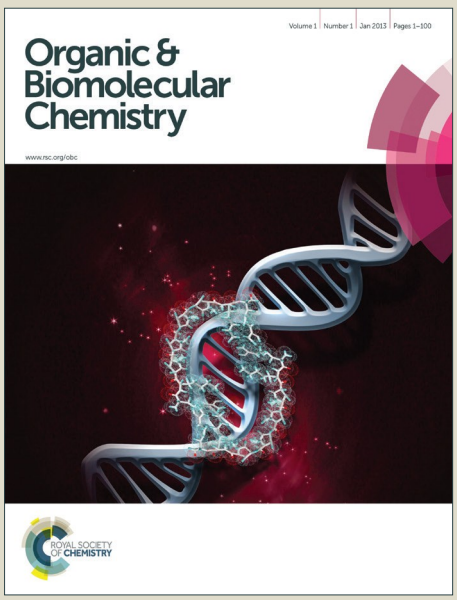
Organic & Biomolecular Chemistry

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/obc

Journal Name

ARTICLE

Novel strategies for the synthesis of unsymmetrical glycosyl disulfides

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Goreti Ribeiro Morais,* Bradley R Springett, Martin Pauze, Lisa Schröder, Matthew Northrop and Robert A Falconer*

Novel strategies for the efficient synthesis of unsymmetrical glycosyl disulfides are reported. Glycosyl disulfides are increasingly important as glycomimetics and molecular probes in glycobiology. Sialosyl disulfides are synthesised directly from the chlorosialoside Neu5Ac2Cl, proceeding via a thiol-disulfide exchange reaction between the sialosyl thiolate and symmetrical disulfides. This methodology was adapted and found to be successfully applicable to the synthesis of unsymmetrical glucosyl disulfides under mild conditions.

Introduction

Although less studied than thioglycosides, interest in glycosyl disulfides has increased significantly over recent years, particularly with their key potential as glycomimetics.[1-3] For instance, glycosyl disulfides have shown affinity to lectin Concanavalin A,[3-6] while other disulfides have been successfully employed as tools to aid understanding of carbohydrate structures.[7, 8] Moreover, the significant utility and advantages of glycosyl disulfides as glycosyl donors in the synthesis of a variety of glycosides[9-13] and oligosaccharides[2, 14, 15] has been demonstrated following the seminal work of the Davis group, with wide applications to the synthesis of glycopeptides[13, 16] and vaccines.[17] Whilst not widely employed to-date, sialosyl disulfides possess equal potential for application to the synthesis of *O*-, *S*- and oligosialosides.

To the best of our knowledge, and contrarily to glycosyl disulfides, there is only one report describing the preparation of sialosyl disulfides (by Hummel). This is achieved by means of a diethyl *S*-azodicarboxylate as a sulfenyl transfer reagent to the *S*-sialosyl moiety.[18] On the other hand, several synthetic methodologies have been developed for the synthesis of glycosyl disulfides. Briefly, symmetrical glycosyl disulfides can be rapidly prepared by the oxidation of the corresponding glycosyl thiols,[4, 19-21] while unsymmetrical glycosyl disulfides can be prepared by reaction of a suitable electrophilic sulfur-based glycosylsulfenyl-tranfer reagent, such as nitropyridine sulfides,[22] alkylthiosulfonates esters,[7-10] selenylsulfides,[2] sulfenamides,[23] and sulfenic acids[24] with thiols. Additionally, the Ramström group successfully extended their phosphine-catalysed disulfide methathesis conditions[25] to the synthesis of unsymmetrical glycosyl disulfides.[26]

These strategies generally require laborious synthesis and purification of the glycosylsulfenylating agents, which are occasionally unstable and often obtained only in moderate yields. We ourselves developed a one-pot synthesis of glycosyl disulfides in good to excellent yields via the use of an *in situ* glycosylsulfenylhydrazine derivative from 1-thiogycosides.[27] Szilágyi *et al*. reported preparation of unsymmetrical glycosyl disulfides using 1-chlorobenzotriazole as oxidising agent, trapping the sulfenyl radicals in an one-pot fashion, albeit under strict temperature control (-78 °C).[28]

Having previously identified the acetyl disulfide sialoside (Neu5Ac2SSAc) **1** as a by-product during the routine synthesis of 2-thioacetyl sialoside (Neu5Ac2SAc) **2** (Figure 1) when 2-chlorosialoside **3** is reacted with KSAc,[29] we were motivated to explore its utility in the preparation of sialosyl disulfides, and to explore whether there were wider applications for glycosyl disulfides.

Results and Discussion

Given that formation of by-product **1** was markedly dependent on the batch of commercial KSAc, with varied levels of oxidation,[29] our initial efforts were focused on the synthesis of this compound. We initially reacted 2-chlorosialoside **3** with KSAc in the presence of an oxidising agent (I_2, Scheme 1). Under these conditions, the partial *in situ* oxidation of KSAc to AcSSAc successfully promoted the formation of **1** from **3** as major product in 80 % yield. An excess of KSAc and longer reaction times (48 h at room temperature) were required to effect complete conversion to **1**. Shortening of reaction times and heating led to several undesirable and unidentified sialic acid-related species and a higher percentage of the 2,3-elimination by-product Neu5Ac2en, which often plagues reactions of this type.[30]

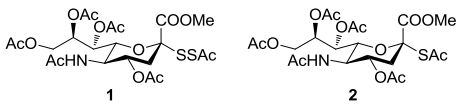
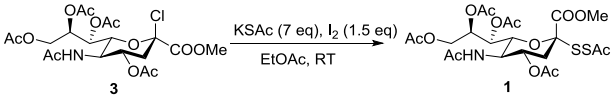
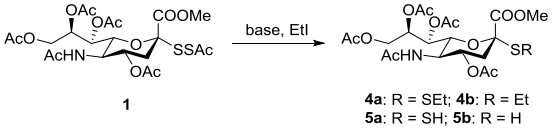


Figure 1

Institute of Cancer Therapeutics, Faculty of Life Sciences, University of Bradford, Bradford BD7 1DP
E-Mail: r.a.falconer1@bradford.ac.uk; gribeiro@bradford.ac.uk
Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x



