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SYNTHESIS OF DAUNOSAMINE-CONTAINING DISACCHARIDES

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ABSTRACT

Treatment of 2,3,6-trideoxy-1,4-di-*O*-(*p*-nitrobenzoyl)-3-(trifluoroacetamido)-L-*lyxo*-hexopyranose (**1**) with benzyl 2,3-dideoxy-D-*glycero*-pentopyranoside and *p*-toluenesulfonic acid gave a mixture of benzyl 2,3,6-trideoxy-4-*O*-*p*-nitrobenzoyl-3-(trifluoroacetamido)-L-*lyxo*-hexopyranoside (49%) and benzyl 2,3-dideoxy-4-*O*-[2,3,6-trideoxy-4-*O*-(*p*-nitrobenzoyl)-3-(trifluoroacetamido)-α-L-*lyxo*-hexopyranosyl]-D-*glycero*-pentopyranoside (**4**, 20%). The structure of the disaccharide **4** was confirmed by a detailed, mass-spectrometric analysis in three modes, namely, negative- and positive-ion, chemical ionization, and electron impact. Similar treatment of the bis(*p*-nitrobenzoate) **1** with ethyl 2,3-dideoxy-D-*glycero*-pentopyranoside gave the ethyl glycoside and the desired disaccharide, showing that the transglycosylation is not restricted to benzyl glycosides. Removal of the *p*-nitrobenzoyl and the benzyl groups from **4** gave the disaccharide 2,3-dideoxy-4-*O*-(2,3,6-trideoxy-3-trifluoroacetamido-α-L-*lyxo*-hexopyranosyl)-D-*glycero*-pentopyranose.

INTRODUCTION

Daunosamine, the sugar component of the antitumor anthracycline antibiotics daunorubicin[1], adriamycin[2], and carminomycin[3], is also found in the form of its dimethyl derivative as a component of the oligosaccharide part of rhodomycin[4,5] and cinerubin[6]. These oligosaccharides contain, in addition to *N*,*N*-dimethyldaunosamine, other deoxy sugars, such as 2,6-dideoxy-L-*lyxo*-hexose and rhodinose (2,3,6-trideoxy-L-*threo*-hexopyranose)[4,5].

We have synthesized anthracycline analogs having sugars other than daunosamine attached to anthracyclinones[7–9], and are now interested in evaluating the antitumor and antibiotic activities of anthracycline analogs possessing a disaccharide chain linked to the anthracyclinones. A logical approach to the synthesis of such disaccharide-containing anthracyclines would be through the reaction of a glycosyl halide with the *N*-trifluoroacetyl derivative of carminomycin or daunorubicin. Unfortunately, such reactions invariably result in transglycosylation, and lead to the direct linkage of the anthracyclinone to the sugar halide. The need, therefore, arose

for the synthesis of disaccharides containing a daunosamine group for use in glycosylation reactions with the desired anthracyclinones.

