Carbohydrate Research 345 (2010) 1211–1215

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Note

Rapid glycosylations under extremely mild acidic conditions. Use of ammonium salts to activate glycosyl phosphites via *P*-protonation

Fumiko Matsumura, Shiro Tatsumi, Natsuhisa Oka, Takeshi Wada *

Department of Medical Genome Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Bioscience Building 702, 5-1-5 Kashiwanoha, Kashiwa, Chiba 277-8562, Japan

ARTICLE INFO

Article history:
Received 3 June 2009
Received in revised form 16 February 2010
Accepted 10 March 2010
Available online 3 April 2010

Keywords:
Glycosylation
Glycosyl phosphites
Ammonium salts
P-Protonation
4,4′-Dimethoxytrityl group

ABSTRACT

Trifluoromethanesulfonic acid salts of tertiary amines were employed as extremely mild acidic activators for rapid glycosylations. Glycosyl phosphite triesters bearing an acid-labile 4,4′-dimethoxytrityl (DMTr) group for transient protection worked as glycosyl donors effectively in the presence of the activators to afford the corresponding disaccharides in good yields without loss of the DMTr group.

Oligosaccharides and their conjugates with other biomolecules (glycoconjugates) play important roles in a wide variety of biological processes and extensive studies are currently in progress to clarify the whole picture.[1–3] Oligosaccharides and glycoconjugates with defined structures are indispensable for such studies.[4–6] Chemical synthesis is a major approach to obtain these materials and advantageous because it can provide pure materials in sufficient quantities at relatively low costs. Oligosaccharides and glycoconjugates containing chemical modifications at defined sites are also available by this approach and used as probes.[7] In addition to the applications to glycobiology, chemically synthesized fragments of these carbohydrates and their modified analogues have been applied to therapeutic studies.[8,9]

Many of oligosaccharides and glycoconjugates have rather complex structures. The complexity comes from the diversity of monosaccharides, which have multiple hydroxy groups and can be connected to each other at different positions through α- or β-glycosyl bonds. To synthesize these compounds, the development of efficient glycosylation reactions that can be performed under mild reaction conditions is strongly required so that a variety of orthogonal protecting groups can be used.[4] With this background, we sought to develop a glycosylation reaction that could be performed under extremely mild acidic conditions and found that salts of tertiary amines consisting of less-nucleophilic components were useful for rapid glycosylations using glycosyl phosphite triesters as glycosyl donors. The results of this study are described in this paper.

Glycosyl phosphite triesters are one of the most reactive glycosyl donors developed so far and have been applied to the synthesis of a wide variety of oligosaccharides and glycoconjugates.[10] Since the early reports on their use as glycosyl donors,[10a–c] strong Lewis acids, typically trimethylsilyl trifluoromethanesulfonate (TMSOTf), 10a,b have been used for their activation. On the other hand, some research groups have reported that the glycosyl phosphites can be activated under mild acidic conditions.[10g–j] For example, Hashimoto et al. have reported that glycosyl phosphites can be activated by 2,6-*tert*-butylpyridinium iodide (DTBPI) to give the corresponding O-glycosylation products in good yields.[10g] However, the relatively long reaction time would be a bottleneck for the synthesis of rather complex oligosaccharides and glycoconjugates. As they have described, the activation of glycosyl phosphites by DTBPI generates the corresponding glycosyl iodides as active intermediates. The resultant glycosyl iodides have modest reactivity to alcohols and react to afford the final glycosylation products typically within 24–48 h under standard reaction conditions.[10g] We anticipated that the reactions of the glycosyl phosphites would be much faster if they are activated only by *P*-protonation under mild acidic conditions in the absence of any nucleophilic catalysts. It has been reported that glycosyl phosphite triesters can be activated via *P*-protonation by using strong Brønsted acids, such as TfOH and Tf_2NH,[10d,k] but it was not clear if the glycosyl phosphites would work as glycosyl donors effectively with a much weaker Brønsted acid, such as tertiary ammonium salts, without any nucleophilic catalysts (Fig. 1).

* Corresponding author. Tel./fax: +81 4 7136 3612.
E-mail address: wada@k.u-tokyo.ac.jp (T. Wada).

0008-6215/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2010.03.009

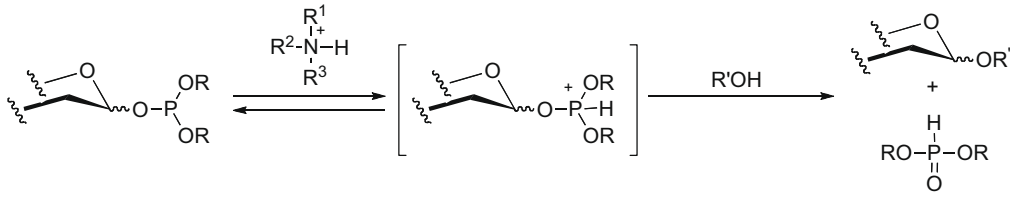
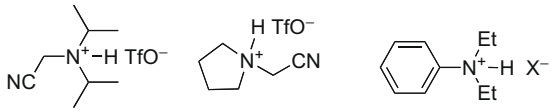


Figure 1. Glycosylation with tertiary ammonium salts via *P*-protonated glycosyl phosphites.

