Oxidative Deamination of *N*-Acetyl Neuraminic Acid: Substituent Effects and Mechanism

Szymon Buda and **David Crich**[*]

Department of Chemistry, Wayne State University, Detroit, MI 48202, USA

Abstract

A study of the mechanism of the oxidative deamination of the *N*-nitroso-*N*-acetyl sialyl glycosides leading with overall retention of configuration to the corresponding 2-keto-3-deoxy-ᴅ-glycero-ᴅ-galacto-nonulopyranosidonic acid (KDN) glycosides is described, making use of a series of differentially *O*-protected *N*-nitroso-*N*-acetyl sialyl glycosides and of isotopic labelling studies. No evidence is found for stereodirecting participation by ester groups at the 4- and 7-positions. Comparisons are drawn with oxidative deamination reactions of 4-amino-4-deoxy and 2-amino-2-deoxy hexopyranosides and a common mechanism is formulated involving the intermediacy of 1-oxabicyclo[3.1.0]hexyl oxonium ions following participation by the pyranoside ring oxygen. A minor reaction pathway has been uncovered by labelling studies in the β-thiosialosides that results in the exchange of the 4-*O*-acetyl group by the glacial acetic acid that serves as external nucleophile in the general oxidative deamination process. A mechanism is proposed for this exchange involving participation by the thioglycoside at the level of an intermediate diazoalkane.

Introduction

The sialic acids, a large group of nine carbon sugar acids based on the 2-keto-3-deoxy-ᴅ-glycero-ᴅ-galacto-nonulopyranosidonic acid framework, are present at the non-reducing end of a myriad of mammalian cell surface glycans.[1-3] *N*-Acetyl neuraminic acid is the most common member of the family, but variation at the 5-position is frequently found with examples including *N*-glycoyl neuraminic, and 2-keto-3-deoxy-ᴅ-glycero-ᴅ-galacto-nonulopyranosidonic acid (KDN). The removal of terminal sialic acid glycosides from cell surface glycans by sialidase enzymes is a key step in the replication cycle of influenza viruses, giving rise to the importance of the sialidase inhibitors as antiviral drugs. Increased levels of sialidation in cell surface glycans have led to the development of cancer vaccine candidates based on tumor-associated carbohydrate antigens (TACAs).[1-4] Modification of the amido group at the 5-position of *N*-amidoneuraminic acid derivatives has proved to be beneficial in the development of sialidase inhibitors and of carbohydrate antigens with greater antigenicity.[5-9] Such modifications have typically been achieved by *N*-deacetylation, its glycosides and derivatives, followed by reinstallation of different acyl groups,[6-7,10-12] or by use of the biosynthetic machinery when various *N*-acyl mannosamines are converted

[*]dcrich@chem.wayne.edu.

enzymatically to the corresponding *N*-acyl neuraminic acids[13] and eventually their glycosides,[14-15] or by a combination of the biosynthetic and chemical methods.[16] Consequently, modifications are typically limited to amide derivatives or other simple substitutions that are compatible with the biosynthetic machinery such as the conversion of 2-deoxy glucose to 5-desacetamido neuraminic acid.[17]

The advent of improved chemical methods for the efficient and stereoselective synthesis of the α-sialyl glycosides based on the use of 4-*O*,5-*N*-oxazolidinone-protected sialyl donors and their *N*-acetyl variants[18-22] gave rise to the possibility of the effective chemical synthesis of C-5 modified sialyl glycosides provided suitable reactions for post-glycosylation removal of the acetamido function could be identified. Toward this end we have described[23-24] the application of the oxidative deamination of neruaminic acid[25-27] and *N*-acetylneuraminic acid[28-29] to the synthesis of C-5 modified α-sialosides. We describe here our investigations into the mechanism of stereospecific oxidative deamination of *N*-nitroso *N*-acetyl neuraminic acid glycosides making use of a series of differentially O-protected derivatives and isotopic labelling studies. Comparisons are made with oxidative deamination reactions in the 4-amino-4-deoxy and 2-amino-2-deoxy hexopyranosides, leading to the formulation of a general mechanism involving participation by the ring oxygen and the intermediacy of 1-oxabicyclo[3.1.0]hexyl oxonium ions, whose fate is determined by the substituent pattern. We also describe a minor reaction pathway found on oxidative deamination of a *N*-nitroso-*N*-acetyl axial sialyl thioglycoside that results in exchange of the 4-O-ester with acetic acid from the medium.

Results

Synthesis

Methyl [methyl (5-acetamido-5-deoxy-β-ᴅ-glycero-ᴅ-galacto-2-nonulopyranosid)onate] **1** was obtained from *N*-acetylneuraminic acid under standard conditions,[30-31] and was converted by standard methods to the known derivatives **2-5**.[30,32-34]

1: X = H
2: X = Ac

3: X = Y = H
4: X = TBDMS, Y = H

5
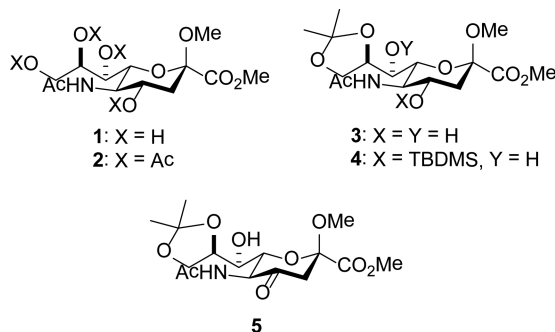
Removal of the isopropylidene group from **4** with trifluoroacetic acid in wet dichloromethane gave the triol **6**, which on acetylation afforded the 4-*O*-silyl-7,8,9-tri-*O*-acetyl derivative **7**. Treatment with tetrabutylammonium fluoride then afforded **8**, which was converted to the corresponding 4-*O*-benzyl ether **9** by reaction with benzyl trichloroacetimidate and TfOH,[35] and to the 4-*O*-*t*-butyloxycarbonyl derivative **10** by stirring with Boc$_2$O in the presence of trimethylamine and 4-dimethylaminopyridine,

aqueous work-up and purification by chromatography over neutral alumina (Scheme 1). It is noteworthy that attempted purification of **10** by chromatography over silica gel resulted in an isolated yield of 28% of the desired product together with 23% of the *N*-acetyl oxazolidinone **11**. This unanticipated cyclization provides a possible alternative entry into the *N*-acetyl oxazolidinones, which have found widespread use in the stereocontreolled synthesis of sialic acid glycosides.[20-22] Isopropylidene cleavage from **5** gave **12** from which **13** was obtained by acetylation. Subsequent Wittig olefination gave the 4-deoxy-4-alkylidene derivative **14** as the *E*-isomer as determined by the NOE correlation of the vinylic hydrogen with the H5. Catalytic hydrogenation over palladium charcoal finally gave **15**, whose configuration rests on coupling constant analysis in the H3,H4,H5 spin system in the ^{1}H NMR spectrum (Scheme 1).

Benzylation of **4** with sodium hydride and benzyl bromide in DMF gave the 7-*O*-benzyl derivative **16**, from which the isopropylidene and silyl groups were removed with acetic acid in methanol yielding **17** and, after acetylation **18** (Scheme 2). Treatment of **1** with di-(*tert*-butyl)-dichlorosilane and imidazole in dichloromethane afforded the 8,9-*O*-silylene derivative **19**, which underwent selective installation of the *tert*-butyldimethylsilyl group on O4 to give **20**. The 7-*O*-Boc group was then installed with Boc$_2$O to give **21**, which on treatment first with TBAF and then acetic anhydride in pyridine afforded **22** (Scheme 2).

