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Synthesis of 6-deoxy-6-halolaminarans and conversion of 6-chloro-6-deoxylaminaran into the 6-amino-6-deoxy derivative

Riaz Khan [a,*], Marco Bosco [a], Paul A. Konowicz [a], Luca Stucchi [a,b], Roberto Rizzo [b]

[a] *POLYtech, Area di Ricerca, Padriciano 99, Trieste 34012, Italy*
[b] *Dipartimento di BBCM, Università degli Studi di Trieste, Trieste, Italy*

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Abstract

The reaction of laminaran, a linear polyglucan with β-(1 → 3)-linkages **1**, with trityl chloride followed by conventional acetylation gave a product that contained predominantly 6-*O*-trityl-2,4-di-*O*-acetate (**3**). Detritylation using hot aqueous acetic acid afforded a product containing 2,6-di-*O*-acetate (**4**), 2,4-di-*O*-acetate (**5**), and 2,4,6-tri-*O*-acetate (**6**) glucosyl residues. Similarly, the 2,4-di-*O*-benzoate (**8**) was prepared and then converted to the 6-bromo-6-deoxy derivative (**9**), using triphenylphosphine and carbon tetrabromide. The benzoyl groups in **9** could not be removed completely under conventional conditions. Direct halogenation of laminaran (**1**) with triphenylphosphine and carbon tetrabromide was unsuccessful. The replacement of the primary hydroxyl groups in **1** by halogens was achieved using methanesulfonyl halide–DMF complex. Syntheses of the 6-bromo-6-deoxy-2,4-di-*O*-formate (**10**) and the 6-chloro-6-deoxy-2,4-di-*O*-formate (**12**) were thus achieved. The formyl groups in **10** and **12** were removed under mild basic conditions to afford **11** and **13**. Treatment of the 6-chloro-6-deoxy-2,4-di-*O*-formate derivative **12** with sodium azide in DMF afforded the free 6-azido-6-deoxy compound **14**. However, when 6-chloro-6-deoxylaminaran (**13**) was treated with sodium azide it gave a compound which contained both the azide and chloro groups at C-6 positions in the polymeric chain. Catalytic hydrogenation of the water soluble 6-azido-6-deoxy-2,4-di-*O*-succinyl derivative **15** to give the desired 6-amino-6-deoxy compound **16** was unsuccessful. Chemical hydrogenation using triphenylphosphine in DMF gave the intermediate 6-triphenylphosphinimine **17**, which on subsequent treatment with aq 30% ammonia afforded a compound containing both the 6-phosphinimino

* Corresponding author.

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and the 6-amino groups. The structures of all the compounds were confirmed by ^{13}C NMR and by FTIR spectroscopy. © 1996 Elsevier Science Ltd.

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1. Introduction

Polysaccharides are renewable, biodegradable, natural polymers. In native and modified form they find multitude of applications in a variety of industry [1]. Some of them have shown interesting biological properties. Fungal polysaccharides such as lentinan and schizophyllan exhibit pronounced immune stimulating activities resulting in an antitumor effect [2]. Medium-molecular-weight sulfated long-chain alkyl oligosaccharides with the alkyl group linked to the reducing end of the molecule have been prepared and shown to be effective as anti-HIV agents [3].

Our objective is to chemically modify polysaccharides to produce new materials for chemical or pharmaceutical applications. In this communication we describe some chemical transformations of laminaran, in particular at C-6 positions leading to trityl, deoxyhalo, azidodeoxy, and aminodeoxy compounds [4].

