Carbohydrate Research 374 (2013) 23–28

Contents lists available at SciVerse ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

The synthesis of a series of deoxygenated 2,3-difluoro-*N*-acteylneuraminic acid derivatives as potential sialidase inhibitors

Stefan Hader, Andrew G. Watts *

Medicinal Chemistry Group, Department of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, United Kingdom

ARTICLE INFO

Article history:
Received 8 January 2013
Received in revised form 4 March 2013
Accepted 23 March 2013
Available online 2 April 2013

Keywords:
Sialic acid
Barton–McCombie deoxygenation
Luperox
Fluorination
Sialidases

ABSTRACT

Here we describe the successful syntheses of a series of 4-, 7-, 8- and 9-deoxygenated 2,3-difluoro-*N*-acetylneuraminic acid derivatives as potential mechanism-based inhibitors of sialidases. The syntheses commenced utilising an enzyme-catalysed aldolase reaction between *N*-acetyl mannosamine and β-fluoropyruvic acid to give 3-fluoro-*N*-acetyl-neuraminic acid. This common intermediate was then used in selective protection protocols and Barton–McCombie deoxygenations to generate the complete set of mono-deoxygenated 3-fluoro-*N*-acetylneuraminic acid derivatives. Finally, a fluorination step utilising (diethylamino)sulfur trifluoride (DAST) was used to successfully generate each of the target difluorides.

1. Introduction

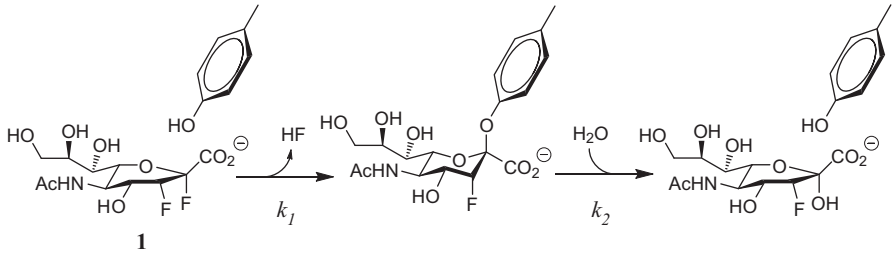
Sialidases are a superfamily of sialic acid-degrading enzymes (EC 3.2.1.18) widely found in higher eukaryotes and in a vast number of microbial pathogens.[1] Owing to the importance that sialidases play in a range of biological processes, the ability to moderate their enzymatic function is associated with numerous therapeutic applications ranging from the treatment of type II diabetes,[2] to their targeting by antiviral[3–5] and cancer[6] therapeutics. Sialidases are known to effect glycoside hydrolysis with a net retention of anomeric stereochemistry (retaining glycosidases) however debate still exists over the nature of the catalytic mechanism through which this hydrolytic reaction is performed. Recently, a number of studies on family GH33 (CAZy)[7] sialidases have demonstrated that these enzymes operate through a two-step, double-displacement mechanism similar to the majority of retaining glycosidases, but involve the participation of a tyrosine residue as the catalytic nucleophile to form, uniquely, a covalent aryl-glycoside intermediate.[8–11] This covalent intermediate was originally observed with *Trypanosoma cruzi* trans-sialidase using the novel mechanism-based inhibitor 2,3-difluoro *N*-acetylneuraminic acid **1**, developed to attenuate glycosylation (k_1) and deglycosylation (k_2) rates in the catalytic cycle of sialidases (Scheme 1).[12,13] The introduction of an electronegative fluorine atom at C-3 serves to destabilise the formation of a positive charge during the transition-state, which reduces the rates of both glycosylation and deglycosylation. A 'good leaving group' at the anomeric position, such as fluorine, selectively rescues the rate of glycosylation allowing the covalently linked sialosyl–enzyme intermediate to be kinetically accessible and accumulate to a high steady-state concentration.

Given the important role of sialidases in numerous diseases it is considered that 2,3-difluoro sialic acids represent a novel class of sialidase inhibitors with the potential to be developed into therapeutics. However, the reported inactivation of *Trypanosoma cruzi* trans-sialidase by 2,3-difluoro-*N*-acetylneuraminic acid **1** required very high concentrations of inhibitor (5 mM), which was considered to be largely attributable to the rapid turn-over of the covalent intermediate (high k_2). This rapid turn-over of the covalent intermediate has also been observed with sialidases from other pathogenic organisms inhibited by difluorosialic acids, suggesting that increasing the stability (half-life) of the covalent intermediate will be necessary to improve the inhibitory properties of this class of compound.[11] Towards this aim, we considered that a detailed understanding of the individual contribution by each hydroxyl group of sialic acid towards transition-state stabilisation of sialidase catalysed hydrolysis would provide valuable information for improving the half-lives of the covalently bound species.

A previous study by Street et al. has investigated substrate/protein interactions through specific modifications of the substrate, creating carefully selected analogues that differ in their electronic properties and hydrogen-bonding capability, but retaining their ability to be accepted and/or processed by the enzyme.[14,15] These studies, performed on glycogen phosphorylase, demonstrated the usefulness of deoxygenated substrates to gain insight into both

* Corresponding author. Tel.: +44 (0) 1225 386788; fax: +44 (0) 1225 386114.
E-mail address: a.watts@bath.ac.uk (A.G. Watts).

0008-6215/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carres.2013.03.026



Scheme 1. The glycosylation (k_1) and deglycosylation (k_2) rate constants are attenuated by fluorine-containing mechanism-based inactivators.

the electronic structure of the enzymatic ground-state Michaelis complex, as well as the transition-state of the reaction.[14,15]

Herein we describe the synthesis of the complete series of mono-deoxygenated 2,3-difluorsialic acids **2**–**5**, designed to facilitate the dissection of individual hydroxyl group contributions towards transition-state stabilisation during the processing of this novel class of inhibitors by sialidases.

