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One-Pot α-Glycosylation Method Using Appel Agents in *N,N*-Dimethylformamide

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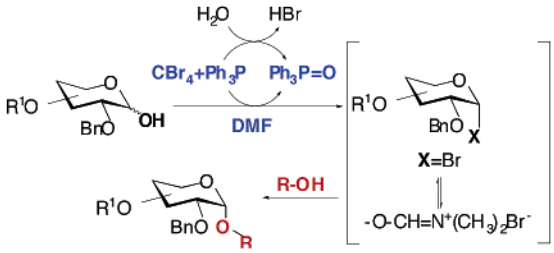
ABSTRACT



A concept for the development of practical glycosylation is presented and demonstrated by one-pot α-glycosylation applying Appel agents for 2-*O*-benzyl-1-OH hexoses in DMF. The reaction, in situ giving the equilibrium of glycosyl bromides and more reactive *O*-glycoside intermediates, accomplishes a near-quantitative α-glycosylation removing the water molecules.

Glycosylation involves coupling reactions between glycosyl donors [G1-X (X = leaving groups)] and alcohol or sugar acceptors (G2-OH) to construct α- or *β*-glycosyl linkages (G1-O-G2). Many types of G1-X are available together with the corresponding promoter agents,[1] and they have contributed enormously to the flexible design of artificial glycoconjugates possessing high medicinal potential.[2,3] In practice, however, chemical glycosylation is still arduous and far from the straightforwardness of biosynthetic and chemo-enzymatic reactions catalyzed by glycosyltransferases.[4] Along with our continuous study on the design of glycoconjugate polymers, we have searched for practical glycosylation methods from both chemical[5] and chemo-enzymatic approaches.[6] In this paper, we communicate a chemical pathway that enables us to prepare α-glycosides easily without special care for dehydration of the reaction system.

For mechanistic reasons, the glycosylation between G1-X and G2-OH must be conducted under anhydrous conditions, except for reactions of simple acceptors under Fisher's conditions or with phase-transfer catalysts.[7] Though the use of molecular sieves under the pressure of drying gas works effectively against the damage of water contamination, moisture-sensitive reactions cause problems for practical syntheses. More seriously, immobilized desiccants are not adaptable to developing technologies of solid-phase[8] and fluorous[9] syntheses.

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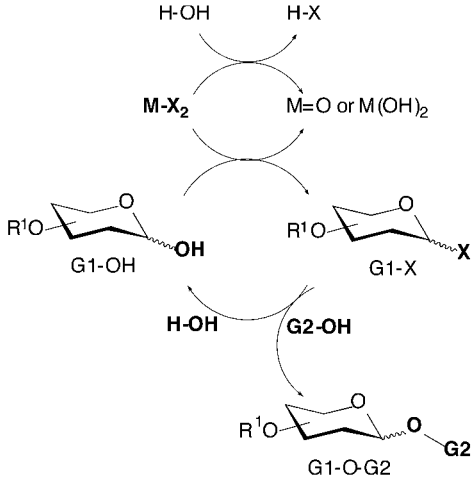
Here, let us hypothesize a dehydrative one-pot glycosylation[10] interfaced with a moisture-sweeping pathway as shown in Scheme 1. There, an M-X_2 reagent transforms

Scheme 1. Glycosylation Interfaced with a Moisture-Sweeping Donor-Generation Pathway

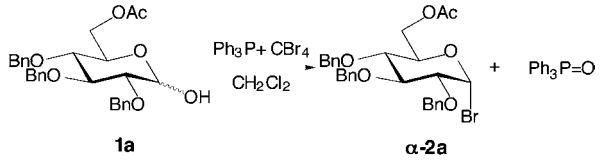


1-hydroxyl donor G1-OH to G1-X trapping 1-OH in the form of M═O. Even if contamination by water molecules decomposes G1-X to G1-OH, the excess agent recovers the active donor. Alternatively, the agent may capture every H-OH in a similar way before the decomposition. In both cases, the M-X_2 agent constructs a reaction system of dehydration, donor generation, and regeneration, which is ready for one-pot glycosylation with G2-OH. It should be noted that the overall pathway assumed here shows an analogy to conjugate reactions in biosynthetic cascades and the cofactor regeneration system developed by Wong et al.[11] for mammalian transferases.

