A Novel Approach to the Construction of Hydroxylamino Interglycosidic Linkages

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A synthetic approach to hydroxylamino interglycosidic linkages, which relies on a glycosylation at the oxygen of a nitrone, produces, after deprotection, the required *N-O*-glycosidic linkage.

An important structural aspect of esperamicin[1]–calicheamicin[2] antibiotics is the presence of an unusual oligosaccharidic moiety, especially the trisaccharide A-B-E, common to both series.[3] It is now recognized that the site-selective cleavage of oligodeoxynucleotides or DNA by these substances is a result of a selective association of the oligosaccharidic appendage in the minor groove of DNA.[3,4] Recent investigations in this structural domain have provided elegant synthetic solutions, notably for the construction of the crucial *N–O* interglycosidic bond.[5–7] All approaches to this problem rely on making the C–N bond last, either by oxime formation from an *O*-glycosyl hydroxylamine and subsequent reduction[5] or by a S_N2 displacement of an axial trifluoromethanesulfonate at C-4 of ring A by the sodium salt of an *O*-glycosyl urethane.[6,7] We now describe how the direct construction of an *N–O*-glycosidic linkage in oligosaccharides is possible by glycosylation of adapted nitrones. The synthesis of oligosaccharidic models incorporating these unique connections are useful for a better understanding of the structural and conformational features important for DNA binding.

During our initial studies, glycosylation with various glycosyl donors at the oxygen atom of hydroxylamine derivatives **1–3** or oxime **4**[8] failed to give any of the desired

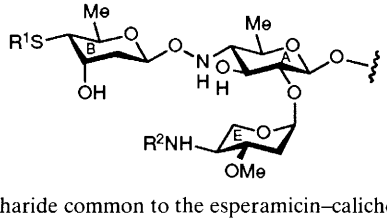
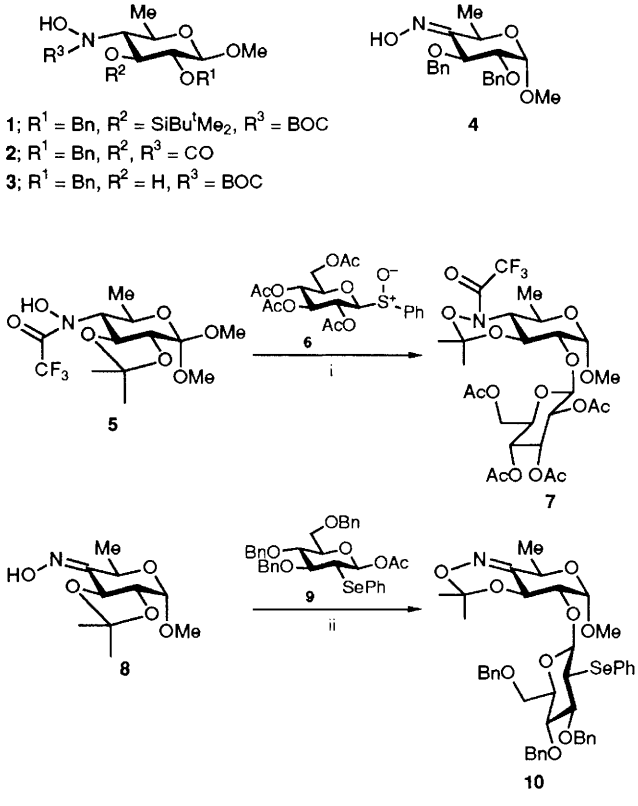
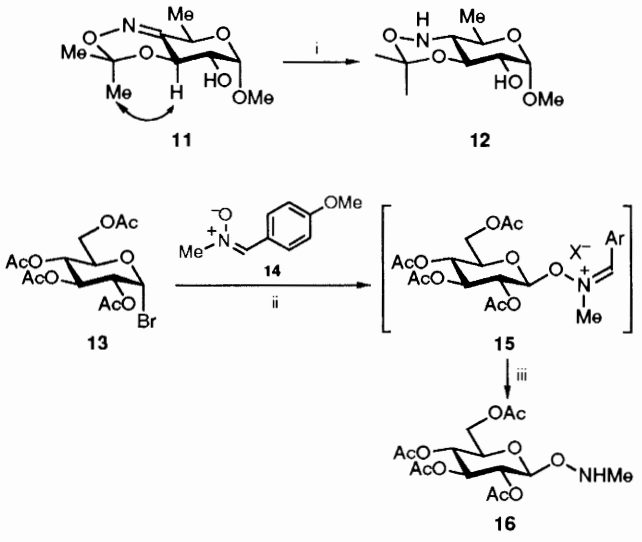