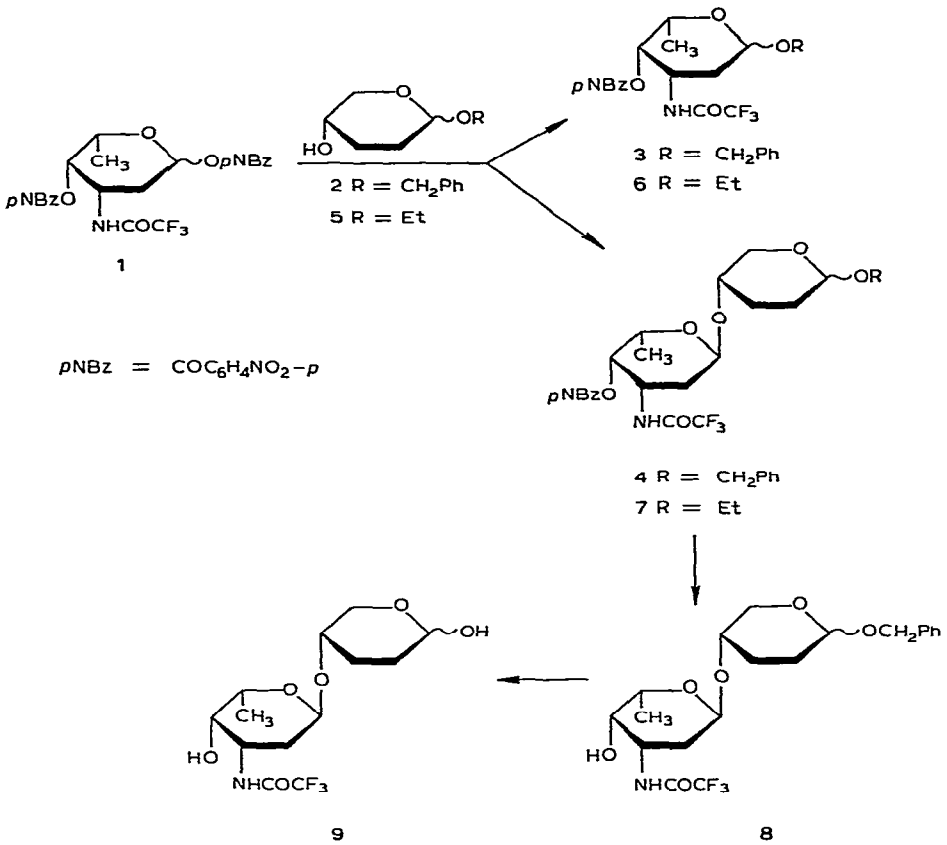
DISCUSSION

Two types of daunosamine-containing disaccharides are possible, those having daunosamine as the (reducing) glycose residue and those having another deoxy sugar residue at the reducing end. This paper deals with the synthesis of disaccharides of the latter type. In principle, such a synthesis would involve the reaction of a daunosamine derivative possessing a good leaving-group on C-1 with a deoxy sugar having only one free hydroxyl group. As the resulting disaccharide would be needed for glycosylation of an anthracyclinone, the protecting group attached to C-1 of the desired disaccharide must be of such a nature that it can be removed without affecting the glycosidic bond linking the two monosaccharides.

In our laboratory, we have recently prepared benzyl 2,3-dideoxy-D-*glycero*-pentopyranoside[10] (**2**), an excellent candidate for the aforementioned glycoside synthesis; it has one free hydroxyl group, and possesses on O-1 a benzyl group that, after formation of the disaccharide, can be removed by hydrogenolysis without affecting the glycosidic bond. As regards the choice of the needed daunosamine derivative possessing a good leaving-group on C-1, we avoided the use of halides, because these compounds are not crystalline, are relatively unstable, and cannot be prepared in high yields. In a recent publication[11], we described the synthesis of daunosamine glycosides from the readily available 2,3,6-trideoxy-1,4-di-*O*-(*p*-nitrobenzoyl)-3-(trifluoroacetamido)-L-*lyxo*-hexopyranose (**1**), using *p*-toluenesulfonic acid as the catalyst.

In the present work, we decided to use the same method to prepare daunosamine-containing disaccharides. The ester **1** readily underwent nucleophilic substitution when treated with benzyl 2,3-dideoxy-D-*glycero*-hexopyranoside (**2**) in the presence of *p*-toluenesulfonic acid as the catalyst. It should be noted that, although compound **2** has only one free hydroxyl group available for linkage with the daunosamine moiety, two reaction-products were obtained. The major one was identified as the previously prepared[11] benzyl 2,3,6-trideoxy-4-*O*-(*p*-nitrobenzoyl)-3-(trifluoroacetamido)-L-*lyxo*-hexopyranoside (**3**), evidently formed by transglycosylation. Careful chromatography of the reaction products yielded, in addition, a small proportion of the desired disaccharide (**4**).

In order to determine whether the undesired reaction was caused by the reactive benzyl group, the reaction was also tested with ethyl 2,3-dideoxy-D-*glycero*-pentopyranoside[12] (**5**). Two products were again obtained, the major one being ethyl 2,3,6-trideoxy-4-*O*-(*p*-nitrobenzoyl)-3-(trifluoroacetamido)-L-*lyxo*-hexopyranoside (**6**) and the minor product, the desired disaccharide (**7**). Efforts to optimize the reaction conditions in order to increase the yield of the disaccharides showed that the transglycosylation products are favored by an excess of the catalyst, whereas decreasing the proportion thereof results in deceleration of the reaction and an increase in the yield



