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A 5-Ureido-Modified Sialyl Donor: A Tool for the Synthesis of α-Sialosides

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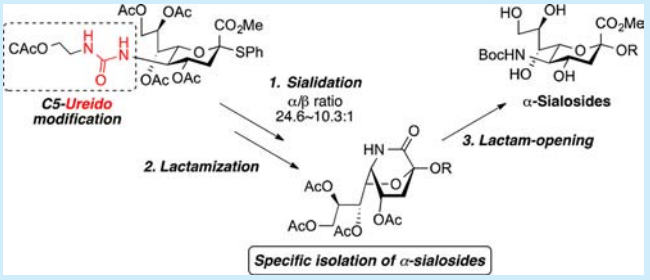
Supporting Information

ABSTRACT: A novel method for the synthesis of α-sialosides using a newly developed 5-ureido-modified sialyl donor is reported. The donor was found to be useful for α-selective sialidation with various glycosyl acceptors, forming α(2,6)Glc, α(2,6)Gal, and α(2,3)Gal linkages in excellent yield and with stereoselectivity. Furthermore, α-sialoside was easily isolated from the reaction mixture by the 1,5-lactamization method under mild conditions. Successful application of the C5-ureido sialyl donor to the synthesis of a sialoside confirmed the usefulness of the present method.



Sialoglycans, which are sialic acid-containing carbohydrate molecules, are mainly found in vertebrates as constituents of glycoproteins and glycosphingolipids and play a significant role in various biological events such as cellular adhesion, regulation of cell proliferation and apoptosis, pathogen infections, and tumor metastasis.[1] Because of their biological significance, research regarding the physiological and pathological implications of sialoglycans has attracted several biologists. Furthermore, the chemical synthesis of sialoglycans is of interest to synthetic chemists because the construction of α-sialoside, which is the only molecule found in nature that features this linkage form (except for cytidine-5′-monophospho-*N*-acetylneuraminic acid (CMP-sialic acid)), is extremely challenging. To date, various strategies for α-selective sialylation have been developed,[2] including stereocontrol by the nitrile solvent effect, varying the nature of the leaving group/promoter, and structural modification of sialyl donors at the C-1, C-3, and C-5 positions. In particular, it has been found in the past decade that modifying the C-5 position[3] to an amide (NHTFAc,[4] NHTCA[5]), imide (NAc_2,[6] NPhth[7]), carbamate (NHTroc,[5,8] NHFmoc,[5] NHAlloc,[5] NHBoc,[5] NHCbz[5]), azide,[9] isothiocyanate,[10] or 5-*N*,4-*O*-oxazolidinone[11] is effective in improving α-selectivity. However, stereoselective sialylation remains a challenge and currently requires laborious column chromatographic separation of glycosylated products, including stereoisomers and other byproducts, to obtain pure α-sialosides. To overcome this issue, our group has developed a unique purification method for α-sialoside by employing 1,5-lactamization, which is used to distinguish α-configured sialosides from mixtures of glycosylated products, allowing specific isolation of α-sialosides.[12] Despite the efficiency of this approach, it has a drawback in that elimination of acyl groups can occur during the lactamization process. Thus, we have attempted to develop a milder lactamization method that proceeds without the elimination of acyl groups. For this purpose, we designed a 5-ureido-modified sialyl donor (**1**), which possesses a 2-hydroxyethyl group as a tail on the ureido functionality, as we anticipated that the strong nucleophilicity of the nitrogen atom in the ureido group would facilitate the cyclization process, allowing a lactam ring to be formed under mild condition. Figure 1 shows the strategy employed in this study for the synthesis of α-sialosides. 1,5-Lactamization following sialidation using **1** can be achieved under mild conditions because the liberation of stable 2-oxazolidinone (**2**) serves as a driving force, affording **3** with intact acyl groups. Thus, we report here an α-selective sialidation using the new 5-ureido-modified sialyl donor and its application to the efficient synthesis of sialosides.

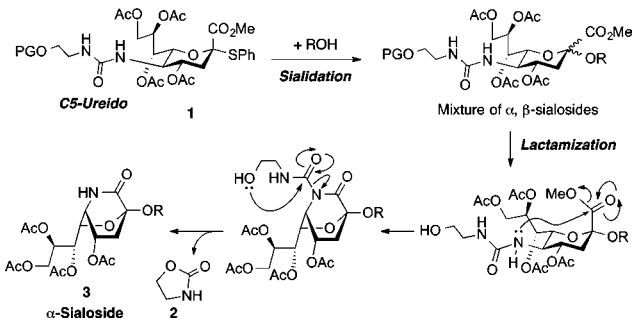


Figure 1. Strategy developed in this study for the synthesis of α-sialosides.