To realize our hypothetical pathway, the choice of M-X_2 agents is a key investigative issue. The reagent should be more reactive to G1-OH and H-OH than to acceptor G2-OH. The reagent itself, M═O, and other possible secondary products should not affect the glycosylation. Among several candidates of M-X_2, we have evaluated the potential of a reagent combination of triphenylphosphine (Ph_3P) and carbon tetrabromide (CBr_4) known as Appel agents.[12]

In our previous study,[13] we found that the reaction of 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-D-glucopyranose **1a** with Appel agents in CH_2Cl_2 yields α-glycosyl bromide α-**2a** (Scheme 2).[14] In this reaction, a hemiacetal 1-OH is trapped in the

Scheme 2



form of Ph_3P═O, showing a feature matching the conversion of M-X_2 to M═O. In the present study, we investigated an optimal access to one-pot glycosylation as well as possible dehydrating pathways.

First, we addressed a halide ion-catalytic α-glycosylation pioneered by Lemieux and co-workers.[15] After a mixture of **1a** and Appel agents was stirred in CH_2Cl_2 for 2.5 h, cholesterol **3a**, *N*,*N*-tetramethylurea,[16] and Et_4NBr were added successively to the mixture. Excitingly, we were able to obtain α-glucosylated cholesterol in high yields and α-selectivity (entry 1, Table 1), though no molecular sieves

Table 1. One-Pot Glycosylation with G1-OH Donors and Appel Agents in Either CH_2Cl_2 or DMF[a]

entry	G1-OH	R^2-OH	conditions	h[b]	α/β (yields, %)[c]
1	**1a**	**3a**	Et_4NBr+TMU/CH_2Cl_2	53	98:2 (92)
2	**1a**	**3a**	TMU /CH_2Cl_2	83	>99:1[d](90)
3	**1a**	**3b**	TMU/CH_2Cl_2	96	>99:1[d](92)
4	**1a**	**3a**	DMF	24	95:5 (96)
5	**1a**	**3b**	DMF	22	>99:1[d](92)
6	**1b**	**3a**	TMU/CH_2Cl_2	58	90:10 (95)
7	**1b**	**3a**	DMF	23	85:15 (95)
8	**1c**	**3a**	DMF	23	94:6 (92)
9	**1d**	**3a**	DMF	23	82:18 (96)
10	**1e**	**3a**	DMF	19	86:14 (95)

[a] All reactions were carried out at room temperature with donors (0.4 mmol), acceptors (1.2 mmol), and a mixture of CBr_4 and Ph_3P (1.2 mmol for each) in given solvent. [b] Reaction times (h) until every donor was consumed. [c] Isolated yields based on the amount of 2-*O*-benzyl-1-OH donors. [d] No β-isomer was detectable in the ^1H NMR spectra of products.

and drying gas were applied. Second, we tried to further simplify the reaction conditions. Entry 2 shows that the α-glycosylation proceeded even in the absence of tetraethylammonium bromide, though it took a prolong time to completion. Entry 3 shows that the simple reaction is valid

(10) Gin and co-workers highlighted a one-pot pathway using G1-OH donors in terms of dehydrative glycosylation. (a) Nguyen, H. M.; Chen, Y.; Duron S. G.; Gin, D. Y. *J. Am. Chem. Soc.* **2001**, *123*, 8766. Reviewed in: (b) Gin, D. *J. Carbohydr. Chem.* **2002**, *21*, 645.

