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Synthesis of aryl sialosides using Mitsunobu conditions

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Abstract—Mitsunobu conditions for the efficient synthesis of aryl α/β-sialosides were developed. An oxidative work-up procedure was employed to avoid a cumbersome chromatographic separation from the 2,3-dehydro derivative of sialic acid, which is formed as a side-product.

Keywords: Mitsunobu conditions; Ketosugars; Glycal; Aryl sialosides, ^{1}H and ^{13}C NMR

1. Introduction

Aryl α-sialosides are often used as chromogenic substrates for detecting and quantifying sialidase activity.[1–3] Sialidases catalyze the hydrolytic cleavage of α-glycosidically linked sialic acid from glycoconjugates that regulate cell-to-cell, cell-to-microorganism, -toxin, and -antibody interactions.[4,5] In addition, aryl sialosides, such as *p*-nitrophenyl sialoside, have also found use as synthetic substrates for sialidases.[6]

In contrast to aldoglycosides, only few methods for the stereoselective synthesis of ketoglycosides, such as aryl sialosides, exist.[2] The most frequently used procedure is a Williamson ether synthesis based on phenolates and 2-halogeno derivatives of sialic acid.[1,3] However, the low solubility of sodium phenolates drastically limits the scope of this method. An alternative method utilizes phase transfer conditions (chloroform–aqueous alkali) for the sialidation of phenols with 2-chloro derivatives of sialic acid.[2] Both approaches result in complete inversion of the anomeric configuration and give moderate to good yields. However, side reactions caused by the basic reaction conditions, for example, hydrolysis of protecting groups or glycal formation by 2,3-elimination, are inevitable.

The Mitsunobu reaction[7] has been widely used for the synthesis of aryl glycosides,[8–17] acyl glycosides,[16–19] alkyl glycosides,[16,20,21] and amino glycosides[16,22] by reacting hemiacetals of aldosugars, predominantly glucose, galactose, and mannose, with weakly acidic acceptors in the presence of diethyl azodicarboxylate (DEAD) and triphenylphosphine (Ph_3P). A major advantage of this direct dehydrative coupling procedure is that activated glycosyl donors do not need to be isolated and all reaction steps—anomerization, activation, and glycosidic bond formation—occur in a single series of events.[23] Due to the neutral conditions, the reaction is compatible with a wide variety of sensitive functionalities, ranging from epoxides and spiroketals to any of the commonly utilized protecting groups.[12,24] The stereochemical outcome depends on the anomeric ratio present in the hemiacetal and its mutarotation occurring under reaction conditions. Phenolic nucleophiles are good acceptors for this glycosylation reaction with aldosugars, leading to aryl glycosides.[8,13,15]

To our knowledge, Mitsunobu conditions have not been used for the glycosylation with ketosugars such as sialic acid, probably because their anomeric centers were regarded as being too sterically hindered.[25] However, since a limited number of successful noncarbohydrate cases have been reported, albeit with moderate yields and stereoselectivities,[26–28] we decided to apply the Mitsunobu reaction to the synthesis of aryl sialosides.

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2. Results and discussion

Treatment of the glycosyl donor methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate with 4-phenoxy-phenol (**2a**) in the presence of NIS-TfOH[29,30] as promoter did not provide the corresponding aryl sialoside **3a**. DMTST[31] also gave unsatisfactory results, providing **3a** in low yield and with poor stereoselectivity.

Gratifyingly, standard Mitsunobu conditions[32] [i.e., addition of DEAD to a solution of hemiketal **1β**,[33] **2a**, and Ph_3P in THF (Table 1, entry 1)] provided an α/β-mixture of sialoside **3a** in 43% yield. Glycal **4**, formed by 2,3-elimination, was isolated as a major side product. In addition, *N*-sialyl-1,2-diethoxycarbonylhydrazine **5**, identified by ESIMS [(*m*/*z*): 672.1 $(M+Na)^+$], was obtained in small quantity. Similar adducts of DEAD to aldosugars have been reported before.[21,22] Since the stereoselectivity at the anomeric center in glycosylation reactions with donors lacking a participating group is markedly influenced by solvent effects, toluene and acetonitrile were examined next. Whereas toluene (entry 2) gave approximately the same results as THF, dramatically improved yields of 75% were obtained in acetonitrile. Surprisingly, although acetonitrile is known for its participating character in glycosylation reactions,[34–36] no improvement of the stereoselectivity could be detected (entry 3).