Scheme 1



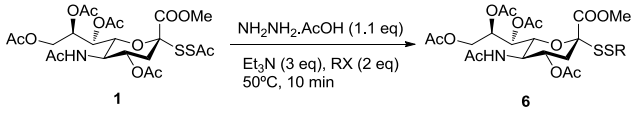
Scheme 2

Furthermore, we have investigated the optimal experimental conditions for the preparation of sialosyl disulfides through the alkylation of **1** with ethyl iodide (Scheme 2). The reactions were monitored by ^{1}H NMR spectroscopy. Despite its instability to diethylamine treatment, Neu5Ac2SSAc **1** was partially alkylated to form the corresponding ethyl disulfide sialoside **4a** (50 %) together with the corresponding thiosialoside **4b** (50 %). Other bases were thus investigated. Triethylamine, diisopropylamine, diisopropylethylamine proved insufficient to promote *S*-deacetylation of the SSAc group, even after 2 h at RT, with **1** being fully recovered from the reaction. Morpholine and hydrazine hydrate successfully promoted *S*-deacetylation of sialoside **1**, but with side reactions, i.e. formation of **5a** and **5b**, and no alkylated products. The use of hydrazine acetate, however, led to the desired SS-alkylated product **4a**, together with non-alkylated disulfide sialoside **5a**. Reaction times longer than 15 minutes also led to the formation of the undesired **4b**. Given these findings, we reasoned that performing the same reaction with both hydrazine acetate and triethylamine simultaneously should yield the desired product, with an absence of break-down products. This proved to be the case: sialoside **1** was fully and efficiently converted into sialosyl disulfide **4a** in only 10 min at 50 ˚C. Acetyl disulfide **1** is thus a convenient and efficient route to sialosyl disulfides under these conditions.

To begin to evaluate the wider applicability of these findings, we explored the scope of this reaction by employing different aryl and alkyl bromides (Table 1). Under these optimised conditions, sialoside **1** proved to be highly reactive to both benzyl- (Entry 2, Table 1) and 4-fluorobenzyl bromide (Entry 5, Table 1) with formation of only the desired product, the respective sialosyl disulfides **6a** and **6b**, obtained in fair to good yields. In addition to DMF, this SS-alkylation reaction was also performed in other solvents such as dichloroethane (Entry 3, Table 1) and acetonitrile (Entry 4, Table 1) at 50 ˚C for 10 min. In these cases conversion of **1** into **6a** was inefficient, with an additional sialic acid-related by-product detected by ^{1}H NMR. DMF was confirmed as the preferred solvent for these reactions. We also confirmed that when either hydrazine acetate or triethylamine were utilised alone with benzyl bromide in dichloroethane, no product was observed, with **1** being fully recovered. When reacting with the less electrophilic cinnamyl bromide (Entry 6, Table 1), however, two products were detected by TLC (we were unable to isolate these products for further investigation). After purification (flash column chromatography), sialosyl disulfide **6c** was obtained in low yield (22 %).

In summary, acetyl disulfide sialoside **1** was demonstrated to be an easily synthesised and useful intermediate for the preparation of some sialosyl disulfides. However, with respect to its limited reactivity to weak electrophiles, associated with side reactions and poor yields, this strategy was deemed sub-optimal as a general method for the synthesis of unsymmetrical sialosyl disulfides. With a view to arriving at a more widely applicable methodology, we thus re-designed our synthetic strategy.

Table 1



Entry	RX	Solvent	Product	Yield (%)
1	ethyl iodide	DMF	**4a**	-[a]
2	benzyl bromide	DMF	**6a**	54[b]
3	benzyl bromide	DCE	**6a**	-[c]
4	benzyl bromide	MeCN	**6a**	-[d]
5	4-fluorobenzyl bromide	DMF	**6b**	79[b]
6	cinnamyl bromide	DMF	**6c**	22[b]

[a.1]H NMR analysis indicated complete conversion of **1** into **4a**; [b.]Isolated yields; [c..1]H NMR analysis indicated 74 % of **6a**; [d..1]H NMR analysis indicated 65% of **6a**.

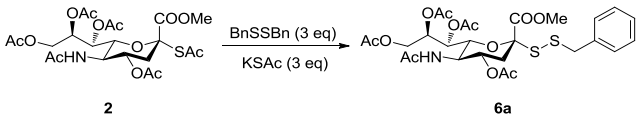
Whilst previously investigating the mechanism by which acetyl disulfide sialoside **1** is formed, we established that 2-chlorosialoside **3** was unreactive towards AcSSAc. This observation ruled out direct nucleophilic attack of a putative acetyl disulfide anion ($AcSS^-$) on the electrophilic anomeric carbon. Repetition of this reaction in the presence of KSAc, however, promoted the formation of Neu5Ac2SSAc **1**, which suggested that the source of **1** is in fact the sialosyl thioacetate, Neu5Ac2SAc **2**.[29] Moreover, we also found that formation of Neu5AcSSAc **1** from pure Neu5Ac2SAc **2** and AcSSAc was only achieved when KSAc was present. This finding suggests that KSAc promotes the selective *S*-deacetylation of the thioacetate group of **2** and that the thiolate anion generated reacts with the electrophilic sulfur of the symmetrical acetyl disulfide to produce the Neu5Ac2SSAc **1** through a thio-disulfide exchange mechanism. Reactions between thiols and symmetrical disulfides, similar to that observed *in vivo* between thiols and glutathione, have been well explored albeit with limited success in organic chemistry for the synthesis of non-glycosidic unsymmetrical disulfides.[31, 32] In the same way, we demonstrated that when **2** is reacted with symmetrical benzyl disulfide BnSSBn in the presence of KSAc, Neu5Ac2SSBn **6a** is formed (Scheme 3). ^{1}H NMR analysis of this reaction showed that after 1 h sialoside **2** had been converted into **6a** in very good yield (75 %).

