Synthesis of 4-*O*-alkylated *N*-acetylneuraminic acid derivatives

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Abstract

The synthesis of 4-*O*-alkyl analogs of Neu5Ac and the scope of the 4-*O*-alkylation reaction are described. The experimental results support the use of activated alkyl halides and sulfonates, in addition to primary alkyl iodides. Primary alkyl bromides and tosylates provide 4-*O*-alkylated compounds in low yields, while primary alkyl chlorides and secondary alkyl bromides fail to afford alkylated products. In addition, we exemplified the utility of the methodology using a thiophenyl Neu5Ac building block to synthesize a 4-*O*-alkyl 2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid (DANA) analog. The results presented expand the toolbox of Neu5Ac chemistry and the findings will be of value in the design of novel tools to study the biology of Neu5Ac binding proteins and as starting points for drug discovery.

Introduction

N-acetylneuraminic acid (Neu5Ac, **1**, Figure 1) is typically found at the terminal end of glycolipids and glycoproteins that decorate the surfaces of all mammalian cell types. Neu5Ac is involved in mediating or modulating a variety of physiological and pathophysiological processes.[1] One of the most well-known roles of Neu5Ac is in the replication cycle of influenza virus.[2] Accordingly, substantial efforts have been placed on the development of Neu5Ac-based antivirals,[3] where modifications of the C4-position of 2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid (DANA, **2**, Figure 1) have been of central importance.[4–12] This culminated in the development of Relenza® (**3**, Figure 1), a C4-modified analog of **2** designed to mimic the transition state of **1** during the neuraminidase catalyzed hydrolysis reaction required for release of virus progeny from infected cells.[5] C4-modified analogs of **2** including nitrogen[4], sulfur[4], and deoxygenated[7] compounds are efficiently accessed via selective ring opening at position 5 of the allylic oxazoline of 2,3-didehydro-*N*-acetylneuraminic acid (**4**, Figure 1).[4] However, in the case of oxygen nucleophiles opening occurs at position 2 of the oxazoline ring.[7] Hence, the method is not applicable to the synthesis of 4-*O*-modified analogs of **2** (or **1**) with retained stereochemistry. C4-deoxy and C4-nitrogen analogs of **1** can however be accessed using the ring-opening methodology but require re-installment of the glycosidic bond which produces two stereoisomers of equal proportions.[13,14]

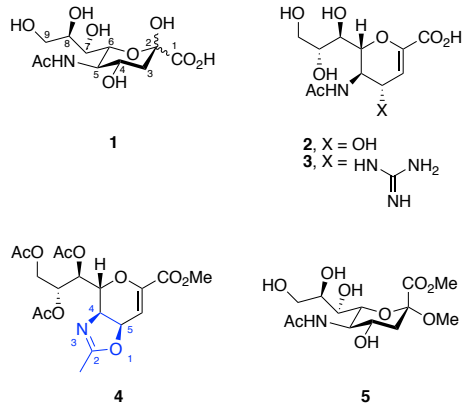


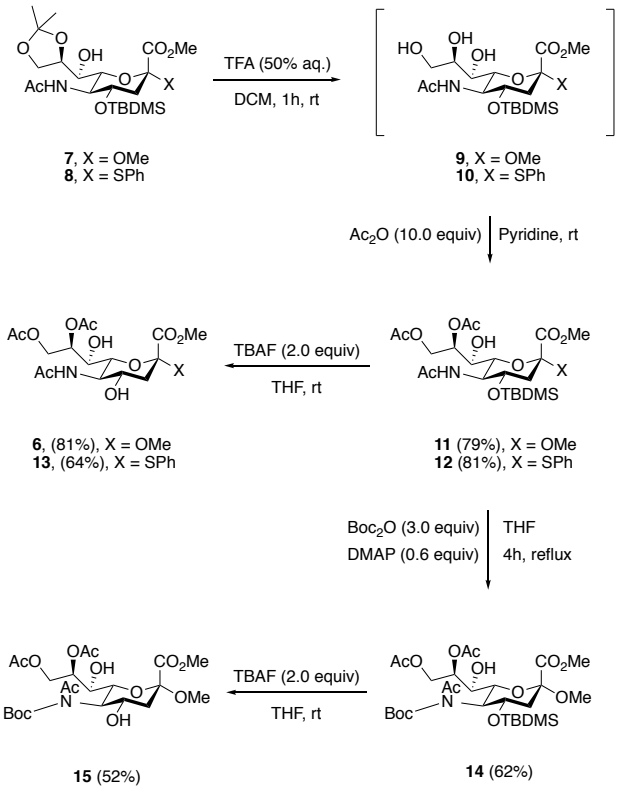
Figure 1. Structure of Neu5Ac and DANA analogs.