Benzylation of **3** with sodium hydride and benzyl bromide in DMF gave the 4,7-di-*O*-benzyl ether **23**, which on removal of the isopropylidene group with toluenesulfonic acid in methanol and subsequent acetylation gave **24** and **25**, respectively (Scheme 3).

Influence of Protecting Groups on Stereoselectivity

The series of sialyl glycosides **2**, **7**, **9**, **10**, **15**, **18**, **22**, **23**, and **25** were converted to a series of *N*-nitroso derivatives **26**-**34** by treatment with NO$^+$BF$_4^-$ in a mixture of pyridine and dichloromethane at −10 °C in essentially quantitative yield and taken forward to the next step immediately.[23-24] Exposure to trifluoroethanol and sodium isopropoxide in dichloromethane and isopropanol at −10 °C followed by addition of acetic acid under the standard conditions gave the corresponding KDN derivatives and their epimers at the 5-position **35-45** as reported in Table 1.

To facilitate interpretation Table 1 is organized in order of decreasing electron-withdrawing effect of their collective protecting group arrays. As observed previously[23-24,29] isolated yields of the oxidative deamination products are typically in the range of 50% (Table 1). Inspection of each reaction mixture by mass spectrometry revealed a number of side products typically arising from i) formal elimination of the nitrosoacetamide function to the give an alkene [M - AcNHNO]$^+$;[28] ii) substitution of the nitrosoacetamide by trifluoroethoxide [M – AcNNO + CF$_3$CH$_2$O]$^+$; and iii) substitution of the nitrosoacetamide by isopropoxide [M – AcNNO + *i*PrO]$^+$. As the reaction mixtures were complex and purification of the byproducts tedious, isolation and characterization of such products was not undertaken. Exceptionally, however, for the *tert*-butyl ester **34**, for which the isolated yield of the oxidative amination product **46** was only 17% (Table 1, entry 10), a sample of the alkene **47** was isolated in 6% yield and characterized. In general the KDN derivatives

with the equatorial acetoxy group at C5 are distinguished from their 5-epi-KDN isomers with the axial group on the basis of the chemical shift and multiplicity of H5, the multiplicity of H4 and the chemical shift of the axial H3 in the ^{1}H NMR spectra. On this basis, the absence of the 5-epimers in Table 1, entries 1-6 and 10 was readily apparent on inspection of the crude reaction mixtures by ^{1}NMR spectroscopy.

47
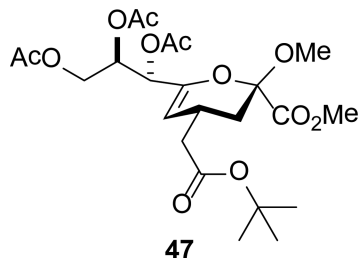
Incorporation of ^{13}C-Labelled Acetic Acid

The oxidative deamination of **26** was also conducted with acetic acid that was 99% enriched at the carbonyl carbon, resulting in the isolation of **35** carrying the labelled acetate group at the 5-position, with no significant loss of label as determined by mass spectrometry (Scheme 4). The identical experiment was conducted on the nitrosoamide **49**, derived from the corresponding α-adamantanyl thioglycoside **48**,[21,24] with comparable clean and complete incorporation of the labelled acetate at the 5-position in the product **50** (Scheme 4). However, with the isomeric nitrosoamide **51**,[24] derived from the β-adamantanyl thioglycoside **52**,[21] mass spectrometric analysis of the crude reaction mixture and of the isolated KDN derivative revealed incorporation of a second isotopically labelled acetoxy group to the extent of approximately 30% (Scheme 4). Careful NMR analysis, particularly correlation of the carbonyl C of the minor enriched acetate with H4 in a HMBC experiment, located this second labelled acetate group at the 4-position of **53** (Scheme 4). The location of the labelled acetate group at the 5-position of the monolabeled products ^{13}C$_1$-**35** and **50** was similarly confirmed by HMBC experiments.

Proton-Deuterium Exchange at the 5-Position

The nitrosylated amide **26** was subjected to the standard Zbiral conditions[23-24,29] with the difference that all protic solvents and reagents employed were deuteriated at the exchangeable position. Thus, a solution of **26** and CF$_3$CH$_2$OD in dichloromethane was treated at −10 °C with a solution of sodium isopropoxide in *i*-PrOD followed by addition of CH$_3$CO$_2$D. Standard work up and chromatographic purification enabled the isolation of the monodeuterio KDN derivative **35**-5*d* in which deuterium was fully and selectively incorporated at the 5-position (Scheme 5).

Discussion

Mechanism of Oxidative Deamination

An mechanistic overview of the oxidative deamination containing the various hypothetical intermediates is presented in Scheme 6 using the per-*O*-acetyl *N*-nitrosoacetamide **26** as example. Trifluoroethoxide generated in situ from trifluoroethanol with sodium

isopropoxide, or added as a prepared salt, serves as a mild nucleophile to bring about the selective deacetylation of the nitrosoamide **26** to give the hydroxydiazine **55**.[36] This step may take place either directly on **26**, which is strongly activated because of the presence of the electron-withdrawing nitroso group, or via the intermediacy of the acetoxydiazene **54** with which it is considered to be in equilibrium.[36] Other nucleophiles that have been used for this purpose in related systems include amine N-oxides,[37] imidazole, and hydroxide.[38] On acidification, either by the addition of suitably acidic nucleophile or by the addition of a Brønsted acid in the presence of a nucleophile,[24] protonation and elimination of water occurs to give the diazonium ion **56** and its conjugate base the diazoalkane **57**.[36] These level 1 nitrogenous intermediates the undergo loss of molecular nitrogen to give one or other of the level 2 intermediates, the carbenium ion **58** and the carbene **59**. These level two intermediates either proceed directly to the elimination byproducts[28] **64** and **65**, and the substitution product **35**, or are stabilized in the form of one or more of the level 3 intermediates **60-63**, which ultimately afford the isolated reaction products. Our goal in initiating this project was to determine the reasons underlying the stereoretentive nature of the oxidative deamination reaction; accordingly the focus of this discussion is on the level 2 and level 3 intermediates.

The high level of incorporation of ^{13}C enriched acetic acid in to the product **35** (Scheme 4) under the typical reaction conditions necessitates an intermolecular process rather than any intramolecular rearrangement with concomitant loss of nitrogen from **26**, **54**, or the ion pair **56**. If the carbenium ion **58** or one of its stabilized forms **60**, **61** and **63** are on the reaction pathway, the complete incorporation of deuterium at the 5-position when the reaction is conducted with deuterated reagents (Scheme 5) requires that the diazonium ion **56** and the diazoalkane **57** be in dynamic equilibrium. Alternatively the incorporation of deuterium (Scheme 5) can be accommodated by the intermediacy of the carbene **59**, or its stabilized equivalent, the ylid **62**. Other carbohydrate-based diazoalkanes generated from N-nitroacetamides have been previously shown to participate in typical carbene-type intermolecular C-H insertions.[39-40] The very high levels of overall retention of configuration typically seen in these reactions, both past[23-24,28-29,41] and present (Table 1, entries 1-6), argue against the formation of the substitution product **35** directly from the carbenium ion **58** or the carbene **59**, although it remains possible that the elimination products **64** and **65** are formed from **58** or **59** by deprotonation or C-H insertion, respectively. Rather, it is likely that the origin of stereoselectivity lies with the intermediacy of one or other of the level 3 intermediates formed either directly from the level 1 intermediates **56** and **57** in a concerted manner or via the transient level 2 intermediates **58** and **59**.