of the disaccharide. Maximum yields of ~20% of disaccharide were obtained with 0.1 molar equivalent of the catalyst and a reaction time of 4 h. Disaccharide **4** was isolated in microcrystalline form, and gave a correct elemental analysis. To confirm its structure, it was subjected to a detailed, mass-spectrometric analysis in three different modes (see Fig. 1). Negative-ion, chemical ionization revealed the molecular ion at mass 582 as the only major ion in the spectrum. The positive-ion, chemical ionization in methane showed a variety of fragments. The highest mass was at 611, which corresponded to M + Et; this was followed by a smaller peak at 597, corresponding to M + Me, and then by an M − 1 peak at 581; a strong peak was observed at 375, corresponding to the daunosamine part of the disaccharide. The other part of the disaccharide molecule was seen at 101. Fragments corresponding to the *p*-nitrobenzoyl group and its further degradation product appeared at 150 and 120, and to the benzyl group, at 91.

As expected, the electron-impact spectrum did not reveal the molecular ion. The largest fragment was at 375, resulting from the daunosamine moiety. The other part of the molecule showed the same peak, at 101, that appears in the chemical-ionization mode, as well as a peak at 177 corresponding to the benzyl furanyl ether.

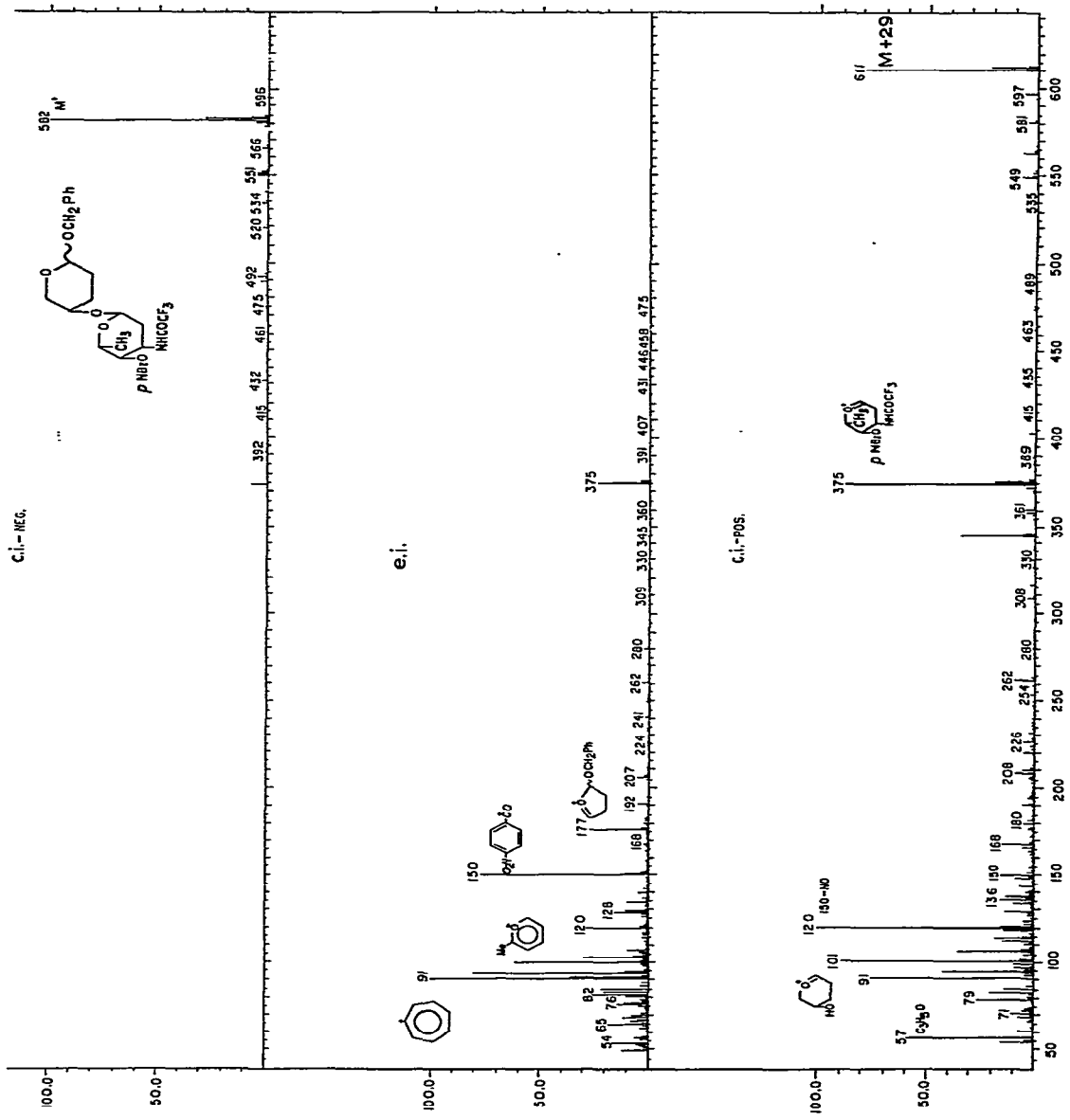


Fig. 1. Mass spectra of disaccharide **4**. (Top, by negative-ion, chemical ionization in methane; middle, by electron impact, and bottom, by positive-ion, chemical ionization in methane.)

The remaining peaks were the same as those appearing at the low-mass end of the chemical-ionization spectrum.

Deprotection of disaccharide **4** was conducted in two steps, the first using methoxide to remove the *p*-nitrobenzoyl group and yield disaccharide **8**. When compound **8** was hydrogenated, with palladium-on-charcoal as the catalyst, it gave the needed disaccharide **9**. The highly-negative, optical rotation of compound **9**

strongly suggests that the interglycosidic linkage has the α-L configuration. The preparation described constitutes the first synthesis of a daunosamine-containing disaccharide.

EXPERIMENTAL