2. Results and discussion

2.1. Synthesis of 5-*N*-acetyl-2,3,4,5-tetradeoxy-2,3-difluoro-D-*erythro*-β-L-manno-non-2-ulopyranosonic acid (2)

The synthesis of 5-*N*-acetyl-2,3,4,5-tetradeoxy-2,3-difluoro-D-*erythro*-β-L-manno-non-2-ulopyranosonic acid **2** began with the formation of 3-fluoro-*N*-acetyl-neuraminic acid **8**, as previously described by Watts and Withers using an aldolase mediated reaction (Scheme 2).[10]

According to this procedure, 2-*N*-acetyl-D-mannosamine **6** and β-fluoropyruvic acid **7** were subjected to Neu5Ac aldolase (EC 4.1.3.3) in aqueous solution at room temperature for 5 days to give 3-fluoro-*N*-acetyl-neuraminic acid **8**. The crude residue was subsequently esterified using trifluoroacetic acid (TFA) in methanol and then treated with 2,2′-dimethoxypropane and catalytic amounts of *p*-toluenesulfonic acid (TSA) in acetone according to the procedure of Ogura et al.[16] to give the 3-fluoro-8,9-*O*-isopropylidene-*N*-acetyl-neuraminic acid methyl ester **9** in 71% overall yield from β-fluoropyruvic acid **7**.

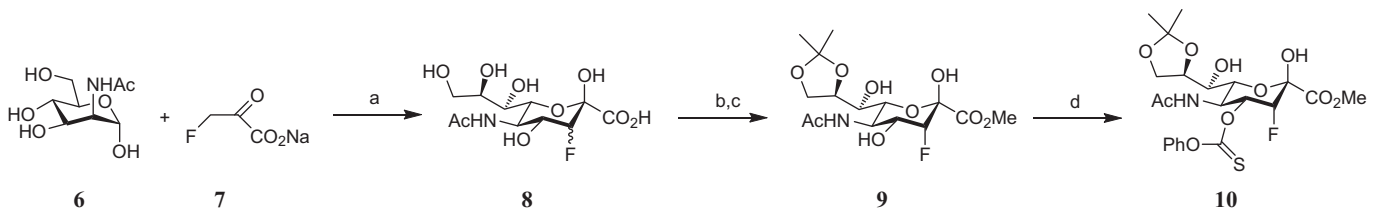
For the subsequent deoxygenation of C-4, we first sought to investigate the use of thionocarbonates and *O*-thiocarbamates as these groups can be introduced under essentially neutral conditions from *O*-phenyl chlorothionoformate and 1,1′-thiocarbonyldiimidazole respectively. Additionally, both thionocarbonates and *O*-thiocarbamates have been shown to react at lower temperatures during radical deoxygenation and have been reported previously for the successful deoxygenation of sialic acid derivatives.[17]

As such, 3-fluoro-8,9-*O*-isopropylidene-*N*-acetyl-neuraminic acid methyl ester **9** was treated with phenyl chlorothionoformate in pyridine and dichloromethane to give the 4-*O*-(phenoxy)thiocarbonyl methyl ester **10** in 76% yield. We next sought to identify suitable conditions for the Barton–McCombie deoxygenation at C-4 to generate the 4-deoxy-3-fluoro methyl ester **11** from the thionocarbonate **10**. Although the deoxygenation of sialic acid at C-4 has been reported previously, the radical initiator used in the vast majority of these reports (AIBN) is now difficult to obtain commercially, prompting us to investigate a variety of radical initiators, radical carriers, solvents and temperatures for this transformation.

Initially, bis(tributyltin) oxide was used to generate tributyltin hydride in situ according to the procedure previously reported of Lopez et al.[18] Here, poly(methylhydrosiloxane) (PMHS) and *n*-butanol in toluene are used to generate two equivalents of tributyltin hydride, with 1,1′ azo bis(cyanocyclohexane) (ACHN) used as the radical initiator. As such, the 4-*O*-(phenoxy)thiocarbonyl derivative **10** was subjected to bis(tributyltin) oxide, *n*-BuOH and azobiscyanocyclohexane in toluene/DMF overnight at 80 °C (Table 1, entry 1).[18] However, under these conditions only the starting compound **10** was recovered. Attempts to drive the conversion forward by increasing the temperature to 90 °C only resulted in decomposition of compound **10** (Table 1, entry 2).

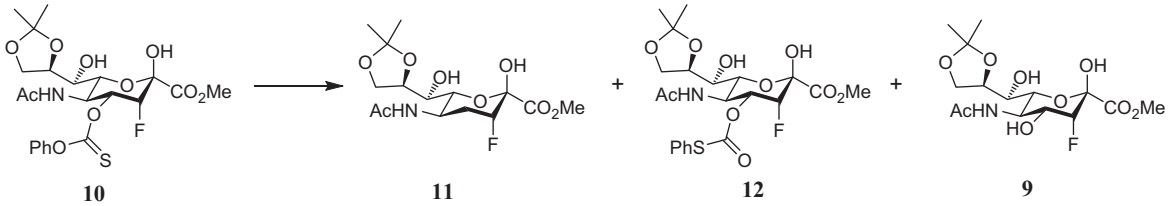
As a consequence of this decomposition, an alternative method reported by Park et al.[19] to effect facile Barton–McCombie deoxygenation was then attempted. Here, the 4-*O*-(phenoxy)thiocarbonyl derivative **10** was subjected to the initiator tetrabutylammonium peroxydisulfate and radical promoter sodium formate in DMF at 60 °C for 24 h as previously described by Park et al. (Table 1, entry 3). Following aqueous work up and purification by silica chromatography (EtOAc/MeOH), the desired product **11** was successfully obtained in 17% yield. However, in addition to the formation of **11**, both starting material **10** and the product of hydrolysis **9** were also observed.[20,21] Several attempts to improve the yield by altering the time and temperature of reaction could only improve the conversion to provide **11** in up to 24% yield (Table 1, entry 4). At this stage it was considered that the reduced rates of conversion observed in comparison to previous reports were likely resulting from the presence of fluorine at C-3 having a significant influence on the progress of the deoxygenation. It was considered that the use of a more effective promoter in tributyltinhydride may overcome the retardive influence of the fluorine.