Stereodirecting neighboring group participation by esters through the intermediacy of dioxalenium ions is a well-established phenomenon.[42-47] Comparable participation of more remote esters through six-membered dioxenium or larger cyclic ions is hotly debated in carbohydrate chemistry,[48-52] but the premise of dioxenium ions as intermediates in simple systems rests securely on a labelling study.[53] In the present oxidative deamination reaction participation through the dioxenium ion **61** would appear to be especially favorable in view of the extended zig-zag conformation of the side chain[54-60] and the ideal location it imposes on the 7-O-acetyl group. Notwithstanding this precedent, the complete retention of

configuration observed when the 4-*O* or 7-*O*-acetyl groups are replaced by benzyl ethers or a silyl group (Table 1, entries 4-6) excludes the need for ester participation through either a dioxalenium or dioxenium ion. That the 4-*O* and 7-*O*-*tert*-butoxycarbonyl protected systems **27** and **28** (Table 1, entries 2 and 3) also afford the standard substitution products in the typical yields with no indication of the type of cyclic carbonates[50,61] anticipated from Boc participation corroborates this conclusion. Similarly, we could find no indication of lactone formation[62] resulting from participation by the *tert*-butyl ester **34** (Table 1, entry 10) with loss of the *tert*-butyl group.

The evidence, therefore, points overwhelmingly toward participation by the ring oxygen as in the oxabicyclo[3.1.0]hexyl oxonium ion **63**. This bicyclic oxonium ion may be formed directly from the diazonium ion **56**, via the intermediacy of the carbenium ion **58**, or by protonation of the carbene-derived ylid **62** (Scheme 6). Indeed, an early study on the oxidative deamination of **1**, under conditions of simple thermal decomposition, reported the isolation of a ring-contracted furanosyl byproduct such as would arise from competing nucleophilic ring opening at the 6-position of **63**, albeit neither characterization data nor a mechanism were advanced.[28] Although we have not isolated and characterized such ring contraction products in the present study, it remains possible that they are present among the several byproducts formed in minor amounts. Kinetic evidence for the intermediacy of oxabicyclo[3.1.0]hexyl oxonium ions in the solvolysis of 2-oxa-6-norbornyl brosylates, in comparison to that of 2-norbornyl brosylates, is found in the work of Kirmse and Mrotzeck.[63]

When both the 4-*O*- and 7-*O*-acetyl groups are replaced by less electron-withdrawing benzyl ethers (**32**, Table 1, entry 7), minor stereochemical leakage is observed in that the still major equatorial acetate **41** was formed in a 4.5:1 ratio with its axial isomer **42**. When additionally the 8-*O*- and 9-*O*-acetyl groups are replaced by a less electron-withdrawing isopropylidene group (**33**, Table 1, entry 8) the stereoselectivity of the reaction is fully compromised and the equatorial and axial acetates **43** and **44**, respectively, are formed in equimolar quantities. These observations support the existence of an equilibrium between carbenium ion **66** and oxabicyclo[3.1.0]hexyl oxonium ions **67** (Scheme 7) with the latter favored under typical conditions in the presence of multiple electron-withdrawing protecting groups. With only two remote electron-withdrawing esters to destabilize it, as in **32**, the carbenium ion is sufficiently populated to cause erosion of stereoselectivity. In the complete absence of electron-withdrawing esters, the carbenium ion is sufficiently stable that it dominates the stereochemical outcome of the reaction. The very high degree of retention of configuration observed with the branched carbon analog **34** (Table 1, entry 1) indicates that the presence of three electron-withdrawing esters in the side chain is sufficient to compensate for the replacement of the C4-O bond by the more electron-donating C-C bond such that the oxonium ion mechanism is still favored for substitution.

In an attempt to prepare and store larger quantities of *N*-nitrosoamides, **33** was accessed on 400 mg scale and stored as a solid in the refrigerator at 4 °C. On examination after 12 days the sample was found to have decomposed to give the typical mixture of substitution and elimination products as determined by mass spectrometry. Inspection of the NMR spectra of the crude mixture of products revealed the substitution product to consist exclusively of the

5-epi-KDN derivative **44**, which was isolated as the derivative **45** in 38% yield after removal of the isopropylidene group and acetylation (Table 1, entry 10). Because it takes place in the solid phase, this rearrangement necessarily arises by an intramolecular process and most likely involves passage through an ion pair related to the level 1 intermediate **56** (Scheme 6) with concerted displacement of nitrogen by the incoming acetate. The possibility of an intramolecular S_N2 process at the level of the *Z*-isomer of the acetoxydiazene related to **54**, formally a 6-endo-tet process,[64] is considered unlikely on the grounds of the endocyclic restriction test.[65-66] It is noteworthy that the corresponding peracetyl *N*-nitrosoamide **26** did not undergo decomposition on standing in the refrigerator at 4 °C for one month, thereby reaffirming the role of protecting groups on the stability of the nitrosoamides.

Comparison with the 4-Deoxy-4-acetamidoglucopyranoside and 2-Deoxy-2-acetamidoglucopyranoside Series

Comparison with older studies on the oxidative deamination of 4- and 2-acetamidopyranosides is instructive. Nitrous acid-promoted deamination of methyl 4-amino-4-deoxy-α-ᴅ-glucopyranoside gives methyl α-ᴅ-glucopyranoside as the major product together with methyl β-L-altrofuranoside and 4,5-anhydro-ᴅ-galactose, all of which are suggestive of the involvement of the ring oxygen and of a bicyclic oxonium ion intermediate (Scheme 8). A further product of this reaction, 3-deoxy-3-*C*-formyl α-ᴅ-xylofuranoside, arises from the migration of C2.[67] Although product distributions were substrate and protecting group dependent, comparable products were observed in the nitrous acid-mediated deamination of methyl 4-amino-4-deoxy-2,3-*O*-isopropylidene-α-ᴅ-rhamnopyranoside.[68] Methyl 4-amino-4,6-dideoxy-ᴅ-mannopyranoside is also reported to give D-rhamnopyranose on nitrous acid deamination and hydrolysis.[69] On the other hand, methyl 4-amino-4,6-dideoxy-2,3-*O*-isopropylidene-α-ᴅ-talopyranoside, with an axial amine at the 4-position, does not undergo ring contraction to a significant extent under the same conditions, implying the absence of participation by the ring oxygen, but gives mainly the products of substitution with inversion of configuration.[68] The analogous dependence on configuration is observed in the NO-mediated deamination of the 4-amino-4-deoxyglycopyranoside residues of various bacterial oligosaccharides.[70]

The ring contraction of 4-*O*-sulfonyl derivatives of rhamnopyranose in the presence of azide in DMF at reflux also is known to give the 5,6-dideoxy-5-azido-talofuranose skeleton as the major product requiring overall inversion at C4 and retention (double inversion) at C5.[71-73] As with the deamination reactions, this ring contraction with inversion of configuration at C4 in suggests participation by the ring oxygen leading again to an intermediate oxabicyclo[3.1.0]hexyl oxonium ion (Scheme 9).

