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Synthetic study on α(2→8)-linked oligosialic acid employing 1,5-lactamization as a key step

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ABSTRACT

An attempt to synthesize α(2→8)-linked oligosialic acid utilizing a 1,5-lactamized sialyl acceptor is described. 1,5-Lactamization was experimentally proven to proceed only for α-sialoside, which was integrated into the synthetic cycle of oligosialic acid as a chemical sorting step to collect the desired α-sialoside and as a transformation step to produce a reactive sialyl acceptor for the next sialylation. Lactamized oligosialyl acceptors served as favorable coupling partners for sialylation, providing high stereoselectivities and high yields.

Sialic acids are expressed mostly on the glycan chains of glycoproteins and glycolipids, which play important roles in biological events such as cell adhesion, cell differentiation, viral infection, and neural network development.[1] In most cases, they occupy the distal end of glycan chains, being commonly linked to a galactose or *N*-acetylgalactosamine residue through an α(2→3) or α(2→6)linkage. In some cases, a sialic acid residue is further connected with another sialic acid at the C-4, C-8, or C-9 position, forming dimeric, oligomeric, or polymeric sialic acid. It is known that the expression of the α(2→8)-linked poly- and oligosialic acids in mammals is developmentally regulated, closely correlating to the stages of neural network formation,[2] and it was also demonstrated that α(2→8)-linked di- or trisialic acid-containing gangliosides showed neurite extension activity.[3]

Based on these impressive biological properties, the chemical synthesis of α(2→8)-linked oligosialic acid has been the subject of intensive investigation in carbohydrate chemistry. So far, α(2→8)-linked disialic acid has been successfully prepared using several approaches,[4] while tri- and tetrasialic acids have been synthesized only by Tanaka et al. whose approach involved utilizing a highly reactive 5-*N*,4-*O*-oxazolidino sialic acid donor and acceptor.[5] Herein, we report an attempt to synthesize α(2→8)-linked oligosialic acid based on a reactive 1,5-lactam sialyl acceptor.

The critical issue in the formation of the α(2→8)linkage between sialic acids is the extremely low reactivity of the C-8 hydroxyl group, which is probably due to the hydrogen bonding with either the C-5 acetamido group or the C-1 methoxy carbonyl group.[6] Recently, we have demonstrated that the reactivity of the C-8 hydroxyl group could be dramatically enhanced by the formation of the 1,5-lactam of sialic acid, and this finding was successfully applied to the synthesis of the glycan moiety of a Hp-s6 ganglioside that possesses a 8-*O*-SO_3H-Neu5Acα(2→8)Neu5Ac sequence.[4f] Based on these results, we developed a synthetic cycle to produce an α(2→8)-linked oligomer of sialic acid as a single anomer (Scheme 1). Simply, the synthesis comprises two reactions—sialylation and 1,5-lactamization. Since the 1,5-lactamization theoretically proceeds in the α-configuration, the lactamization was expected to function as a chemical sorting step to collect the desired α-glycoside exclusively as the lactamized sialyl acceptor for the next sialylation. Repetition of this cycle was expected to afford β-anomer-free α(2→8)oligosialic acids.

To carry out this cycle, we designed key sialyl units **6** and **8**, which bear a phenylthio[7] or trifluoroacetimidate group[8] at the anomeric position as a leaving group. A trifluoroacetyl group was used both to enhance the reactivity of the sialyl donor and to act as a tentative protecting group for the C-5 amino group.[4e,9] Benzyl groups were incorporated as persistent protecting groups during the chain extension, and the C-8 hydroxyl group was capped with a chloroacetyl group. The synthesis of sialyl donors **6** and **8** began

