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Synthesis of (1→6)-β-D-glucosamine hexasaccharide, a potential antitumor and immunostimulating agent

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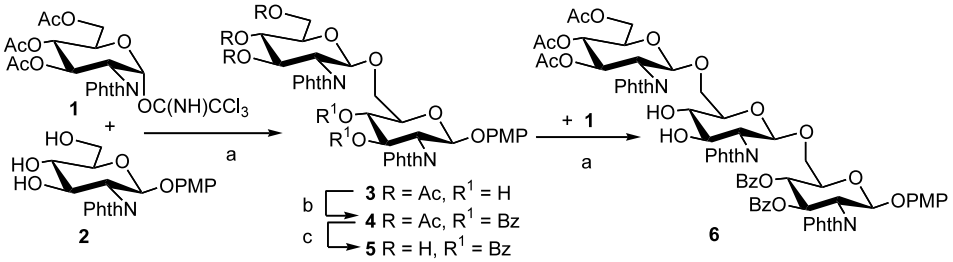
Abstract—(1→6)-β-D-Glucosamine hexasaccharide was synthesized convergently using isopropyl thioglycosides as glycosyl donors in all coupling steps. The target compound showed good antitumor activity based on mice S_{180} model studies.

Linear oligosaccharides are widely distributed in natural products. For example, linear D-mannan from *Candida albicans* and *Pseudomonas Syringae pv. Ciccaronei*, interacts with mannan-binding proteins of higher animals and elicits a specific immune response with formation of antibodies, which recognize oligosaccharide fragments of the mannose polymer.[1] (1→3)-β-D-Glucans are believed to have immunomodulatory activity.[2] Sulfated linear D-galactopyranose derivatives exhibit anti-HIV activity in vitro.[3] Furthermore, β-D-(1→4)-*N*-acetyl glucosamine has been used as a bifidus factor to promote animal growth.[4] The synthesis of linear sugar chains has been extensively explored,[5] but to the best of our knowledge, (1→6)-β-D-hexaglucosamine has not been synthesized so far.[6] Herein we report the first synthesis of (1→6)-β-D-glucosamine hexasaccharide and its bioactivities as an antitumor and an immunostimulating agent.

The initial strategy was designed based on our previous work[7] on the regioselective synthesis of a (1→5)-linked octa-furanoside using an unprotected glycoside as the acceptor. Thus, coupling of imidate **1** and partially protected acceptor **2**[8] under standard glycosylation conditions furnished disaccharide **3** in 30% yield (Scheme 1). About 54% of **2** was recovered after column separation. Compound **3** was benzoylated and subsequently deacetylated with 5% HCl (gas) in methanol to give triol **5** (76%). The critical regioselective glycosylation of **1** and **5** was not straightforward and a 35% yield of trisaccharide **6** was obtained. We realized that the rapid consumption of donor **1** might be responsible for the low yields. Adding a second portion of **1** to the reactions did indeed improve the yields (~50%). Unfortunately, the removal of 4-methoxyphenyl (PMP) from **6** using DDQ or CAN[9] was also a low yielding step in this strategy.

We then turned our attention to the stepwise synthesis as shown in Scheme 2. *N*-Phthaloyl glucosamine **7** was regioselectively silylated on C-6 with TBSCl in pyridine and acetylated in situ. $BF_3{\cdot}Et_2O$-catalyzed desilyation



Scheme 1. *Reagents and conditions*: (a) TMSOTf, CH_2Cl_2/CH_3CN (4:1), −15 to 0°C, 30% (**3**); 35–50% (**6**); (b) BzCl, Py; (c) 5% HCl (gas), MeOH, 76%.

Keywords: carbohydrate; glycosylation; glucosamine; antitumor agent; immunostimulant.