Based on the finding that KSAc, which is crucial for the preparation of Neu5Ac2SAc **2**, also efficiently promotes the reaction of **2** with symmetrical disulfide BnSSBn, we thought it interesting to attempt formation of Neu5Ac2SSBn **6a** directly from chlorosugar Neu5Ac2Cl **3**. This would remove the need for separate thiosialoside synthesis. ^{1}H NMR experiments indicated that when **3** and BnSSBn were reacted in the presence of KSAc, no Neu5Ac2SSBn **6a** was observed after a 3 h reaction, and that **3** had not been completely converted

into **2**. When **3** and KSAc were reacted for several hours prior to addition of BnSSBn, however, Neu5Ac2SSBn **6a** was formed in 33 % after 9 h. This two-step one-pot reaction was drastically improved when a base (diethylamine) was added with the BnSSBn. Neu5Ac2Cl **3** was completely converted into Neu5Ac2SSBn **6a** in 1 h. These conditions could also be applied to the synthesis of other benzyl and phenyl sialosyl disulfides (entries 2 and 4, Table 2).

This methodology could be utilised to successfully prepare the forementioned cinnamyl sialosyl disulfide **6c** (entry 3, Table 2). As expected, aliphatic symmetrical disulfides were found to be less reactive towards chlorosugar **3**, with longer reaction times (2 h to overnight) required (entries 6, 7, and 9, Table 2). Hydroxyl-functionalised disulfides were also amenable in these conditions, with **7d** being obtained in good yield after a 2 hr reaction (entry 7, Table 2). When a heteroaromatic disulphide (entry 8) was reacted under these conditions, the respective thiosialoside was also observed in equal amounts to the disulphide **7e**. This presumably results from the competitive nucleophilic attack of the *in situ* formed (and relatively more reactive) 5-chloropyridin-2-mercaptan to the unreacted chlorosialosyl **3**. Formation of this thiosialoside was minimised by first reacting chlorosialoside **3** with KSAc followed by the addition of 5-chloropyridin-2-disulfide and base after 6 h.
We have thus identified convenient conditions for the synthesis of sialosyl disulfides, starting from either the 2-chlorosialoside **3** or 2-thioacetate **2**.

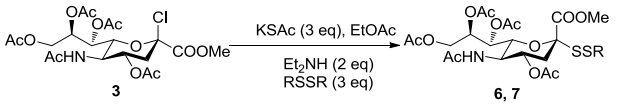
We subsequently sought to establish wider applicability to the broader glycoside field. D-glucose was selected as being generally representative. In this case, diethylamine was found to be insufficient to promote the synthesis of glucosyl disulfides. Other organic bases such as diisopropylethylamine and morpholine proved to be equally insufficient. Hydrazine hydrate efficiently promoted the thiol-disulfide exchange reaction between glucosyl thioacetate **8** and the symmetrical disulfide of interest, however. The method proved successful for a similar range of alkyl and aryl disulfides, resulting in glucosyl disulfides being successfully synthesised from 1-thioacetate derivative **8** in good yields (Table 3). Previous reports describing the synthesis of glucosyl disulfides from glucosyl thioacetate **8** employed sodium hydroxide, which leads also to complete hydrolysis of *O*-acetate protecting groups.[33] Hydrazine hydrate is more suitable in this regard.



2 **6a**

Scheme 3

Table 2

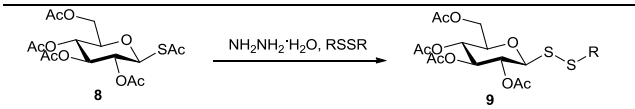


3 **6, 7**

Entry	R	Product	Yield (%)
1	benzyl	**6a**	65
2	4-fluorobenyl	**6b**	62
3	cinnamyl	**6c**	56
4	phenyl	**7a**	75
5	per-*O*-acetyl-glucose	**7b**	61
6	methyl	**7c**	71
7	2-hydroxyethyl	**7d**	64
8	5-chloropyridin-2-yl	**7e**	69[a]
9	cyclohexyl	**7f**	60

[a].Corrected yield by ^{1}H NMR

Table 3



8 **9**

Entry	R	Product	Yield (%)
1	2-hydroxyl ethyl	**9a**	49
2	cyclohexyl	**9b**	58
3	phenyl	**9c**	65
4	benzyl	**9d**	60[a]
5	4-fluorobenzyl	**9e**	52[b]
6	methyl	**9f**	65
7	5-chloropyridin-2-yl	**9g**	66

[a]Yield calculated by ^{1}H NMR; [b]1H NMR of the crude indicated full conversion of **8** into **9e**

Conclusions

In summary, we report novel strategies for the synthesis of unsymmetrical sialosyl and glycosyl disulfides, offering significant advantages over existing methodologies, employing readily utilised intermediates. This methodology offers a convenient route to compounds of biological interest, with potential as carbohydrate probes or enzyme inhibitors. Given the increasing awareness of the importance of carbohydrates in biological processes, e.g. cancer progression and metastasis,[34, 35] the field of synthesis of carbohydrate mimetics and probes such as glycosyl disulfides will continue to gain pace.

Experimental

General information

NMR spectra were generated on a JEOL ECA-600 and Bruker AMX 400 operating at 600 MHz and 400 MHz respectively. Chemical shifts are reported in ppm downfield relative to tetramethylsilane (solvent $CDCl_3$). Low resolution mass spectra (LRMS) were generated using a Micromass Quattro Ultima. High resolution mass

spectrometry was performed at the National Mass Spectrometry Centre Swansea using MAT95 or MAT900 in the electrospray ionisation (ESI) mode. Analytical thin-layer chromatography (TLC) was performed on precoated silica plates 60 F_{254} (Merck). Visualisation of the plates was carried out using UV light (254 nm), and/or a solution of permanganate or sulphuric acid followed by heating. Flash column chromatography was carried out on Merck 9385 silica gel 60 (40-63 μm) (Merck). All solvents were of reagent grade.

General method for the synthesis of sialosyl disulfides

A solution of 3 (0.1 mmol) in ethyl acetate (3 ml) was mixed with KSAc (0.3 mmol) followed by the addition of symmetrical disulfide (0.3 mmol) and diethylamine (0.2 mmol) at RT. After 30 min, the reaction mixture was filtered and the filtrate was concentrated under vacuum. Column chromatography on silica gel (ethyl acetate 100%) afforded desired sialosyl disulfides.