Nitrosylation of *N*-acetyl-β-ᴅ-glucosamine tetraacetate gives an isolable *N*-nitroso acetamide that on standing undergoes decomposition to give β-ᴅ-glucose pentaacetate, by a process considered to involve rearrangement to the diazoacetate and subsequent S_Ni, possibly with participation by the ring oxygen.[74-75] On standing in chloroform *N*-acetyl-*N*-nitroso-α-ᴅ-glucosamine tetraacetate, but not its β-anomer, similarly affords β-ᴅ-glucose pentacetate leading to the suggestion of neighboring group participation and ultimate migration by the axial anomeric acetate.[76] Alternatively, treatment of *N*-acetyl-*N*-nitroso-α- or β-ᴅ-

glucosamine tetraacetate with potassium isopropoxide followed by acetylation gives 3,4,5-tri-*O*-acetyl-1,2-dideoxy-D-erythro-pent-1-ynitol by a process considered to involve diazoalkane formation, loss of nitrogen to give the carbene, hydrogen migration and C1-C2 bond cleavage (Scheme 10).[74-75] In aqueous acetone both anomers of *N*-acetyl-*N*-nitroso-D-glucosamine tetraacetate undergo ring contraction with inversion of configuration at C2 to give triacetyl-2,5-anhydro-D-mannose (chitose) implying transannular attack by the ring oxygen and the intermediacy of an oxabicyclo[3.1.0]hexyl oxonium ion (Scheme 10).[76] *N*-Acetylglucosamine and its glycosides undergo the analogous rearrangement on *N*-nitrosylation;[77] the process has been employed in the depolymerization of glycoaminoglycans[70] and in the cleavage of ring I from the aminoglycoside antibiotic paromomycin.[78]

Overall, a consistent pattern emerges in which a tetrahydropyran carrying an equatorial leaving group undergoes transannular participation by the ring oxygen with inversion of configuration to give an 1-oxoabicyclo[3.1.0]hexyl oxonium ion (Scheme 11). In most cases participation by the ring oxygen predominates over participation by ester substituents on the tetrahydropyran framework. The subsequent chemistry of the 1-oxoabicyclo[3.1.0]hexyl oxonium ion is dictated by the reaction conditions and above all by the substituent pattern. Nucleophilic attack at the bridgehead carbon leads to the substitution product with overall inversion of configuration, whereas nucleophilic attack in the smaller bridge affords ring contraction products. The partitioning between these two pathways depends on the overall substituent pattern, the nucleophile, and the reaction conditions. In the sialic acid series, with the larger more heavily substituted side chain in the small bridge, attack at the bridgehead predominates (Table 1 and Scheme 6). Conversely, in the 4-aminoglucosyl series and related compounds (Schemes 8 and 9) with a smaller and less electron-withdrawing side chain in the small bridge, ring contraction is more common. In the 2-aminoglucosyl series (Scheme 10), the small bridge is the anomeric carbon and ring contraction is facilitated by the presence of glycosidic oxygen.

Influence of the anomeric Substituent and Configuration

Finally we return to the formation of the doubly labelled KDN derivative $^{13}C_2$-**53** identified as byproduct on use of ^{13}C-labelled acetic acid as nucleophile in the oxidative deamination of the β-thiosialoside **51**, and the absence of comparable products in the reactions of the epimeric substrate **48** or the *O*-glycoside **26** (Scheme 4). We suggest that incorporation of the label at the 4-position is best explained by the series of equilibria outlined in Scheme 12. Thus the diazoalkane **A**, whose formation is dictated by the deuterium incorporation experiment (Scheme 6), exists in equilibrium with the zwitterion **B** arising from participation by the axial thioglycoside. O→N Acetate transfer then occurs via a six-membered cyclic transition state from O4 to give the *N*-acyl diazene **C**, which undergoes deacetylation on reaction with the labelled acetic acid present in the reaction mixture to give **D** and ^{13}C-labelled acetic anhydride. The reverse process returns either **A** or $^{13}C_1$-**A** depending on which of the two carbonyl groups in the mono ^{13}C-labelled acetic anhydride serves as electrophile. With the label introduced at O4, $^{13}C_1$-**A** then enters the standard mechanism in parallel with its isotopomer **A** leading overall to the formation of $^{13}C_2$-**53** and $^{13}C_1$-**53**, respectively.

Conclusion

The stereoretentive oxidative deamination of the *N*-nitroso-*N*-acetyl sialyl glycosides derives its stereospecificity from participation by the pyranosyl ring oxygen through the intermediacy of a 1-oxabicyclo[3.1.0]hexyl oxonium ion and not via participation by esters at either the 4- or 7-positions. In general the oxidative deamination of the sialic acids and the 4-amino-4-deoxy- and 2-amino-2-deoxy glycopyranosides proceeds through a common pathway with the differences in product distribution, mainly substitution verses ring contraction, determined by the substituents at the level of the 1-oxabicyclo[3.1.0]hexyl oxonium ion intermediate. A minor reaction pathway in the β-thiosialosides has been identified by isotopic labelling studies in which the 4-*O*-acetate undergoes exchange with external acetic acid in the medium, for which a mechanism is proposed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References

1. Angata T, Varki A. Chem. Rev. 2002; 102:439–469. [PubMed: 11841250]

2. Chen X, Varki AP. ACS Chem. Biol. 2010; 5:163–176. [PubMed: 20020717]

3. Varki A, Gagneux P. Ann. N. Y. Acad. Sci. 2012; 1253:16–36. [PubMed: 22524423]

4. Crocker, PR.; Kelm, S. Carbohydrates in Synthesis and Biology. Ernst, B.; Hart, GW.; Sinaÿ, P., editors. Vol. 4. Wiley-VCH; Weinheim: 2000. p. 579-596.

5. Thomson, R.; von Itzstein, M. Carbohydraye-Based Drug Discovery. Wong, C-H., editor. Vol. 2. Wiley-VCH; Weinheim: 2003. p. 831-861.

6. Hsu Y, Ma H-H, Lico LS, Jan J-T, Fukase K, Uchinashi Y, Zulueta MML, Hung S-C. Angew. Chem. Int. Ed. 2014; 53:2413–2416.

7. El-Deeb IM, Guillon P, Winger M, Eveno T, Haselhorst T, Dyason JC, von Itzstein M. J. Med. Chem. 2014; 57:7613–7623. [PubMed: 25198831]

8. Rillahan CD, Schwartz E, McBride R, Fokin VV, Paulson JC. Angew. Chem. Int. Ed. 2012; 51:11014–11018.

9. Kiefel MJ, von Itzstein M. Chem. Rev. 2002; 102:471–490. [PubMed: 11841251]

10. Smith PW, Starkey ID, Howes PD, Sollis SL, Keeling SP, Cherry PC, von Itzstein M, Wu WY, Jin B. Eur, J. Med. Chem. 1996; 31:143–150.