The interest in Neu5Ac analogs and their roles in biological systems is constantly increasing. Therefore, efficient methods that allow site-selective modifications of the Neu5Ac-template are of great utility for studying Neu5Ac biology and for drug discovery. Methods to selectively access C4-modified analogs of **1** are scarce, with relatively few reported examples. These include carba[15,16], keto[16], ether[14, 17–20], nitrogen[13], and deoxygenated[21] derivatives. A potential drawback in the development of direct methods could be the competing formation of intramolecular lactams[22–24] and lactones[25] that occur under both basic and acidic conditions. To date, examples of selectively 4-*O*-modified Neu5Ac analogs include 4-*O*-Ac, -benzyl[26–30], -allyl[23], -silyl[15], -methyl[14, 17, 18], -ethyl[18], -cyanomethyl[19], and -tertbutoxyacetate[20] groups. The electrophiles used to produce these 4-*O*-modified analogs all have in common that they are activated, highly reactive, and with few exceptions lack the presence of β-hydrogens. Further, the commercial availability of suitable electrophiles remains limited. Herein, we set out to study the scope and the 4-*O*-alkylation of Neu5Ac.

Results and discussion

In an ongoing research project, we were interested in studying the potential of 4-*O*-alkyl analogs of **5** (Figure 1) as probes targeting cell attachment during adenovirus[31-33] and coxsackievirus infections[34, 35]. We hypothesized that **6** (Scheme 1) would be a suitable substrate to study *O*-alkylation. This previously described protective group strategy is straightforward, high yielding, and allow removal of the protective groups in a final single step.[15] Propargyl bromide was selected as the model electrophile. The lack of β-protons minimizes the competing E2 reaction, thus providing a fair measure of the effectiveness of the S_N2 reaction. In addition, the generated alkynyl product can be further modified under mild conditions[36, 37]. Compound **5**[38] (Figure 1) was obtained from **1**, and then converted by standard methods to the known derivative **7**[39] (Scheme 1). Treatment of **7** with wet trifluoroacetic acid in DCM afforded **9** which upon acetylation gave the fully protected derivative **11** in 79% yield over two steps. Treatment of **11** with TBAF in THF afforded **6** in 81% yield.

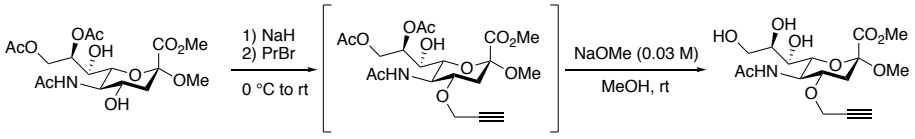
Scheme 1. Synthesis of Neu5Ac derivates selectively protected at the 7-, 8-, and 9-positions.



Attempts to alkylate **6** using $Ba(OH)_2$/BaO in DMF[23, 30, 40], K_2CO_3 in THF, or $CsCO_3$ in MeCN, resulted in minimal amounts of **16**. However, promising observations were made in THF using KHMDS or NaH, with NaH providing superior conversion and product formation. Standard *O*-alkylation conditions were screened by treating **6** with NaH on ice prior to the addition of propargyl bromide (entries 1 and 2, Table 1). In DMF, this resulted in nearly complete decomposition and only trace amounts of **16** (entry 1). However, in THF the 4-*O*-propargylated derivative **17** was isolated in 50% yield over two steps (entry 2). The *O*-deacetylation was performed to compensate for formation of hydrolyzed species during the reaction (Figure S1), and thus facilitate isolation of the desired 4-*O*-alkyl product. The *O*-deacetylation of purified **16** gave **17** in 85% yield, the value that was used to estimate the yield for the *O*-alkylation (Table 1).

Table 1. Screening and optimization of reaction conditions.

[a]Estimated yields are based on the isolated yield (85%) of the *O*-deacetylated of **17**. [b]Solubility issues. N.d = not determined.



