Mild elimination of a glycosidically linked –O $CH_2CH_2CH_2NH_2$ spacer-arm

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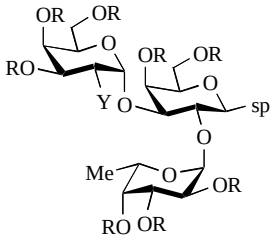
The spacer-arm –$OCH_2CH_2CH_2NH_2$ of complex oligosaccharides can be removed by oxidative deamination followed by alkaline -elimination.

The application of oligosaccharides to various chemical and biochemical studies often requires that these were prepared with the free 1-OH group or as spacered glycosides, *i.e.*, as glycosides of alcohols whose second function may serve for conjugation with macromolecules or labels. Spacering is one of the key problems of oligosaccharide synthesis strategy. The introduction of a spacer or prespacer group can be performed (i) at the final stage of the synthesis (by glycosidation of the oligosaccharide with a spacer alcohol) or (ii) at the initial stage when the spacer also serves as a 1-O-protecting group. Both approaches have advantages and drawbacks. The former makes it possible to obtain both free and spacered oligosaccharides; however, spacering at the final stage usually leads to a loss of yield, especially when a microscale synthesis is performed. The second approach is more economical; however, only the spacered product can be obtained.



1a sp = $OCH_2CH_2CH_2NHCOCF_3$, R = H, Y = NHAc
1b sp = $OCH_2CH_2CH_2NHCOCF_3$, R = H, Y = OH
2a sp = OAc, R = Ac, Y = NHAc
2b sp = OAc, R = Ac, Y = OAc
3a sp = $OCH_2CH_2CH_2NH_2$, R = H, Y = NHAc
3b sp = $OCH_2CH_2CH_2NH_2$, R = H, Y = OH
4a sp = $OCH_2CH_2CH_2NHAc$, R = Ac, Y = NHAc
4b sp = $OCH_2CH_2CH_2NHAc$, R = Ac, Y = OAc

Here we describe a methodology based on the second approach, namely, the removal of the spacer group –OCH_2CH_2–CH_2NH_2, which was often employed in our studies.[1,†]

A simple one-stage removal of the spacer-arm by acid hydrolysis or acid acetolysis in oligosaccharides **1a** and **1b** was unsuccessful because of cleavage of the acid-labile Fuc 1-2Gal bond.[‡]

In searching for a mild despacering procedure, we examined the applicability of the following two-stage approach. The spacer-arm –O $CH_2CH_2CH_2NH_2$ in compound **3a** or **3b** was subjected sequentially to the Corey method[3] and alkaline -elimination by treatment of the compounds with 3,5-di-*tert*-butyl-1,2-benzoquinone in methanol and acidification of intermediate azomethines **5** with oxalic acid. The final acetylation with acetic anhydride in pyridine simplified the chromatographic purification (see Scheme 1).

In this way, the azomethine obtained from the spacered blood group trisaccharide B gave rise to 41% aldehyde **6**[§] and 39% –OC $H_2CH_2CH_2NHAc$ derivative **4b**. The azomethine obtained from spacered blood group trisaccharide A under the same conditions gave rise to benzoxazole **7**[§] (70%). Although according to Corey and Achiva[3] primary amines can be converted into either carbonyl compounds or benzoxazole depending on carbon chain branching, it is surprising that trisaccharides having only minor differences in the sites distant from the reaction centre behaved in such a different fashion under the same conditions[‡,¶].

The next stage of the proposed method has to be alkaline -elimination of the free trisaccharide from derivatives **6** and **7**. However, the treatment with aqueous alkali solutions would result in splitting off the monosaccharide from the position O-3 of the despacered Gal moiety due to additional -elimination, where the deprotected 1-OH group plays a role of the aldehyde group. To avoid the secondary -elimination (so-called peeling), we used the conditions of base-catalysed acetolysis/acetylation (AcOH + AcONa + Ac_2O) described earlier for the elimination of complex oligosaccharides from the protein core:[4] acetic anhydride converts 1-OH into 1-OAc and thus blocks it. Thus, the treatment of compounds **6** and **7** with an AcOH–AcONa–A c_2O mixture at 110 °C for 48 h[††] gave despacered derivative **2b** or **2a** in 68 or 93% yield, respectively.

Note that we did not optimise the conditions of either the Corey reaction or -elimination and did not perform a one-pot process, which can increase the yields of final oligosaccharides.

Thus, we have demonstrated that 3-aminopropyl glycosides of complex oligosaccharides can be converted into despacered forms in non-acidic conditions.