As such, the 4-*O*-(phenoxy)thiocarbonyl derivative **10** was subjected to a general Barton–McCombie protocol utilising tributyltin hydride and ACHN in toluene under reflux conditions (Table 1, entry 5). Following aqueous workup and purification, the desired



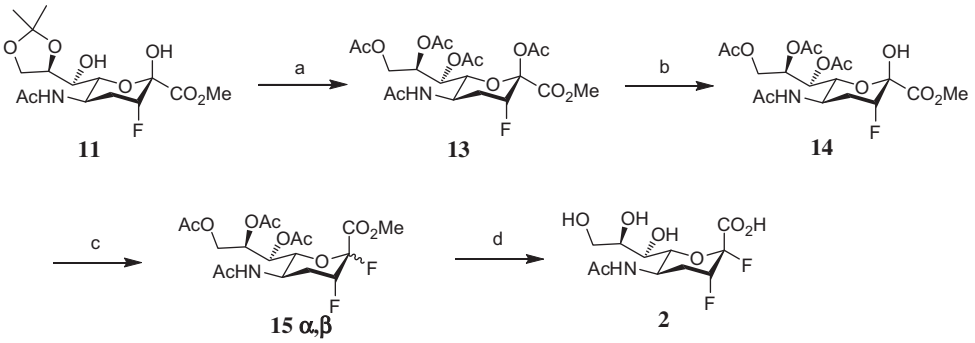
Scheme 2. Reagents and conditions: (a) *N*-Acetylneuraminic aldolase, H_2O, 5 d, rt; (b) MeOH, TFA, 16 h, rt; (c) 2,2-dimethoxypropane, *p*-toluenesulfonic acid, Et_3N, acetone, 4 h, 50 °C, 71% (over 3 steps); (d) phenyl chlorothionoformate, pyridine/CH_2Cl_2, 4 h, −40 °C → rt, 76%.

Table 1
Overview of the different conditions used for the synthesis of 4-deoxy-3-fluoro-8,9-*O*-isopropylidene-*N*-acetylneuraminic acid methyl ester **11**



Entry	Initiator (equiv)	Promotor (equiv)	Solvent	Time (h)	*T* (°C)	Product	Yield[a] (%)
1	ACHN[b] (0.15)	$(Bu_3Sn)_2O$ (0.037) PMHS (5) *n*-BuOH (5.5)	Toluene/DMF	O/N	80	s.m.	n.d.
2	ACHN[b] (0.15)	$(Bu_3Sn)_2O$ (0.037) PMHS (5) *n*-BuOH (5.5)	Toluene/DMF	3.5	90	dec	n.d.
3	$(Bu_4N)_2S_2O_8$ (3)	$NaHCO_3$ (6)	DMF	24	60	**11**	17
4	$(Bu_4N)_2S_2O_8$ (3)	$NaHCO_3$ (6)	DMF	5	70	**11**	24
5	ACHN[b] (0.3)	Bu_3SnH (3.7)	Toluene	24	120	**11**	24
6	BTBPB[c] (0.45)	Bu_3SnH (3.7)	1,4-Dioxane	4	100	**11**	81

dec = Decomposition.
n.d. = Not determined.
[a] Isolated yields after silica chromatography.
[b] 1,1′ Azo bis(cyanocyclohexane).
[c] 50% 2,2-Bis(*tert*-butylperoxy)-butane mineral oil-solution.



Scheme 3. Reagents and conditions: (a) (i) AcOH/H_2O, 2 h, 60 °C, (ii) Ac_2O, pyridine, 3 d, 40 °C, 76% (2 steps); (b) hydrazine acetate, MeOH/CH_2Cl_2, 20 h, 4 °C, 51%; (c) DAST, CH_2Cl_2, 1.5 h, −40 °C → −10 °C, α-anomer: 45%, β-anomer: 30%; (d) (i) NaOMe, MeOH, 30 min, rt, (ii) 1 M NaOH, pH 12, 1 h, rt, 52% (2 steps).

product **11** was isolated in 24% yield. A major side product resulting under these conditions was also identified as the sulfur–oxygen exchanged carbonate **12**. The rearrangement of thionocarbonates under Barton–McCombie conditions, giving derivatives such as the *O*-thiocarbonyl **12**, has been described previously by Powers and Tarbell.[22] Hence, in an effort to reduce the degree of rearrangement, 2,2-bis(*tert*-butylperoxy)-butane (BTBPB) was substituted as the radical initiator to shorten reaction times while using 1,4-dioxane as solvent. Here, 3-fluoro-8,9-*O*-isopropylidene-4-*O*-(phenoxy)thiocarbonyl-*N*-acetylneuraminic acid methyl ester **10** was then subjected to radical Barton–McCombie conditions using tributyltin hydride in the presence of BTBPB in 1,4-dioxane under reflux to provide compound **11** in 81% yield (Table 1, entry 6).[23]

Having successfully deoxygenated the C-4 position, compound **11** was then subjected to 80% aqueous acetic acid according to the procedure of Anazawa et al.[24] to remove the *O*-8,9-isopropylidene group, followed by acetylation using acetic anhydride in pyridine to give the *per*-*O*-acetylated 4-deoxy-3-fluoro-*N*-acetylneuraminic acid **13** in 76% yield over the two steps (Scheme 3).