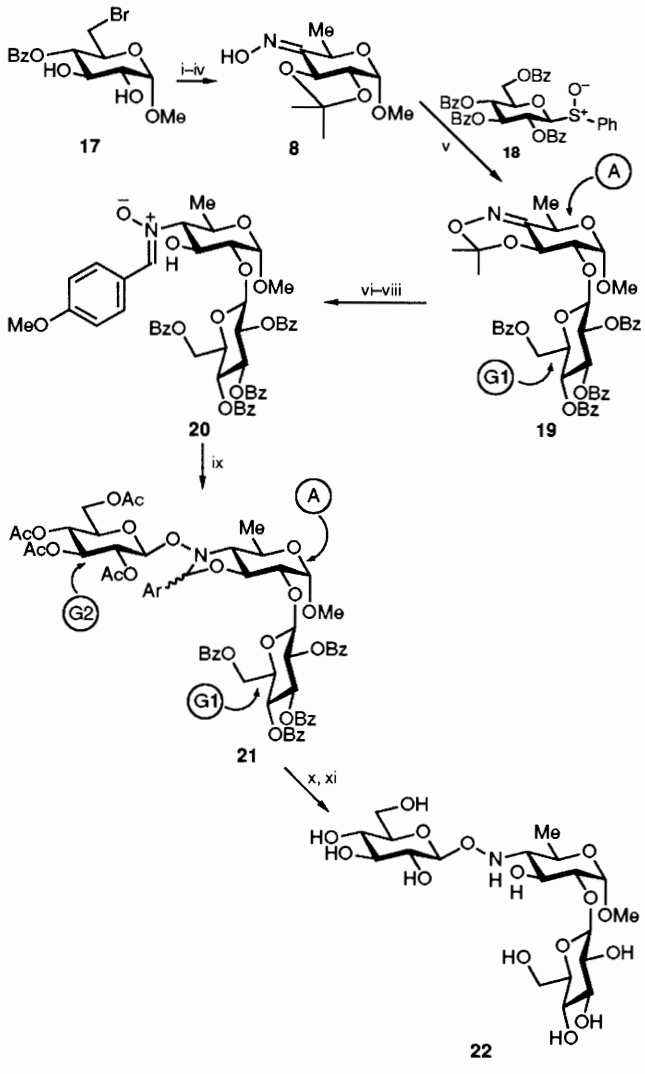
Fig. 1 Trisaccharide common to the esperamicin–calicheamicin series



Scheme 1 *Reagents and conditions*: i, 2 equiv. of **6**, 2 equiv. of Tf_2O, −78 to −35 °C, 1 h; ii, 1.2 equiv. of **9**, 0.1 equiv. of TMSOTf, MS 4 Å, 0 °C, 20 min



Scheme 2 *Reagents and conditions*: i, 6 equiv. of $NaCNBH_3$, MeOH–HCl, pH 3, room temp., 4 h; ii, 1.1 equiv. of **14**, 2 equiv. of AgOTf, CH_2Cl_2, −10 °C; iii, 0.1 mol dm^{-3} HCl–THF, room temp.



Scheme 3 *Reagents and conditions*: i, Raney Ni, H_2, MeOH : AcOEt : Et_3N, 6 : 6 : 7, 85%; ii, 2-methoxypropene, CSA cat., DMF, 60 °C, 82%; iii, MeONa, MeOH, room temp., 80%; iv, PCC, MS 3 Å, AcONa, CH_2Cl_2 then NH_2OH, HCl, EtOH–pyridine, 60 °C, 74%; v, 1.5 equiv. of **18**, 1.5 equiv. of Tf_2O, −78 to −35 °C, 1.5 h, 95%; vi, 6 equiv. $NaCNBH_3$, MeOH–HCl, pH 3, room temp., 60% plus 38% recovered starting material; vii, 0.3 mol dm^{-3} HCl, MeOH : H_2O, 2 : 1, room temp.; viii, 1.3 equiv. of *p*-anisaldehyde, pyridine : EtOH, 2 : 1, room temp., 0.5 h, 80% for the two steps; ix, 1.2 equiv. of **13**, 2.4 equiv. of AgOTf, CH_2Cl_2, −10 °C, 1 h, 80%; x, 0.1 mol dm^{-3} aqueous HCl, MeOH : CH_2Cl_2, 4 : 1, 0 °C, 2.5 h, 86%; xi, MeONa–MeOH, 0 °C, 2 h, 97%

disaccharides (Scheme 1). In the course of these investigations we found, however, that hydroxylamine **5** gave disaccharides when treated with various glycosyl donors *without base*, *e.g.* sulfoxides **6** leading to **7**† (61% yield) under Kahne's conditions.[9] Likewise, treatment of oxime **8** with acetoxy selenide **9**[10] gave disaccharide **10** (57% yield). In these transformations, the promoter (Tf_2O or TMSOTf) first catalyses the acetal rearrangement making the hydroxy group at position 2 available for glycosylation.

This clean rearrangement led us to consider acetal **8** as a pivotal intermediate for a sequential glycosylation at position 2 then 4. This strategy might be viable provided that (*i*) a stereoselective oxime reduction in **8** producing an equatorial C–N bond at C-4 in unit A could be set up, and (*ii*) a protected form of the hydroxylamino group suitable for an efficient glycosylation could be found.