† As a rule, this spacer is used as the trifluoroacetamidopropyl group –O $CH_2CH_2CH_2NHCOCF_3$, which can be quantitatively deblocked by an alkali.[2]

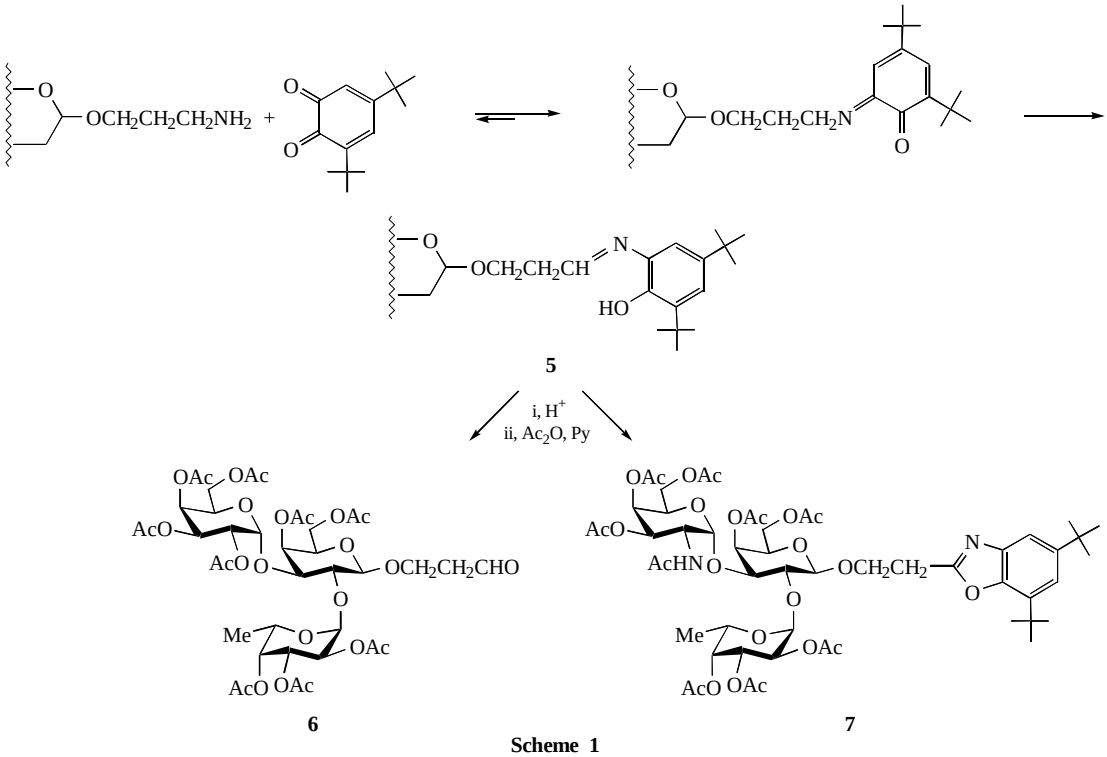
‡ No cleavage of the glycosidic bond Gal -sp leading to desired peracetate **2a** or **2b** was observed when acid acetolysis of the corresponding trifluoroacetamidopropyl glycosides of trisaccharides **1a** or **1b** (AcOH/Ac_2O/H_2SO_4, 100:100:1, 0–20 °C) was carried out. More severe conditions (an increase in the amount of sulfuric acid or a higher temperature) led to the cleavage of the Fuc 1-2Gal bond. Analogous results were obtained upon acid acetolysis (AcOH/Ac_2O/H_2SO_4, 100:100:1, 5 °C) of A trisaccharide acetamidopropyl glycoside **4a**. However, under the same conditions, the similar B trisaccharide glycoside **4b** was converted into peracetate **2b**, 90%.

§ A solution of amine **3** (0.34 mmol) and 3,5-di-*tert*-butyl-1,2-benzoquinone (83 mg, 0.37 mmol) in 30 ml of MeOH was stirred under argon at room temperature. The colour of the reaction mixture changed from dark brown to green in 1 h. The stirring was continued for 24 h; next, oxalic acid dihydrate was added to pH 2, the reaction mixture was evaporated to dryness *in vacuo*, and the solid residue was washed with a 2:1 ethyl acetate–benzene mixture to remove the remaining benzoquinone. The residue was conventionally acetylated with Ac_2O in Py for 24 h, and product **6** or **7** was separated by column chromatography. Selected spectral data:

6: ^{1}H NMR ($CDCl_3$) δ: 1.19 (d, 3H, Me′), 1.9–2.18 (27H, 9OAc), 3.37 (m, 2H, CH_2CHO), 3.67 dd (1H, H^2, $J_{2,1}$ 7.5 Hz, $J_{2,3}$ 10 Hz), 3.78 (m, 1H, H^5), 3.98, 4.08 (2H, H^6), 4.09, 4.30 (2H, H$^{6'}$), 4.10 (m, 1H, OCHCH$_2$), 4.41 (1H, H$^{5''}$), 4.45 (m, 1H, OCH'CH$_2$), 4.46 (d, 1H, H^1), 4.51 (m, 1H, H$^{5'}$), 5.12 (1H, H$^{3''}$), 5.18 (1H, H$^{2''}$), 5.23 (1H, H$^{4''}$), 5.34 (dd, 1H, H$^{2'}$, $J_{2',1'}$ 3 Hz, $J_{2',3'}$ 10 Hz), 5.37 (d, 1H, H$^{1'}$), 5.39 (d, 1H, H^4, $J_{4,3}$ 3.5 Hz), 5.46 (dd, 1H, H$^{3'}$), 5.52 (d, 1H, H$^{1''}$, $J_{1'',2''}$ 3.5 Hz), 5.61 (dd, 1H, H$^{4'}$). FAB MS, *m*/*z*: 922 (M$^+$).