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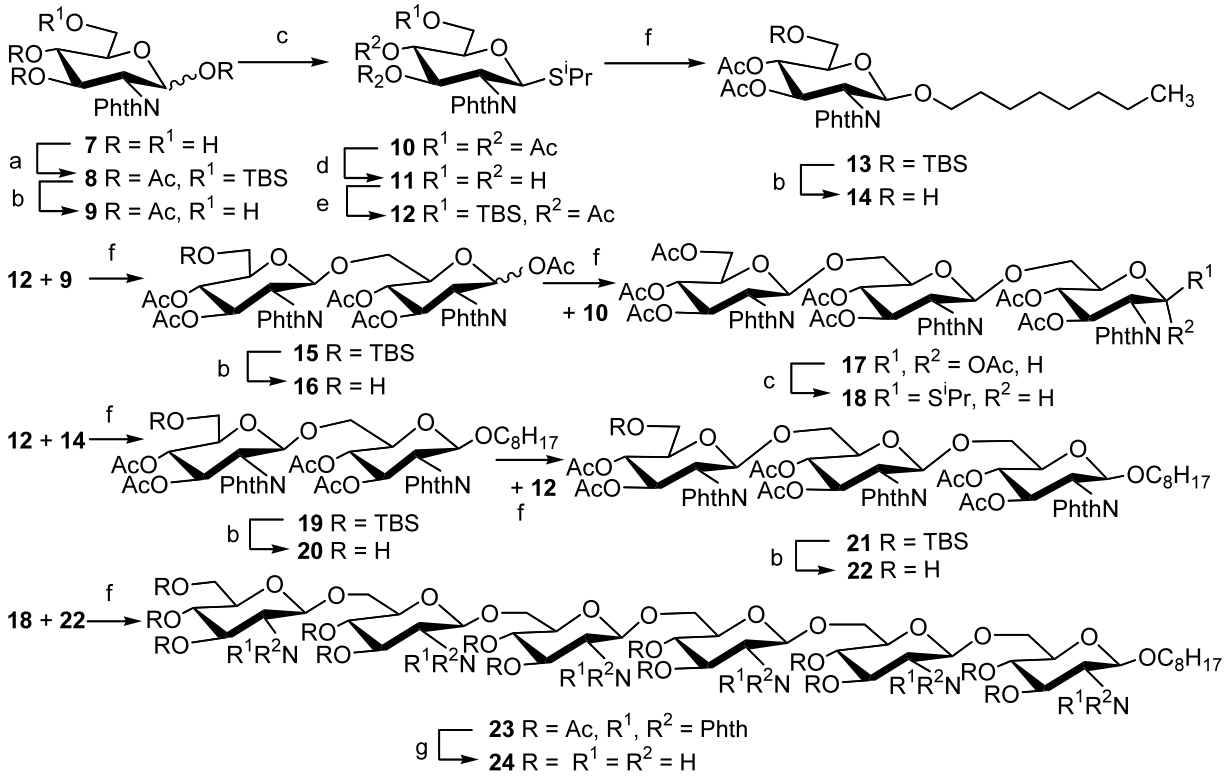

Scheme 2. *Reagents and conditions*: (a) NaOMe, MeOH; TBSCl, Py; Ac_2O, 70%; (b) $BF_3{\cdot}Et_2O$, CH_2Cl_2, 95% (**9**); 89% (**14**); 84% (**16**); 88% (**20**); 85% (**22**); (c) from **7**: Ac_2O, Py; 2-propanethiol, $BF_3{\cdot}Et_2O$, CH_2Cl_2, 86%; (d) NaOMe, MeOH; (e) TBSCl, Py; Ac_2O, 78%; (f) NIS/TMSOTf, CH_2Cl_2, −20°C, 95% (**13**), 94% (**15**), 86% (**17**), 80% (**19**), 92% (**21**), 90% (**23**); (g) NH_3, MeOH, 91%.

of **8** gave acceptor **9** smoothly. It is noteworthy that TBAF promoted desilylation causes acyl migration from C-4 to C-6. A modified Helfrich reaction of fully acetylated **7** and 2-propanethiol in CH_2Cl_2 under reflux gave isopropyl thioglycoside **10** (86%) and protection group manipulation furnished the latent glycosyl donor **12** in 78% yield. Condensation of **12** and **9** was achieved with great success and disaccharide **15** was obtained in 94% yield. Cleavage of TBS from **15** using 2.0 equiv. of $BF_3{\cdot}Et_2O$ was carried out smoothly and further glycosylation with **10** yielded **17** which was converted to isopropyl thioglycoside **18** (67%) as described in the preparation of **10**. Convergently, **12** was reacted with octanol in the presence of NIS and TMSOTf to give **13** (95%) which was treated with $BF_3{\cdot}Et_2O$ to yield **14** (89%). Reiterative coupling and desilylation of **12** and **14** gave trisaccharide acceptor **22** in 55% overall yield (from **14**). *N*-Iodosuccinimide (NIS) and TMSOTf-catalyzed condensation of **18** and **22**, followed by deacylation in ammonia-saturated methanol afforded hexasaccharide **24**[10] in excellent yield (82%, two steps).