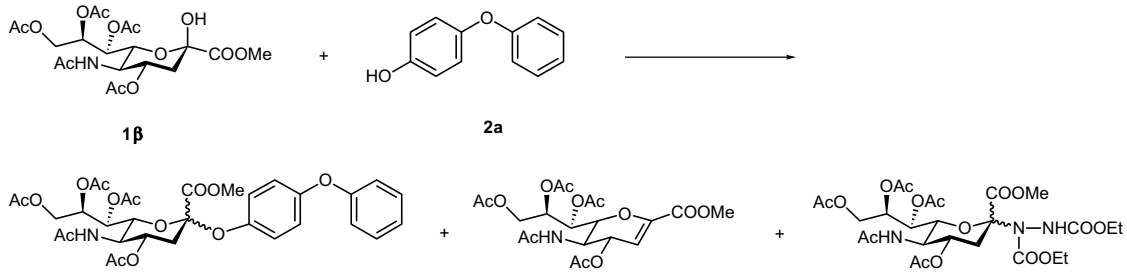
To further improve yield and stereoselectivity, other reaction variables such as temperature, order of addition, and additives, were investigated.

Smith et al.[18] reported that the anomeric ratio in the Mitsunobu acylation can be improved by starting the reaction at −50 °C. However, these conditions led to a marked decrease in yield and stereoselectivity (entry 4). Also at −25 °C (entry 5) or rt (entry 6) the yield of sialoside **3a** was reduced and the side product **4** was favored instead.

According to Voyle's report,[14] the outcome of the reaction can be influenced by the order of addition. However, we were unable to observe an improvement in yield or stereoselectivity when we allowed the zwitterionic P–N adduct from Ph_3P and DEAD to form, before adding the hemiketal and the nucleophile (entries 4 and 7).

Elimination is a common side reaction in Mitsunobu reactions, especially when the emerging double bond is conjugated.[25] As a consequence, sialic acid poses a significant challenge, and glycal **4** is formed under various sialidation conditions. According to Wovkulich et al.,[38] the dehydration pathway in the Mitsunobu

Table 1. Exploration of variant reaction conditions for the sialidation under Mitsunobu conditions



Entry[a]	Solvent	Temp (°C)	Time (h)	**3a** (α:β)[b]	**3a**:**4**:**5**[c]
1	THF	0	6	43% (62:38)	1:1:0.3
2	Toluene	0	3	40% (60:40)	1:1.2:0.4
3	CH_3CN	0	3	75% (64:36)	1:0.3:0.1
4[d]	CH_3CN	−45	3	23% (55:45)	1:1.3:1
5	CH_3CN	−25	16	61% (57:43)	1:0.4:0.2
6	CH_3CN	rt	3	62% (63:37)	1:0.5:0.1
7[e]	CH_3CN	0	3	67% (60:40)	1:0.3:0.2
8[f]	CH_3CN	0	3	46% (51:49)	1:0.3:0.1

[a] Standard conditions: **1β** (1.0 equiv), **2a** (2.0 equiv), Ph_3P (1.5 equiv), DEAD (1.5 equiv), molecular sieves 3 Å.

[b] Isolated yields of product mixture (α and β), ratios of isomers were determined by ^1H NMR[37a,b] of the crude product prior to oxidative treatment (vide infra).

[c] Ratios of **3a**, **4**, and **5** were determined according to ^1H NMR of the crude products: [**3a**-H_{3e}(α,β):**4**-H_3(6.02 ppm):**5**-H_{3e}(α,β:2.55,2.44 ppm)].