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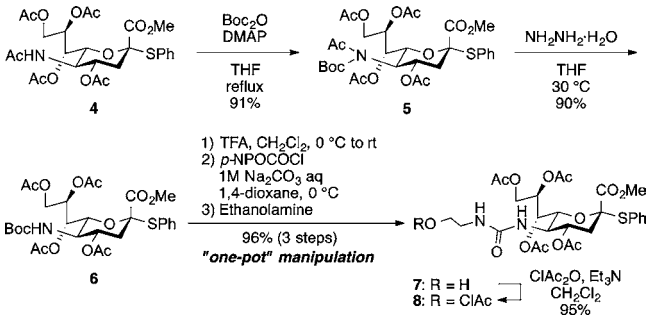
The C5-modified sialyl donor **8** was prepared as illustrated in Scheme 1. First, a Boc group was introduced to the acetamide

Scheme 1. Preparation of the C5-Ureido-Modified Sialyl Donor



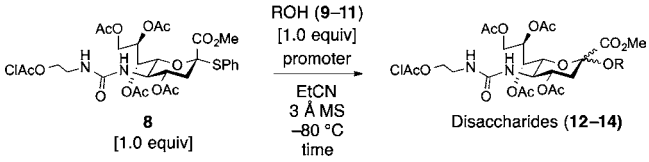
group at C5 of thioglycoside **4**[13] to afford **5**, and the *N*-acetyl group was then chemoselectively removed by hydrazine to afford **6** in good yield.[14] The replacement of the carbamate with the ureido functionality was achieved over three steps in a one-pot manner, with removal of the Boc group, formation of *p*-nitrophenylcarbamate, and subsequent nucleophilic substitution by ethanolamine (along with the elimination of *p*-nitrophenol), affording **7** in excellent yields. Finally, the distal hydroxyl group was protected as a chloroacetate to give the donor **8**, which was then assayed in sialidation experiments with various glycosyl acceptors.

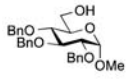
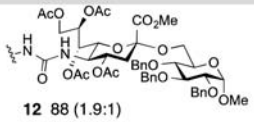
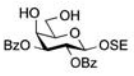
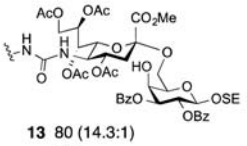
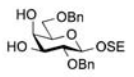
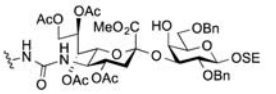
Table 1 shows the results of the sialidation of **8** with three glycosyl acceptors (**9**–**11**). All glycosylations were performed in EtCN at −80 °C, except that reported in entry 1. The stereochemistry of the products was confirmed by ^{1}H NMR and heteronuclear multiple-bond correlation spectroscopies.

First, the 6-OH of glucosyl acceptor **9**[15] was sialylated using the NIS-TfOH promoter system[16] in CH_2Cl_2. The reaction was started at −80 °C, and then the temperature was increased to −60 °C after 2 days. As a result, the corresponding sialoside **12** was obtained in 88% yield with low α-selectivity (α/β ratio, 1.9:1, entry 1). The same sialidation was attempted in EtCN; however, 88% of the acceptor was recovered (entry 2). Note that, despite the low yield of this reaction, the α-selectivity was clearly improved compared to entry 1, probably through the nitrile solvent effect.[17] In the reaction reported in entry 3, *p*-NO_2PhSOTf, which was developed by Crich et al.,[18] was used as the promoter. The reaction proceeded smoothly, even in EtCN at −80 °C, and afforded sialoside **12** in good yield (79%) with high α-stereoselectivity (α/β ratio, 10.4:1). Although this promoter system exerted a powerful activating effect in this sialidation, some byproducts, including the dechloroacetylated compound, were generated, presumably due to the use of excess AgOTf (2.5 equiv). Next, the IBr-AgOTf promoter system was examined.[19] Surprisingly, the reaction was completed in 15 min, affording **12** in 80% yield. The stereoselectivity (α/β ratio, 10.3:1) was almost the same as that in entry 3, and importantly, almost no byproduct was observed (entry 4). Therefore, we decided to use the IBr-AgOTf system as the promoter for the present 5-ureido-type donor **8** in all subsequent reactions.