11. Wang Q, Guo Z. ACS Med. Chem. Lett. 2011; 2:373–378. [PubMed: 21691430]

12. Pazynina G, Tyrtysh T, Nasonov V, Belyanchikov I, Paramonov A, Malysheva N, Zinin AI, Kononov LO, Bovin N. Synlett. 2013:226–230.

13. Horstkorte, R.; Keppler, OT.; Reutter, W. Carbohydrate-Based Drug Discovery. Wong, C-H., editor. Vol. 2. Wiley-VCH; Weinheim: 2003. p. 863-873.

14. Chokhawala HA, Huang S, Lau K, Yu H, Cheng J, Thon V, Hurtado-Ziola N, Guerrero JA, Varki A, Chen X. ACS Chem. Biol. 2008; 3:567–576. [PubMed: 18729452]

15. Sugiarto G, Lau K, Qu J, Li Y, Lim S, Mu S, Ames JB, Fisher AJ, Chen X. ACS Chem. Biol. 2012; 7:1232–1240. [PubMed: 22583967]

16. Rillahan CD, Macauley MS, Schwartz E, He Y, McBride R, Arlian BM, Rangarajan J, Fokin VV, Paulson JC. Chem. Sci. 2014; 5:2398–2406. [PubMed: 24921038]

17. Starkey ID, Mahmoudian M, Noble D, Smith PW, Cherry PC, Howes PD, Sollis SL. Tetrahedron Lett. 1995; 36:299–302.

18. Tanaka H, Nishiura Y, Takahashi T. J. Am. Chem. Soc. 2006; 128:7124–7125. [PubMed: 16734441]

19. Farris MD, De Meo C. Tetrahedron Lett. 2007; 48:1225–1227.

20. Crich D, Li W. J. Org. Chem. 2007; 72:2387–2391. [PubMed: 17338570]

21. Crich D, Li W. J. Org. Chem. 2007; 72:7794–7797. [PubMed: 17824651]

22. Hsu C-H, Chu K-C, Lin Y-S, Han J-L, Peng Y-S, Ren C-T, Wu C-Y, Wong C-H. Chem. Eur. J. 2010; 16:1754–1760. [PubMed: 20066711]

23. Crich D, Navuluri C. Angew. Chem. Int. Ed. 2010; 49:3049–3052.

24. Navuluri C, Crich D. Angew. Chem. Int. Ed. 2013; 52:11549–11552.

25. Isemura M, Schmid K. Biochem. J. 1971; 124:591–604. [PubMed: 5135244]

26. Mononen I. Carbohydr. Res. 1981; 88:39–50.

27. Inoue S, Kitajima K. Glycoconj. J. 2006; 23:277–290. [PubMed: 16897172]

28. Shirai R, Nakamura M, Hara S, Takayanagi H, Ogura H. Tetrahedron Lett. 1988; 29:4449–4452.

29. Schreiner E, Zbiral E. Liebigs Annalen der Chemie. 1990:581–586.

30. Kuhn R, Lutz P, Macdonald DL. Chem. Ber. 1966; 99:611–617. [PubMed: 5930124]

31. Chopra P, Thomson RJ, Grice D, von Itzstein M. Tetrahedron Lett. 2012; 53:6254–6256.

32. Baggett N, Marsden BJ. Carbohydr. Res. 1982; 110:11–18. [PubMed: 7172166]

33. Zbiral E, Phadtare S, Schmid W. Liebigs Ann. Chem. 1987:39–43.

34. Hartmann M, Zbiral E. Monat. Chem. 1989; 120:899–906.

35. Iversen T, Bundle DR. J. Chem. Soc., Chem. Commun. 1981:1240–1241.

36. Kirmse W. Angew. Chem. Int. Ed. 1976; 15:251–320.

37. Nikolaides N, Godfrey AG, Ganem B. Tetrahedron Lett. 1990; 31:6009–6012.

38. Challis BC, Jones SP. J. Chem. Soc., Perkin Trans. 1979; 2:703–706.

39. Sarabia F, Martín-Ortiz L, López-Herrera FJ. Org. Biomol. Chem. 2003; 1:3716–3725. [PubMed: 14649903]

40. Sarabia-García F, López-Herrer FJ. Tetrahedron. 1996; 52:4757–4768.

41. Zunk M, Williams J, Carter J, Kiefel MJ. Org. Biomol. Chem. 2014; 12:2918–2925. [PubMed: 24687140]

42. Frush HL, Isbell HS. J. Research Natl. Bur. Standards. 1941; 27:413–428.

43. Winstein S, Grunwald E, Ingraham LL. J. Am. Chem. Soc. 1948; 70:821–828.

44. Capon, B.; McManus, SP. Neighboring Group Participation. Plenum; New York: 1976.

45. Paulsen H, Herold C-P. Chem. Ber. 1970; 103:2450–2462.

46. Crich D, Dai Z, Gastaldi S. J. Org. Chem. 1999; 64:5224–5229.

47. Nukada T, Berces A, Zgierski MZ, Whitfield DM. J. Am. Chem. Soc. 1998; 120:13291–13295.

48. Komarova, BS.; Ustyuzhania, NE.; Tsvetkov, YE.; Nifantiev, NE. Modern Synthetic Methods in Carbohydrate Chemistry; From Monosaccharides to Complex Glycoconjugates. Werz, DB.; Vidal, S., editors. Wiley; Weinheim: 2014. p. 125-160.

49. Kim KS, Suk D-H. Top. Curr. Chem. 2011; 301:109–140. [PubMed: 21229347]

50. Crich D, Hu T, Cai F. J. Org. Chem. 2008; 73:8942–8953. [PubMed: 18939876]

51. Baek JY, Kwon H-W, Myung SJ, Park JJ, Kim MY, Rathwell DCK, Jeon HB, Seeberger PH, Kim KS. Tetrahedron. 2015; 71:5315–5320.

52. Ma Y, Lian G, Li Y, Yu B. Chem. Commun. 2011; 47:7515–7517.

53. Wilen SH, Delguzzo L, Saferstein R. Tetrahedron. 1987; 43:5089–5094.

54. Grindley, TB. Glycoscience: Chemistry and Chemical Biology. Fraser-Reid, B.; Tatsuta, K.; Thiem, J., editors. Vol. 1. Springer; Berlin: 2001. p. 3-51.