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(14) An analogous reaction was reported by Khatuntseva et al.[12d] who applied the Appel agents for 1-bromination of L-fucose derivatives and one-pot glycosylation in the presence of $HgBr_2$, $Hg(CN)_2$, and molecular sieves.

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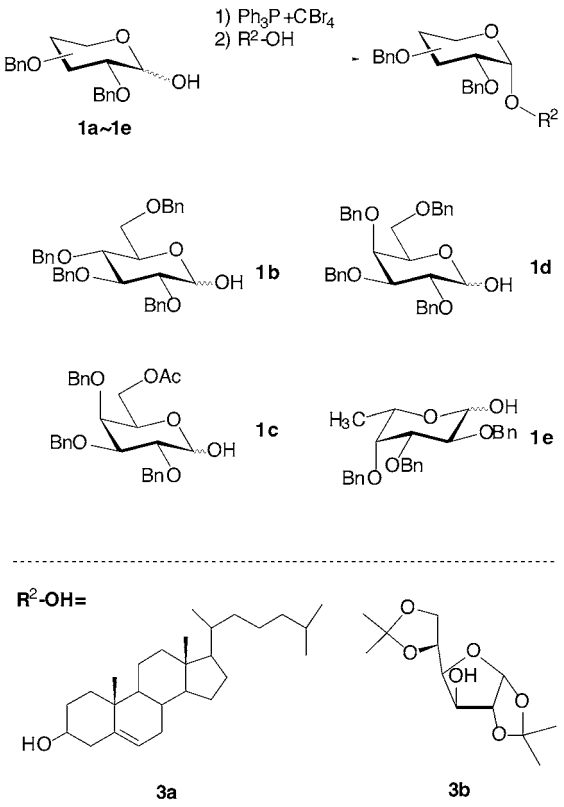
also for a sugar acceptor **3b** with a hindered 3-OH, proving its potential for oligosaccharide synthesis.

When the overall reaction was carried out in DMF, glycosylation proceeded even in the absence of tetraethylammonium bromide, the one-pot α-glycosylation could be additionally simplified and facilitated (entries 4 and 5). In this case, *N*,*N*-tetramethylurea was replaced with the solvent itself. Entries 6−10 show that every D-*gluco*- (**1a**, **1b**), D-*galacto*- (**1c**, **1d**), and L-*fucosyl* **1e** donor gave the corresponding α-glycosides efficiently. When the α-selectivity of **1a** (entry 3−5) and **1c** (entry 8) with a 6-*O*-acyl group is compared with that of **1b** and **1d** with a 6-*O*-benzyl group, we can see the effect of the 6-*O*-acyl group to promote α-selectivity (Scheme 3).[17]

Scheme 3



To search for the underlying dehydration pathways, we undertook ^{1}H NMR study for the reaction of **1a** and Appel agents. After the 1-bromination was carried out for **1a** in CH_2Cl_2, the mixture was concentrated and dissolved again in either $CDCl_3$, DMF-d_7, or ca. 1% D_2O−DMF-d_7 solvent. The ^{1}H NMR spectrum of the $CDCl_3$ solution enabled us to confirm that the reaction gave α-**2a** exclusively (H-1, δ 6.40 ppm, doublet, $J_{1,2}$ = 4.0 Hz, Figure 1a). On the other hand, the spectrum of the DMF-d_7 solution showed a feature different from that of α-**2a**. The H-1 signals shifted to a lower field, while it kept the vicinal coupling constants characteristic for α-D-glucopyranose with $^4C_1$-ring conformation (H-1, δ 7.08 ppm, d, $J_{1,2}$ = 4.0 Hz: H-2, δ 3.68 ppm, dd, $J_{2,3}$ = 10.0 Hz). This indicates that the glycosyl bromide α-**2a** is transformed to an alternative α-glycosyl species α-**2a**-**X** in the DMF-d_7 solution. Another experiment reacting **1a** and Appel agents in an NMR glass tube allowed us to determine that α-**2a**-**X** is derived in DMF solvent but not in CH_2Cl_2. In Figure 1b, it is shown that α-**2a**-**X** is unstable in an aqueous DMF-d_7 solvent and returns to a mixture of α-**2a** and **1a** in a reversible manner. This also shows that the new species is more sensitive to water than α-**2a**. Figure 1c shows that an additional introduction of the Appel agents regenerates the labile species, removing every water molecule.