[d] To a solution of Ph_3P in dry CH_3CN at −45 °C, DEAD was added. The mixture was stirred for 10 min, then **1β** was added, and stirring continued for 10 min before addition of **2**. The mixture was allowed to warm slowly to rt over a period of 3 h and kept at rt until TLC indicated complete consumption of **1β**.

[e] DEAD was dissolved in dry CH_3CN, and Ph_3P was added, after 5 min **2a** was added, then **1β**.

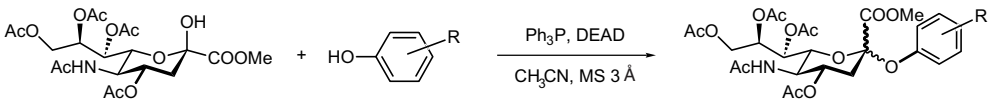
[f] The same procedure as (entry 3), except 1.0 equiv of pyridine was added to the reaction mixture.

reaction can be minimized by performing the reaction in the presence of 1.0 equiv of pyridine. However, in our hands, this additive not only reduced the yield of glycal **4**, but also that of the desired product **3a** (entry 8).

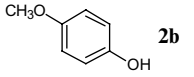
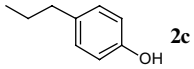
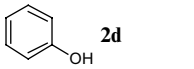
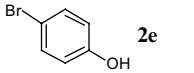
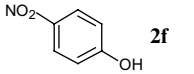
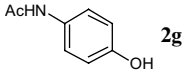
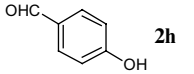
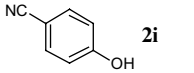
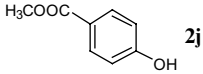
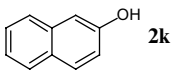
The often formed glycal side product is especially aggravating, since it is usually difficult to separate from the desired product.[39,40] One approach to avoid the separation problem is the chemical transformation of the side product into a derivative with modified polarity. Goto and co-workers[41] converted glycals into 2-bromo-3-hydroxy or 2-bromo-3-methoxy sialic acid derivatives by reaction with NBS in CH_3CN–H_2O or CH_3OH. However, even though the reaction is mild and high yielding, the polarity of the products is not changed significantly and therefore did not allow a separation from the sialosides. The conditions for the oxidation of glycals reported by Marra and Sinay[33] ($NaIO_4$ in CH_3CN–CCl_4–H_2O in the presence of a catalytic amount of $RuCl_3{\cdot}H_2O$) are in fact incompatible with a variety of functional groups such as olefins, alcohols, aromatic rings, and ethers,[42] but allowed when applied only for 2–5 min to selectively oxidize glycal **4** without affecting the yield of the desired sialosides. The polar products of the glycal oxidation could then readily be removed by extraction with water.

With optimized reaction conditions and oxidative work-up procedure for model compound **3a** in hand (entry 3), we applied the protocol to other phenols. In most cases, high yields and α/β-selectivities in the range of 2:1 were obtained (Table 2). It is noteworthy that the yields correlate with the acidity of the glycosyl acceptors. Phenols bearing electron-withdrawing substituents

Table 2. Formation of aryl sialosides **3b–k** under Mitsunobu conditions



1β **2b-k** **3b-k**

2b–k	Temp (°C)/time (h)	**3b–k** (α:β)[a]	**3**:**4**:**5**[b]	**3b–k** (α-H_{3e},β-H_{3e}) (ppm)
 **2b**	0/3	50% (50:50)	1:0.8:0.1	2.69, 2.65
 **2c**	0/3	73% (58:42)	1:0.3:tr	2.68, 2.64
 **2d**	0/3	76% (59:41)	1:0.3:0.1	2.71, 2.65
 **2e**	0/6	72% (64:36)	1:0.2:tr	2.67, 2.61
 **2f**	0/3	90% (55:45)	1:tr:tr	2.75, 2.69
 **2g**	0/3	65% (62:38)[c]	1:0.3:0.2	2.70, 2.64
 **2h**	0/3	89% (60:40)[d]	1:0.1:tr	2.73, 2.67
 **2i**	0/3	83% (62:38)	1:0.1:tr	2.73, 2.66
 **2j**	0/3	87% (46:54)	1:0.1:tr	2.70, 2.65
 **2k**	0/4	76% (74:26)	1:0.4:tr	2.76, 2.72

tr = trace.