Methyl 2-(benzylsulfanyl) 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,3,5-trideoxy-2-thio-β-D-*glycero*-D-*galacto*-2-nonulopyranosonate (**6a**) – ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.88 (s, 3H, NAc), 1.94, 2.03, 2.17 (4s, 12H, 4OAc), 2.23 (dd, 2H, H-3ax, $J_{3ax,4}$ 12.5 Hz, $J_{3ax,3eq}$ 12.6 Hz), 2.69 (dd, 1H, H-3eq, $J_{3eq,4}$ 4.2 Hz, $J_{3ax,3eq}$ 12.6 Hz), 3.81 (s, 3H, COOMe), 4.00 – 4.14 (m, 5H), 4.36 (d, 1H, H-9, $J_{9,9'}$ 12.5 Hz), 4.89 (ddd, 1H, H-4), 5.26 (d, 1H, H-6), 5.34 (d, 1H, H-7), 5.39 (m, 1H, H-8), 7.24 – 7.33 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.30, 20.79, 20.91, 20.96, 21.25, 23.32, 37.54, 44.71, 49.44, 53.25, 62.23, 67.47, 69.43, 69.79, 75.01, 89.47, 127.77, 128.66 (2C), 129.69 (2C), 136.41, 168.00, 170.05, 170.21, 170.32, 170.73, 171.09; MS (ES^+) $C_{27}H_{35}NO_{12}S_2$ (629) *m/z* (%) 630.3 [M+H]+ (100); HRMS (ES^+) Found 630.1668, calcd for $C_{27}H_{36}NO_{12}S_2$ 630.1673 $[M+H]^+$

Methyl 2-[(4'-fluorobenzyl)sulfanyl] 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,3,5-trideoxy-2-thio-β-D-*glycero*-D-*galacto*-2-nonulopyranosonate (**6b**) - ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.93 (s, 3H, NAc), 1.96, 2.04, 2.13, 2.19 (4s, 12H, 4OAc), 2.22 (dd, 1H, H-3a, $J_{3ax,3eq}$ 12.5Hz), 2.71 (dd, 1H, H-3eq, $J_{3eq,4}$ 4.6 Hz, $J_{3ax,3eq}$ 12.5Hz), 3.81 (s, 3H, COOMe), 3.99 (d, 1H, ^2J 12.1 Hz), 4.02-4.07 (m, 3H, H-5, NH, -CH), 4.11 (dd, 1H, H-9a, $J_{8,9a}$ 5.4 Hz, $J_{9a,9b}$ 12.5 Hz), 4.35 (dd, 1H, H-9b, $J_{8,9b}$ 2.7 Hz, $J_{9a,9b}$ 12.5 Hz), 4.89 (m, 1H, H-4), 5.27 (d, 1H, H-6, $J_{5,6}$ 9.3 Hz), 5.32 (dd, 1H, H-7, $J_{6,7}$ 1.5 Hz, $J_{7,8}$ 8.2 Hz), 5.39 (m, 1H, H-8), 7.00 (d, 1H, ^3J 8.5 Hz), 7.01 (d, 1H, ^3J 8.5 Hz), 7.32 (d, 1H, ^3J 8.5 Hz), 7.33 (d, 1H, ^3J 8.5 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.78, 20.86, 20.92, 21.24, 23.18, 37.48, 43.82, 49.73, 53.32, 62.08, 67.36, 68.94, 69.58, 74.75, 89.58, 114.21, 115.48, 115.65, 120.75, 131.36, 131.41, 167.86, 170.09, 170.20, 170.73, 171.15, 171.29; MS (ES^+) $C_{27}H_{34}FNO_{12}S_2$ (647.69) *m/z* (%) 648.4 [M+H]+ (20), 665.2 $[M+NH_4]$+ (100), 670.1 [M+Na]+ (40); HRMS (ES^+) Found 648.1567, calcd for $C_{27}H_{35}FNO_{12}S_2$ 648.1579$[M+H]^+$

Methyl 2-(cynnamylsulfanyl) 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,3,5-trideoxy-2-thio-β-D-*glycero*-D-*galacto*-2-nonulopyranosonate (**6c**) - ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.90 (s, 3H, NAc), 1.99, 2.03, 2.13, 2.18 (4s, 12H, 4OAc), 2.24 (dd, 1H, H-3ax, $J_{3ax,4}$ 11.8 Hz, $J_{3ax,3eq}$ 12.5 Hz), 2.70 (dd, 1H, H-3eq, $J_{3ax,4}$ 4.6 Hz, $J_{3ax,3eq}$ 12.5 Hz), 3.62 (m, 2H), 3.81 (s, 3H, COOMe), 4.00 (d, 1H, NH, $J_{NH,5}$ 10.8 Hz), 4.06 (m, 1H, H-5), 4.13 (dd, 1H, H-9a, $J_{8,9a}$ 5.5 Hz, $J_{9a,9b}$ 12.3 Hz), 4.38 (d, 1H, H-9a, $J_{9a,9b}$ 12.3 Hz), 4.88 (m, 1H, H-4), 5.23 (d, 1H, H-6, $J_{5,6}$ 9.7 Hz), 5.32 (d, 1H, H-7, $J_{7,8}$ 8.1 Hz), 5.37 (m, 1H, H-8), 6.20 (m, 1H), 6.56 (d, 1H, ^EJ 15.4 Hz), 7.24 (m, 1H), 7.30 (m, 2H), 7.39 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.85, 20.88, 20.94, 21.24, 23.25, 37.50, 42.63, 49.50, 53.28, 62.39, 67.46, 69.46, 69.71, 74.71, 89.15, 123.32, 126.60 (2C), 127.88, 128.68 (2C), 134.99, 136.65, 162.97, 168.01, 170.09, 170.15, 170.72, 170.74, 171.12; MS (ES^+) $C_{29}H_{37}NO_{12}S_2$ (655.18) *m/z* (%) 656.33 [M+H]+ (20), 678.24 [M+Na]+ (55); HRMS (ES^+) Found 673.2086, calcd for $C_{29}H_{37}NO_{12}S_2$ 673.2095$[M+NH_4]^+$