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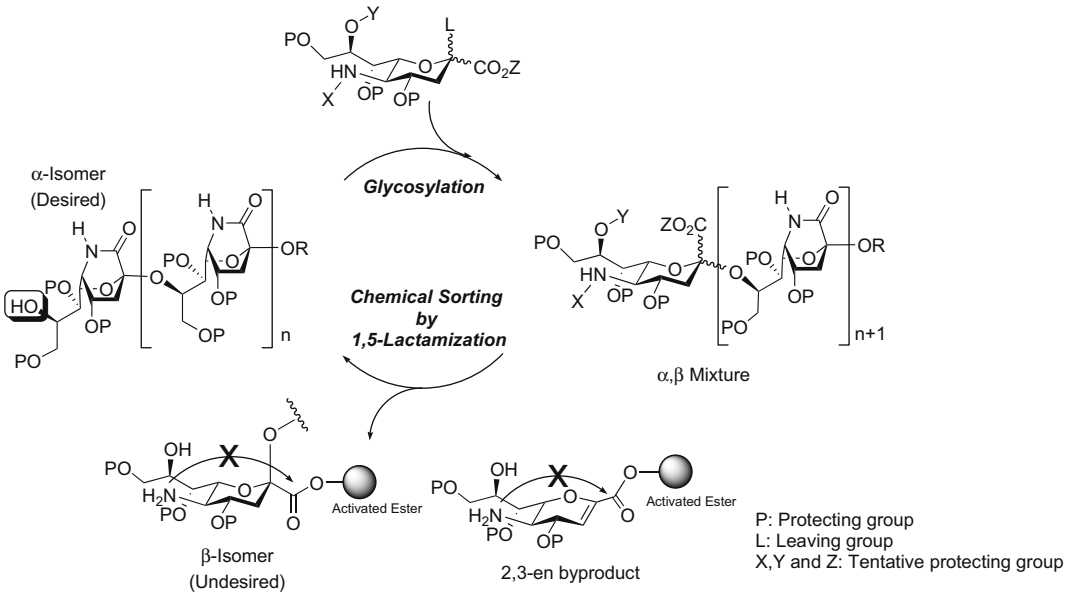
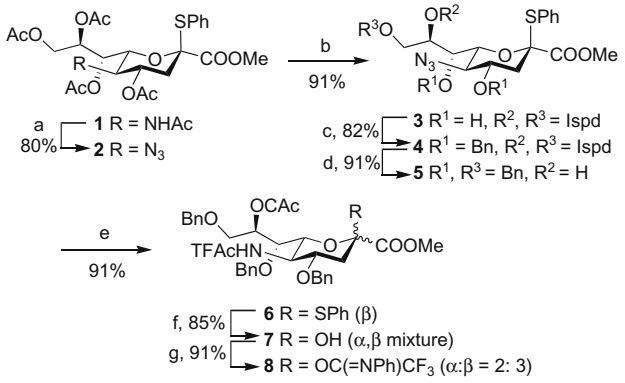


Scheme 1.

with the complete deacetylation of *N*-Ac thioglycoside **1** with MsOH in MeOH, followed by a diazo transfer and *O*-acetylation to provide 5-azide derivative **2**[10] (80%, 3 steps) (Scheme 2). Compound **2** was then subjected to de-O-acetylation, 8,9-O-isopropylidenation, and 4,7-di-O-benzylation to give compound **4** (75%, 4 steps). Subsequent removal of the 8,9-*O*-isopropylidene acetal and selective 9-O-benzylation via a stannylene acetal intermediate afforded compound **5** (91%, 3 steps). Then, azido derivative **5** was converted into *N*-TFAc sialyl donor **6** through a reaction sequence including: (1) reduction of the azide group to an amino group with 1,3-propanedithiol and NEt_3 in MeOH[11]; (2) N-trifluoroacetylation; and (3) O-chloroacetylation (91%, 3 steps). Furthermore, the treatment of **6** with NBS in wet acetone afforded hemiketal **7** (85%), which, upon reaction with *N*-(phenyl)trifluoroacetimidoyl chloride