The first condition was readily confirmed on acetal **11** (Scheme 2), prepared in 90% yield by rearrangement of **8** (TMSOTf, 0.1 equiv., CH_2Cl_2, 0 °C, 15 min), which, by classical cyanoborohydride reduction, stereoselectively provided hydroxylamine **12**‡ (69% yield, 96% based on the recovered starting material). None of the D-galacto isomer with an axial C–N bond could be isolated. Whereas reduction of oxime **8** under the same conditions provided an 85% yield of a 2 : 3 mixture of the D-gluco and D-galacto isomers, this stereochemical outcome can easily be explained by the efficient steric shielding of the α-face by the isopropylidene group.§

For the second condition, we felt that nitrones could be considered *N*-protected hydroxylamines, in spite of the fact that their *O*-alkylation producing *O*-alkylated nitrone salts is a reaction that has virtually been unexplored.[11] The *p*-methoxy benzylidene protection in the form of a *N*-*p*-methoxy benzylidene nitrone was selected for its potential flexibility in the deprotection sequence. In a simple model, we found that treatment of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bro-

† All new compounds gave satisfactory spectral and analytical data. The structure of di-(tri)saccharides was assigned by using a combination of one- and two-dimensional NMR spectroscopy, including NOE measurements.

‡ Selected ^1H NMR data ($CDCl_3$, 300 MHz): **12**: δ 2.09 (d, 1 H, $J_{OH,2}$ = 8.8 Hz, OH_2), 2.85 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.8 Hz, H_4), 5.16 (bs, 1 H, NH). **19**: δ 3.78 (dd, 1 H, $J_{1,2}$ = 3.6, $J_{2,3}$ = 9.9 Hz, A ring H_2), 4.40 (d, 1 H, $J_{2,3}$ 9.9 Hz, A ring H_3), 4.92 (d, 1 H, $J_{1,2}$ 3.6 Hz, A ring H_1), 5.02 (d, 1 H, $J_{1,2}$ 7.9 Hz, G1 ring H_1). **20**: δ 2.70 (d, 1 H, $J_{OH,3}$ 3.1 Hz, A ring OH_3), 3.37 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.8 Hz, A ring H_4), 4.81 (d, 1 H, $J_{1,2}$ 3.6 Hz, A ring H_1), 5.23 (d, 1 H, $J_{1,2}$ 7.9 Hz, G1 ring H_1), 7.30 (s, 1 H, vinylic H). **21**: δ for the major isomer: 2.69 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.9 Hz, A ring H_4), 4.42 (d, 1 H, $J_{1,2}$ 8.2 Hz, G2 ring H_1), 4.83 (d, 1 H, $J_{1,2}$ 3.3 Hz, A ring H_1), 5.14 (s, 1 d, C*H*Ar), 5.16 (d, 1 H, $J_{1,2}$ 7.9 Hz, G1 ring H_1). **22**: δ 2.32 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.9 Hz, A ring H_4); 4.47 (d, 1 H, $J_{1,2}$ 8.0 Hz, G1 or G2 ring H_1), 4.51 (d, 1 H, $J_{1,2}$ 7.8 Hz, G2 or G1 ring H_1), 4.87 (d, 1 H, $J_{1,2}$ 3.8 Hz, A ring H_1).

§ The half-chair conformation of the dioxazinyl ring is shown in structure **11**, as demonstrated by NOE measurements (NOE contact between the axial methyl of the isopropylidene group and H_3).

mide **13** with simple nitrone **14** using silver trifluoromethanesulfonate as a promoter provided the unstable *O*-β-D-glucosylated nitrone salt **15** which gave, on acidic hydrolysis, the *O*-glucosyl-*N*-methyl hydroxylamine **16** (68% yield from **13**, Scheme 2).

These concepts were applied in a straightforward synthesis of a model trisaccharide incorporating the central sugar A of the natural products. Treatment of oxime **8**, prepared in five steps from the known[12] bromobenzoate **17** (Scheme 3) with the benzoylated sulfoxides **18** furnished the 2-*O*-linked oxime disaccharide **19**‡ in 95% yield.¶ As seen above for **11**, cyanoborohydride reduction of oxime **19** furnished exclusively the equatorial hydroxylamine in 60% yield (98% based on the recovered starting material).

Acid hydrolysis and *p*-anisaldehyde treatment readily provided nitrone **20**‡ in 80% yield. Coupling of nitrone **20** with glycosyl bromide **13** promoted by silver trifluoromethanesulfonate in dichloromethane afforded regio- and stereo-selectively the trisaccharides **21**‡ in 80% yield (ratio of 6 : 1 at the *N,O*-acetalic carbon). In this notable reaction, the most probable *O*-glucosyl nitrone salt resulting from a regioselective glycosylation at the nitrone's oxygen was immediately stabilized by an intramolecular nucleophilic attack of the hydroxy group at position 3.

Trisaccharides **21** were then deprotected in two steps by mild acidic hydrolysis (86%) and deacylation (97%) to give **22**.‡ The chemistry described here could provide a useful alternative to the interesting strategies recently developed[5–7] and render other oligosaccharides of this type readily accessible for biochemical investigations.[13]

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¶ In this reaction **18** was found to be superior to the corresponding acetylated or pivaloylated sulfoxides,[9] which provided the corresponding disaccharides in 32 and 51% yields, respectively, together with the isomeric *ortho*disaccharides (15 and 8%, respectively).

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