Glycosyl phosphites with an acid-labile 4,4′-dimethoxytrityl (DMTr) group at the *O*-6 position (**5**, **6**) were synthesized as glycosyl donors for this study via regioselective tritylation of the *O*-6-position of reducing sugars (**1**,[11] **2**[12]) in the presence of silver nitrate and subsequent *O*-1 phosphitylation (Scheme 1). The DMTr group is routinely used for transient protection of primary hydroxy groups in nucleic acid synthesis[13] for its advantages, such as the regioselective protection of primary hydroxy groups, stability under various reaction conditions, acid lability for facile deprotection under mild acidic conditions, and the strong UV–vis absorption of the resultant DMTr cation, which is used for quantitation.[14] Despite these advantages, the DMTr group has been rarely used for the synthesis of oligosaccharides,[11,15] mainly because of its incompatibility with the common glycosylation conditions using strong Brønsted or Lewis acids. The development of glycosylation reactions which proceed rapidly under mild acidic conditions would allow the synthesis of oligosaccharides using more extensive protecting groups than those currently used; the DMTr group would be representative of acid-labile protecting groups. We also expected that the bulky DMTr group at the 6-position might work as an α-directing group in glycosylation reactions.[11,16]

Trifluoromethanesulfonic acid salts of tertiary amines (Fig. 2, **7**–**9**[17]) were employed as activators for the following reasons: (1) the activators must have a mild but sufficient acidity to protonate the phosphorous atom of phosphite triesters and must be less-nucleophilic so that the formation of stable N-glycosides, such as pyridinium glycosides,[18] would be avoided. The suitable acidity and extremely low nucleophilicity of **7** and **8** have already been demonstrated by using them to activate *P*-stereogenic diastereopure phosphoramidite derivatives via protonation to give the corresponding phosphite derivatives in a stereospecific manner.[17b,19] The *N*,*N*-diethylanilinium salt **9** was also employed because of its modest acidity and low nucleophilicity. (2) The counter anion must also be less nucleophilic. TfO^- would be a suitable counter anion because the resultant glycosyl trifluoromethanesulfonates have been reported to be extremely reactive even at −78 °C and act as the corresponding oxocarbenium cations at ambient temperatures.[20] An *N*,*N*-diethylanilinium salt **10** containing a nucleophilic iodide anion was used as a control.

Glycosylation reactions of appropriately protected monosaccharides **11** and **12** were then carried out by using the glycosyl donors (**5**, **6**) and the activators (**7**–**10**) (Table 1). The glycosylations of the sterically less-hindered **11** were completed within 5–10 min when the less-nucleophilic activators **7**–**9** were used and the desired



7 **8**

9: $X^- = TfO^-$
10: $X^- = I^-$

Figure 2. Tertiary ammonium salts containing less-nucleophilic triflate anion (**7**–**9**) or highly-nucleophilic iodide anion (**10**).

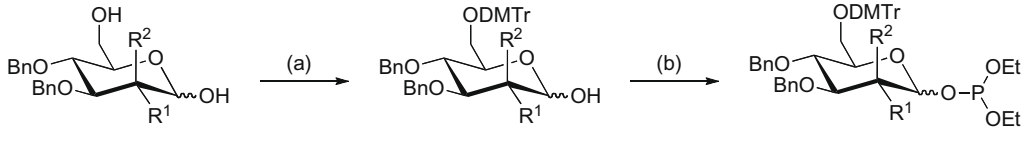
disaccharides **13** and **14** were isolated in good to excellent yields (entries 1–5 and 7). The reactions were slightly slower in polar solvents (entries 3 and 4) than in less polar CH_2Cl_2 (entry 2). In sharp contrast, the glycosylation proceeded very slowly when promoted by *N*,*N*-diethylanilinium iodide **10** due to the formation of the glycosyl iodide intermediate **19**, though the α-selectivity was excellent as reported in the literature (entry 6).[10g] The formation of the glycosyl iodide **19** from **5** and **10** was also confirmed by ^1H NMR.[21] Removal of the *O*-6-DMTr group of the fully protected disaccharides **13** and **14** was achieved by using 3 vol % dichloroacetic acid in CH_2Cl_2 within 2 min in the presence of Et_3SiH as a scavenger of the liberated DMTr cation[22] to afford the detritylated disaccharides **16**[23] and **17**,[24] respectively, virtually quantitatively (entries 5 and 7). In the cases of the sterically more-hindered glycosyl acceptor **12**, the reaction was completed within 5 min by using the activator **8** (entry 8), whereas it was sluggish when the activator **9** was used (entry 9). The resultant disaccharide **15**[11] was detritylated to afford **18**[25] in modest yields (entries 8 and 9). Only the α-isomer of **18** was obtained in both of these reactions most likely due to the α-directing effect of the *O*-6-DMTr group.

In summary, rapid glycosylations were achieved with glycosyl phosphites as glycosyl donors and less-nucleophilic trifluoromethanesulfonic acid salts of tertiary amines as activators. The absence of nucleophilic catalysts was essential for rapid reactions. Glycosyl donors having an acid-labile DMTr group were compatible with the present reaction conditions, indicating that a wide range of acid-labile substrates and protecting groups can be used in this method.

1. Experimental

1.1. General methods

IR spectra were recorded on a JASCO FT/IR-480 Plus spectrophotometer. NMR spectra were recorded on a Varian Mercury 300. ^1H



1: R^1 = OBn, R^2 = H
2: R^1 = H, R^2 = OBn

3: R^1 = OBn, R^2 = H, 81%
4: R^1 = H, R^2 = OBn, 88%

5: R^1 = OBn, R^2 = H, 77% (α:β = 58:42)
6: R^1 = H, R^2 = OBn, 55% (α:β = 93:8)

Scheme 1. Synthesis of glycosyl donors **5** and **6**. Reagents and conditions: (a) DMTrCl, Et_3N, $AgNO_3$, THF–DMF (1:1, v/v); (b) $(EtO)_2PCl$, Et_3N, CH_2Cl_2.