55. Christian R, Schulz G, Brandstetter HH, Zbiral E. Carbohydr. Res. 1987; 162:1–11. [PubMed: 3594474]

56. Brown EB, Brey WS Jr. Weltner W Jr. Biochim. Biophys. Acta. 1975; 399:124–130. [PubMed: 1148272]

57. Flippen JL. Acta Cryst. 1973; B29:1881–1886.

58. Veluraja K, Rao VSR. Biochim. Biophys. Acta. 1980; 630:442–446. [PubMed: 7397233]

59. Sabesan S, Bock K, Lemieux RU. Can. J. Chem. 1984; 62:1034–1045.

60. Kancharla PK, Crich D. J. Am. Chem. Soc. 2013; 135:18999–19007. [PubMed: 24261615]

61. Bartlett PA, Meadows JD, Brown EG, Morimoto A, Jernstedt KK. J. Org. Chem. 1982; 47:4013–4018.

62. Wen P, Crich D. J. Org. Chem. 2015; 80:12300–12310. [PubMed: 26565923]

63. Kirmse W, Mrotzeck U. Chem. Ber. 1988; 121:485–492.

64. Baldwin JE. J. Chem. Soc., Chem. Commun. 1976:734–736.

65. Tenud L, Farooq S, Seibl J, Eschenmoser A. Helv. Chim. Acta. 1970; 53:2059–2069.

66. Beak P. Acc. Chem. Res. 1992; 25:215–222.

67. Ng Ying Kin NMK, Williams JM, Horsington A. J. Chem. Soc. C. 1971:1578–1583.

68. Al-Radhi AK, Brimacombe JS, Tucker LCN. J. Chem. Soc, Perkin Trans. 1972; 1:315–320.

69. Lee C-H, Schaffner CP. Tetrahedron Lett. 1966; 7:5837–5840. [PubMed: 5980011]

70. Duan J, Kasper DL. Glycobiology. 2011; 21:401–409. [PubMed: 21030538]