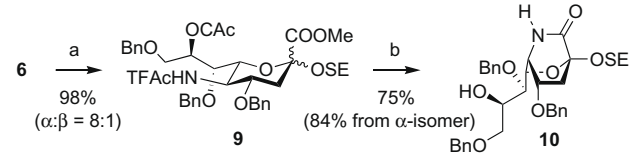


Scheme 2. Reagents and conditions: (a) (i) MsOH/MeOH, reflux; (ii) TfN_3, DMAP/MeOH, rt; (iii) Ac_2O, pyr, 0 °C→rt, 80% (3 steps); (b) (i) NaOMe/MeOH, rt; (ii) DMP, CSA/MeCN, rt, 91% (2 steps); (c) (i) BnBr, TBAI, NaH/DMF, rt; (ii) NaOMe/MeOH, rt, 82% (2 steps); (d) (i) 80% aq AcOH, 80 °C; (ii) DBTO/PhMe, 120 °C; (iii) BnBr, TBAI/PhMe, 120 °C, 91% (3 steps); (e) (i) 1,3-propanedithiol, Et_3N/MeOH, reflux; (ii) TFAcOMe, NEt_3, THF–MeOH (1:1), rt; (iii) CAcOH, DCC, DMAP/CH_2Cl_2, rt, 91% (3 steps); (f) NBS, wet acetone, rt, 85%; g) $CF_3C(=NPh)Cl$, K_2CO_3/acetone, rt, 91%. MsOH = methanesulfonic acid, TfN_3 = trifluoromethanesulfonyl azide, DMAP = 4-dimethylaminopyridine, DMP = 2,2-dimethoxypropane, CSA = (±)-10-camphorsulfonic acid, TBAI = tetra-*n*-butylammonium iodide, DBTO = di-*n*-butyltin oxide, TFAc = trifluoroacetyl, CAc = chloroacetyl, DCC = *N*,*N*-dicyclohexylcarbodiimide, Ispd = isopropylidene.

in the presence of K_2CO_3 in acetone,[8] was converted into imidate donor **8** in 91% yield as a mixture of α- and β-isomers (α:β = 2:3).

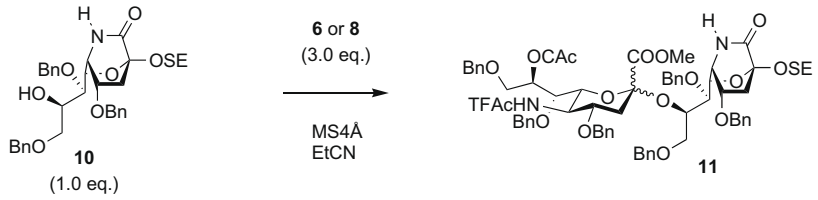
To assess the feasibility of the synthetic cycle, the sialyl unit **6** was subjected to glycosidation and subsequent 1,5-lactamization (Scheme 3). Then, sialyl donor **6** was reacted with 2-(trimethylsilyl)ethanol in the presence of NIS and TfOH[12] with the assistance of the nitrile solvent effect[13] at −40 °C to provide sialoside **9** as a anomeric mixture (α:β = 8:1) in 98% yield. Next, the resulting anomeric mixture was directly saponified and subsequently 1,5-lactamized by the treatment with HBTU and DIEA to afford acceptor **10** as a single isomer in 75% yield (84% as a conversion yield of the α-isomer). In this reaction, the β-isomer was transformed into several unidentified highly polar compounds, which may include polymerized products,[14] thereby greatly facilitating the chromatographic separation of the lactamized α-sialoside. In addition, from a mixture containing the corresponding 2,3-ene derivative, lactamized α-sialoside **10** was easily collected by silica gel column chromatography. These results proved that 1,5-lactamization functions as a chemical sorting step for α-sialoside.

Thus, with the lactamized acceptor **10** in hand, we then incorporated it into the cycle. First, we investigated the sialylation reaction of 1,5-lactamized acceptor **10** with donors **6** and **8** (Table 1). All reactions were carried out in EtCN as a stereocontrolling medium. The initial coupling of acceptor **10** with thioglycoside donor **6** using NIS–TfOH as the activator afforded the desired disaccharide **11** in 27% yield as an anomeric mixture (α:β = 16:1) (entry 1). The anomeric configuration of the major product in the mixture



Scheme 3. Reagents and conditions: (a) SEOH, NIS, TfOH, MS 3 Å/MeCN, −40 °C, 98% (α:β = 8:1); (b) (i) 1.0 M aq NaOH–THF–MeOH (1:2:2), rt; (ii) HBTU, DIEA/MeCN, rt; (iii) 1.0 M aq NaOH–THF–MeOH (1:2:2), rt, 75% (3 steps). SE = 2-(trimethylsilyl)ethyl, NIS = *N*-iodosuccinimide, TfOH = trifluoromethane sulfonic acid, HBTU = *O*-(benzotriazol-1-yl)-*N*,*N*,*N*',*N*'- tetramethyluronium hexafluorophosphate, DIEA = *N*,*N*-diisopropylethylamine.