2. Results and discussion

In our initial strategy to obtain 6-deoxyhalolaminaran we chose to prepare the *O*-2,4-protected laminaran as an intermediate by way of the 6-*O*-trityl-2,4-*O*-diester (**3**) compound. Laminaran (**1**) was treated with trityl chloride in a mixture of DMF and pyridine in the presence of a catalytic amount of 4-dimethylaminopyridine at 100 °C for 1 h and then at room temperature for 24 h to give the trityl ether **2** which on conventional acetylation afforded the expected 2,4-di-*O*-acetate (**3**). ^{13}C NMR spectra of **2** and **3** were consistent with their structures. In the spectrum of **2** the aromatic carbons appeared between δ 126–129 and the quaternary carbon at δ 143.5, indicating the presence of the trityl group. Detritylation of **3** with aq 98% acetic acid at 100 °C gave, according to ^{13}C NMR, a compound containing 2,6-di-*O*-acetate (**4**), 2,4-di-*O*-acetate (**5**), and 2,4,6-tri-*O*-acetate (**6**) glucosyl residues. The presence of **6** indicated that tritylation was not quantitative. In the ^{13}C NMR spectrum the three signals at δ 60.4, 61.8, and 62.8 were assigned to C-6 due to **5**, **6**, and **4**, respectively. The presence of **4** could be due to the migration of the acetyl groups. The C-4 → C-6 acetyl migration was confirmed by treatment of a solution of the above compound in DMF with *tert*-butylamine and water at 80 °C for 2 h and then at room temperature for 12 h. The ^{13}C NMR spectrum of the resulting product showed the disappearance of the peak at δ 60.4 due to C-6 of the 2,4-di-*O*-acetate **5**, and the presence of the two C-6 resonances at δ 61.8 and 62.8 due to **6** and **4** (see Table 1). Unlike acetyl groups, benzoyl groups usually do not migrate during detritylation. Hence, compound **2** was benzoylated to give the 6-*O*-trityl-2,4-di-*O*-benzoate (**7**) and then detritylated with aq acetic acid to afford the 2,4-di-*O*-benzoate (**8**). The ^{13}C NMR spectrum of **8** revealed a single peak at δ 60.83 due to C-6,

Table 1
^{13}C NMR data (Me_2SO-d_6) of the substituted glucosyl carbon atoms of laminaran derivatives

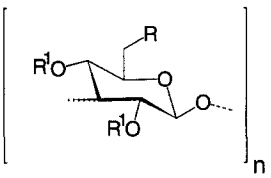
Compound	δ (ppm)					
	C-1	C-2	C-3	C-4	C-5	C-6
1	103.93	72.83	86.25	68.47	76.47	60.93
2	103.30	72.62	86.11	68.39	74.82	63.01
3	99.49	72.54	86.14	68.46	77.40	62.80
4	99.62	70.23	78.10	67.67	72.76	62.77
5	99.75	70.34	78.24	67.75	73.66	60.40
6	99.75	70.33	78.23	67.71	72.18	61.76
7	99.34	73.00	86.32	70.03	76.95	63.32
8	99.55	73.41	80.60	69.75	77.00	60.82
9	103.47	73.28	80.62	69.70	76.24	33.47
11	102.50	72.82	84.56	69.67	73.99	33.55
12	99.62	71.27	81.61	68.54	74.03	44.13
13	102.62	72.87	84.95	68.95	74.51	44.56
14	102.62	73.16	84.37	68.73	74.31	51.9
15	99.37	71.69	78.16	68.77	77.37	43.29
16	102.70	72.93	84.86	69.54	74.53	42.49
17	102.70	72.93	84.86	69.54	74.53	44.56

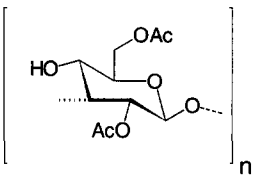
indicating that no detectable C-4 → C-6 benzoyl migration had occurred. Addition of trichloromethylisocyanate to the ^1H NMR solution of **8** revealed the appearance of a singlet at δ 10.3 due to the proton of the carbamate, thereby indicating the presence of a single OH group in the glucosyl residues.

