Pergamon

Tetrahedron Letters, Vol. 36, No. 38, pp. 6839-6842, 1995
Elsevier Science Ltd
Printed in Great Britain
0040-4039/95 $9.50+0.00

0040-4039(95)01442-X

Steric Control of N-Acetylgalactosamine In Glycosidic Bond Formation

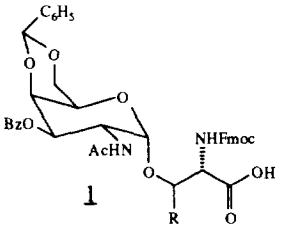
John E. Yule, Ting C. Wong, Sham S. Gandhi, Dongxu Qiu, Marc A. Riopel and R. Rao Koganty*

Biomira Inc., 2011-94 Street, Edmonton, Alberta, Canada T6N 1H1

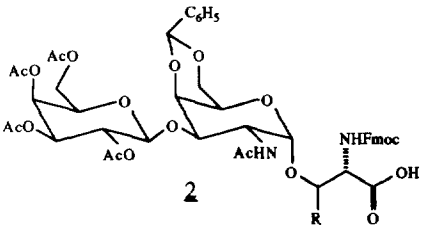
Abstract: N-Acetylgalactosamine, protected with a 4,6-cyclic acetal followed by selective acylation at 3-OH, provides an excellent donor for the synthesis of α-glycosides, particularly the cancer associated antigens such as Tn, TF, Sialyl-Tn and Sialyl-TF. This fast and efficient synthesis is easily adaptable for commercial production of mucin type glycopeptides with O-linked carbohydrate structures which are currently being investigated as vaccines against cancers.

N-Acetylgalactosamine predominantly exists in nature as an α-glycoside particularly in mucins where most O-linked oligosaccharide biosynthesis is initiated with the attachment of α-N-acetylgalactosamine to serines and threonines.[1] Mucins that are expressed by cancer cells have been receiving increasing attention with the identification of carbohydrate structures such as Tn, TF and STn as tumor associated.[2-5]

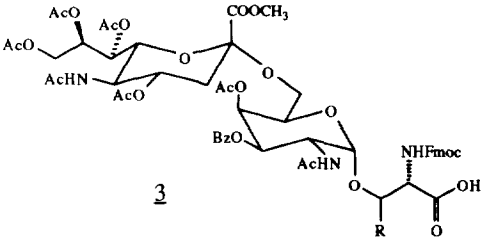
Segments of membrane anchored tumor mucin MUC-1, in the form of glycopeptides, have been under investigation as possible vaccines for the immunotherapy of a variety of cancers of epithelial origin such as cancers of breast, ovaries, lung, pancreas and colon etc. Consequently, the commercial production of glycopeptides depends a great deal on the availability of glycosylated serines and threonines (**1-3**), as suitable building blocks.



1



2

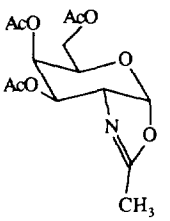


3

Ser, R=H
Thr, R=CH_3

We report here a novel synthesis of these valuable building blocks which can be adapted for the commercial production of glycopeptides that need α-GalNAc based O-linked carbohydrate structures whose synthesis hitherto has not been commercially viable. Use of N-acetylgalactosamine for the synthesis of α-N-acetylgalactosaminides has not been possible due to the collapse of the donor during glycosylation reactions

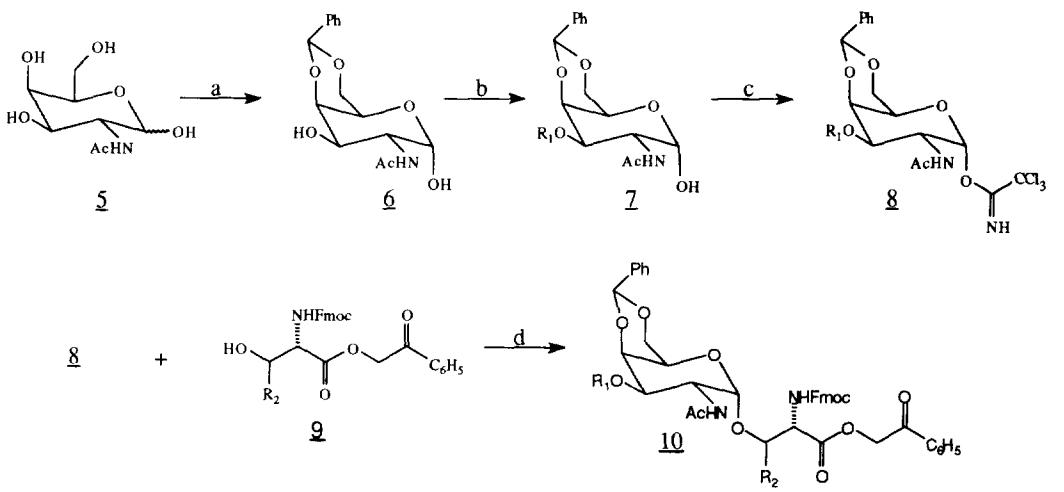
to bicyclic oxazoline[6] derivatives (**4**). Paulsen[7] and Lemieux[8] devised an elegant method to overcome the interference of 2-acetamido group with the anomeric carbon during the glycosidic bond formation, through the use of 2-azido precursor.