Methyl 2-[phenylsulfanyl] 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,3,5-trideoxy-2-thio-β-D-*glycero*-D-*galacto*-2-nonulopyranosonate (**7a**) ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.87 (s, 3H, NAc), 2.02, 2.03, 2.05, 2.12 (4s, 12H, OAc), 2.19 (dd, 1H, H-3ax, $J_{3ax,4}$ 12.4 Hz, $J_{3ax,3eq}$ 12.8 Hz), 2.75 (dd, 1H, H-3eq, $J_{3eq,4}$ 4.8 Hz, $J_{3eq,3ax}$ 12.8Hz), 3.48 (s, 3H, COOMe), 3.91 (m, 3H, H-5, H-6, H-9a), 4.25 (dd, 1H, H-9b, $J_{8,9}$ 2.8 Hz, $J_{9a,9b}$ 12.4 Hz), 4.85 (m, 1H, H-4), 5.10 (m, 1H, H-8), 5.24 (m, 2H, H-7, NH), 7.21 (m, 1H), 7.32 (m, 2H), 7.55 (d, 2H, ^3J 7.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.74, 20.87, 21.15, 23.21, 37.18, 49.25, 52.90, 61.71, 67.11, 69.46, 69.63, 74.66, 76.72, 77.04, 77.36, 86.49, 127.15, 128.01, 128.75, 136.12, 167.67, 169.89, 170.08, 170.26, 170.70, 170.94; MS (ES^+) $C_{26}H_{33}NO_{12}S_2$ (615) m/z 616.29 $[M+H]^+$ (47) 638.28 $[M+Na]^+$ (100); HRMS (ES^+) Found 638.1330, calcd for $C_{26}H_{33}NO_{12}S_2Na$ 638.1336 $[M+Na]^+$

Methyl 2-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)] 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,3,5-trideoxy-2-thio-β-D-*glycero*-D-*galacto*-2-nonulopyranosonate (**7b**) - ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.86 (s, 3H, NAc), 1.98, 2.01, 2.03, 2.05, 2.08, 2.09, 2.14, 2.19 (s, 24 H, 8 OAc), 2.79 (dd, 1H, H-3eq$_{Neu}$, $J_{3ax,4}$ 4.4 Hz, $J_{3ax,3eq}$ 12.8 Hz), 3.79 (s, 3H, OMe), 3.88-4.17 (m, 5H, H-5$_{glu}$, NH, H-5$_{Neu}$, H-7$_{Neu}$, H-9a$_{Neu}$), 4.33-4.39 (m, 2H, H-6$_{Neu}$, H-9b$_{Neu}$), 4.64 (d, 1H, H-1$_{glu}$, $J_{1,2}$ 10.0 Hz), 4.85 (m, 1H, H-4$_{Neu}$), 4.94 (dd, 1H, H-2glu, $J_{1,2}$ 10.0 Hz, $J_{2,3}$ 9.2 Hz), 5.12 (m, 2H, H-4$_{glu}$ and H-6a$_{glu}$), 5.31 (m, 2H, H-6b$_{glu}$ and H-8$_{Neu}$), 5.55 (dd, 1H, H-3$_{glu}$, $J_{2,3}$ 9.2 Hz, $J_{3,4}$ 9.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.16, 20.55, 20.57, 20.66, 20.71, 20.79, 20.98, 23.14, 38.42, 49.16, 52.89, 50.34, 61.71, 62.36, 67.46, 68.08, 68.83, 69.72, 70.46, 73.51, 75.05, 75.60, 87.08, 90.81, 167.04, 169.40, 169.43, 169.99, 170.07, 170.13, 170.32, 170.60, 170.71, 170.82, 171.07; MS (ES^+) $C_{34}H_{47}NO_{21}S_2$ (869.2) *m/z* (%) 870.22 [M+H]+ (36), 892.20 [M+Na]+ (100); HRMS (ES^+) Found 887.2414, calcd for $C_{34}H_{47}NO_{21}S_2NH_4$ 887.2420 $[M+NH_4]^+$

Methyl 2-[methylsulfanyl] 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,3,5-trideoxy-2-thio-β-D-*glycero*-D-*galacto*-2-nonulopyranosonate (**7c**) - ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.88 (s, 3H, NAc), 2.03, 2.04, 2.12, 2.15 (s, 12 H, 4 OAc), 2.22 (dd, 1H, H-3ax, $J_{3ax,4}$ 12.0 Hz, $J_{3ax,3eq}$ 12.8 Hz), 2.50 (s, 3H, SSMe), 2.70 (dd, 1H, H-3eq, $J_{3ax,4}$ 4.7 Hz, $J_{3ax,3eq}$ 12.8 Hz), 3.81 (s, 3H, OMe), 4.01 (m, 2H, H-5, H-6), 4.13 (m, 1H, H-9a), 4.39 (m, 1H, H-9b), 4.89 (m, 1H, H-4), 5.21 (d, 1H, NH, $J_{5,NH}$ 9.6 Hz), 5.31 (m, 2H, H-6, H-8); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.72, 20.79, 20.99, 21.05, 23.15, 24.74, 37.36, 49.41, 53.03, 62.15, 67.46. 69.52, 69.72, 74.91, 89.12, 168.00, 169.95, 170.08, 170.15, 170.59, 170.89; MS (ES^+) $C_{21}H_{31}NO_{12}S_2$ (553.6) *m/z* (%) 554.26 [M+H]+ (18), 576.25 [M+Na]+ (100); HRMS (ES^+) Found 554.1348, calcd for $C_{21}H_{32}NO_{12}S_2$ 554.1348 $[M+H]^+$

Methyl 2-[(2-hydroxyethyl)methylsulfanyl] 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,3,5-trideoxy-2-thio-β-D-*glycero*-D-*galacto*-2-nonulopyranosonate (**7d**) - ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.87 (s, 3H, NAc), 2.02, 2.06, 2.13, 2.15 (4s, 12H, 4OAc), 2.24 (dd, 1H, H-3ax, $J_{3ax,4}$ 12.0 Hz, $J_{3ax,3eq}$ 12.6 Hz), 2.68 (dd, 1H, H-3eq, $J_{3ax,4}$ 4.8 Hz, $J_{3ax,3eq}$ 12.6 Hz), 2.76 (t, 1H, OH, *J* 6.3 Hz), 2.94 (m, 1H, S$CH_2$$CH_2$OH), 3.08 (m, 1H, S$CH_2$$CH_2$OH), 3.81 (s, 3H, COOMe), 3.85 (m, 2H, $CH_2$$CH_2$OH), 4.01 (m, 3H, H-5, H-6, H-9a), 4.39 (dd, 1H, H-9b, $J_{8,9}$ 2.8

