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Synthesis of *N*-acetyllactosamine based branched hexasaccharide

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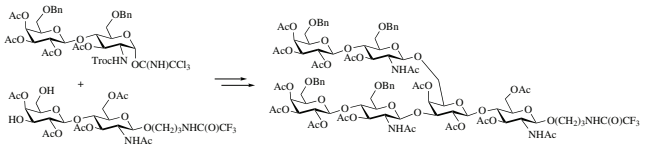
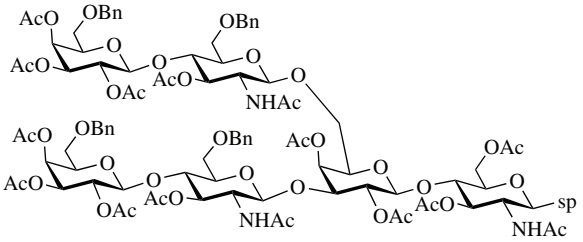
The protected spacer-armed branched tri-*N*-acetyllactosamine has been synthesized as a building block for the synthesis of biantennary ABH blood group antigens. The key synthetic step is the simultaneous glycosylation of the 3′- and 6′-OH groups of an *N*-acetyllactosamine glycosyl acceptor by *N*-Troc-lactosamine trichloroacetimidate.



ABH blood group antigens are presented on human red blood cells (RBCs) as terminal fragments of complex oligosaccharides belonging to glycolipids and glycoproteins. The minimal structural fragments common to all A or B antigens represent the terminal A trisaccharide GalNAcα1-3(Fucα1-2)Galβ or its B counterpart Galα1-3(Fucα1-2)Galβ, which are formed from H disaccharide Fucα1-2Galβ through the addition of GalNAcα or Galα units, respectively. The core structures of ABH glycans, related to RBCs, consist predominantly of the repeating *N*-acetyllactosamine units and are called type 2 core chains. The diversity of the ABH glycans can be illustrated by their simplest representatives that consist of single A or B tetrasaccharide antigenic determinant and one LacNAc core unit, as well as by the complex ones, originated from polyglycosylceramides or glycoproteins, that include up to eight antigenic determinants and have highly branched core chains with up to 20 LacNAc residues, the branching point being always 3,6-substituted galactose with an additional chain at the O-6 position.[1]

Chemical and chemoenzymatic syntheses of the ABH glycans are well developed. Nevertheless, these glycans are typically obtained in the form of single, or monovalent, antigens with either A or B antigenic determinant.[2–9] In scope of our plans to synthesize biantennary divalent A and B oligosaccharides with branched trilactosamine core and two A or B tetrasaccharide fragments, intended for further investigation of the core chains influence on the antigenicity of ABH glycans, the branched trilactosamine building block **1** has been required.

The reported synthetic scheme for 3,6-branched trilactosamine[10] employed peracetylated *N*-Troc-lactosamine bromide as a glycosyl donor as well as *N*-acetyllactosamine as a glycosyl acceptor with the free OH group at C-3′ and the OBn group at C-6′, and represented a stepwise glycosylation procedure including (i) glycosylation of the 3′-OH group, (ii) *N*-Troc → *N*-Ac transformation, (iii) hydrogenolysis of the 6-*O*-benzyl group at internal Gal of the resulting bislactosamine, and finally (iv) glycosylation of the deprotected 6-OH group by the same starting peracetylated *N*-Troc-lactosamine bromide. The reported approach appeared to be unsuitable for this work, because the 6-*O*-benzyl groups were necessary at the terminal galactose residues of compound **1** to enable further deblocking of the 3-OH groups at the same residues through a 3,4-orthoester moiety installation and its subsequent opening, thus making the hydrogenolysis step (iii) inappropriate.