Selective deprotection at the C-2 position of compound **13** was then performed according to a known procedure using hydrazine acetate in dichloromethane/MeOH,[10,25–27] to afford the hemiketal **14** in 51% yield. Subsequent fluorination by treatment with DAST in dichloromethane at −40 to −10 °C for 1.5 h resulted in the formation of compounds **15**-**α,β** as an anomeric mixture which proved separable by silica chromatography.

Unfortunately, the assignment of absolute stereochemistry to the two compounds on the basis of coupling constants, by either ^1H or ^{19}F NMR analysis, was not possible. As such, small molecule X-ray crystallographic analysis of the less polar compound was utilised to confirm that the fluorine atom at position C-2 for this compound was axial, being labelled F1 in the X-ray structure shown (Fig. 1). Upon this basis, it was determined that the desired α-anomer was isolated in 45%, along with the β-anomer isolated in 30% yield.

The desired α-*per*-*O*-acetyl-4-deoxy-2,3-difluoro-*N*-acetyl-neuraminic acid methyl ester **15**-**α** was then globally deacetylated following Zemplén[28] conditions using sodium in methanol at room

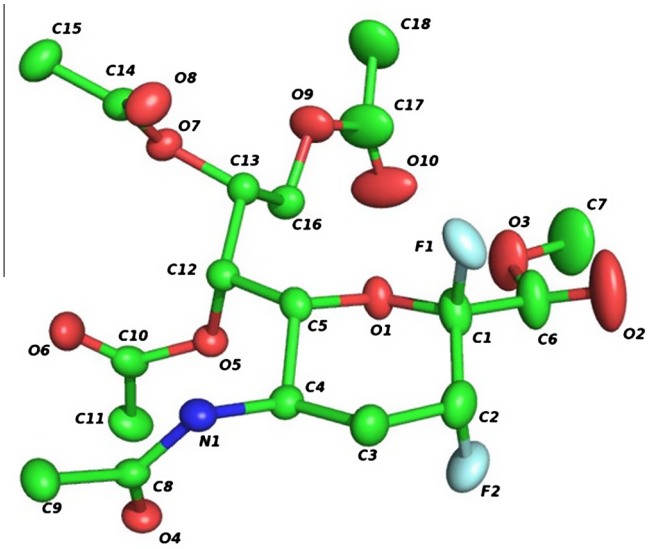


Figure 1. X-ray crystallographic structure of the C-2 axial fluorine containing *per-O*-acetyl-4-deoxy-2,3-difluoro-*N*-acetyl-neuraminic acid methyl ester **15-β**.

temperature, followed by saponification of the crude residue using 0.5 M aqueous sodium hydroxide and purification by silica chromatography to give the target compound, 5-*N*-acetyl-2,3,4,5-tetradeoxy-2,3-difluoro-D-*erythro*-β-L-manno-non-2-ulopyranosonic acid **2**, in 52% yield from **15**-α.

2.2. Synthesis of 5-*N*-acetyl-2,3,5,7-tetradeoxy-2,3-difluoro-D-*erythro*-β-L-manno-non-2-ulopyranosonic acid (3)

For the synthesis of 5-*N*-acetyl-2,3,5,7-tetradeoxy-2,3-difluoro-D-*erythro*-β-L-manno-non-2-ulopyranosonic acid **3**, the previously generated intermediate **9** was treated with benzoyl chloride in dichloromethane and pyridine at −40 °C for 90 min to afford 2,4-di-*O*-benzoyl-3-fluoro-8,9-*O*-isopropylidene-*N*-acetylneuraminic acid methyl ester **16** in 54% yield (Scheme 4). A major side product also formed under these conditions was identified as the 2,4,7-tri-*O*-benzoyl-3-fluoro-8,9-*O*-isopropylidene-*N*-acetyl-neuraminic acid methyl ester **17**, which was isolated in 18% yield. The introduction of an *O*-thionocarbonate at OH-7 was then performed using phenyl chlorothionoformate under conditions previously employed for the synthesis of **10**, which yielded the desired product **18** along with the product of sulfur–oxygen exchange **19**, previously observed by Powers and Tarbell.[22] as an inseparable mixture in a ratio of 1:1.

Given that the attempted introduction of a thionocarbonate at O-7 had resulted in formation of an inseparable mixture, it was considered that the use of a thiocarbamate according to the method of Miyazaki et al.[17] could prove more successful. Hence, the 2,4-di-*O*-benzoyl-3-fluoro-8,9-*O*-isopropylidene-sialic acid methyl ester **16** was treated with 1,1′-thiocarbonyldiimidazole in anhydrous dichloromethane to provide the 2,4-di-*O*-benzoyl-3-fluoro-8,9-*O*-isopropylidene-7-*O*-thiocarbamate-*N*-acetylneuraminic acid methyl ester **20** in 95% yield (Scheme 5). The 7-*O*-thiocarbamate derivative **20** was then subjected to Barton–McCombie deoxygenation conditions using Luperox® 101 as a radical initiator and tributyltin hydride in 1,4-dioxane under reflux conditions to give the 2,4-di-*O*-benzoyl-7-deoxy-3-fluoro-8,9-*O*-isopropylidene-*N*-acetylneuraminic acid methyl ester **21** in 89% yield. Here, Luperox® 101 was found to have advantages over BTBPB as a radical initiator such as ease of use and increased stability. Compound **21** was then subjected to sodium in methanol and the resultant crude mixture then treated with 80% aqueous acetic acid, followed by acetylation using acetic anhydride in pyridine to give the *per*-*O*-acetyl-7-deoxy-3-fluoro-*N*-acetylneuraminic acid methyl ester **22** in 83% yield over the 3 steps.