Hz, $J_{9a,9b}$ 12.4 Hz), 4.88 (m, 1H, H-4), 5.23 (m, 1H, NH, $J_{5,NH}$ 9.6 Hz), 5.26 (dd, 1H, H-7, *J* 1.6 Hz, *J* 8.0 Hz), 5.36 (m, 1H, H-8); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.75, 20.79, 21.09, 21.38, 23.14, 37.41, 39.98, 49.34, 53.15, 60.41, 60.50, 62.73, 67.45, 69.00, 74.76, 89.37, 167.87, 170.11, 170.17, 170.41, 170.91, 171.11;MS (ES^+) $C_{22}H_{33}NO_{13}S_2$ (583.6) *m/z* (%) 584 [M+H]+ (14), 606.24 [M+Na]+ (100); HRMS (ES^+) Found 601.1722, calcd for $C_{22}H_{33}NO_{13}S_2NH_4$ 601.1732 $[M+NH_4]^+$

Methyl 2-[(5-chloropyridin-2-yl)sulfanyl]-5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,3,5-trideoxy-2-thio-β-D-*glycero*-D-*galacto*-2-nonulopyranosonate (**7e**) ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.86 (s, 3H, NAc), 2.01, 2.03, 2.03, 2.11 (4s, 12H, 4OAc), 2.24 (dd, 1H, H-3ax, $J_{3ax,4}$ 12.0 Hz, $J_{3ax,3eq}$ 12.4 Hz), 2.68 (dd, 1H, H-3eq, $J_{3ax,4}$ 4.8 Hz, $J_{3ax,3eq}$ 12.8 Hz), 3.73 (s, 3H, COOMe), 4.01 (m, 3H, H-5, H-6, H-9a), 4.39 (dd, 1H, H-9b, $J_{8,9}$ 2.8 Hz, $J_{9a,9b}$ 12.4 Hz), 4.91 (m, 1H, H-4), 5.01 (m, 1H, H-8), 5.18 (d, 1H, NH, $J_{5,NH}$ 8.0 Hz), 5.27 (m, 1H, H-7), 7.75 (dd, 1H, H4`, $^4J$ 2.2 Hz $^3J$ 8.6 Hz), 7.78 (d, 1H, H3`, ^3J 8.6 Hz), 8.38 (d, 1H, H6`, ^4J 2.2 Hz); 7.68, 7.80, 8.40, (m, 3H, H-2', H-3', H-4') ; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.16, 20.55, 20.70, 20.77, 20.98, 23.11, 37.41, 49.28, 53.24, 60.35, 62.00, 67.20, 68.86, 69.37, 74.70, 87.57, 121.58, 136.82, 147.64, 157.35, 167.69, 169.82, 169.85, 170.14, 170.53, 170.81; MS (ES^+) $C_{25}H_{31}ClN_2O_{12}S_2$ (650.1) *m/z* (%) 651.27 [M+H]+ (100), 673.11 [M+Na]+ (22); HRMS (ES^+) Found 651.1069, calcd for $C_{25}H_{32}ClN_2O_{12}S_2$ 651.1080 $[M+H]^+$

Methyl 2-[(cyclohexyl)methylsulfanyl] 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,3,5-trideoxy-2-thio-β-D-*glycero*-D-*galacto*-2-nonulopyranosonate (**7d**) - ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.31 (m, 7H), 1.66 (m, 1H), 1.82 (m, 2H), 1.90 (s, 3H, NAc), 2.07, 2.10, 2.14, 2.17 (4s, 12H, 4OAc), 2.l (dd, 1H, H-3ax, $J_{3ax,4}$ 12.0 Hz, $J_{3ax,3eq}$ 12.8 Hz), 2.68 (dd, 1H, H-3eq, $J_{3ax,4}$ 4.8 Hz, $J_{3ax,3eq}$ 12.8 Hz), 2.95 (m, 1H), 3.83 (s, 3H, COOMe), 3.93 (m, 2H, H-5, H-6), 4.07 (dd, 1H, H-9a, $J_{8.9a}$ 5.2 Hz, $J_{9a,9b}$ 12.6 Hz), 4.41 (dd, 1H, H-9b, $J_{8,9}$ 2.4 Hz, $J_{9a,9b}$ 12.6 Hz), 4.92 (m, 1H, H-4), 5.34 (m, 3H, NH, H-7, H-8); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.55, 14.63, 21,27, 21.29, 21.51, 21.86, 23.64, 26.16, 37.73, 40.45, 43.32, 49.84, 50.15, 53.49, 62.47, 67.92, 69.93, 70.36, 75.24, 88.96, 168.81, 170.32, 170.38, 170.64, 171.01, 171.38; MS (ES^+) $C_{26}H_{39}NO_{12}S_2$ (621.7) *m/z* (%) 622.26 [M+H]+ (8), 644.32 [M+Na]+ (100); HRMS (ES^+) Found 622.1974, calcd for $C_{26}H_{33}NO_{12}S_2$ 622.1986 $[M+H]^+$.

General method for the synthesis of glycosyl disulfides – To a solution of 8 (0.1 mmol) in ethyl acetate (2 ml) was added hydrazine hydrate (0.3 mmol) followed by symmetrical disulfide (0.5 mmol) at RT. After 4-5, the reaction mixture was quenched with sat sol $NaHCO_3$ (20 ml) and extracted ethyl acetate (3 x 20 ml). The organic phase was dried over $MgSO_4$, filtered and the filtrate concentrated under vacuum. Column chromatography on silica gel (petroleum ether/ethyl acetate 2:1) afforded desired glycosyl disulfides.