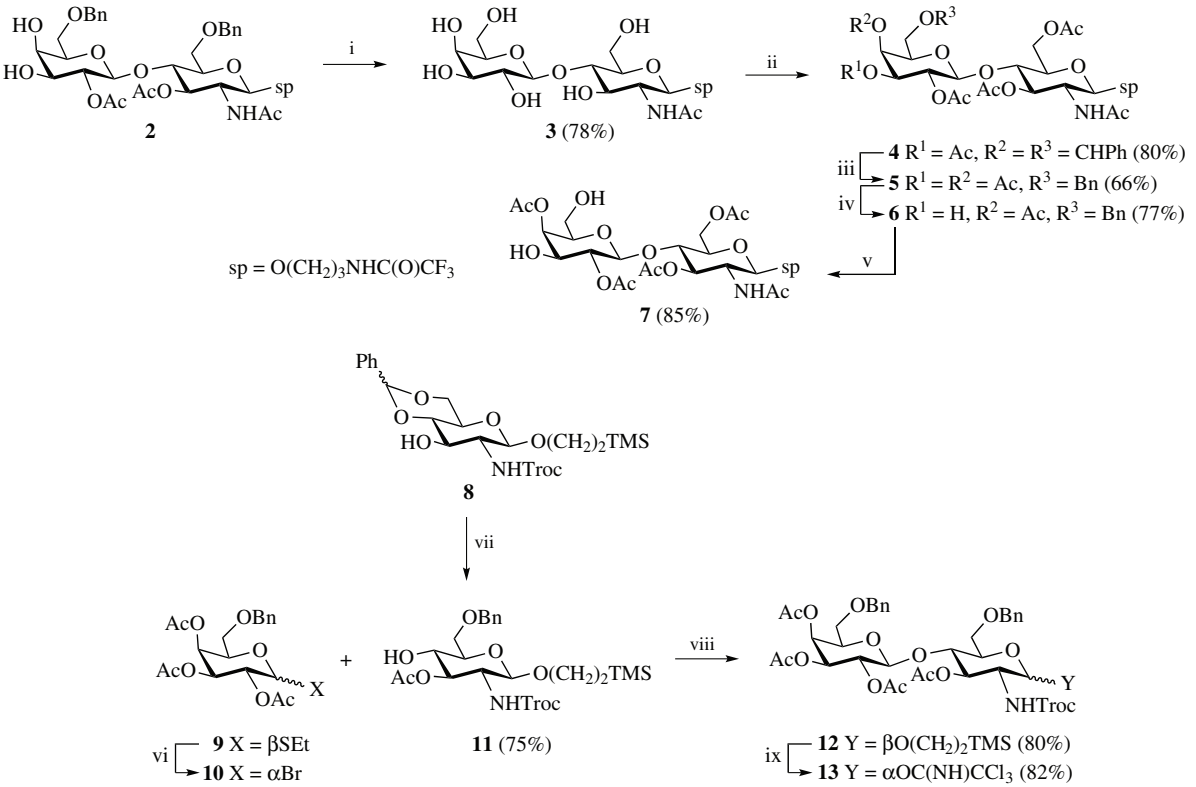
1

sp = $O(CH_2)_3NHC(O)CF_3$

For the synthesis of compound **1**, we proposed an alternative approach based on simultaneous glycosylation of the 3′- and 6′-hydroxyl groups of *N*-acetyllactosamine. To perform this reaction, we first synthesized glycosyl acceptor **7** and glycosyl donor **13** (Scheme 1).

A spacer-armed lactosamine **2**[2] was a starting reactant in the synthesis of glycosyl acceptor **7**. Compound **2** was deacetylated by MeONa in MeOH and then the benzyl groups were removed by H_2 on Pd/C, affording completely deblocked glycoside **3** in 78% yield. Further benzylidenation by $PhCH(OMe)_2$ in the presence of TsOH with the following acetylation by Ac_2O in pyridine resulted in compound **4** in 80% yield. Reductive opening of the benzylidene acetal moiety in disaccharide **4** with $NaBH_3CN$ and MsOH,[11] followed by acetylation, led to the lactosamine derivative **5** in 66% yield. To deblock its 3′-OH group, compound **5** was deacetylated by MeONa in MeOH and then treated with $MeC(OEt)_3$ in the presence of TsOH, affording the cyclic 3′,4′-orthoester. The following acetylation by Ac_2O in pyridine and the opening of the orthoester cycle by 80% AcOH resulted in the 3′-OH derivative **6** (77% yield). Finally, hydrogenolysis of compound **6** afforded 3′,6′-diol **7** in 85% yield. The presence of free hydroxyls at C-3′ and C-6′ of compound **7** was confirmed by ^1H NMR spectroscopy. The signals of H-3′, H-6′a and H-6′b were observed in a high field with δ 3.82, 3.48 and 3.67 ppm, respectively (see Online Supplementary Materials for details).