[a] Isolated yields of product mixture (α and β), ratios of isomers were determined by ^{1}H NMR[37a,b] of the crude products before oxidative treatment (vide infra).

[b] Ratios of **3**, **4**, and **5** were determined according to ^{1}H NMR of the crude products: [**3**-$\mathbf{H}_{3e}$(α,β):**4**-$\mathbf{H}_3$(6.02 ppm):**5**-$\mathbf{H}_{3e}$(α,β:2.55,2.44 ppm)].

[c] Oxidative treatment was not necessary because of the different polarity between glycal and the product.

[d] According to the mass and ^{1}H NMR spectrum, trace of carboxy acid derivative was formed after oxidative treatment.

gave excellent yields [e.g., 4-nitrophenol (**2f**)], while phenols substituted with electron-donating groups resulted in lower yields and increased amounts of side products (e.g., **2b**). Analogous observations were made when methyl 2,3,4-tri-*O*-acetyl-D-glucopyranuronate was reacted with substituted phenols under Mitsunobu conditions.[14] A correlation of the pK_a of the acceptor with the stereochemical outcome of the Mitsunobu reaction, as proposed by Myer et al.,[17] was not observed in our sialidation study.

The sialosides can be formed by two mechanistic pathways of the Mitsunobu reaction.[7,25] Firstly, the reaction of the quaternary phosphonium salt, formed from Ph_3P and DEAD in the presence of a weak acid, with the hemiketal **1α** or **1β** yields the corresponding oxophosphonium species **6α** or **6β**. The triphenylphosphine oxide leaving group is then displaced by the weakly acidic nucleophile through a S_N2 mechanism to yield the sialosides **8α** or **8β**. Alternatively, **8α** and **8β** can be formed from **6α** and **6β** in a S_N1-type mechanism by elimination of triphenylphosphine oxide (⇒**7**) followed by addition of the nucleophile.

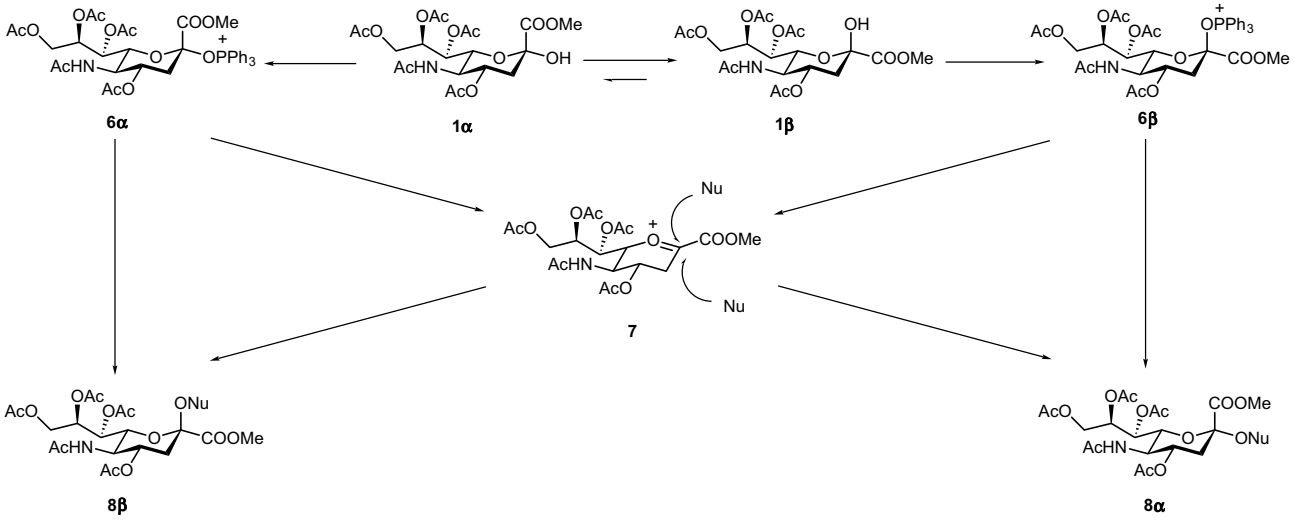
The best stereoselectivity (α:β ≈ 3:1) was observed with 2-naphthol (entry *k*) as acceptor, while all other cases gave only modest or no diastereoselectivity (3:2 to 1:1). This low selectivity suggests the partial involvement of a S_N1 reaction mechanism via the oxonium ion **7** (Scheme 1). However, the fact that the anomeric ratio does not significantly improve when CH_3CN is used as a solvent (Table 1, entry 3), compared to THF or toluene (entries 1 and 2), does not support this reaction pathway. On the other hand, the stereochemical outcome does not reflect the anomeric ratio present in hemiketal **1** (α:β ≈ 5:95 in CH_3CN).[43] Therefore, it is likely that the low diastereoselectivity is a consequence of the kinetically favored reaction of **1α** to the phosphonium ion **6α**, which by S_N2 displacement by the nucleophile is then transformed into the sialoside **8β**.