General. — Melting points were determined with a Kofler block and are uncorrected. Optical rotations were measured at 20° with a Bendix series 1100 polarimeter. N.m.r. spectra were recorded with a Varian EM-360 spectrometer, with tetramethylsilane as the internal standard, and $CDCl_3$ as the solvent. Thin-layer chromatography was conducted on Eastman Kodak 13181 silica-gel plates. Columns for chromatography were packed with Sargent–Welch SC 14608 silica gel (60–200 mesh). Microanalyses were performed in the microanalysis laboratory of the Department of Chemistry and Chemical Engineering by Mrs. S. Brotherton. Petroleum ether refers to the fraction boiling at 30–60°. Mass spectra were made available by Finnegan Instruments, a division of Finnegan Corporation.

*Benzyl 2,3,6-trideoxy-4-*O-(p-*nitrobenzoyl*)-*3-(trifluoroacetamido)*-α-L-lyxo-*hexopyranoside* (**3**) *and benzyl 2,3-dideoxy-4-*O-[*2,3,6-trideoxy-4-*O-(p-*nitrobenzoyl*)-*3-(trifluoroacetamido)*-α-L-lyxo-*hexopyranosyl*]-D-glycero-*pentopyranoside* (**4**). — A solution of the benzyl glycoside **2** (200 mg, 0.95 mmol) in benzene (15 mL) and nitromethane (10 mL) was dried by concentration to about half its original volume. It was then cooled to room temperature and the *p*-nitrobenzoate **1** (400 mg, 0.74 mmol) was added together with *p*-toluenesulfonic acid (18 mg, 0.1 mmol) as the catalyst. The mixture was stirred for 1 h at room temperature, a further amount of **1** (50 mg, 0.09 mmol) was added, and the mixture was stirred for an additional 1.5 h. Ethyl acetate was then added, and the mixture was successively washed with saturated sodium hydrogencarbonate solution and water, dried (sodium sulfate), and evaporated to a syrup that was applied to a column of silica gel and eluted with 2:1 ether–petroleum ether. The first fraction was the transglycosylation product, benzyl 2,3,6-trideoxy-4-*O*-(*p*-nitrobenzoyl)-3-(trifluoroacetamido)-α-L-*lyxo*-hexopyranoside (**3**), m.p. 118–124°, yield 175 mg (44%).

Continued elution with the same solvent-mixture gave the disaccharide **4** (100 mg, 20%), isolated as a syrup. A small, crystalline sample was obtained from the syrup by addition of ether–petroleum ether; m.p. 68–74°, $[\alpha]_D$ −84° (*c* 0.3, chloroform).

Anal. Calc. for $C_{27}H_{29}F_3N_2O_9$: C, 55.67; H, 4.98; N, 4.81. Found: C, 55.36; H, 5.07; N, 5.04.