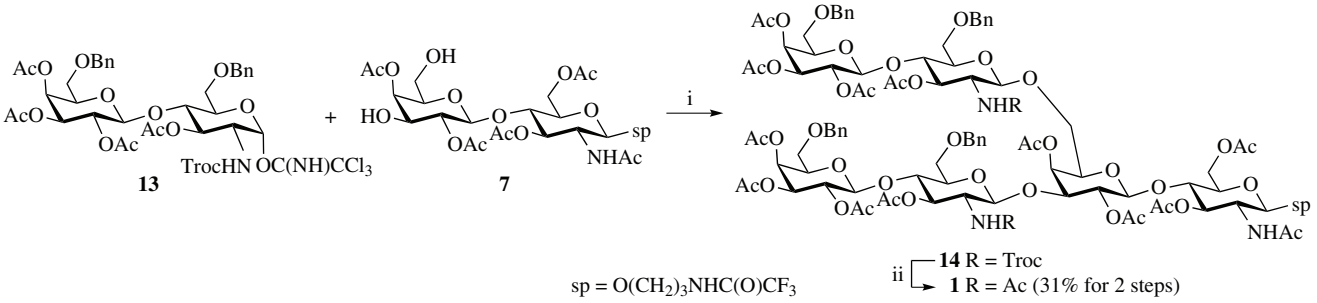
The synthesis of glycosyl donor **13** started from the known *N*-Troc-derivative of glucosamine **8**,[12] which was acetylated by



Scheme 1 *Reagents and conditions*: i, MeONa, MeOH, then H_2, Pd/C, MeOH; ii, $PhCH(OMe)_2$, TsOH, DMF, then Ac_2O, pyridine; iii, $NaBH_3CN$, MsOH, THF, then Ac_2O, pyridine; iv, MeONa, MeOH, then $MeC(OEt)_3$, TsOH, MeCN, then Ac_2O, pyridine, then 80% AcOH; v, H_2, Pd/C, MeOH; vi, Ac_2O, pyridine, then $NaBH_3CN$, MsOH, THF; vii, Br_2, CH_2Cl_2; viii, AgOTf, TMU, MS 4 Å, CH_2Cl_2; ix, TFA, CH_2Cl_2, then Cl_3CCN, DBU, CH_2Cl_2.

Ac_2O in pyridine and then subjected to reductive opening of the benzylidene acetal moiety by $NaBH_3CN$ and MsOH, thus affording glycosyl acceptor **11** in 75% yield. The 4-OH group of compound **11** was glycosylated with bromide **10**, obtained in turn from the corresponding known[13] SEt glycoside **9**. The glycosylation was carried out using AgOTf as a promoter and led to *N*-Troc-lactosamine **12** in 80% yield (H-1′: 4.45 ppm, $J_{1,2}$ 7.94 Hz). The anomeric protecting group $TMSCH_2CH_2$ in compound **12** was then cleaved under acidic conditions by TFA in CH_2Cl_2,[14] and the resulted hemiacetal was converted into trichloracetimidate **13** by treatment with Cl_3CCN in the presence of DBU[15,16] (yield 82%). The structure of compound **13** was confirmed by ^{1}H NMR spectroscopy. The singlet of the C(NH) CCl_3 proton at 8.73 ppm and the doublet of H-1 at 6.41 ppm with $J_{1,2}$ 3.6 Hz proved the formation of α-trichloracetimidate (see Online Supplementary Materials).

The key step on the route towards compound **1** was the glycosylation of both 3′- and 6′-OH groups of acceptor **7** with trichloracetimidate **13** (Scheme 2). The reaction was carried out in CH_2Cl_2 using 3.5 fold excess of compound **13**, *i.e.* 1.75 fold excess for each hydroxyl group, and TMSOTf as a promoter, and resulted in a mixture of glycosides containing the required product **14**. To simplify the isolation of the product, the mixture was treated with TBAF in THF and then acetylated by Ac_2O in pyridine, thus the *N*-Troc protecting groups were converted into *N*-Ac groups.[17] The target branched tri-*N*-acetyllactosamine building block **1** was then isolated in 31% yield for the two steps, namely glycosylation and *N*-Troc → *N*-Ac conversion. The structure of compound **1** was confirmed by ^{1}H NMR spectroscopy (for the complete spectra, see Online Supplementary Materials). The signals of H-1 protons of the nonreducing GlcNAc moiety appeared as doublets at 4.51 and 4.56 ppm with $J_{1,2}$ 8.0 and 9.0 Hz, respectively, which corresponded to the β-configuration of the two formed glycosidic bonds. The signals of the H-3 and H-6 protons of the disubstituted Gal residue appeared in a high field at 3.89, 3.43 and 3.71 ppm for H-3 II, H-6a II and H-6b II, respectively, while the signals of H-2 and H-4 appeared in a low field at 4.71 and 5.32 ppm for H-2 II and H-4 II, respectively. This confirmed, that no migration of acetyl groups took place, as well as that both 3′- and 6′-hydroxyls of compound **7** were glycosylated. The appearance of three singlets related to the NC(O)Me groups at 1.68, 1.75 and 1.76 ppm corresponded to the complete *N*-Troc → *N*-Ac conversion.