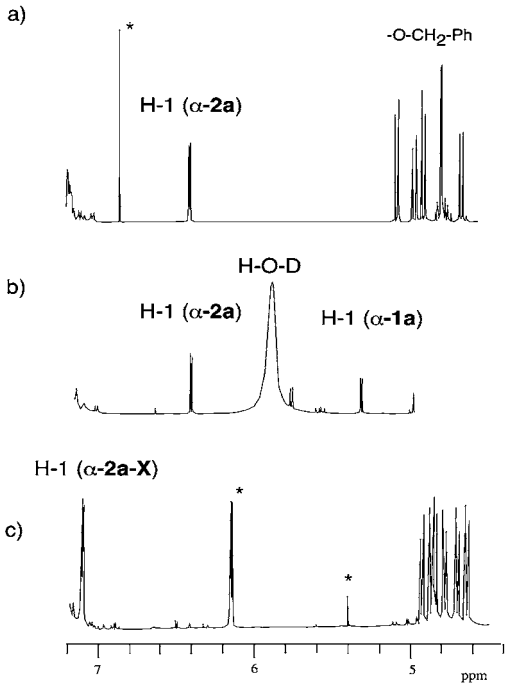


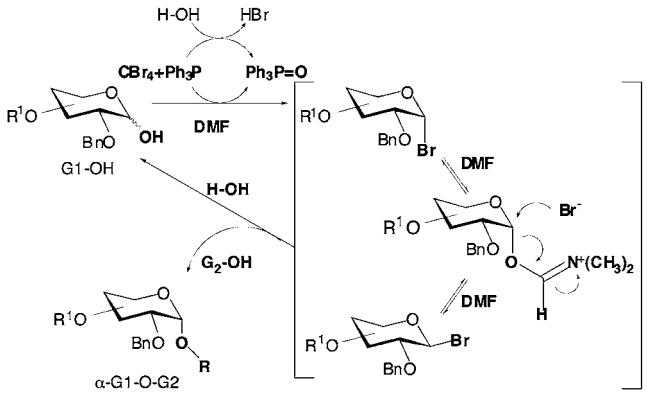
Figure 1. ^{1}H NMR spectra (500 MHz) of a mixture of **2a** and Appel agent dissolved again in $CDCl_3$ (a) and 1% D_2O−DMF solvent (b). [Spectrum c was recorded after Appel agents (9 mol equiv for **1a**) were added to the ca. 1% D_2O−DMF solution. Asterisk (∗) notes signals of unidentified nonsugar components.]

In these NMR experiments, we observed some intriguing phenomena as described below. (1) The reagents begin the conversion of **1a** to α-**2a**-**X** after they dry up the solvent system. (2) The capacity of water absorption is obviously larger than what we expect for the conversion of Ph_3P to Ph_3P═O. It is obvious that CBr_4 is also used for the dehydration of DMF probably to yield CO_2 or CO_3^{2-} in a final form. (3) The ^{1}H-signals of α-**2a** are hardly detectable on the way leading to α-**2a**-**X**, while they appear upon the addition of D_2O to α-**2a**-**X** in the DMF solution leading back to **1a** as can be seen in Figure 1b. (4) Every conversion among the three sugar components, i.e., **1a**, α-**2a**, and α-**2a**-**X**, is reversible and with negligible side reactions affording irreversible sugars.

(17) Nishida, Y.; Takamori, Y.; Matsuda, K.; Ohrui, H.: Kobayashi, K. *J. Carbohydr. Chem.* **1999**, *18*, 65, and references therein.