Bromination of **8** with triphenylphosphine–carbon tetrabromide–pyridine at 80 °C for 3.5 h gave, according to ^{13}C NMR, the 6-bromo-6-deoxy derivative **9** as a minor product. Attempts to completely remove the benzoyl groups using sodium hydroxide were unsuccessful. Therefore, in order to prepare 6-deoxy-6-halolaminaran, the above approach was abandoned in favour of a direct halogenation reaction. Primary hydroxyl groups in carbohydrates have been halogenated using reagents such as carbon tetrahalides–triphenylphosphine–pyridine [5], triphenylphosphine–*N*-halosuccinimides [6] and DMF–methanesulfonyl halide [7]. Reaction of laminaran with carbon tetrahalides–triphenylphosphine–pyridine or triphenylphosphine–*N*-halosuccinimides to give the corresponding 6-halo derivatives were unsuccessful. Treatment of laminaran with a combination of mesyl chloride–DMF gave predominantly the 6-chloro-6-deoxy-2,4-di-*O*-formate (**12**). Interestingly, chlorination at C-4 was not observed. In the ^{13}C NMR spectrum of **12** the signal due to C-6 was shifted from δ 60.93 to 44.13, indicating the presence of a chlorine atom at C-6. Similarly, treatment of laminaran with mesyl bromide–DMF complex gave the 6-bromo-6-deoxy-2,4-di-*O*-formate (**10**). The formyl ester groups in **10** and **12** were cleaved under mild basic conditions to afford the free 6-deoxyhalo compounds **11** and **13**, respectively.

The S_N2 displacement reaction of 6-chloro-6-deoxylaminaran **13** with sodium azide in DMF yielded a product which contained both the 6-azido-6-deoxy- (**14**) and the 6-chloro-6-deoxy- (**13**) glucosyl residues. This was confirmed by its ^{13}C NMR spectrum in which the C-6 signals appeared at δ 44.3 for **13** and δ 50.9 for **14**. When the azide

displacement reaction was performed with the 6-chloro-6-deoxy-2,4-di-*O*-formate **12**, the 6-azido **14** was obtained in a nearly quantitative yield. The formate ester groups were completely removed at the end of the reaction. The structure of **14** was confirmed by FTIR spectroscopy (2100 cm^{-1}, azide) and by ^{13}C NMR spectroscopy (δ 50.9, C-6).





4

	R	R^1
1	OH	H
2	OTr	H
3	OTr	Ac
5	OH	Ac
6	OAc	Ac
7	OTr	Bz
8	OH	Bz
9	Br	Bz
10	Br	HCO
11	Br	H
12	Cl	HCO
13	Cl	H
14	N_3	H
15	N_3	$CO(CH_2)_2CO_2Na$
16	NH_2	H
17	$N{=}PPh_3$	H

Compound **14** was conventionally succinylated to give a water soluble derivative **15** which was then hydrogenated in the presence of palladium-on-carbon as a catalyst. However, the reduction reaction was unsuccessful. In amylose the primary azide group has been reduced almost quantitatively to the corresponding amines using triphenylphosphine and ammonia [8]. However, treatment of **14** with triphenylphosphine in DMF followed by hydrolysis with aq 30% ammonia gave a product which contained both 6-amino-6-deoxy- (**16**) and 6-triphenylphosphinimine- (**17**) glucosyl residues. In the ^{13}C NMR spectrum the minor signal at δ 42.5 was due to C-6 linked to a NH_2 group and the major at δ 44.6 linked to the phosphinimino group. The presence of a phosphin-

imino group linked to the polysaccharide was confirmed by ^{31}P NMR decoupled spectra and a 1D hetero-correlated, proton detected experiment (INVSAT2) [9]. The CH_2 protons coupled to the phosphorus gave an inverse doublet at high field in the region of δ 3.2–3.4. According to FTIR, no signal for the azido group at 2100 cm^{-1} was observed. When the above reaction was performed in dimethyl sulfoxide, instead of DMF, similar results were obtained. Incomplete conversion of **14** to **16** indicates that the triphenylphosphinimine intermediate **17** is more stable than the corresponding amylose intermediate [8].