7: ^{1}H NMR ($CDCl_3$) δ: 1.07 (d, 3H, Me′), 1.35 (s, 9H, 3Me-q), 1.5 (s, 9H, 3Me-q), 3.30 (t, 2H, CH_2C=), 3.81 (dd, 1H, H^2, $J_{2,1}$ 7 Hz, $J_{2,3}$ 9 Hz), 3.89 (dd, 1H, H^3, $J_{3,4}$ 3 Hz), 4.13 (m, 1H, OCHCH$_2$), 4.26 (1H, H$^{5'}$), 4.43 (m, 1H, OCH'CH$_2$), 4.5 (1H, H$^{2'}$), 4.51 (d, 1H, H^1), 5.02 (dd, 1H, H$^{3'}$, $J_{3',2'}$ 11 Hz, $J_{3',4'}$ 3 Hz), 5.2 (1H, H$^{5''}$), 5.24 (d, 1H, H$^{1'}$, $J_{1',2'}$ 3 Hz), 5.25 (1H, H$^{3''}$), 5.32 (dd, 1H, H$^{2''}$, $J_{2'',3''}$ 11 Hz, $J_{2'',1''}$ 3.5 Hz), 5.37 (dd, 1H, H^4), 5.46 (dd, 1H, H$^{4'}$), 5.51 (d, 1H, H$^{1''}$), 6.17 (d, 1H, NHAc, $J_{NH,2'}$ 9 Hz), 7.54 (d, 1H, H-q), 7.55 (d, 1H, H-q). FAB MS, *m*/*z*: 1123 (M$^+$).

¶ Corey and Achiva[3] also described the formation of a benzoxazole product without an explanation of the over-oxidation.



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6 **7**

Scheme 1

† Base-catalysed acetolysis of **7**. A mixture of **7** (100 mg, 89 mol), 500 mg of anhydrous sodium acetate, 2 ml of acetic anhydride and 2 ml of acetic acid was kept in a 5 ml sealed tube for 48 h at 110 °C. The reaction mixture was poured onto ice and extracted with chloroform. The organic layer was washed with water, a saturated aqueous solution of $NaHCO_3$ and water, dried and concentrated *in vacuo*. Column chromatography using an acetone–hexane eluent gave 75 mg (93%) of peracetate **2a**, FAB MS, *m/z*: 908 (M^+). Pure - and -acetates were isolated by HPLC.

-**2a**: ^{1}H NMR ($CDCl_3$) δ: 1.13 (d, 3H, Me′), 1.9–2.1 (30H, 10OAc), 4.05 (dd, 1H, H^3), 4.11 (1H, H^6), 4.06 (1H, H^6), 4.2 (dd, 1H, H^2), 4.51 (ddd, 1H, H$^{2'}$, $J_{2',1'}$ 3 Hz, $J_{2',3'}$ 7.5 Hz, $J_{2',NH}$ 9.5 Hz), 4.8 (dd, 1H, H$^{3'}$), 5.18 (1H, H$^{3''}$), 5.23 (1H, H$^{4''}$), 5.25 (d, 1H, H$^{1'}$), 5.30 (1H, H$^{1''}$), 5.35 (1H, H$^{2''}$), 5.46 (dd, 1H, H^4, $J_{4,3}$ 3 Hz, $J_{4,5}$ 1 Hz), 6.06 (d, 1H, NHAc), 6.33 (d, 1H, H^1, $J_{1,2}$ 3 Hz).

-**2a**: ^{1}H NMR ($CDCl_3$) δ: 1.08 (d, 3H, Me′), 1.9–2.1 (30H, 10OAc), 3.86 (dd, 1H, H^2, $J_{2,1}$ 7 Hz, $J_{2,3}$ 9 Hz), 3.9 (dd, 1H, H^5, $J_{5,6}$ 7 Hz, $J_{5,4}$ 1 Hz), 4.2 (dd, 1H, H^3), 4.27 (dd, 1H, H$^{5''}$), 4.38 (1H, H$^{5'}$), 4.61 (ddd, 1H, H$^{2'}$, $J_{2',1'}$ 3.5 Hz, $J_{2',3'}$ 11 Hz, $J_{2',NH}$ 11 Hz), 5.1 (dd, 1H, H$^{3'}$), 5.25 (d, 1H, H$^{1'}$), 5.35 (1H, H$^{4''}$), 5.42 (1H, H^4), 5.54 (1H, H$^{4'}$), 5.56 (d, 1H, NHAc), 5.62 (d, 1H, H^1, $J_{1,2}$ 7 Hz).

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