The presented procedure for the synthesis of aryl sialosides expands the existing pool of methods. Furthermore, the employed glycosyl donor is easily synthesized. The reaction conditions are mild and the yields are good to excellent. The improvement of the stereoselectivity is currently further investigated.

3. Experimental

3.1. General methods

NMR spectra were recorded on a Bruker Avance DMX-500 (500 MHz) spectrometer. Assignment of ^1H and ^{13}C NMR spectra was achieved using 2D methods (COSY, HSQC). Chemical shifts are expressed in ppm using residual $CHCl_3$ as references. Optical rotations were measured using a Perkin–Elmer Polarimeter 241. Reactions were monitored by TLC using glass plates coated with silica gel 60 F_{254} (Merck) and visualized by using UV light and/or by charring with a molybdate solution (0.02 M solution of ammonium cerium sulfate dihydrate and ammonium molybdate tetrahydrate in aqueous 10% H_2SO_4). Column chromatography was performed on silica gel (Uetikon, 40–60 mesh). Pyridine was freshly distilled under argon over KOH. Acetonitrile and toluene, were dried by filtration over Al_2O_3 (Fluka, type 5016 A basic). THF was dried by refluxing with sodium, benzophenone and distilled immediately before use. Molecular sieves (3 Å) were activated in vacuo at 500 °C for 2 h immediately before use.



Scheme 1. Postulated mechanistic pathway of sialidation using Mitsunobu conditions.

3.2. Representative procedure

3.2.1. Methyl (*p*-phenoxyphenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (3a). DEAD (47.5 μL, 0.3 mmol) was added to a stirred solution of compound **1β**[33] (100 mg, 0.2 mmol), Ph_3P (80 mg, 0.3 mmol), and compound **2a** (76 mg, 0.4 mmol) in dry CH_3CN (6 mL) at 0 °C under argon. The reaction mixture was stirred at 0 °C for 3 h, diluted with CH_2Cl_2 (15 mL), and filtrated through a pad of Celite. The Celite was washed with CH_2Cl_2 (3–5 mL) and the combined filtrates were concentrated under reduced pressure, then dried at high vacuum. A catalytic amount of $RuCl_3{\cdot}H_2O$ was added to a vigorously stirred biphasic solution of the residue in $NaIO_4$ (~0.1 g), CCl_4 (0.8 mL), CH_3CN (0.8 mL), and H_2O (1.2 mL). After 2–5 min at rt, the thick, yellowish-green mixture was diluted with CH_2Cl_2 (25 mL), and washed with H_2O (2 × 10 mL). The organic layer was dried over Na_2SO_4, filtrated, and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (0.25% gradient MeOH in CH_2Cl_2) to afford sialoside **3a** (101 mg, 75%, α:β = 76:24) as a white foam. The diastereomeric mixtures of **3a** could be separated by flash chromatography on silica gel (5% gradient EtOAc in toluene), yielding **3a-α** (60 mg, 45%) and **3a-β** (16 mg, 12%) as white foams.