Table 1

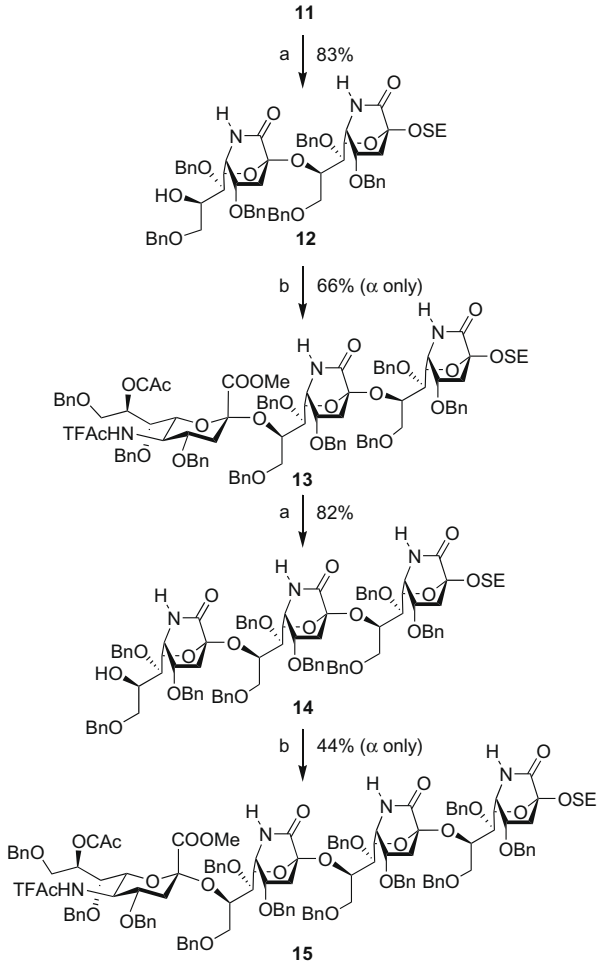


Entry	Donor	Promoter	Temp (°C)	Time (h)	Yield (%)	α:β[a]
1	**6** (β)	NIS (4.5 equiv), TfOH (0.9 equiv)	−80 to 0	9	27	16:1
2	**6** (β)	PhSeBr (9.0 equiv), AgOTf (9.0 equiv) DTBMP (9.0 equiv)	−80 to −70	4	0	—
3	**6** (β)	Ph_2SO (9.0 equiv), Tf_2O (3.3 equiv) DTBMP (6.0 equiv)	−80 to rt	12	0	—
4	**8** (α,β)	TMSOTf (0.15 equiv)	−80	72	86	16:1

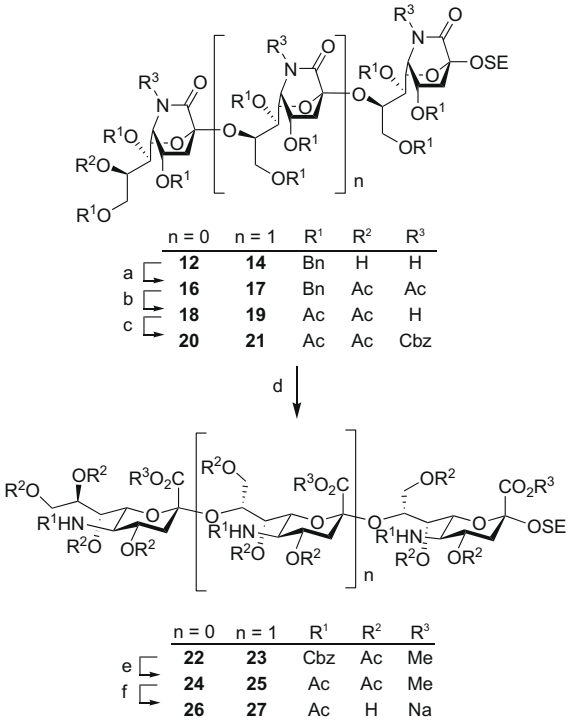
[a] Determined by ^{1}H NMR analysis of the α/β mixture.