Sialidation with galactosyl acceptors **10**[20] and **11**[21] (entries 5 and 6) afforded predominantly the α-sialosides **13** (α/β ratio, 14.3:1) and **14** (α/β ratio, 14.8:1), respectively. The use of excess **8** (2.0 equiv) led to improvements in both yield (94%) and stereoselectivity (α/β ratio, 24.6:1, entry 7). We attribute

Table 1. Results of Sialidation of 8 with Various Glycosyl Acceptors



entry	ROH	promoter[a]	time [h]	disaccharides yield/% (α:β ratio)[b]	ROH[c] [%]
1[d]	 **9**	A	96	 **12** 88 (1.9:1)	8
2	**9**	A	120	**12** 5 (7.9:1)	88
3	**9**	B	2	**12** 79 (10.4:1)	8
4	**9**	C	0.25	**12** 80 (10.3:1)	20
5	 **10**	C	1	 **13** 80 (14.3:1)	18
6	 **11**	C	1	 **14** 64 (14.8:1)	25
7[e]	**11**	C	1	**14** 94 (24.6:1)	0

[a]Promoter: A, NIS-TfOH; B, *p*-NO_2PhSCl-AgOTf; C, IBr-AgOTf. [b]Determined by ^{1}H NMR analysis. [c]Recovered acceptor. [d]The reaction was performed in CH_2Cl_2. [e]2.0 equiv of **8** was used.

the elevation of α-selectivity in entry 7 to an increase in the presence of the β-triflate intermediate, associated with the increased amount of AgOTf.

We next examined 1,5-lactamization to verify the concept of this study. As a model experiment, α-sialosides **7** and **15** were selected; the results of 1,5-lactamization are presented in Table 2. First, the effect of the base in the lactamization was investigated in reactions conducted in toluene at reflux temperature (entries 1–4). Evaluation of 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU), 1,5-diazabicyclo[4.3.0]non-5-ene (DBN), and 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) as the base revealed that DBU was the most effective base for the formation of the lactamized product **16** (57% yield). No lactamization occurred without base (entry 1). The effect of the solvent was also examined (entries 5 and 6) at 100 °C, and DMF gave the best result in terms of both yield and reaction time (68% in 1 h, entry 6). When the reaction was conducted at higher temperature (120 °C), it was completed in only 40 min and the yield was improved to 75% (entry 7). Subsequently, the effect of substrate concentration in lactamization was examined. Decreasing the concentration to 10 mM improved the yield to 83% (entry 8), with the reaction TLC analysis indicating a yield of more than 83% because of no observation of other spots. We found that deacetylated products were produced during the evaporation process prior to column chromatography. As shown in entry 9, quenching of the reaction by the addition of acetic acid led to the best yield (93%). Finally, the lactamization of disaccharide **15**, which was derived from **12** through the removal of the chloroacetyl group, was conducted using the same procedure as that given in entry 9, affording the lactamized disaccharide **17** in excellent yield (entry 10). Note that the

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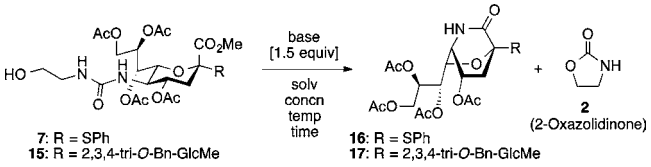
Table 2. Optimization of the 1,5-Lactamization of 5-Ureido−Sialyl Acid Derivatives



entry	SM[a]	base	solv[b]	concn [mM]	t [°C]	time [h]	prod	yield/%[c]
1	7	none	T	50	reflux	24	**16**	0
2	7	DBU	T	50	reflux	2	**16**	57
3	7	DBN	T	50	reflux	24	**16**	16
4	7	TBD	T	50	reflux	0.75	**16**	7
5	7	DBU	N	50	100	1.5	**16**	53
6	7	DBU	D	50	100	1	**16**	68
7	7	DBU	D	50	120	0.75	**16**	75
8	7	DBU	D	10	120	1	**16**	83
9[d]	7	DBU	D	10	120	1	**16**	93[e]
10	**15**	DBU	D	10	120	4.5	**17**	89[e]

[a]SM: starting material. [b]Solvent: T, toluene; N, nitromethane; D, DMF. [c]Estimated from the mixture of **16** and **2** based on ^{1}H NMR analysis. [d]The reaction was quenched by the addition of AcOH. [e]Isolated yield.

formation of 2-oxazolidinone (**2**) was confirmed in all reactions, whose observation was helpful in elucidating the reaction mechanism of lactamization.