Compound **22** was then selectively deprotected at the C-2 position using hydrazine acetate in a mixture of dichloromethane and methanol at 4 °C to afford the hemiketal **23** in 84% yield. Treatment of the hemiketal **23** with DAST in dichloromethane at −40 to −10 °C according to the method described previously, then afforded the products of fluorination as a mixture of α- and β-anomers. Despite exhaustive efforts, it was not found to be possible to separate the α- and β-anomers using silica chromatography at this stage, though it was considered that purification might be achieved following deacetylation of the mixture.

As such, the crude anomeric mixture of difluorides was deacetylated using sodium in methanol at room temperature to give the 7-deoxy-2,3-difluoro-*N*-acetyl-neuraminic acid methyl esters **24**-α,β. Subsequent purification by silica chromatography provided the desired α-anomer in 41% yield along with the β-anomer in 20% yield, over the two steps.

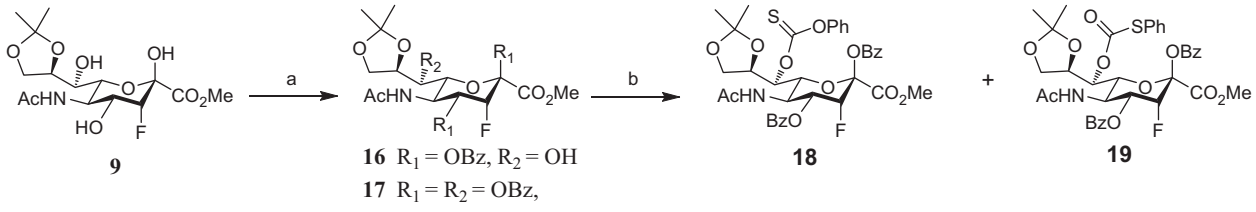
Finally, saponification of the desired methyl ester **24**-α with 0.5 M aqueous sodium hydroxide at room temperature yielded the target compound **3** in 49% yield.

2.3. Synthesis of 5-*N*-acetyl-2,3,5,8-tetradeoxy-2,3-difluoro-D-*erythro*-β-L-manno-non-2-ulopyranosonic acid (4)

For the synthesis of 5-*N*-acetyl-2,3,5,8-tetradeoxy-2,3-difluoro-D-*erythro*-β-L-manno-non-2-ulopyranosonic acid **4**, the previously synthesised methyl ester derivative **9** was subjected to acetic anhydride in pyridine for 7 days to give the 2,4,7-tri-*O*-acetyl-3-fluoro-8,9-*O*-isopropylidene-*N*-acetyl-neuraminic acid methyl ester **25** in 91% yield (Scheme 6).

Next, compound **25** was hydrolysed using 80% aqueous acetic acid, followed by the addition of benzoyl chloride in pyridine at room temperature to give selectively the O-9-benzoyl derivative **26** in 70% yield over the two steps. Compound **26** was then treated with phenyl chlorothionoformate in pyridine at 0 °C to afford the 2,4,7-tri-*O*-acetyl-9-*O*-benzoyl-3-fluoro-8-*O*-(phenoxy)thiocarbonyl-*N*-acetylneuraminic acid methyl ester **27** in 84% yield. Subsequent Barton–McCombie deoxygenation of compound **27** using Luperox® 101 and tributyltin hydride in 1,4-dioxane under reflux as described earlier, provided the 2,4,7-tri-*O*-acetyl-9-*O*-benzoyl-8-deoxy-3-fluoro-*N*-acetyl-neuraminic acid methyl ester **28** in 80% yield.

The selective anomeric deprotection of compound **28** was then accomplished using hydrazine acetate in dichloromethane and metha-