Scheme 2 *Reagents and conditions*: i, TMSOTf, MS 4 Å, CH_2Cl_2; ii, TBAF, THF, then Ac_2O, Py.

The synthesized tri-*N*-acetyllactosamine building block **1** is considered valuable for its conversion into the corresponding 3′,3′-diol with the hydroxyl groups at C-3-positions of the terminal Gal residues. This synthesis can be realized *via* the orthoester installation/opening procedure, as described above for compound **5**. Further glycosylation of the 3′,3′-diol will open the way to complex biantennary oligosaccharides related to the ABH blood system. The advantages of the presented block synthesis as compared to the known stepwise one are the following: (i) simplicity and time economy due to the simultaneous glycosylation of 3′- and 6′-OH groups in the lactosamine acceptor, as well as the synchronous *N*-Troc → *N*-Ac transformation in the two glucosamine units of the resulting hexasaccharide; and (ii) the possibility to use product **1** for the synthesis of complex biantennary glycans, in particular ABH oligosaccharides.

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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.2019.11.026.

References

1 J. Kościelak, *Transfusion Medicine*, 2001, **11**, 267.
2 G. V. Pazynina, T. V. Tyrtysh and N. V. Bovin, *Mendeleev Commun.*, 2002, **12**, 143.
3 P. J. Meloncelli and T. L. Lowary, *Carbohydr. Res.*, 2010, **345**, 2305.
4 P. J. Meloncelli, L. J. West and T. L. Lowary, *Carbohydr. Res*., 2011, **346**, 1406.
5 P. J. Meloncelli and T. L. Lowary, *Aust. J. Chem.*, 2009, **62**, 558.
6 G. Karki, V. N. Mishra and P. K. Mandal, *Glycoconjugate J.*, 2016, **33**, 63.
7 P. Zimmermann, U. Greilich and R. R. Schmidt, *Tetrahedron Lett*., 1990, **31**, 1849.
8 I. M. Ryzhov, E. Yu. Korchagina, I. S. Popova, T. V. Tyrtysh, A. S. Paramonov and N. V. Bovin, *Carbohydr. Res.*, 2016, **430**, 59.
9 O. Blixt and N. Razi, *Methods Enzymol*., 2006, **415**, 137.
10 V. V. Severov, I. M. Belyanchikov, G. V. Pazynina and N. V. Bovin, *Russ. J. Bioorg. Chem*., 2007, **33**, 122 (*Bioorg. Khim.*, 2007, **33**, 131).
11 A. I. Zinin, N. N. Malysheva, A. M. Shpirt, V. I. Torgov and L. O. Kononov, *Carbohydr. Res.*, 2007, **342**, 627.
12 V. V. Severov, G. V. Pazynina, T. V. Ovchinnikova and N. V. Bovin, *Russ. J. Bioorg. Chem.*, 2015, **41**, 147 (*Bioorg. Khim.*, 2015, **41**, 170).
13 S. Sato, Y. Ito and T. Ogawa, *Carbohydr. Res*., 1986, **155**, C1.
14 K. Jansson, T. Frejd, J. Kihlberg and G. Magnusson, *Tetrahedron Lett.*, 1988, **29**, 361.
15 K. Kalidasan, Y. Su, X. Wu, S. Q. Yao and M. Uttamchandani, *Chem. Commun.*, 2013, **49**, 7237.
16 R. R. Schmidt and W. Kinzy, in *Advances in Carbohydrate Chemistry and Biochemistry*, ed. D. Horton, Elsevier, Amsterdam, 1994, vol. 50, pp. 21–123.
17 C.-y. Huang, N. Wang, K. Fujiki, Y. Otsuka, M. Akamatsu, Y. Fujimoto and K. Fukase, *J. Carbohydr. Chem.*, 2010, **29**, 289.

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