4

However, we found that 4,6-O-benzylidenyl-N-acetylgalactosamine (**6**) can be converted to a stable donor which reacts with an alcohol, including serine and threonine, forming α-glycosides in moderate to high yields depending on the reactivity of the acceptor.[9] The scheme shows the reaction sequence to α-N-acetylgalactosaminides of serine and threonine[10] suitable for the Fmoc based solid phase glycopeptide synthesis.

Scheme



9a, $R_2 = H$

9b, $R_2 = CH_3$

10a, $R_1 = CH_3CO/C_6H_5CO$, $R_2 = H$

10b, $R_1 = CH_3CO/C_6H_5CO$, $R_2 = CH_3$

a. Acetonitrile, p-toluene sulfonic acid, benzaldehyde dimethyl acetal; b. Pyridine, benzoyl or acetyl chloride, -25°C; c. CH_2Cl_2, CCl_3CN, DBU, 0°C; d. THF, $BF_3{\cdot}OEt_2$, Drierite®, -10°C.

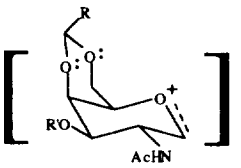
Commercially available N-acetylgalactosamine (**5**) is converted to its 4,6-O-benzylidenyl analogue (**6**) in 80-85% yield using acetonitrile as a solvent. Though, initially N-acetylgalactosamine is insoluble, the solubility increases as the reaction proceeds. The product is purified by crystallization from 95% ethanol. **6** is selectively acylated at 3-OH using either benzoyl chloride or acetyl chloride at -20°C, in 60-70% yield of 3-O-acyl-4,6-O-benzylidenyl-N-acetylgalactosamine (**7**). The 1-OH derivative is quantitatively converted to the donor trichloroacetimidate (**8**). α-Glycosides[10] of suitably protected serine and threonine (**9**) are formed in a typically BF_3-etherate catalysed reaction, in moderate yields of about 45-50%. A significant aspect of this reaction is that only traces of β-glycoside and oxazoline are formed. This remarkable stereoselectivity may be attributed to the cyclic 1,3-dioxane ring which positions the O^4 and O^6 to stabilize the oxycarbonium ion transient (**11**) which probably promotes the formation of the thermodynamically favored α-linkage. Once the 4- and 6- hydroxy groups are derivatized to cyclic acetal, the anomeric hydroxy group completely realigns to α-configuration (**6**).[11] On the other hand, 4,6-benzylidenyl N-acetylglucosamine exists as a mixture of α, β anomers, while the donor of which yields the predominantly β-glycosides. The 3-OH of **6** can be protected with an ester or a sialyl ether (TBDMS) which have very little influence on the yield of α-glycoside. Introduction of sialyl ethers or other selectively cleavable groups at 3-position may offer advantages in the synthetic extensions of the glycoside. Synthesis can be further extended by deblocking the 4,6-benzylidenyl protecting group, to sialyl Tn structure.[10] Additionally, solvents seem to have significant influence in determining the yield and distribution of α and β glycosides. Solvents, particularly those with strong donor character such as tetrahydrofuran seem to influence the α:β ratio, in addition to the reactivity of the glycosyl acceptor (table). In solvents that stabilise intermediate (**11**), α-glycoside is a predominant product irrespective of the reactivity of the alcohol, while in solvents like methylene chloride the reactivity of the alcohol seems to determine the stereochemistry of glycosidic bond. Crotyl alcohol yields predominantly β-glycoside while less reactive serine and threonine yield only α-glycoside.

The conditions for glycosylations are simple. To a reaction involving 1 to 2 g of the donor (**8**) and 1.5 equivalents of an acceptor in dry THF, 0.1 equivalents of $BF_3{\cdot}OEt_2$ in THF is added with stirring. The reaction is completed in about an hour. The yield of the glycoside is lower with threonine (20-30%) compared to serine (45-55%). However, irrespective of the yield, both form only α-glycoside.

