continues our previous works on the synthesis of glycoconjugates bearing pneumococcal oligosaccharide antigens,[9] included acylation of spacered compounds **1**–**4** with the active ester **5**[3] to afford the target molecular probes **6**–**9**. Their structures were confirmed by ^{1}H NMR data (see Online Supplementary Materials). Mass spectrometry is the most sensitive method of selective determination of complex organic compounds in various matrices.[10] To understand processes occurring during mass spectrometric analyses of complex carbohydrates, the dependence of fragmentation on the structure of these compounds and the ionization method used has to be revealed. These studies were started by Professors N. K. Kochetkov and O. S. Chizhov more than fifty years ago in application to EI MS of methyl ethers of simple glycosides and other monosaccharide derivatives[11] and are still in progress in our institution.

ESI and MALDI mass spectra of some spacered, biotinylated reagents were described in two publications in the last decade. Thus, Banoub *et al*. interpreted high-resolution mass spectra (the second and the pseudo-third orders) of five biotinylated linkers, *i.e.*, four photoreactive agents and the dimer in which biotin residues were linked with pentaethylene glycol.[12] The latter one and the psoralen–biotin conjugate with a triethylene glycol linker may be considered as analogues of our molecular probes. Kapkova has studied MALDI TOF and ESI–ion trap low-resolution mass spectra of different carbohydrate reagents prepared from mono-, di-, and oligosaccharides related to N-chains of glycoproteins by treatment with biotinamidocaproyl hydrazide.[13] In this work we studied ESI MS of the spacered molecular probes **6**–**9** (Figure 1) to reveal regularities in collision-induced dissociation (CID).†

First-order positive mode ESI mass spectra of compounds **6**–**9** consist of peaks of singly and doubly charged adducts of the molecules with proton, ammonium, sodium, and potassium and their combinations, and negative ion mode mass spectra contain $[M-H]^-$, $[M-2H]^{2-}$, and $[M-3H]^{3-}$ (Table S1, Online Supplementary Materials).

The corresponding CID MS/MS of $[M+H]^+$ and $[M+NH_4]^+$ were similar (activation of $[M+NH_4]^+$ gives $[M+H]^+$, further fragmentation of the latter results in intense peaks), secondary-order mass spectra of metallized molecules differs from MS/MS

† High resolution mass spectra were measured on a Bruker maXis instrument.[10(*a*),14] The measurements were performed in a positive ion mode (interface capillary voltage, –4.5 kV) or in a negative ion mode (3.3 kV); ISCID energy 0.0 eV, scanning mass range from *m*/*z* 50 to 3000. External and internal calibrations (both MS and MS/MS) were done with Electrospray Tuning Mix™ (Agilent). A syringe injection was used for solutions of studied compounds in an acetonitrile/water (50/50 vol%) mixture. Nitrogen was applied as a dry gas (4.0 dm^3 min^{-1}), sheath gas (4 bar), and a collision gas; interface temperature was set at 180 °C. No additives (acids, metal salts, *etc*.) were used. TuneLow method was used to observe small ions (lower than *m*/*z* 300). Compositions of primary and fragment ions were calculated from their accurate masses using the corresponding option of the Bruker Compass 1.3 program. The description of mass spectrometric fragmentation of oligosaccharides is given as generally accepted.[15,16]



Scheme 1

of $[M+H]^+$ or $[M+NH_4]^+$.[17] Nevertheless, in all of them for **6–9** one can observe elimination of all of the carbohydrate residues from the 'non-reducing' end to the aglycone (Figure 1, Figures S1–S4 and Tables S2–S19 in Online Supplementary Materials) in accordance with well-established regularities of fragmentations of positive ions of oligosaccharides and glycosides (B and Y series).[15,16]