In conclusion, halogenation using triphenylphosphine–carbon tetrabromide or *N*-chlorosuccinimide in pyridine gave a product with a low degree of halogenation at C-6 presumably due to steric hindrance. However, less hindered halogenating reagents such as methanesulfonyl chloride (or bromide)–DMF gave laminaran derivatives in which a high degree of the glucosyl residues were halogenated at C-6. An explanation for this behaviour may be that the conformation of laminaran, a β-(1 → 3)-linked polysaccharide, is more rigid than amylose and is more sensitive to steric effects. This conclusion was further supported by the observation that, unlike amylose [8], complete reduction of the intermediate phosphinimino derivative of laminaran could not be achieved.

3. Experimental

General methods.—All evaporations were carried out under reduced pressure. TLC was performed on silica gel 60 F_{254} (1.05554, Merck), with detection by charring with ethanolic 5% H_2SO_4. ^1H, ^{13}C, and ^{31}P NMR spectra were recorded at 200.13, 50.23, and 80.97 MHz, respectively, with a Bruker AC200 spectrometer. Spectra were recorded in Me_2SO-d_6 and $CDCl_3$ with Me_4Si as internal standard (δ 0.00). FTIR experiments were performed on a Perkin Elmer model 1750 with single beam. Laminaran (Mw. 5500) was purchased from Protan, Norway. Prior to reactions laminaran was taken in about 15 mL of *N*,*N*-dimethylformamide (DMF) and then the solvent was removed under reduced pressure, and this operation was repeated three times. ^{13}C NMR data for all compounds, except **10**, are given in Table 1. Methanesulfonyl bromide was synthesized using the general procedure of Hearst and Noller [10]. Calculations of the molar equivalents of polysaccharide derivatives are based on the molecular weight of a constituent glucosyl residue, 162.15 in the case of laminaran.

6-O-Triphenylmethyllaminaran (**2**).—A solution of **1** (0.5 g, 3.1 mmol) in pyridine (10 mL) was treated with triphenylmethyl (trityl) chloride (1.0 g, 3.6 mmol) in the presence of 4-dimethylaminopyridine (20 mg) with stirring at 100 °C for 3 h and then at room temperature for 24 h. TLC (9:1 acetone–water) showed on charring a yellowish black faster moving spot. Approximately 50% of the solvent was removed under reduced pressure, and then the product was precipitated from Et_2O and purified by removing the unreacted reagents and low molecular weight organic impurities by thoroughly washing with EtOAc (**2**; 0.85 g, 62.5%).

2,4-Di-O-acetyl-6-O-trityllaminaran (**3**).—A solution of **2** (0.5 g, 1.14 mmol) in pyridine (20 mL) was treated with acetic anhydride (2.5 mL, 22.6 mmol) in the presence of 4-dimethylaminopyridine (50 mg) with stirring at room temperature for 24 h and

100 °C for 1 h. The solvent was removed under reduced pressure, the resultant residue was taken in CH_2Cl_2, washed successively with cold 2 M HCl, aq $NaHCO_3$, water, and the organic layer was dried (Na_2SO_4), and concentrated to give **3** (360 mg, 61%).

2,6-Di- (**4**) *and 2,4-di-* (**5**) *O-acetyllaminaran.*—Compound **3** (0.5 g, 0.95 mmol) was treated with 98% HOAc (15 mL) at 100 °C for 1 h. TLC (4:1 acetone–water) showed a slow moving product. Approximately 50% of the solvent was removed under reduced pressure and the products were precipitated from Et_2O. Unreacted reagents and low molecular weight organic impurities were removed by thoroughly washing with EtOAc to give a compound containing both **4** and **5** glucosyl residues (0.21 g, 90%).

C-4 → C-6 Acetyl migration of compound containing **4** *and* **5**.—A solution of the compound (0.05 g) in DMF was treated with *tert*-butylamine (0.4 mL) and a few drops of water at 80 °C for 2 h and then at room temperature for 12 h. The solvent was removed under vacuum and the product was recovered by precipitation from Et_2O (0.04 g).