6 **16** **17**

Entry	Solvent	Br (equiv)	NaH (equiv)	[6] M	*O*-alkylation %[a] (estimated)	Yield %
1	DMF	5.0	1.1	0.06	traces	-
2	THF	5.0	1.1	0.3	59	50
3	DMF	5.0	2.0	0.1	47	40
4	THF	5.0	2.0	0.1	61	52
5	1,4-Dioxane	5.0	2.0	0.1	51	43
6	MeCN	5.0	2.0	0.1	41	35
7	Toluene	5.0	2.0	0.1[b]	n.d	n.d
8	THF	1.1	2.0	0.1	25	22
9	THF	2.0	2.0	0.1	45	39
10	THF	10.0	2.0	0.1	51	44
11	THF	5.0	1.0	0.1	56	47
12	THF	5.0	5.0	0.1	37	31
13	THF	5.0	1.1	0.1	56	47
14	THF	5.0	1.1	0.05	39	33
15	THF	5.0	1.1	0.3	82	70
16	THF	5.0	1.1	1.0[b]	52	45
17	THF	5.0	1.5	0.3	79	67

Alkoxide formation was studied by mixing compound **6** with NaH in DMF-d_7 and in THF-d_8, respectively, and recording ^{1}H-NMR spectra at two different time points (Figure 2A-D, and Figure S2A-F). Within 10 minutes, compound **6** was essentially consumed in DMF-d_7 resulting in a complex mixture of products (Figure 2A and B). In contrast, only minimal signs of degradation were observed in THF-d_8 10 minutes post-addition of NaH (Figure 2C and D), and the majority of **6** was largely intact after 1 hour (Figure S2F). This prompted us to reverse the addition order, **6** was mixed with propargyl bromide in the selected solvent on ice before adding NaH. Furthermore, the stoichiometry of NaH was increased from 1.1 to 2.0 equivalents to assure complete deprotonation of both the hydroxyl and acetamide of **6.** These modifications drastically improved the yield of **17** in DMF (40%; entry 3, Table 1), while no significant effect was observed in THF (52% yield; entry 4). This highlights the importance of avoiding pre-formation of the alkoxide in DMF. The 4-*O*-alkylated product **17** was confirmed by 2D NMR analysis, and by treatment with acetic anhydride in pyridine which afforded **16** in 60% yield.

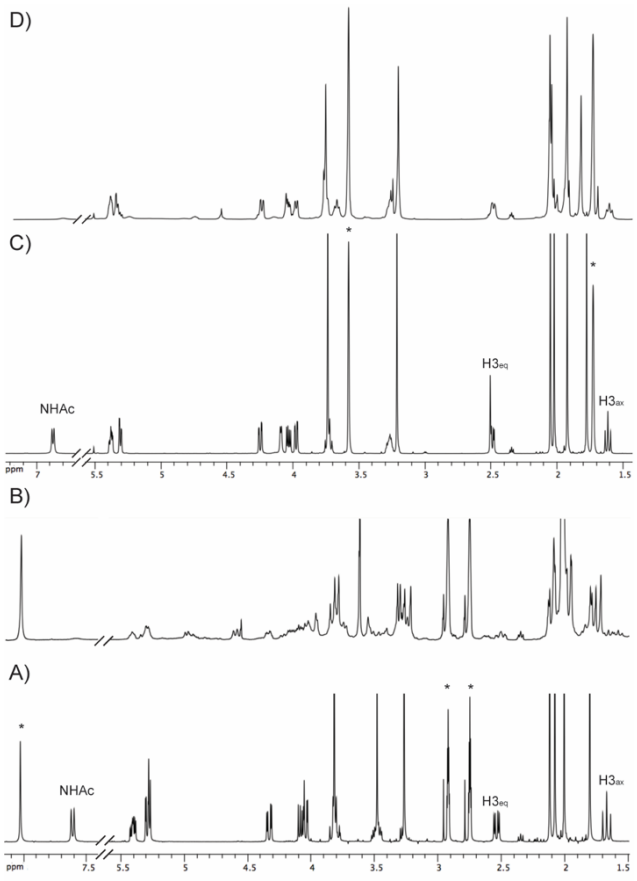
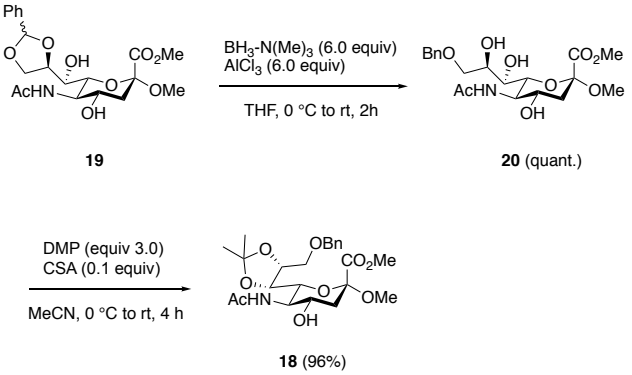


Figure 2. ^{1}H-NMR spectra of **6** in DMF-d_7 (A-B) and THF-d_8 (D-C). A and C) reference, no added base. B and D) directly upon addition of NaH. *solvent residual peak.