Compound **3a-α**: $[\alpha]_D^{25}$ −2.2° (*c* 1.0, $CHCl_3$); R_f 0.15 (toluene–EtOAc, 1:3); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.89 (s, 3H, NHAc), 2.01, 2.03, 2.11, 2.12 (4s, 12H, 4OAc), 2.18 (t, 1H, $J_{3a,3e}$, $J_{3a,4}$ 12.6 Hz, H-3a), 2.70 (dd, 1H, $J_{3e,4}$ 4.7 Hz, H-3e), 3.68 (s, 3H, OCH_3), 4.08 (q, 1H, $J_{4,5}$, $J_{5,6}$, $J_{5,NH}$ 10.4 Hz, H-5), 4.15 (dd, 1H, $J_{8,9a}$ 4.7, $J_{9a,9b}$ 12.2 Hz, H-9a), 4.30–4.33 (m, 2H, H-6, H-9b), 4.94 (ddd, 1H, H-4), 5.31 (d, 1H, NH), 5.33–5.37 (m, 2H, H-7, H-8), 6.91–6.93, 6.95–6.98, 7.02–7.08, 7.29–7.32 (m, 9H, Ar); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.72, 20.82, 20.94 (4C, 4OAc), 23.17 (NHAc), 37.74 (C-3), 49.31 (C-5), 52.88 (OCH_3), 62.04 (C-9), 67.40 (C-7), 68.86 (C-4), 69.31 (C-8), 73.29 (C-6), 100.33 (C-2), 118.28, 119.85, 122.01, 122.95 129.66 (9C, CH-Ar), 149.35, 153.54, 157.80 (C-Ar), 167.77, 169.95, 169.98, 170.25, 170.59, 170.92 (6CO); HRMS: Calcd for $C_{32}H_{37}NO_{14}$+Na 682.2214 $[M+Na]^+$; Found *m/z* 682.2212.

Compound **3a-β**: $[\alpha]_D^{25}$ −31.3° (*c* 1.0, $CHCl_3$); R_f 0.19 (toluene–EtOAc, 1:3); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.86, 1.87, 1.97, 2.05, 2.15 (5s, 15H, 4OAc, NHAc), 1.99 (m, 1H, H-3a), 2.66 (dd, 1H, $J_{3e,4}$ 4.9, $J_{3a,3e}$ 12.9 Hz, H-3e), 3.74 (s, 3H, OCH_3), 4.10 (dd, 1H, $J_{6,7}$ 2.3 Hz, $J_{5,6}$ 10.6 Hz, H-6), 4.14 (dd, 1H, $J_{8,9a}$ 7.1, $J_{9a,9b}$ 12.6 Hz, H-9a), 4.22 (q, 1H, $J_{4,5}$, $J_{5,NH}$ 10.4 Hz, H-5), 4.69 (dd, 1H, $J_{8,9b}$ 2.2 Hz, H-9b), 4.89 (ddd, 1H, $J_{7,8}$ 3.9 Hz, H-8), 5.37 (dd, 1H, H-7), 5.43 (d, 1H, NH), 5.46 (ddd, 1H, $J_{3a,4}$ 11.1 Hz, H-4), 6.86–6.90, 6.94–7.05, 7.06–7.08, 7.29–7.32 (m, 9H, Ar); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.69, 19.75, 19.81. 19.88 (4OAc), 22.13 (NHAc), 37.34 (C-3), 48.12 (C-5), 52.14 (OCH_3), 61.17 (C-9), 67.17 (C-7), 67.51 (C-4), 71.21 (C-8), 71.54 (C-6), 98.18 (C-2), 117.21, 117.29, 119.29. 122.01, 128.79 (9C, CH-Ar), 148.44, 151.38, 156.56 (C-Ar), 166.29, 169.11. 169.28, 169.47, 169.60, 170.03 (6CO); HRMS: Calcd for $C_{32}H_{37}NO_{14}$+Na 682.2214 $[M+Na]^+$; Found *m/z* 682.2225.

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