*Ethyl 2,3,6-trideoxy-4-*O-(p-*nitrobenzoyl*)-*3-(trifluoroacetamido)*-α-L-lyxo-*hexopyranoside* (**6**) *and ethyl 2,3-dideoxy-4-*O-[*2,3,6-trideoxy-4-*O-(p-*nitrobenzoyl*)-*3-(trifluoroacetamido)*-α-L-lyxo-*hexopyranosyl*]-D-glycero-*pentopyranoside* (**7**). — A solution of the ethyl glycoside **5** (90 mg) in benzene (10 mL) and nitromethane (5 mL) was dried, as before, by concentration to about two-thirds of its original volume. It was allowed to cool to room temperature before adding *p*-nitrobenzoate **1**

(214 mg) and the catalyst (*p*-toluenesulfonic acid, 11 mg). The resulting suspension was stirred for 2 h at room temperature. Ethyl acetate was then added, and the mixture was successively washed with saturated sodium hydrogencarbonate solution and water, dried (sodium sulfate), and evaporated to a syrup that was chromatographed on silica gel. Elution with 2:1 ether–petroleum ether gave a product (54 mg) that was found to be identical with ethyl 2,3,6-trideoxy-4-*O*-(*p*-nitrobenzoyl)-3-(trifluoroacetamido)-L-*lyxo*-hexopyranoside (**6**), m.p. 177–180° and mixed m.p. 176–180°.

Continued elution with the same solvent-mixture gave the disaccharide (**7**) (24 mg), $[\alpha]_D$ −107° (*c* 0.4, chloroform). Rechromatography of the intermediate fractions gave an additional sample of the monosaccharide derivative; overall yield of the disaccharide, 24%. A microcrystalline sample of compound **7** was obtained by addition of ether–petroleum ether, m.p. 62–65°.

Anal. Calc. for $C_{22}H_{27}F_3N_2O_9$: C, 50.77; H, 5.19; N, 5.38. Found: C, 51.07; H, 5.30; N, 5.13.

Benzyl 2,3-dideoxy-4-O-[*2,3,6-trideoxy-3-(trifluoroacetamido)*-α-L-lyxo-*hexopyranosyl*]-D-glycero-*pentopyranoside* (**8**). — The disaccharide derivative **4** (80 mg) was treated with a few drops of M sodium methoxide solution in methanol (3 mL) for 2 h at room temperature. The base was then neutralized with dilute acid, and the solution evaporated, to give an amorphous residue which was purified by chromatography on silica gel. Elution with 39:1 dichloromethane–methyl alcohol and evaporation of the eluate afforded a white solid (*p*-nitrobenzoic acid). Continued elution with the same solvent-mixture gave the title compound, isolated as a syrup (50 mg, 84%), $[\alpha]_D$ −56° (*c* 0.25, chloroform).

Anal. Calc. for $C_{20}H_{22}F_2NO_6$: C, 55.42; H, 6.00; N, 3.23. Found: C, 55.70; H, 6.25; N, 3.08.

2,3-Dideoxy-4-O-[*2,3,6-trideoxy-3-(trifluoroacetamido)*-α-L-lyxo-*hexopyranosyl*]-D-glycero-*pentopyranose* (**9**). — Compound **8** (110 mg) was dissolved in 80% ethyl alcohol (50 mL) and hydrogenated overnight at 65 lb.in.$^{-2}$ in the presence of 10% Pd–C catalyst (110 mg). T.l.c. of the products with 9:1 dichloromethane–methyl alcohol showed the presence of some starting material and two products. The catalyst was filtered off, and fresh catalyst (130 mg) was added to the mixture, which was then hydrogenated for an additional 24 h at 60 lb.in.$^{-2}$. T.l.c. at this stage showed only a trace of the starting material. Removal of the catalyst by filtration, and evaporation of the filtrate, gave a syrupy residue which was chromatographed on silica gel. Elution with 39:1 dichloromethane–methyl alcohol yielded, first, a small amount of the starting material. Subsequent elution with 19:1 dichloromethane–methyl alcohol gave a syrupy mixture of the anomers of disaccharide **9**: yield 51 mg (58%), $[\alpha]_D$ −95° (*c* 0.63, methyl alcohol; equil.). Addition of ether and petroleum ether gave a microcrystalline sample, m.p. 60–65°.

Anal. Calc. for $C_{13}H_{20}F_3NO_6$: C, 45.48; H, 5.83; N, 4.08. Found: C, 45.77; H, 5.91; N, 4.16.

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