Common solvents for *O*-alkylation reactions were screened and the yields of **17** were lower in both 1,4-dioxane (43%; entry 5, Table 1) and MeCN (35%; entry 6) in comparison to the reference reaction (entry 4). The reaction in toluene (entry 7) was slow, with incomplete conversion after 72 hours of stirring, likely due to poor solubility, and was not processed further. Decreasing the stoichiometry of propargyl bromide to 1.1 and 2.0 equivalents afforded **17** in 22% and 39% yields, respectively (entries 8 and 9), while increased stoichiometry gave **17** in 44% yield (10 equiv; entry 10) and resulted in larger concentration of side products. Reduced stoichiometry of NaH gave **17** in 47% yield (1.0 equiv; entry 11, Table 1) with incomplete conversion, while increased stoichiometry provided **17** in 31% yield (5.0 equiv; entry 12) with larger amounts of side products, suggesting the stoichiometry of NaH should be greater than one but less than two equivalents to assure complete conversion and minimize formation of side products. Indeed, 1.1 equivalents of NaH gave a clean reaction and complete conversion, albeit without improvement of the yield (47%; entry 13). Decreased substrate concentration gave **17** in 33% yield (0.05 M; entry 14). Pleasingly, increased concentration produced **17** in 70% yield (0.3 M; entry 15), corresponding to a 35% improvement in comparison to the reference reaction. Higher concentration was associated with solubility issues, but provided **17** in 45% yield (1.0 M; entry 16). With the optimized conditions at hand the stoichiometry of NaH was adjusted to 1.5 equivalents as conversion was incomplete in some reactions when using 1.1 equivalents. This resulted in complete

conversion of **6**, providing **17** in 67% yield (entry 17). To conclude, the optimal conditions are a concentration of 0.3 M (in THF), 5.0 equivalents of propargyl bromide, and 1.1–1.5 equivalents of NaH.

Scheme 2. Orthogonal protection of Neu5Ac derivates at the 7-, 8-, and 9-positions.

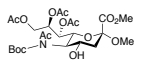
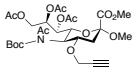
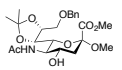
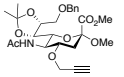
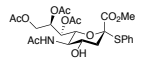
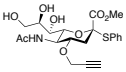
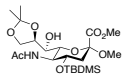
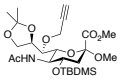
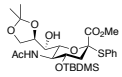
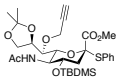
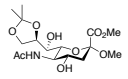
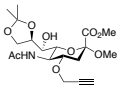
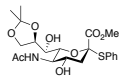
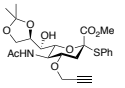


19 **20** (quant.)

18 (96%)

In an attempt to further improve the yields of the *O*-alkylation, compounds **15** and **18** were prepared (Scheme 1 and 2, respectively). Compound **15** with its tertiary amide renders it resistant toward potential side products arising from lactamization[22–24]. In two steps, **15** was accessed from **11** by treatment with Boc anhydride and DMAP in THF followed by cleavage of the TBDMS group using TBAF (Scheme 1). Surprisingly, the reactivity of **15** was completely abolished towards *O*-alkylation (entry 1, Table 2). Prolonged reaction times (2.5 h), heating (60 °C for 16 h), and irradiation in a microwave reactor (100 °C for 20 min) were inefficient in causing conversion. Compound **18** was prepared from the known derivative **19**[41] including selective reduction with borane-trimethyl amine and aluminum chloride in THF affording **20** that upon treatment with 2,2-dimethoxypropane and camphor sulfonic acid in MeCN gave the 9-*O*-benzyl-7,8-acetonide protected **18** in 96% yield. This protective group strategy is orthogonal allowing site-selective removal and functionalization of the glycerol side chain (C7, C8, and C9). Further, the protective groups have increased resistance toward hydrolysis under basic conditions. Upon *O*-alkylation **18** gave **22** in 57% yield (entry 2). Compound **13** was prepared in analogous manner to **6** (Scheme 1), and upon *O*-alkylation afforded **23** in 74% yield (entry 3, Table 2). Compound **23,** and analogs thereof, significantly broaden the scope due to their potential for modifications at the C2-position via glycosylation, or elimination to access 4-*O*-alkyl DANA analogs.[19] The developed conditions were applied to synthetic intermediates **7** and **8** which provided **24** and **25** in 48% and 71% yields, respectively (entries 4 and 5, Table 2). Thus, supporting access to 7-*O*-alkylated species. Synthetic intermediates **26** and **27** selectively afforded the 4-*O*-alkylated products **28** and **29** in 31% and 49% yields (entries 6 and 7, Table 2), respectively. Thus, significantly decreasing the number of steps to access 4-*O*-alkylated analogs of **18.**