9

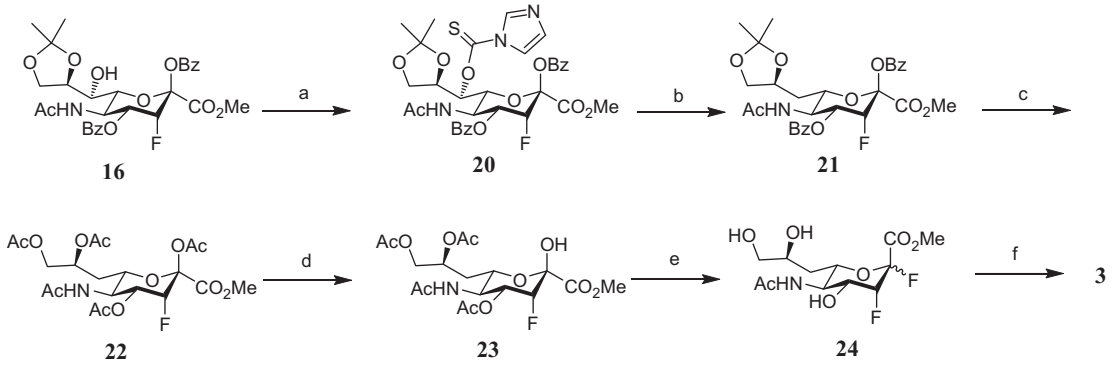
16 R_1 = OBz, R_2 = OH

17 R_1 = R_2 = OBz,

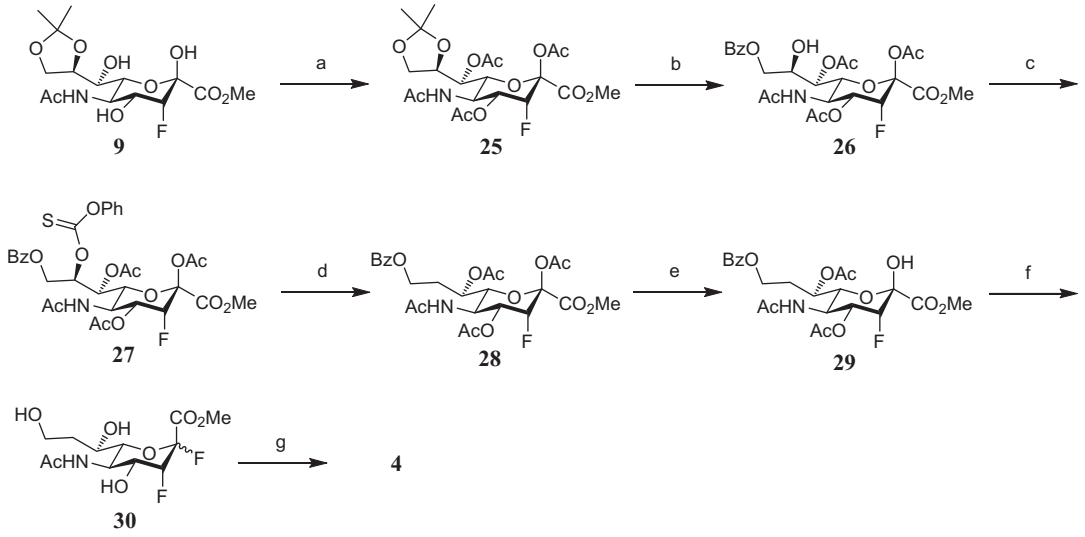
18

19

Scheme 4. Reagents and conditions: (a) Benzoylchloride, CH_2Cl_2/pyridine, 1.5 h, −40 °C → −20 °C, **16**: 54%, **17**: 18%; (b) phenyl chlorothionoformate, CH_2Cl_2/pyridine, O/N, rt, **18**:**19**; 1:1.



Scheme 5. Reagents and condtions: (a) 1,1′ Thiocarbonyldiimidazole, CH_2Cl_2, 20 h, 40 °C, 95%; (b) Luperox® 101, Bu_3SnH, 1,4-dioxane, 4 h, 100 °C, 89%; (c) (i) NaOMe, MeOH, 36 h, 40 °C, (ii) 80% AcOH/H_2O, 2 h, 60 °C, (iii) Ac_2O, DMAP, pyridine, O/N, rt, 83% (3 steps); (d) hydrazine acetate, MeOH/CH_2Cl_2, 20 h, 4 °C, 84%; (e) (i) DAST, CH_2Cl_2, 1.5 h, −40 °C → −10 °C, (ii) NaOMe, MeOH, 3 h, rt, α-anomer: 41% (2 steps), β-anomer: 20% (2 steps); (f) 1 M NaOH, pH 12, 1 h, rt, 49%.



Scheme 6. Reagents and conditions: (a) Ac_2O, pyridine, 7 d, rt, 91%; (b) (i) 80% AcOH/H_2O, 2 h, 60 °C, (ii) benzoylchloride, pyridine, O/N, rt, 70% (2 steps); (c) phenyl chlorothionoformate, pyridine, 15 h, 0 °C → rt, 84%; (d) Luperox® 101, Bu_3SnH, 1,4-dioxane, 4 h, 100 °C, 80%; (e) hydrazine acetate, MeOH/CH_2Cl_2, 20 h, 4 °C, 83%; (f) (i) DAST, CH_2Cl_2, 1.5 h, −40 °C → −10 °C, (ii) NaOMe, MeOH, O/N, rt, (iii) TFA, MeOH, O/N, rt, α-anomer: 25%, β-anomer: 11% (3 steps); (g) 1 M NaOH, pH 12, 1 h, rt, 22%.

nol at 4 °C to give the hemiketal **29** in 83% yield. The subsequent treatment of **29** with DAST in dichloromethane at −40 to −10 °C then afforded the difluorides **30**-**α**,**β** as an inseparable mixture. Deacetylation of **30**-**α**,**β** using sodium in methanol at room temperature, followed by silica chromatography, then provided the desired α-anomer in 25% yield, as well as the β-anomer in 11% yield, over the 3 steps.

Finally, ester saponification of the desired compound **30**-**α** using 0.5 M aqueous sodium hydroxide then gave the target compound **4** in 22% yield from **29**.

2.4. Synthesis of 5-*N*-acetyl-2,3,5,9-tetradeoxy-2,3-difluoro-D-*erythro*-β-L-manno-non-2-ulopyranosonic acid (5)

For the synthesis of 5-*N*-acetyl-2,3,5,9-tetradeoxy-2,3-difluoro-D-*erythro*-β-L-manno-non-2-ulopyranosonic acid **5**, the previously prepared methyl ester **17** was hydrolysed using 80% aqueous acetic acid and the crude mixture then treated with 1,1′-thiocarbonyldiimidazole in anhydrous dichloromethane to give the desired 2,4,7-tri-*O*-benzoyl-8,9-*O*-carbonothioate-3-fluoro-*N*-acetylneuraminic acid methyl ester **31** in 81% yield over the 2 steps (Scheme 7).