2-Hydroxyethyl-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)disulfide (**9a**) ^{1}H NMR ($CDCl_3$, 400 MHz,) δ 1.97, 1.99, 1.99, 2.05 (s, 12H, OAc), 2.38 (bs, 1H,OH), 2.88 (m, 2H, CH_2CH_2OH), 3.73 (ddd, 1H, H-5, $J_{5,6a}$ 2.0 Hz, $J_{5,6b}$ 4.7 Hz, $J_{4,5}$ = 9.6 Hz,), 3.83 (t, *J* = 5.9 Hz, 2H, CH_2CH_2OH), 4.14 (dd, 1H, H-6a. $J_{5,6a}$ 2.0 Hz, $J_{6a,6b}$ 12.4 Hz), 4.21 (dd, 1H, H-6b, $J_{5,6b}$ 4.7 Hz, $J_{6a,6b}$12.5 Hz), 4.52 (d, 1H, H-1, $J_{1,2}$ 9.6 Hz),), 5.06 (dd, 1H, H-4, $J_{3,4}$ 9.6 Hz, $J_{4,5}$ 9.6 Hz), 5.24 (m, 2H, H-2, H-3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.48, 20.51, 20.57, 20.63, 41.91, 60.02, 61.78, 67.97, 69.09, 73.75, 76.24, 87.14, 169.10, 169.30, 170.11, 170.61; MS (ES^+) $C_{15}H_{22}O_{10}S2$ (426) *m/z* (%) 463.21 [M+Na]+ (100);

4-Flurorobenzyl-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-disulfide (**9e**) - ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.01, 2.02, 2.04, 2.07 (4s, 12H, OAc), 3.75 (m, 1H, H-5) 3.99 (s, 2H, SSCH_2PhF), 4.19 (dd, 1H, H-6a, $J_{5,6a}$ 2.4 Hz, $J_{6a,6b}$ 12.4 Hz), 4.27 (dd, 1H, H-6b, $J_{5,6b}$ 4.8 Hz, $J_{6a,6b}$ 12.4 Hz), 4.53 (d, 1H, H-1, $J_{1,2}$ 9.6 Hz), 5.13 (dd, 1H, H-4, $J_{3,4}$ 9.6 Hz, $J_{4,5}$ 9.6 Hz), 5.25 (dd, 1H, H-3, $J_{2,3}$ 9.6 Hz, $J_{3,4}$ 9.6 Hz), 5.32 (dd, 1H, H-2, $J_{1,2}$ 9.6 Hz, $J_{2,3}$ 9.6 Hz), 7.00 (dd, 2H, H3`, ^3J 8.4 Hz, $J_{H,F}$ 8.8 Hz). 7.27 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.53, 20.55, 20.60, 20.67, 43.53, 62.09, 68.11, 69.16, 73.83, 87.79, 115.28 (2C), 131.10 (2C), 169.07, 169.36, 170.13, 170.41; MS (ES^+) $C_{21}H_{25}FO_9S_2$ (504) *m/z* (%) 527.14 [M+Na]+ (100); HRMS (ES^+) Found 522.1249, calcd for $C_{21}H_{29}FO_9S_2N$ 522.1262 $[M+NH_4]^+$.

Methyl-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-disulfide (**9f**) – ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.01, 2.03, 2.08 (3s, 12H, 4OAc), 2.48 (s, 3H, SSCH_3), 3.74 (m, 1H, H-5), 4.17 (dd, 1H, H-6a, $J_{5,6a}$ 2.4 Hz, $J_{6a,6b}$ 12.4 Hz), 4.23 (dd, 1H, H-6b, $J_{5,6b}$ 4.8 Hz, $J_{6a,6b}$ 12.4 Hz), 4.57 (d, 1H, H-1, $J_{1,2}$ 9.6 Hz), 5.11 (dd, 1H, H-4, $J_{3,4}$ 9.6 Hz, $J_{4,5}$ 9.6 Hz), 5.25 (dd, 1H, H-3, $J_{2,3}$ 9.6 Hz, $J_{3,4}$ 9.6 Hz), 5.30 (dd, 1H, H-2, $J_{1,2}$ 9.6 Hz, $J_{2.3}$ 9.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.54, 20.57, 20.63, 20.66, 24.62, 60.35, 62.09, 68.15, 69.11, 73.89, 88.03, 169.11, 169.35, 170.18, 170.49; MS (ES^+) $C_{15}H_{22}O_9S_2$ (410.07) *m/z* (%) 433.20 [M+Na] + (100); HRMS (ES^+) Found 428.1036, calcd for $C_{15}H_{22}O_9S_2$ HRMS (ES^+) Found 418.1036, calcd for $C_{15}H_{26}O_9S_2N$ 428.1043 $[M+NH_4]^+$.

5-chloropyridin-1-sulfanyl -(2,3,4,6-tetra-O-acetyl-β-d-glucopyranosyl)disulphide (**9g**) -^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.00, 2.02, 2.10, 2.13 (4s, 12H, OAc), 3.79 (m, 1H, H-5), 4.22 (dd, 1H, H6a, $J_{5,6a}$ 2.0 Hz $J_{6a,6b}$ 12.4 Hz), 4.25 (dd, 1H. H6b, $J_{5,6b}$ 4.4 Hz, $J_{6a,6b}$ 12.4 Hz), 4.66 (d, 1H, H-1, $J_{1,2}$ 10.0 Hz)), 5.06 (dd, 1H, H-4, $J_{4,5}$ 9.6 Hz, $J_{3,4}$ 10.0 Hz, 5.17 (d, 1H, H-2, $J_{2,3}$ 9.6 Hz, $J_{1,2}$ 10.0 Hz), 5.25 (dd, 1H, H-3, $J_{2,3}$ 9.6 Hz, $J_{3,4}$ 10.0 Hz), 7.34 (d, 1H, ^3J 8.0 Hz), 7.42 (d, 1H, ^3J 8.0 Hz), (7.66 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.17, 20.53, 20.62, 20.75, 21.00, 60.37, 61.60, 67.94, 69.78, 73.89, 76.19, 87.28, 138.14, 169.16, 169.28, 170.09, 170.68, 210.01; MS (ES^+) $C_{19}H_{22}ClNO_9S_2$ (507) *m/z* (%) 508.17 $[M+H]^+$(100), 503.16 [M+Na]+ (24); HRMS (ES^+) Found 508.0489, calcd for $C_{19}H_{22}ClNO_9S_2$ 508.0497 $[M+H]^+$

Acknowledgements

The authors acknowledge EPSRC for a DTA studentship awarded to RAF to support BRS. Yorkshire Cancer Research is additionally thanked for financial support of RAF. The EPSRC UK National Mass Spectrometry Facility, Swansea University, is thanked for HRMS measurements.