To experimentally validate the 1,5-lactamization mechanism, three types of sialic acid derivatives (**4**, **6**, and **18**) were treated with DBU for different times (Figure 2). The 5-amide derivative

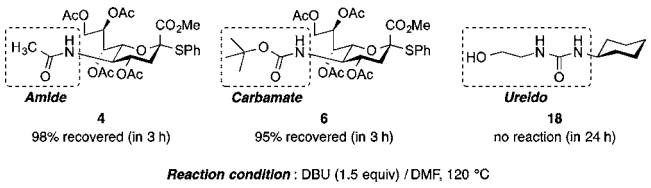


Figure 2. Results of experiments to elucidate the reaction mechanism of lactamization.

4 and 5-carbamate derivative **6** were treated with DBU in DMF at 120 °C, which are the optimized conditions as reported in Table 2. However, no lactamized product was obtained in 3 h, and almost all starting materials were recovered in both cases. Next, we performed another experiment using the ureido derivative **18**[22] to establish the lactamization mechanism. We wondered if the liberation of 2-oxazolidinone occurred before or after the cyclization step to form the lactam ring. If the cyclization step occurred after the liberation of **2**, cyclohexylamine would be obtained as the product. However, **18** did not produce any product even after stirring for 24 h.

Having examined the significance of the ureido functionality on lactamization, we propose the reaction mechanism for 1,5-lactamization as illustrated in Figure 3. First, cyclization takes place under the relatively high temperature required for the ring-flip from the chair form ($^2C_5$) to the boat form ($^{2,5}B$). Second, 2-oxazolidinone is released with the assistance of a base-like DBU, affording the lactam ring. Finally, the anion on the lactam receives a proton to complete 1,5-lactamization. As an alternative mechanism, concomitant lactam formation and oxazolidinone generation and liberation might be available.

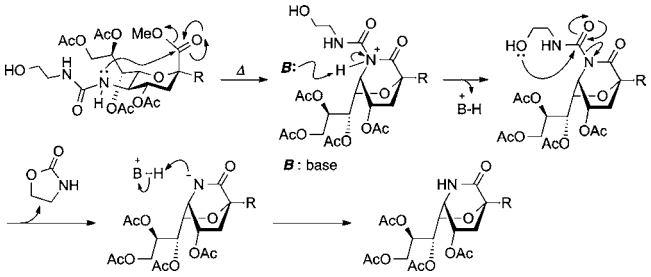
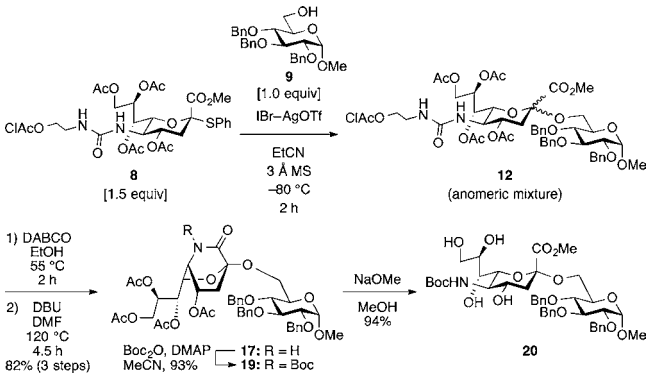


Figure 3. Proposed reaction mechanism for 1,5-lactamization.

Application of the present methodology to the synthesis of a sialoside was carried out (Scheme 2). The sialidation of **8** with **9**

Scheme 2. Application of the Present Methodology to the Synthesis of an α-Sialoside



was performed in the presence of IBr-AgOTf in EtCN at −80 °C, and the resulting mixture was roughly purified by gel filtration column chromatography, affording **12** as a mixture of stereoisomers. The chloroacetyl group on **12** was removed by treatment with DABCO in EtOH at 55 °C,[23] followed by lactamization, affording **17** in excellent yield (82% over three steps). Introduction of the Boc group onto the lactam ring was easily achieved using Boc_2O and DMAP in MeCN to afford **19** in 93% yield. Finally, the lactam derivative **19** in boat form was converted into **20** in chair form as the natural conformation in excellent yield.

In conclusion, a novel method for the synthesis of sialosides employing a C5-ureido-modified sialyl donor has been developed. The newly developed donor was found to be useful for the formation of sialosides in a highly α-selective manner using the IBr-AgOTf and *p*-NO_2PhSCl-AgOTf promoter systems. Furthermore, C5-ureido functionality-specific 1,5-lactamization enabled specific isolation of the α-sialoside from the reaction mixture after sialidation. This α-specific isolation method may be applied to the successful linear synthesis of structurally complex sialoglycans, which is currently considered to be highly difficult. Our current research involves the application of this methodology to the synthesis of more complex sialoglycans containing di- and trisialic acid residues.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00403.

Synthetic procedures, NMR spectra of all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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