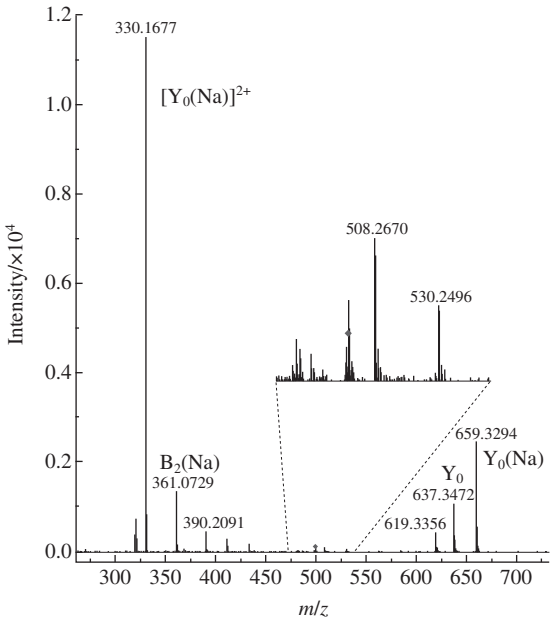


Figure 1 High-resolution ESI MS/MS of an $[M+H+Na]^{2+}$ ion at *m/z* 499.2 of compound **6** (activation energy 20 eV).

The fragmentation of the aglycone occurs predominantly at the distant from the biotin fragment C–O bond in the HEG spacer resulting in the formation of $C_{22}H_{42}N_3O_8S^+$ (*m/z* 508, from protonated molecules), $C_{22}H_{41}N_3O_8SNa^+$ (*m/z* 530, from $[M+Na]^+$) (Figure 1, inset), and $C_{22}H_{42}N_3O_8SK^+$ (*m/z* 546, from $[M+K]^+$, Table S4). In Figure 2 the corresponding cleavage is marked by an asterisk. For $[M+2H]^{2+}$ ion of **6**, small peaks of ions corresponding to lowest homologues (one, two or three C_2H_4O groups less than the fragment mentioned above, Table S5) were present. At low *m/z*, a small peak of biotin-containing ion at *m/z* 270 ($C_{12}H_{20}N_3O_2S^+$) was observed, the latter was previously described by Banoub *et al.*[12]

Note that in the negative ion mode CID MS/MS of $[M-H]^-$ and $[M-2H]^{2-}$ of **6**, a cleavage of the same C–O bond in the HEG spacer distant from biotin fragment (the ion corresponds to $C_{22}H_{40}N_3O_8S^-$, calc. *m/z* 506.2542, Tables S7 and S8, marked by an asterisk, see also Figure 2) was observed. Carbohydrate-containing fragments resulting from the cleavage of the same C–O bond in the HEG spacer (*i.e.*, in opposite direction) are marked by a double asterisk (Table S15). In the CID MS/MS of the $[M-2H]^{2-}$ ion, the most abundant are doubly charged ions resulting from elimination of H_2S and H_2CS (**6**, Figure S1, Table S8).

In summary, HEG-spacered carbohydrate molecular probes give conclusive ESI MS and CID MS/MS. In the latter, along with expected B and Y series of peaks, unusual cleavage of the

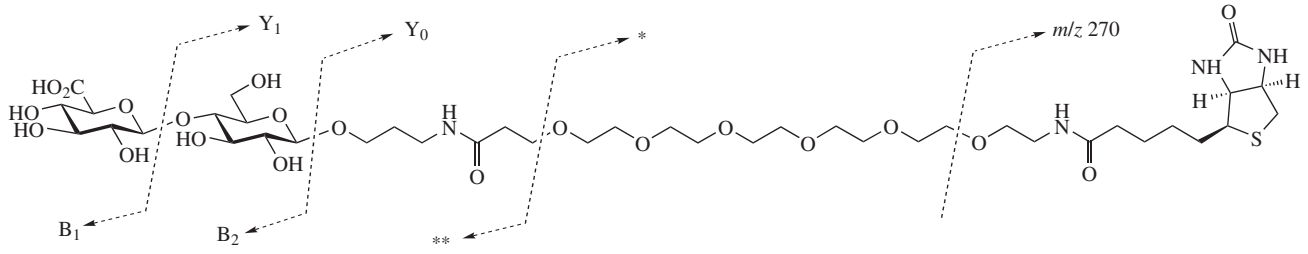


Figure 2 Fragmentation of the biotinylated molecular probe **6** under CID MS/MS.

most distant from biotin moiety C–O bond was found for both positive and negative ion modes. This cleavage may be considered as a characteristic CID fission for HEG-spacered biotin probes (the general results will be published soon). Similar fragmentation was also observed in the case of biotinylated conjugates bearing HNK-1 antigenic trisaccharide ligands.[18]

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2015.11.020.