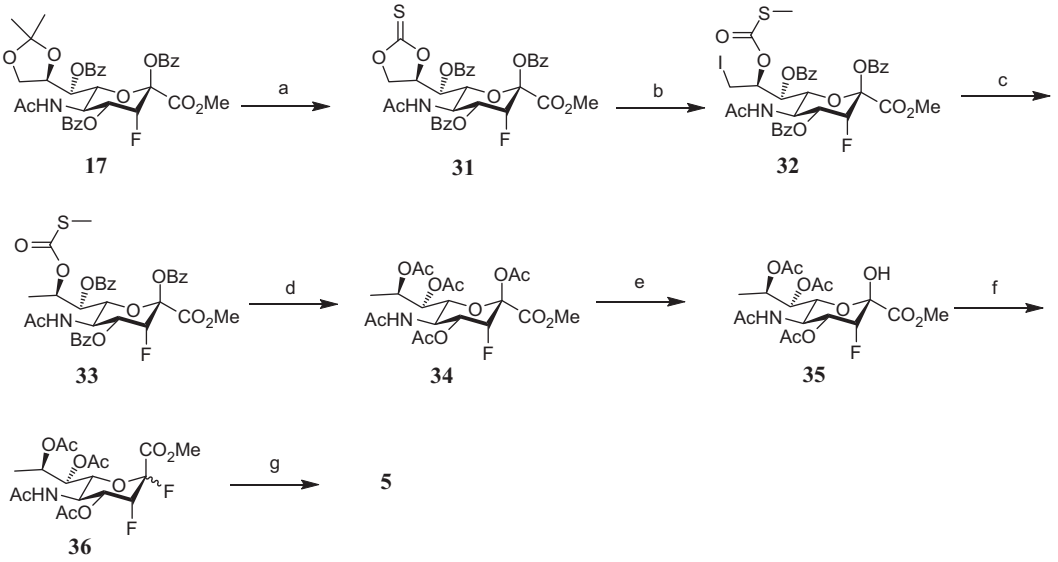
Compound **31** was then subjected to freshly distilled iodomethane at 56 °C overnight as previously reported by Schreiner et al.[29] to give the 9-iodo-8-*O*-methylthiocarbonyl-*N*-acetylneuraminic acid derivative **32** in 95% yield. The 8-*O*-methylthiocarbonyl derivative **32** was then subjected to Barton–McCombie deoxygenation using Luperox® 101 and tributyltin hydride in 1,4-dioxane under reflux as described previously, to yield the 9-deoxy-3-fluoro-8-*O*-methylthiocarbonyl-*N*-acetylneuraminic acid methyl ester **33** in 98% yield. The 9-deoxy derivative **33** was then treated with sodium in methanol and the subsequent product then acetylated using acetic anhydride in pyridine to give the 9-deoxy methyl ester derivative **34** in 77% yield over the 2 steps.[28]

Next, compound **34** was selectively deprotected at position C-2 using hydrazine acetate in dichloromethane and methanol at 4 °C to afford the hemiketal derivative **35**. Treatment of compound **35** with DAST in dichloromethane at −40 to −10 °C as mentioned previously, afforded **36**-**α**,**β** as a 1:1 mixture of the α- and β-fluorides, each isolated in 38% yield at this stage following silica chromatography.

The methyl ester derivative **36**-**α** was then deacetylated using sodium in methanol, followed by saponification of the resulting crude residue using 0.5 M aqueous sodium hydroxide to give the desired 9-deoxy-2,3-difluoro-*N*-acetylneuraminic acid **5** in 99% over the 2 steps.

3. Conclusions

Herein we have described the successful syntheses of the four novel monodeoxygenated 2,3-difluorosialic acids **2**–**5** as potential inhibitors and mechanistic probes for sialidases. A number of approaches were investigated for the synthesis of the 4-deoxy deriv-



Scheme 7. Reagents and conditions: (a) (i) 80% AcOH/H_2O, 2 h, 60 °C, (ii) 1,1′ thiocarbonyldiimidazole, CH_2Cl_2, 2 d, 40 °C, 81% (2 steps); (b) CH_3I, 20 h, 56 °C, 95%; (c) Luperox® 101, Bu_3SnH, 1,4-dioxane, 4 h, 100 °C, 98%; (d) (i) NaOMe, MeOH, O/N, rt, (ii) Ac_2O, DMAP, pyridine, O/N, rt, 77% (2 steps); (e) hydrazine acetate, MeOH/CH_2Cl_2, 20 h, 4 °C, 68%; (f) DAST, CH_2Cl_2, 1.5 h, −40 °C → −10 °C, α-anomer: 38%, β-anomer: 38%; (g) (i) NaOMe, MeOH, 3 h, rt, (ii) 1 M NaOH, pH 12, 1 h, rt 99% (2 steps).

ative **2**, where a Barton–McCombie protocol using the radical carrier tributyltin hydride under reflux conditions in 1,4-dioxane was found to be effective and proved to be generally applicable to the synthesis of the remaining monodeoxygenated 2,3-difluorosialic acid derivatives **3**–**5**. Analysis of the small molecule X-ray structure of the difluoride **15** proved necessary for the unambiguous assignment of its anomeric stereochemistry, enabling the successful synthesis of **2**. The Barton–McCombie conditions identified for the synthesis of **2** were also applied to the syntheses of targets **3**–**5**, with the exception that Luperox 101 was employed as a free radical initiator in preference to BTBPB. The peroxide Luperox 101 is a commonly used initiator for free radical polymerisation reactions, though its use has not been reported previously for application under Barton–McCombie deoxygenation conditions. Here, we found Luperox 101 to be an effective radical initiator for the Barton–McCombie deoxygenation of sialic acids, suggesting that this reagent may prove to be generally useful as an alternative to the traditional initiator AIBN for tinhydride mediated deoxygenations.