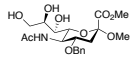
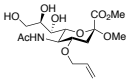
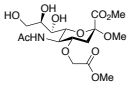
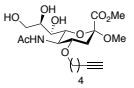
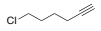
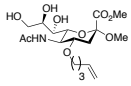
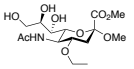
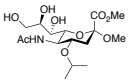
Table 2. *O*-alkylation of diversely protected Neu5Ac building blocks. All reactions were conducted in THF (0.3 M substrate), and performed by treating a stirred solution of 5.0 equivalents of propargyl bromide and substrate with 1.1–1.5 equivalents of NaH (specific details in SI).
[a]Yield over two steps.

Entry	Substrate		Product		Yield %
1		**15**		**21**	no reaction
2		**18**		**22**	57
3		**13**		**23**	74[a]
4		**7**		**24**	48
5		**8**		**25**	71
6		**26**		**28**	31
7		**27**		**29**	49

Representative examples of commercial alkyl halides and sulfonates were then screened to study the scope of the 4-*O*-alkylation of **6** (Table 3). As expected, the activated alkyl bromides benzylbromide, allyl bromide and ethyl bromoacetate afforded the corresponding 4-*O*-ethers **30** (62% yield; entry 1), **31** (52% yield, entry 2), and **32** (78% yield, entry 3), respectively. An initial reaction with 6-iodo-1-hexyne gave **33** in a mere 10% yield (entry 4). Dipolar aprotic solvents are known to increase the rate of substitution due to their ability to solvate cations[42] and the use of DMF indeed afforded **33** in 45% yield (entry 4). Using 6-chloro-1-hexyne resulted in trace amounts of **33** (entry 5), and addition of TBAI, or KI, did not result in the isolation of **33** in either THF or DMF. Upon *O*-alkylation 5-bromo-1-pentene afforded **34** in 17% yield (entry 6). Ethyl tosylate gave **35** in poor yield (8%; entry 7) with substantial amounts of hydrolyzed starting material. However, propargyl mesylate gave **17** in 58% yield (entry 8), thus supporting the use of activated alkyl sulfonates. Last, we attempted to substitute 2-bromopropane which resulted in trace amounts of **36** (entry 9) in line with the fact that 2° halides are less reactive in *O*-alkylation reactions due to excess β-protons favoring an E2 pathway over the desired substitution reaction.[43]

Table 3. Scope of the *O*-alkylation reaction.

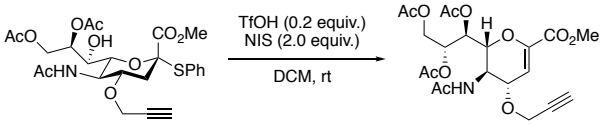
[a]THF as solvent. [b]DMF as solvent. [c]KI or TBAI as additives. [d]Purification by HPLC, 80% pure.

Entry	Electrophile	Product		Yield %
1			**31**	64[a]
2			**32**	52[a]
3			**33**	79[a]
4			**34**	10[a] 45[b]
5			**34**	traces[abc]
6			**35**	17[b]
7			**36**	8[d]
8			**18**	58
9			**37**	traces[ab]

To exemplify the utility of the developed methodology we purified intermediate **37** and treated it with TfOH and NIS in DCM[44] affording the 4-*O*-propargyl DANA analog **37** in 87% yield (Scheme 3), thus confirming access to 4-*O*-alkyl DANA analogs[19], via C3-elimination. These compounds have potential as antivirals towards human parainfluenza type-1.[45–49]

In summary we have systematically studied *O*-alkylation of Neu5Ac derivatives and provided insights in the scope of the reaction for preparation of tool compounds and starting points for drug discovery.

Scheme 3. Synthesis of 4-*O*-propargyl DANA analog.



37 **38** (87%)

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Supporting information Available

General experimental procedures and NMR-spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

Notes

The authors declare no competing financial interest.

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