71. Stevens CL, Glinski RP, Taylor KG, Blumbergs P, Sirokman F. J. Am. Chem. Soc. 1966; 88:2073–2074.

72. Hanessian S. Chem. Comm. 1966:796–798.

73. Stevens CL, Glinski RP, Taylor KG, Sirokman F. J. Org. Chem. 1970; 35:592–596.

74. Horton D, Loh W. Carbohydr. Res. 1974; 36:121–130. [PubMed: 4412633]

75. Horton D, Loh W. Carbohydr. Res. 1974; 38:189–203.

76. Llewellyn JW, Williams JM. Carbohydr. Res. 1973; 28:339–350.

77. Horton D, Philips KD. Carbohydr. Res. 1973; 30:367–374.

78. Cassinelli G, Julita P, Arcamone F. J. Antibiotics. 1978; 31:382–384. [PubMed: 659337]
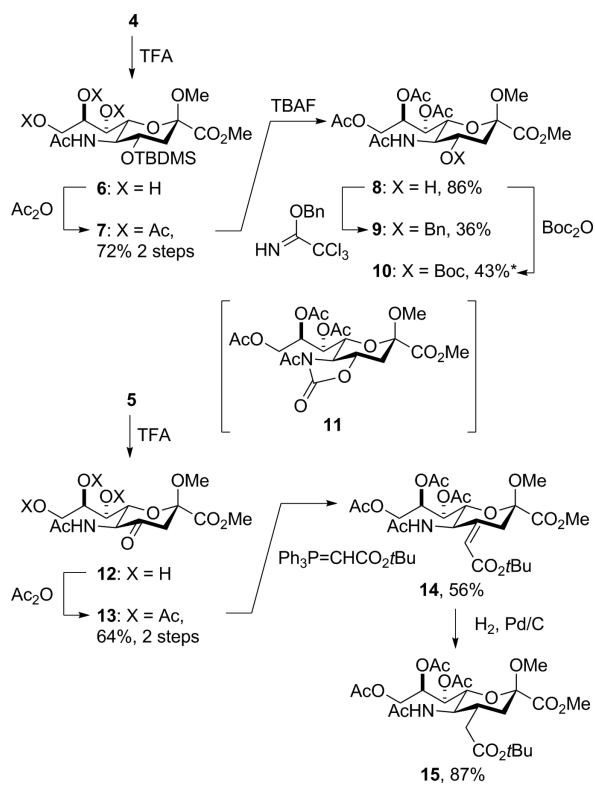
Scheme 1.
Synthesis of NeuAc Derivatives Selectively Functionalized at the 4-Position.
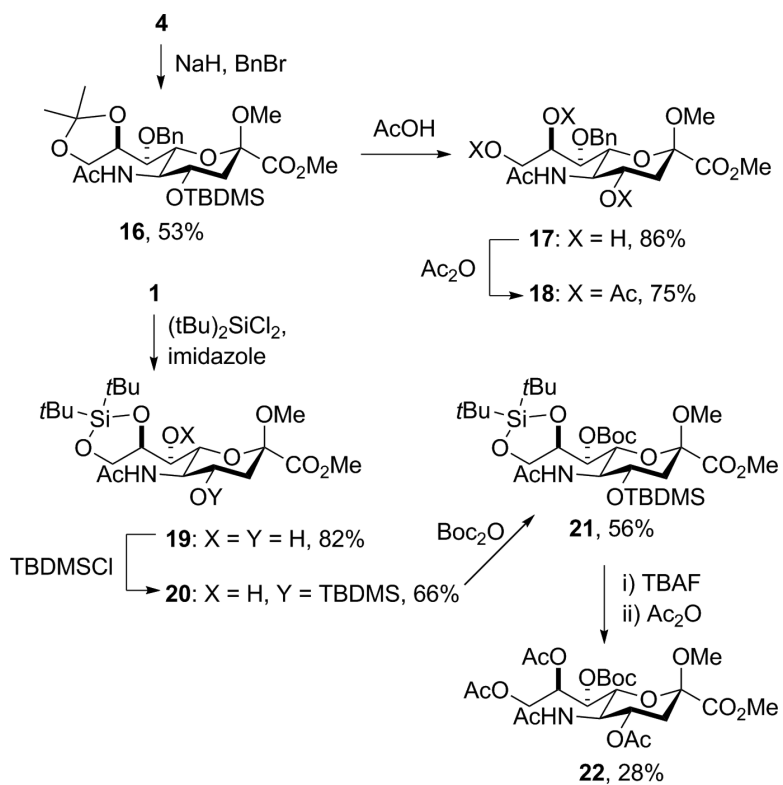
Scheme 2.
Synthesis of NeuAc Derivatives Selectively Protected at the 7-Position.
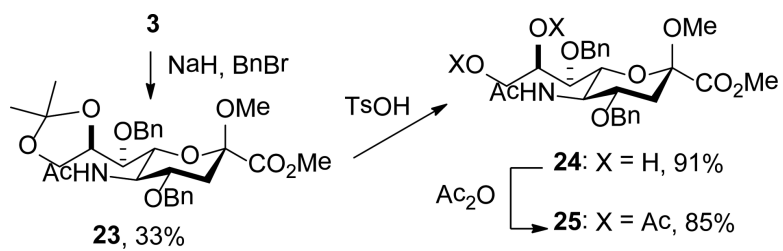
Scheme 3.
Synthesis of a NeuAc Derivative Selectively Protected at the 4- and 7-Positions.
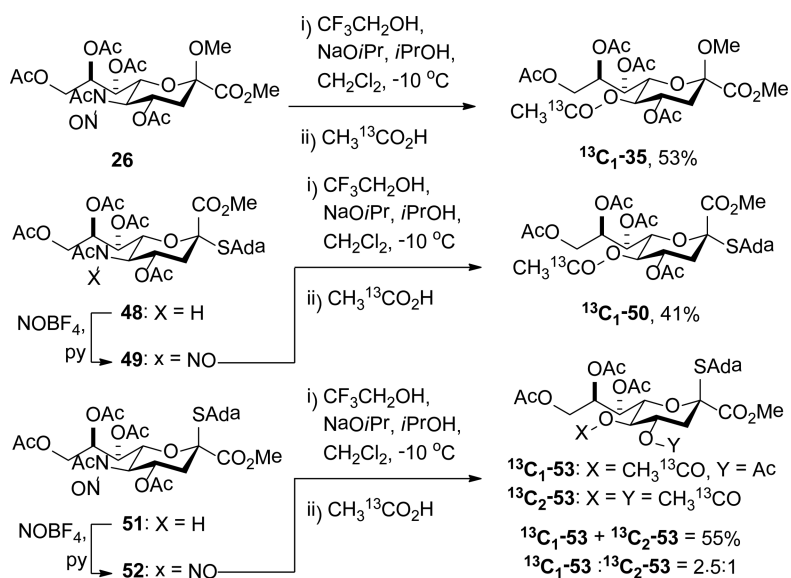
Scheme 4.
Incorporation of ^{13}C-Labelled Acetate.
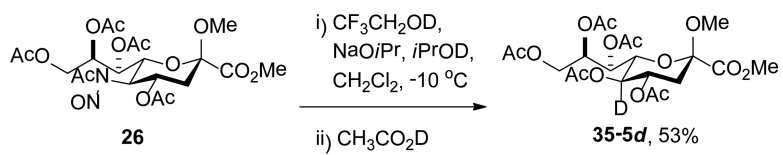
Scheme 5.
Deuterium Incorporation at the 5-Position of KDN.
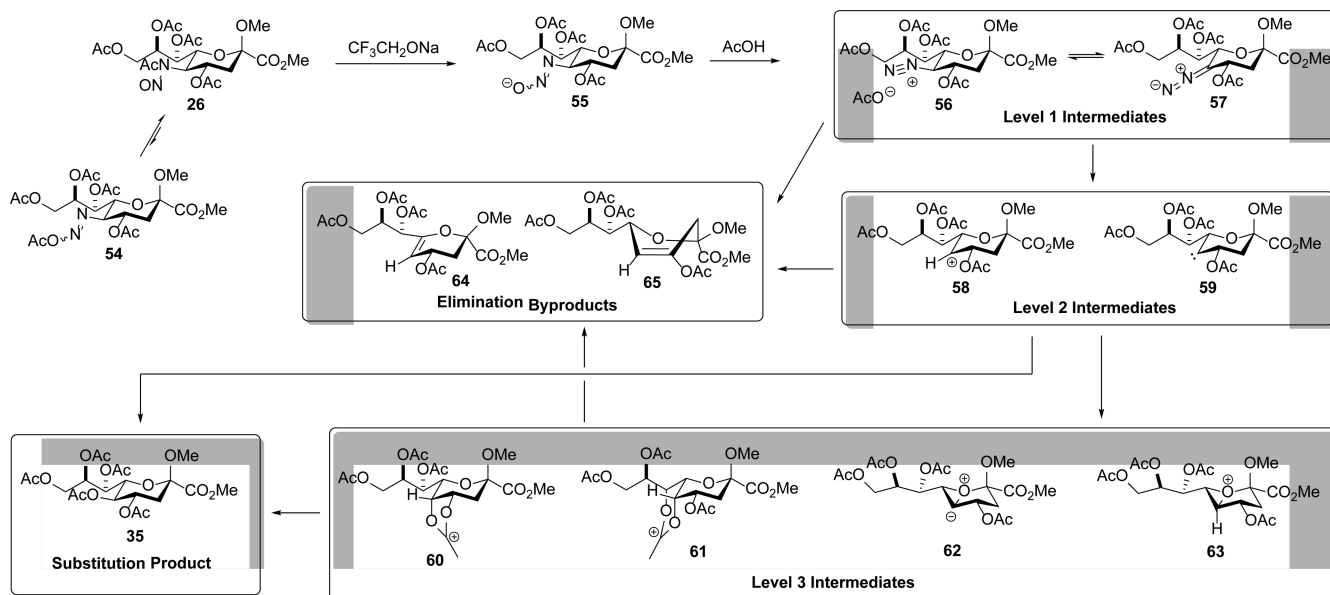
Scheme 6.
Global Mechanism for Oxidative Deamination with Three Levels of Hypothetical
Intermediates