Another notable observation is the variability in the ratio of anomeric fluorides generated here from treatment of the hemi-ketals with DAST. Previous studies generating 2,3-difluoro sialic acid derivatives consistently report over 80% yield of the desired α-anomer when DAST fluorinations were performed at a constant temperature of −30 °C.[10,11] We consider that variable ratios of α-anomer observed here (50%-α for **36** to 70%-α for **24**) may result from our modified procedure where the DAST fluorinations were performed over a temperature range from −40 to −10 °C.

The series of deoxygenated difluorosialic acids **2**–**5** are currently being investigated as inhibitors against a host of sialidases of bacterial, trypanosomal and viral origin, with the results of these kinetic and crystallographic studies to be presented in the future.

Acknowledgments

S.H. was supported by an MRC capacity building studentship. A.G.W. was supported by MRC Grant G0600514. We gratefully acknowledge Dr. Tim Woodman (Department of Pharmacy and Pharmacology, University of Bath) for NMR support and Dr. Mary Mahon (Department of Chemistry, University of Bath) for performing the X-ray crystallographic analysis.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carres.2013.03.026.

References

1. Vimr, E.; Lichtensteiger, C. *Trends Microbiol.* **2002**, *10*, 254–257.
2. Treadway, J. L.; Mendys, P.; Hoover, D. J. *Expert Opin. Invest. Drugs* **2001**, *10*, 439–454.
3. Groopman, J. E. *Rev. Infect Dis.* **1990**, *12*, 908–911.
4. Zitzmann, N.; Mehta, A. S.; Carrouee, S.; Butters, T. D.; Platt, F. M.; McCauley, J.; Blumberg, B. S.; Dwek, R. A.; Block, T. M. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 11878–11882.
5. Laver, W. G.; Bischofberger, N.; Webster, R. G. *Sci. Am.* **1999**, *280*, 78–87.
6. Gloster, T. M.; Davies, G. J. *Org. Biomol. Chem.* **2010**, *8*, 305–320.
7. Cantarel, B. L.; Coutinho, P. M.; Rancurel, C.; Bernard, T.; Lombard, V.; Henrissat, B. *Nucleic Acids Res.* **2009**, *37*, D233–D238.
8. Koshland, D. E. *Biol. Rev. Camb. Philos. Soc.* **1953**, *28*, 416–436.
9. Zechel, D. L.; Withers, S. G. *Acc. Chem. Res.* **2000**, *33*, 11–18.
10. Watts, A. G.; Withers, S. G. *Can. J. Chem.* **2004**, *82*, 1581–1588.
11. Watts, A. G.; Oppezzo, P.; Withers, S. G.; Alzari, P. M.; Buschiazzo, A. *J. Biol. Chem.* **2006**, *281*, 4149–4155.
12. Watts, A. G.; Damager, I.; Amaya, M. L.; Buschiazzo, A.; Alzari, P.; Frasch, A. C.; Withers, S. G. *J. Am. Chem. Soc.* **2003**, *125*, 7532–7533.
13. Buchini, S.; Buschiazzo, A.; Withers, S. G. *Angew. Chem., Int. Ed.* **2008**, *47*, 2700–2703.
14. Street, I. P.; Armstrong, C. R.; Withers, S. G. *Biochemistry* **1986**, *25*, 6021–6027.
15. Street, I. P.; Rupitz, K.; Withers, S. G. *Biochemistry* **1989**, *28*, 1581–1587.
16. Ogura, H.; Furuhata, K.; Sato, S.; Anazawa, K.; Itoh, M.; Shitori, Y. *Carbohydr. Res.* **1987**, *167*, 77–86.
17. Miyazaki, T.; Sato, H.; Sakakibara, T.; Kajihara, Y. *J. Am. Chem. Soc.* **2000**, *122*, 5678–5694.
18. Lopez, R. M.; Hays, D. S.; Fu, G. C. *J. Am. Chem. Soc.* **1997**, *119*, 6949–6950.
19. Park, H. S.; Lee, H. Y.; Kim, Y. H. *Org. Lett.* **2005**, *7*, 3187–3190.
20. Tsuda, Y.; Sato, Y.; Kanemitsu, K.; Hosoi, S.; Shibayama, K.; Nakao, K.; Ishikawa, Y. *Chem. Pharm. Bull.* **1996**, *44*, 1465–1475.
21. Barton, D. H. R.; Dalko, P. I.; Gero, S. D. *Tetrahedron Lett.* **1992**, *33*, 1883–1886.
22. Powers, D. H., Jr.; Tarbell, D. S. *J. Am. Chem. Soc.* **1956**, *78*, 70–71.
23. Barton, D. H. R.; McCombie, S. W. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1574–1585.
24. Anazawa, K.; Furuhata, K.; Ogura, H. *Chem. Pharm. Bull.* **1988**, *36*, 4976–4979.
25. Excoffier, G.; Gagnare, D.; Utille, J. P. *Carbohydr. Res.* **1975**, *39*, 368–373.
26. Alais, J.; David, S. *Carbohydr. Res.* **1992**, *230*, 79–87.
27. Iida, M.; Endo, A.; Fujita, S.; Numata, M.; Sugimoto, M.; Nunomura, S.; Ogawa, T. J. *Carbohydr. Chem.* **1998**, *17*, 647–672.
28. Zemplen, G.; Pacsu, E. *Ber. Dtsch. Chem. Ges.* **1929**, *62B*, 1613–1614.
29. Schreiner, E.; Christian, R.; Zbiral, E. *Liebigs Ann. Chem.* **1990**, 93–97.