References

1 *Carbohydrates in Chemistry and Biology. Part I: Chemistry of Saccharides, vols. 1 and 2, Part II: Biology of Saccharides, vols. 3 and 4*, eds. B. Ernst, G. W. Hart and P. Sinay, Wiley-VCH, Weinheim, 2000.
2 E. Diamandis and T. K. Christopoulos, *Clin. Chem*., 1991, **37**, 625.
3 Y. E. Tsvetkov, M. Burg-Roderfeld, G. Loers, A. Arda, E. V. Sukhova, E. A. Khatuntseva, A. A. Grachev, A. O. Chizhov, H.-C. Siebert, M. Schachner, J. Jimenes-Barbero and N. E. Nifantiev, *J. Am. Chem. Soc*., 2012, **134**, 426.
4 V. P. Ananikov, E. A. Khokhlova, M. P. Egorov, A. M. Sakharov, S. G. Zlotin, A. V. Kucherov, L. M. Kustov, M. L. Gening and N. E. Nifantiev, *Mendeleev Commun.*, 2015, **25**, 75.
5 O. E. Galanina, M. Mecklenburg, N. E. Nifantiev, G. V. Pazynina and N. V. Bovin, *Lab Chip*, 2003, **4**, 260.
6 E. A. Kurbatova, D. S. Vorobiov, I. B. Semenova, E. V. Sukhova, D. V. Yashunsky, Y. E. Tsvetkov and N. E. Nifantiev, *Biochemistry (Moscow)*, 2013, **78**, 818 (*Biokhomiya*, 2013, **78**, 1046).
7 M. L. Gening, E. A. Kurbatova, Yu. E. Tsvetkov and N. E. Nifantiev, *Russ. Chem. Rev*., 2015, **84**, 1100.
8 (*a*) D. F. Lefeber, J. P. Kamerling and J. F. G. Vliegenthart, *Chem. Eur. J*., 2001, **20**, 4411; (*b*) E. V. Sukhova, D. V. Yashunsky, Yu. E. Tsvetkov, E. A. Kurbatova and N. E. Nifantiev, *Russ. Chem. Bull., Int. Ed*., 2014, **63**, 511 (*Izv. Akad. Nauk, Ser. Khim.*, 2014, 511).
9 (*a*) N. K. Kochetkov, N. E. Nifant'ev and L. V. Backinowsky, *Tetrahedron*, 1987, **43**, 3109; (*b*) N. E. Nifant'ev, L. V. Backinowsky and N. K. Kochetkov, *Carbohydr. Res.*, 1988, **174**, 61.
10 (*a*) P. A. Belyakov, V. I. Kadentsev, A. O. Chizhov, N. G. Kolotyrkina, A. S. Shashkov and V. P. Ananikov, *Mendeleev Commun*., 2010, **20**, 125; (*b*) A. K. Przybył and Z. Nowakowska, *Mendeleev Commun.*, 2013, **23**, 101.
11 N. K. Kochetkov and O. S. Chizhov, *Adv. Carbohydr. Chem*., 1966, **21**, 39.
12 S. Sioud, B. Genestie, F. Jahouh, P. Martin and J. Banoub, *Rapid Commun. Mass Spectrom*., 2009, **23**, 1941.
13 P. Kapkova, *Rapid Commun. Mass Spectrom*., 2009, **23**, 2775.
14 R. E. Ardrey, *Liquid Chromatography – Mass Spectrometry: An Introduction*, John Wiley & Sons, Chichester, 2003.
15 B. Domon and C. E. Costello, *Glycoconjugate J*., 1988, **5**, 397.
16 J. Zaia, *Mass Spectrom. Rev*., 2004, **23**, 161.
17 L. M. Teesch and J. Adams, *Org. Mass Spectrom*., 1992, **27**, 931.
18 A. O. Chizhov, E. V. Sukhova, E. A. Khatuntseva, M. L. Gening, Y. E. Tsvetkov and N. E. Nifantiev, *Carbohydr. Res.*, 2015, **417**, 15.

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