Notes and references

1. I. Hamachi, T. Nagase and S. Shinkai, *J. Am. Chem. Soc.*, 2000, **122**, 12065-12066.
2. D. P. Gamblin, P. Garnier, S. van Kasteren, N. J. Oldham, A. J. Fairbanks and B. G. Davis, *Angew. Chem. Int. Edit.*, 2004, **43**, 828-833.
3. S. Andre, Z. C. Pei, H. C. Siebert, O. Ramstrom and H. J. Gabius, *Bioorg. Med. Chem.*, 2006, **14**, 6314-6326.
4. Z. C. Pei, T. Aastrup, H. Anderson and O. Ramstrom, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 2707-2710.
5. Z. C. Pei, R. Larsson, T. Aastrup, H. Anderson, J. M. Lehn and O. Ramstrom, *Biosens. Bioelectron*, 2006, **22**, 42-48.

6. B. N. Murthy, S. Sinha, A. Surolia, N. Jayaraman, L. Szilagyi, I. Szabo and K. E. Kover, *Carbohyd. Res.*, 2009, **344**, 1758-1763.
7. L. Szilagyi, T. Z. Illyes and P. Herczegh, *Tetrahedron Lett.*, 2001, **42**, 3901-3903.
8. I. Brito, M. Lopez-Rodriguez, A. Benyei and L. Szilagyi, *Carbohyd. Res.*, 2006, **341**, 2967-2972.
9. B. G. Davis, S. J. Ward and P. M. Rendle, *Chem. Commun.*, 2001, 189-190.
10. E. J. Grayson, S. J. Ward, A. L. Hall, P. M. Rendle, D. P. Gamblin, A. S. Batsanov and B. G. Davis, *J. Org. Chem.*, 2005, **70**, 9740-9754.
11. D. N. Harp and J. G. Gleason, *J. Am. Chem. Soc.*, 1971, **93**, 2437-2445.
12. R. J. Ferrier, R. H. Furneaux and P. C. Tyler, *Carbohyd. Res.*, 1977, **58**, 397-404.
13. G. J. L. Bernardes, E. J. Grayson, S. Thompson, J. M. Chalker, J. C. Errey, F. El Oualid, T. D. W. Claridge and B. G. Davis, *Angew. Chem. Int. Edit.*, 2008, **47**, 2244-2247.
14. C. F. Liang, M. C. Yan, T. C. Chang and C. C. Lin, *J. Am. Chem. Soc.*, 2009, **131**, 3138-+.
15. C. F. Liang, T. C. Kuan, T. C. Chang and C. C. Lin, *J. Am. Chem. Soc.*, 2012, **134**, 16074-16079.
16. M. Fernandez-Gonzalez, O. Boutureira, G. J. L. Bernardes, J. M. Chalker, M. A. Young, J. C. Errey and B. G. Davis, *Chem. Sci.*, 2010, **1**, 709-715.
17. E. J. Grayson, G. J. L. Bernardes, J. M. Chalker, O. Boutureira, J. R. Koeppe and B. G. Davis, *Angew. Chem. Int. Edit.*, 2011, **50**, 4127-4132.
18. G. Hummel and O. Hindsgaul, *Angew. Chem. Int. Edit.*, 1999, **38**, 1782-1784.
19. M. J. Kiefel, R. J. Thomson, M. Radovanovic and M. von Itzstein, *J. Carbohyd. Chem.*, 1999, **18**, 937-959.
20. S. Knapp, E. Darout and B. Amorelli, *J. Org. Chem.*, 2006, **71**, 1380-1389.
21. M. Adinolfi, D. Capasso, S. Di Gaetano, A. Iadonisi, L. Leone and A. Pastore, *Org. Biomol. Chem.*, 2011, **9**, 6278-6283.
22. W. M. Macindoe, A. H. van Oijen and G. J. Boons, *Chem. Commun.*, 1998, 847-848.
23. T. Z. Illyes, T. Szabo and L. Szilagyi, *Carbohyd. Res.*, 2011, **346**, 1622-1627.
24. M. C. Aversa, A. Barattucci and P. Bonaccorsi, *Eur. J. Org. Chem.*, 2009, 6355-6359.
25. R. Caraballo, M. Rahm, P. Vongvilai, T. Brinck and O. Ramstrom, *Chem. Commun.*, 2008, 6603-6605.
26. R. Caraballo, M. Sakulsombat and O. Ramstrom, *Chem. Commun.*, 2010, **46**, 8469-8471.
27. G. Ribeiro Morais and R. A. Falconer, *Tetrahedron Lett.*, 2007, **48**, 7637-7641.
28. N. Stellenboom, R. Hunter, M. R. Caira and L. Szilagyi, *Tetrahedron Lett.*, 2010, **51**, 5309-5312.
29. G. Ribeiro Morais, Oliveira I F, Humphrey A J and R. A. Falconer, *Carbohyd. Res.*, 2010, **345**, 160-162.
30. G. Ribeiro Morais, R. S. Oliveira and R. A. Falconer, *Tetrahedron Lett.*, 2009, **50**, 1642-1644.
31. B. Mandal and B. Basu, *Rsc Adv*, 2014, **4**, 13854-13881.
32. M. Musiejuk and D. Witt, *Org. Prep. Proced. Int.*, 2015, **47**, 95-131.
33. J. J. Bailey and D. R. Bundle, *Org. Biomol. Chem.*, 2014, **12**, 2193-2213.
34. R. A. Falconer, R. J. Errington, S. D. Shnyder, P. J. Smith and L. H. Patterson, *Curr. Cancer Drug Targets*, 2012, **12**, 925-939.
35. Y. M. Al-Saraireh, M. Sutherland, B. R. Springett, F. Freiberger, G. Ribeiro Morais, P. M. Loadman, R. J. Errington, P. J. Smith, M. Fukuda, R. Gerardy-Schahn, L. H. Patterson, S. D. Shnyder and R. A. Falconer, *PloS One*, 2013, **8**, e73366.