2,4,6-Tri-O-acetyllaminaran (**6**).—A solution of **1** (0.5 g, 3.1 mmol) in DMF (20 mL) and pyridine (10 mL) was treated with acetic anhydride (2.5 mL, 22.6 mmol) in the presence of 4-dimethylaminopyridine (50 mg) with stirring at room temperature for 24 h and 100 °C for 1 h. The solvent was removed under reduced pressure, the resultant residue was taken in CH_2Cl_2, washed successively with cold 2 M HCl, aq $NaHCO_3$, water, and the organic layer was dried (Na_2SO_4), and concentrated to give **6** (450 mg, 50%).

2,4-Di-O-benzoyl-6-O-trityllaminaran (**7**).—A solution of **2** (1.0 g, 2.27 mmol) in pyridine (20 mL) was treated with benzoyl chloride (1.3 mL, 7.6 mmol) in the presence of 4-dimethylaminopyridine (0.1 g) with stirring at room temperature for 24 h and 100 °C for 1 h. The solvent was removed under reduced pressure, the resultant residue was taken in CH_2Cl_2, washed successively with cold 2 M HCl, aq $NaHCO_3$, water, and the organic layer was dried (Na_2SO_4), and concentrated to give **3** (1.3 g, 84%).

2,4-Di-O-benzoyllaminaran (**8**).—Compound **7** (0.5 g, 0.74 mmol) was treated with 98% HOAc (15 mL) at 100 °C for 1 h. TLC (4:1 acetone–water) showed a slow moving product. Approximately 50% of the solvent was removed under reduced pressure and the product was precipitated from Et_2O. Unreacted reagents and low molecular weight organic impurities were removed by thoroughly washing with EtOAc (**8**; 0.21 g, 71%).

Bromination of 2,4-di-O-benzoyllaminaran (**8**).—A solution of **8** (0.5 g, 1.25 mmol) in pyridine (20 mL) was treated with triphenylphosphine (0.93 g, 3.55 mmol) and CBr_4 (1.46 g, 4.4 mmol) at 5 °C for 1 h and then at 80 °C for 3.5 h. The polymer was precipitated from Et_2O, the solid residue was taken in CH_2Cl_2 and washed with cold 2 M HCl and then with water. The organic layer was dried (Na_2SO_4) and concentrated to give the product (0.35 g) in which the 2,4-di-*O*-benzoyl-6-bromo-6-deoxylaminaran **9** glucosyl residue was present, according to ^{13}C NMR in low yield.

Bromination of **1** *using methanesulfonyl bromide–DMF reagent.*—A suspension of **1** (0.5 g, 3.1 mmol) in DMF (5 mL) was treated with CH_3SO_2Br (4.0 mL, 12.9 mmol), initially at −40 °C for 1 h, then at room temperature for 2 h and then at 70 °C for 12 h. The reaction mixture was neutralized with Na_2CO_3 and dialyzed against distilled water (6 × 1 L) for 72 h to give 6-bromo-6-deoxy-2,4-di-*O*-formyllaminaran **10**. The solid residue was filtered, treated with 4 M NaOH to pH 11, and then the solution was

dialyzed against distilled water (6 × 1 L) for 72 h. The solid residue was filtered off, dried in vacuo at 40 °C over P_2O_5 to afford 6-bromo-6-deoxylaminaran (**11**; 0.57 g, 82%).

Chlorination of **1**.—A suspension of **1** (5.0 g, 3.1 mmol) in DMF (50 mL) was treated with CH_3SO_2Cl (20 mL, 258 mmol), initially at −40 °C for 1 h then at room temperature for 2 h, and then at 80 °C for 12 h. The reaction mixture was neutralized with Na_2CO_3 and then dialyzed against distilled water (6 × 2 L) for 72 h. The precipitate formed in the dialysis tube was filtered, washed thoroughly with water, dried in vacuo at 40 °C over P_2O_5 to afford 6-chloro-6-deoxy-2,4-di-*O*-formyllaminaran (**12**; 4.5 g, 61%).