was determined to be α from its $^3J_{C1,H3ax}$ coupling constant (6.9 Hz)[15] while that of the minor product was less than 1.0 Hz, indicating it to be β.[16] When other activators[17,18] for thioglycoside **6** were used, none of the desired disaccharide **11** was produced (entries 2 and 3). It was found that these oxidative conditions were not well suited for the sialylation of lactamized sialyl acceptor **10**. In entry 1, the corresponding *N*-hydroxy derivative of **10** was isolated as a byproduct, which was produced probably as a result of N-iodination within the lactam by NIS followed by hydrolysis during the aqueous work-up. As shown in entry 4, the coupling of **10** with the imidate donor **8** catalyzed by TMSOTf provided a high yield of **11** (86%) with excellent α-selectivity (α:β = 16:1).

Next, the disaccharide **11** as an anomeric mixture was saponified and 1,5-lactamized to afford the acceptor **12** in pure form (Scheme 4). In the next cycle, to our delight, the coupling of **8** and **12** provided trisialic acid **13** as a single isomer



Scheme 4. Reagents and conditions: (a) (i) 1.0 M aq NaOH–THF–MeOH (1:2:2), rt; (ii) HBTU, DIEA/MeCN, rt; (iii) 1.0 M aq NaOH–THF–MeOH (1:2:2), rt, 83% (**12**, 3 steps), 82% (**14**, 3 steps); (b) **8**, TMSOTf/EtCN, −80 °C, 66% (**13**, α only), 44% (**15**, α only).



Scheme 5. Reagents and conditions: (a) (i) Ac_2O, DMAP, pyr, rt, 89% (**16**), 94% (**17**), (ii) H_2, $Pd(OH)_2$–C/EtOAc–EtOH (1:1), 40 °C; (iii) Ac_2O, DMAP, pyr, rt; (iv) hydrazine acetate/THF, rt, 84% (**18**, 3 steps), 85% (**19**, 3 steps); (b) CbzOSu, DMAP, pyr, rt, 61% (**20**), 56% (**21**); (c) (i) NEt_3–MeCN–H_2O (1:9:5), 40 °C; (ii) MeI, K_2CO_3/DMF, rt; (iii) Ac_2O, DMAP, pyr, rt, 37% (**22**, 3 steps), 22% (**23**, 3 steps); (d) H_2, $Pd(OH)_2$–C/Ac_2O–EtOAc (1:1), rt, 71% (**24**), 61% (**25**); (e) 1.0 M aq NaOH, 40 °C, 97% (**26**), 41% (**27**). CbzOSu = *N*-(benzyloxycarbonyloxy)succinimide.

($^3J_{C1,H3ax}$ = 6.9 Hz)[15] in 66% yield. Once again, **13** could be converted into 1,5-lactamized acceptor **14**, which was sialylated with **8** under the same reaction conditions to furnish the tetrasaccharide **15** again as a pure α-isomer ($^3J_{C1,H3ax}$ = 5.8 Hz)[15] in 44% yield.

Finally, we attempted to convert the fully lactamized di- and trisaccharide **12** and **14** into their natural *N*-acetyl form (Scheme 5). According to our reported procedure,[4f] compounds **12** and **14** are predisposed for lactam opening. Thus, the following reaction sequences supplied the suitably protected **20** and **21** in 46% and 45% yields, respectively: acetylation, debenzylation, acetylation, N-deacetylation, and N-benzyloxycarbonylation. Then, basic hydrolysis of **20** and **21** followed by methylation of the resulting carboxylic acids and acetylation afforded products **22** and **23**. Finally, debenzylation and acetylation produced the *N*-Ac derivatives **24** and **25** in 71% and 61% yields, which were subjected to hydrolysis providing **26** and **27**.

In conclusion, the synthesis of α(2→8) trisialoside has been completed by a route incorporating 1,5-lactamization reactions. Since it was proven that the process of 1,5-lactamization is selective for α-sialoside in a glycosylation mixture, this principle could be applied not only in sialoside synthesis in the liquid phase but also in syntheses in the solid phase. Applications of this methodology to the synthesis of gangliosides will be reported in the future.

Acknowledgments

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Supplementary data

Supplementary data (^{1}H and ^{13}C NMR spectra of compounds **11**, **12**, **13**, **14**, **15**, **26**, and **27**) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.05.057.

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