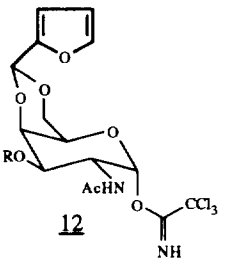
Table

Solvent	Alcohol	α:β	% Yield of α & β
Methylene chloride	Crotyl	1:9	80
	Serine	20:1	30
THF	Crotyl	9:1	80
	Serine	20:1	55

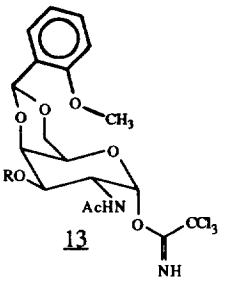
In an effort to enhance the yield of α-glycosides, we have synthesised furaldehyde (**12**) and anisaldehyde (**13**) protected donors. In both cases the protecting groups are expected to have a stabilising influence on the intermediate (**11**) thereby enhancing the yield of α-glycoside. The results are either ambiguous or there was only



11



12



13

12. R = CH_3CO/C_6H_5CO 13. R = CH_3CO/C_6H_5CO

a marginal increase in the yield of α-glycosides, depending on the solvent used. In methylene chloride as solvent, there is no difference in α-glycoside yield with (**12**) or (**13**) compared to the benzylidene protected donor. But in THF, there has been a modest increase in the yield of α-glycosides of about 15% compared to donor (**8**). The evidence indicates that the 2-acetamido group does not interfere with the formation of the glycosidic bond, though the reasons are not clear.

REFERENCES AND NOTES

1. Schachter, H.; Brockhausen, I. The Biosynthesis of Serine (Threonine)-N-Acetylgalactosamine Linked Carbohydrate Moieties. In *Glycoconjugates: Composition, Structure and Function*; Allen, H.J.; Kisailus, E.C. Eds.; Marcel Dekker, Inc.: New York, **1992**, 263-332.
2. Springer, G.F. *Science* **1984**, 224, 1198-1206.
3. Itzkowitz, S.H.; Bloom, E.J.; Kokal, W.A.; Modin, G.; Hakomori, S.-I.; Kim, Y.S. *Cancer* **1990**, 66, 1960-1966.
4. Kobayashi, H.; Toshihiko,T.; Kawashima, Y. *J.Clinical Oncology* **1992**, 10, 95-101.
5. a. Fung, P.Y.S.; Madej, M.; Koganty, R.R.; Longenecker, B.M. Cancer Res. **1990**, 50, 4308-4314. b. Longenecker, B. M.; McLean, G. D. *The Immunologist* **1993**, 1, 89-93.
6. Jacquinet, J.C.; Zurabayan, S.E.; Khorlin, A. Ya. *Carbohydrate Res.* **1973**, 32, 137-143.
7. Paulsen, H.; Kolar, C.; Stenzel, W. *Chem. Ber.* **1978**, 111, 2358-2369.
8. Lemieux, R.U.; Ratcliffe, R.M. *Can. J. Chem.* **1979**, 57, 1244-1251.
9. U.S. Patent (USSN 08/208,268) pending.
10. NMR data for compound **10a** ($R_1=C_6H_5CO$) ^1H ($CDCl_3$) - 7.35 - 8.1 (m, 24H, Ar), 6.55 (d, 1H, J=9.0 Hz, NH), 5.85 (d, 1H, J=8.5 Hz, NH), 5.63 (d, 1H, J=16.0 Hz, CH_2COPh), 5.51 (s, 1H, PhCH), 5.3 - 5.43 (m, 2H), 5.2 (d, 1H, $J_{1,2}$=3.5 Hz, H-1), 5.15 - 5.07 (m, 2H), 4.79 (brs, 1H, Serα-H), 4.2 - 4.58 (m, 7H), 3.84 - 3.99 (m, 2H); 1.8 (s, 3H, NAc), ^{13}C-nmr δ:99.52 (C-1), 100.76 (Ph CH), 155.92 (NHCO), 169.75 ($COOCH_2$), 191.57 (CH_2COPh). ^1H nmr (D_2O) of deblocked sialyl-Tn synthesised from **10a**. 4.84 (d, 1H, $J_{1,2}$=3.5 Hz), 4.14 (dd, 1H, $J_{1,2}$=3.5 Hz, $J_{2,3}$=12.0 Hz, H-2), 2.73 (dd, 1H, J=4.5 Hz, 12.5 Hz, H-3 eq), 2.07 (s, 3H, NHAc), 2.03 (s, 3H, NHAc), 1.66 (dd, 1H, J=12.4 Hz, H-3ax). Agrees with lit. Iijima and Ogawa. Carbohydrate Res. **1986**, 172, 183-193.
11. The diol **9** exists totally as α-hydroxide in organic solvents as indicated by the nmr data. ^1H (DMSO-d_6+CD_3OD) δ 5.12 (d, 1H, H-1, $J_{1,2}$=3.0 Hz); ^{13}C (DMSO-d6) 91.35 (C-1).

(*Received in USA* 8 *May* 1995; *revised* 14 *July* 1995; *accepted* 26 *July* 1995)