6-Chloro-6-deoxylaminaran (**13**).—A solution of **12** (4.5 g, 19 mmol) in DMF (10 mL) was stirred with M NaOH (13.5 mL) at pH 10 for 6 h and then the solution was dialyzed against distilled water (6 × 1 L) for 3 days. The solid residue was filtered off, dried in vacuo at 40 °C over P_2O_5 to give 6-chloro-6-deoxylaminaran (**13**; 2.12 g, 62%). Anal. Calcd for Cl, 17.85%; Found: 15.62%.

Reaction of **13** *with* NaN_3.—A solution of **12** (5.75 g, 29.1 mmol) in DMF (100 mL) was treated with NaN_3 (4.75 g, 73 mmol) at 100 °C for 16 h. The solution was then dialyzed against distilled water (6 × 2 L) for 72 h. The solid residue was filtered off, dried in vacuo at 40 °C over P_2O_5 to afford a product which contained both the 6-azido- (**14**) and 6-chloro- (**13**) groups (5.1 g).

Reaction of **12** *with* NaN_3.—A solution of **12** (1.0 g, 4.2 mmol) in DMF (20 mL) was treated with NaN_3 (0.66 g, 10.2 mmol) at 100 °C for 16 h. The solution was then dialyzed against distilled water (6 × 2 L) for 72 h. The solid residue was filtered off, dried in vacuo at 40 °C over P_2O_5 to afford predominantly 6-azido-6-deoxylaminaran (**14**; 0.38 g, 48%).

*6-Azido-6-deoxy-2,4-di-*O*-succinyllaminaran* (**15**).—A solution of **14** (0.1 g, 0.53 mmol) in pyridine (10 mL) was treated with succinic anhydride (0.15 g, 1.5 mmol) in the presence of a catalytic amount of 4-dimethylaminopyridine at 100 °C for 24 h. The solution was neutralized with M NaOH, dialyzed against aq $NaHCO_3$, and compound **15** was recovered by lyophilization (0.22 g, 95%); IR (KBr): ν 2100 (azido) and 1740 (ester) cm^{-1}.

Catalytic hydrogenation of **15**.—A solution of **15** (100 mg, 0.23 mmol) in water (100 mL) and Pd–C (10 mg) was shaken under H_2 in a PARR hydrogenator for 24 h. The catalyst was filtered off and the solution lyophilized to give the unreacted starting material **15** (84 mg).

Reduction of 6-azido-6-deoxylaminaran (**14**) *with triphenylphosphine in DMF*.—A solution of **14** (0.2 g, 1.07 mmol) in DMF (15 mL) was treated with triphenylphosphine (0.59 g, 2.25 mmol) with stirring at room temperature for 1 h. To this was added 7.6 M aq NH_3 (10 mL) and the mixture was stirred for 2 h at room temperature and then neutralized with 2 M HCl. The product was precipitated from Et_2O, washed with EtOAc and dried in vacuo at 40 °C over P_2O_5 to afford the compound which contained both the 6-amino-6-deoxy- (**16**) and the 6-triphenylphosphinimine- (**17**) glucosyl residues (0.07 g).

Reduction of 6-azido-6-deoxylaminaran (**14**) *with triphenylphosphine in* Me_2SO.—A solution of **15** (200 mg, 1.07 mmol) in Me_2SO (15 mL) was treated with triphenylphos-

phine (0.59 g, 2.25 mmol) with stirring at room temperature for 1 h. To this was added 7.6 M aq NH_3 (10 mL) and the mixture was stirred for 2 h at room temperature and then neutralized with 2 M HCl. The product was precipitated from Et_2O, washed with EtOAc and dried in vacuo at 40 °C over P_2O_5 to afford a compound which contained both the 6-amino-6-deoxy- (**16**) and the 6-triphenylphosphinimine- (**17**) glucosyl residues (0.01 g).

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