The above ^{1}H NMR study as well as mechanistic considerations allow us to assign the chemical structure of α-**2a**-**X** as *O*-[6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl]-methylene-*N*,*N*-dimethylammonium bromide, a kind of Vilsmeier−Haack intermediate.[18] The FAB-mass spectrum of a mixture of α-**2a**-**X** and the Appel agents in DMF supported this assignment [$(M + Na)^+ = 650$]. Because of its highly labile property, the spectral assignment of α-**2a**-**X** may remain incomplete. However, the formation of such an intermediate can rationalize not only the NMR observations but also the glycosylation reactions accelerated in DMF. Though Lemieux and co-workers[15] reported a similar solvent effect, they did not indicate the occurrence of such DMF-glycosyl adducts. Thus, it seems appropriate to propose a novel α-glycosylation pathway where the reactive *O*-glycoside is in situ formed and associated (Scheme 4). We

Scheme 4. Overview of One-Pot α-Glycosylation Using Appel Agents in DMF



speculate that the intermediate may accelerate the formation of β-glycosyl bromide leading to α-selective glycosylation. It is also probable that the glycosyl imidate like α-**2a**-**X** is derived not only from glycosyl bromide but also directly from 1-OH sugar in the reaction with a Vilsmeier reagent produced in the Appel agents-DMF system. It is obvious in any case that the Appel agents play multiple roles and thus allow a simple α-glycosylation.

In conclusion, we have demonstrated a general strategy to develop practical glycosylation, in which the choice of M-X_2 type reagent for G1-OH type donors is a key investigative issue. In this paper, we have described the convenient use of Appel agents in DMF and thereby proposed a one-pot α-glycosylation method. The glycosylation via a glycosyl imidate looks similar to the *O*-trichloroacetimidate method established by Schmidt.[1b] More exactly, the dehydrative method using 2-*O*-benzyl-1-OH donors is closer to one-pot dehydrative glycosylation of Gin and co-workers,[10] Koto et al.,[19] Leroux and Perlin,[20] and Pavia et al.[21] Our method is, however, distinct from these methods in that the reaction takes place in the universal, nonhalogenated, but hygroscopic solvent DMF without special care for dehydration.[22] This property will have implications in scaled-up, solid-phase, and other glycosylation processes.

Acknowledgment. This study was supported in part by a grant of the 21st Century COE program "Nature-Guided Materials Processing" of the Ministry of Education, Culture, Sport, Science and Technology of Japanese Government.

Supporting Information Available: ^{1}H NMR and FAB-MS data of glycosyl products and ^{1}H NMR spectra supporting reversible conversion among **1a**, α-**2a**, and α-**2a**-**X**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(19) Koto, S.; Morishima, N.; Zen, S. *Carbohydr. Res.* **1984**, *130*, 73.

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(21) Pavia, A. A.; Ung-Chhun, S. N. *Can. J. Chem.* **1981**, *59*, 482.

(22) **A General Protocol for One-Pot α-Glycosylation in DMF.** Overall, reactions are conductible at ambient temperature (15−20 °C) in a glass vessel closed with a septum cap. No molecular sieves or drying gas is required as long as the vessel is dried at 120 °C in an oven prior to the use. Typical procedure is as follows: A 2-*O*-benzylated sugar G1-OH in DMF (molecular sieves 4A inclusive, 99.8% grade, water content <0.1%) is treated first with a mixture of Ph_3P (3 mol equiv) and CBr_4 (3 mol equiv) until G1-OH sugar is consumed completely (observed as glycosyl bromide on silica gel TLC, *n*-hexane/ethyl acetate = 5:1) (2−3 h) and then with an acceptor alcohol (2−3 molar equiv). The reaction is stirred at room temperature until the donor is consumed (8−25 h). The reaction mixture is diluted in toluene, washed with saturated $NaHCO_3$ and water, concentrated, and subjected to purification on silica gel column.