66
- Carbenium Ion
- Unselective
- Populated with Multiple Ether Groups R

67
- Bicyclic Oxonium Ion
- Stereoselective
- Favored by Multiple Electron-Withdrawing Groups R
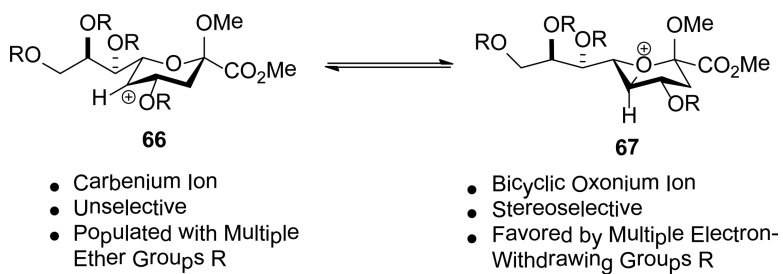
Scheme 7.
Equilibrating Carbenium and Bridged Oxonium Ions and the Influence of Protecting Groups.
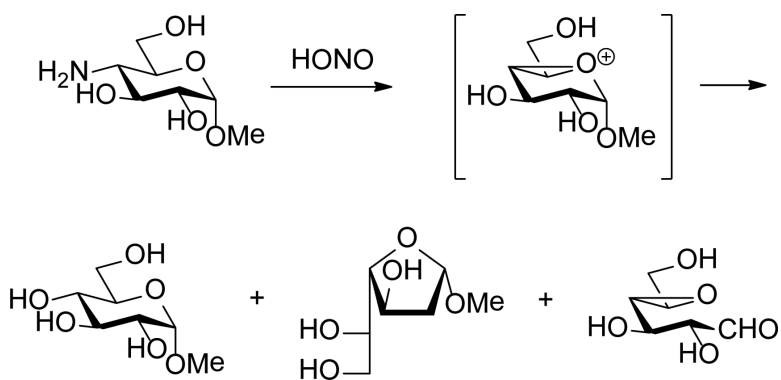
Scheme 8.
Deamination Reactions in the 4-Amino-4-deoxyglucose Series
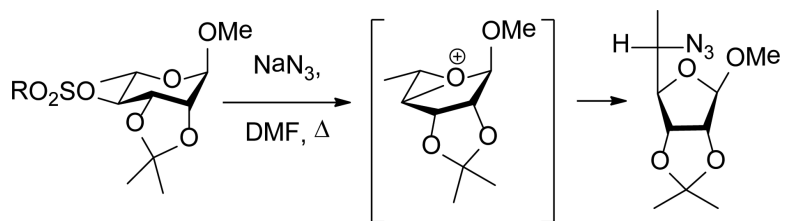
Scheme 9.
Ring Contraction of a Rhamnosyl 4-*O*-Sulfonate
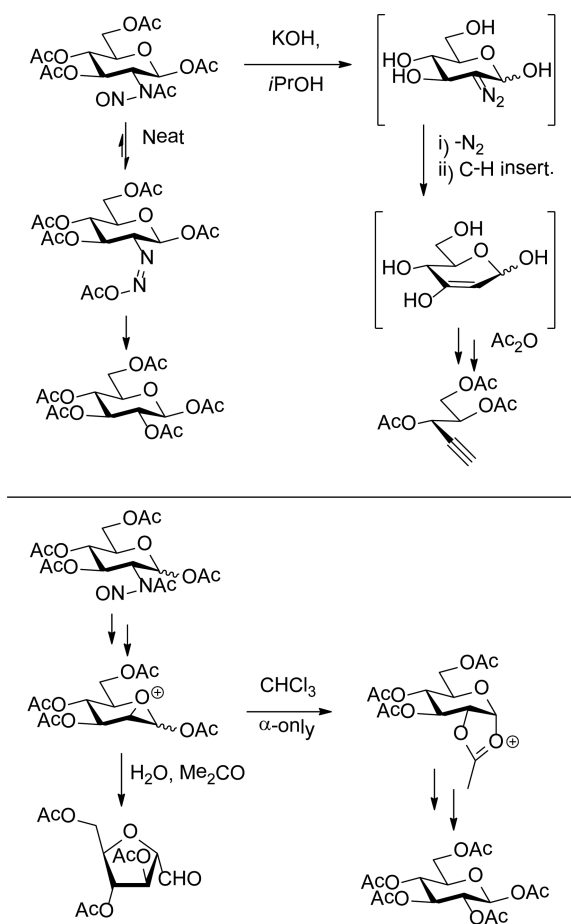
Scheme 10.
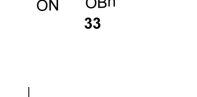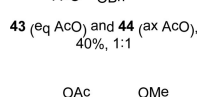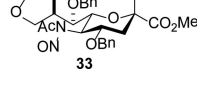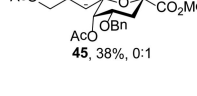Deamination Reactions in the Glucosamine Series
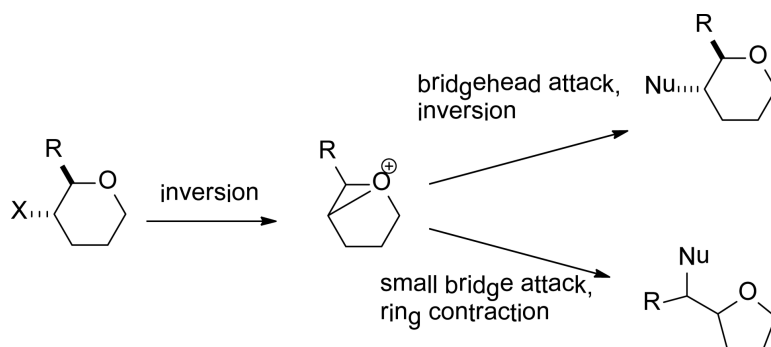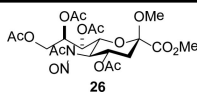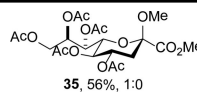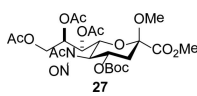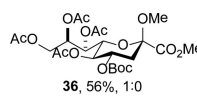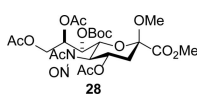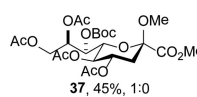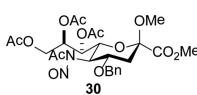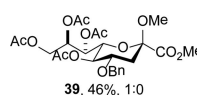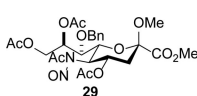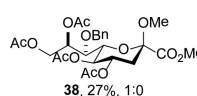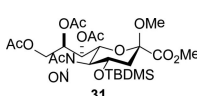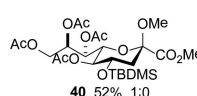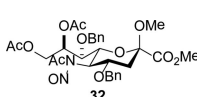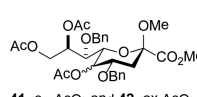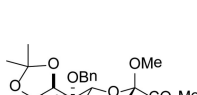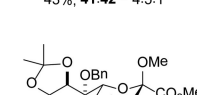
Scheme 11.
1-Oxabicyclo[3.1.0]hexyl Oxonium Ion Formation and Opening.
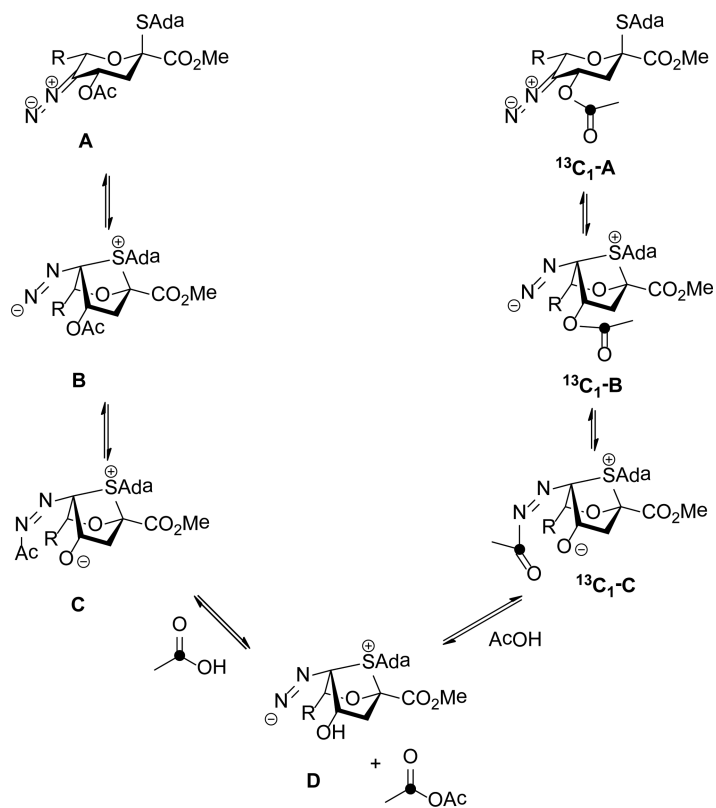
Scheme 12.
Incorporation of ^{13}C-Labelled Acetate at O4 in the Axial Thioglycoside **53**.

Table 1

Influence of Protecting Groups on Stereoselectivity of KDN Formation.[a]

Entry	Nitrosylated Substrate	Product, Yield, selectivity (KDN:5-epi-KDN)
1	**26**	**35**, 56%, 1:0
2	**27**	**36**, 56%, 1:0
3	**28**	**37**, 45%, 1:0
4	**30**	**39**, 46%, 1:0
5	**29**	**38**, 27%, 1:0
6	**31**	**40**, 52%, 1:0
7	**32**	**41** (eq AcO) and **42** (ax AcO), 43%, **41:42** = 4.5:1
8	**33**	**43** (eq AcO) and **44** (ax AcO), 40%, 1:1
9[b,c]	**33**	**45**, 38%, 0:1
10[d]	**34**	**46**, 17%, 1:0

[a]With the exception of entry 9 all reactions were conducted under a standard set of conditions involving treatment of the nitrosoamide in CH_2Cl_2 at −10 °C with trifluoroethanol and then sodium isopropoxide in isopropanol followed by addition of glacial acetic acid and warming to 0 °C.

[b]The nitrosamide was stored neat at 4 °C in the refrigerator for 12 days. No external reagents were added.

[c]To facilitate isolation from a complex mixture the isopropylidene group was cleaved the initial product **44** and the residual diol acetylated to give **45**.

[d]Isolated yield of pure **46** is only 17% owing to difficulties in isolation.