Kun Min mice weighing about 20 g were used for the bioassay. Seven-day-old Sarcoma-180 ascites (0.05 mL, about 6×10^6 cells) was transplanted into the right groins of mice. The test samples, dissolved in distilled water, were injected daily for 14 days starting 24 h after tumor implantation. At the end of the 14th day, the mice were killed, and the tumors were extirpated and weighted. Haemoglobin (Hb), red blood cell (RBC), white blood cell (WBC) and marrow cell counts were recorded on SYSMEX. The results (Table 1), compared to lentinan and CTX in the parallel test, suggest that compound **24** may be a potent antitumor agent.

Table 1. Preliminary studies on antitumor activity of hexaglucosamine **24**

Sample	Dose (mg/kg mouse)	Tumor growth inhibition (%)	Body weight (g)	Hb (g/L)	RBC ($\times10^{12}$)	WBC ($\times10^8$)	Marrow cell ($\times10^8$)
Control	0	0	27.4±4.09	116.6±13.0	7.2±1.01	12.7±2.86	5.0±1.45
CTX[a]	70	75	22.1±3.87	108.2±15.80	6.9±0.78	5.8±2.12	1.9±0.94
24	2	50	29.8±3.91	128.8±18.80	8.6±1.13	12.9±3.12	5.9±1.63
Lentinan	5	25	26.0±3.23	92.8±12.03	6.3±0.70	6.6±2.26	5.6±1.25
CTX+**24**[b]	70+2	75	25.7±3.39	107.1±12.38	7.4±0.89	12.7±6.28	3.7±1.35

[a] 35 mg on the first and third days, respectively.
[b] 36 mg on the first and third days, respectively.

In conclusion, a highly efficient and practical method was developed for the preparation of (1→6)-β-D-glucosamine oligosaccharides. Isopropyl thioglycosides were shown to be suitable donors in target synthesis compared to the corresponding trichloroacetimidates. Acceptors with anomeric acetyl groups, which could simplify the protection strategy, are compatible to NIS/TMSOTf promoted coupling reactions. Using this procedure to build up more complicated glucosamine oligosaccharides is currently underway in our group.

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10. (a) Selected physical data for **23**: $[\alpha]_D^{25}$ +37 (*c* 1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 0.81 (t, 3 H, *J* 7.2 Hz, CH_2CH_3), 0.85–1.28 (m, 12 H, 6 CH_2), 1.770, 1.776, 1.778, 1.788, 1.794, 1.870, 1.936, 1.937, 1.942, 1.945, 1.950, 2.052, 2.164 (13 s, 39 H, $COCH_3$), 3.20 (m, 1 H, OCH_aH_b), 3.46 (dd, 1 H, *J* 10.8, 6.4 Hz), 3.16 (m, 1 H, OCH_aH_b), 3.40–3.80 (m, 14 H), 3.90–3.96 (m, 2 H), 4.11–4.24 (m, 6 H), 4.36–4.41 (m, 2 H), 4.70–4.82 (m, 3 H), 4.93 (t, 1 H, *J* 9.2 Hz), 5.13 (d, 1 H, *J* 8.4 Hz, H-1^I), 5.21 (t, 1 H, *J* 9.2 Hz), 5.25 (d, 1 H, *J* 8.4 Hz, H-1^{II}), 5.27 (d, 2 H, *J* 8.4 Hz, H-1^{III}, H-1^{IV}), 5.33 (d, 1 H, *J* 8.4 Hz, H-1^V), 5.51 (d, 1 H, *J* 8.4 Hz, H-1^{VI}), 5.57–5.67 (m, 5 H), 6.07 (dd, 1 H, *J* 10.8, 9.0 Hz), 7.71–7.92 (m, 24 H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 97.51, 97.56, 97.60 (2C), 97.66, 98.00 (6 C-1). MALDI TOF-MS calcd for $C_{118}H_{122}N_6O_{50}$: 2422.72 [M]. Found: 2445.6 $[M+Na]^+$, 2461.6 $[M+K]^+$; (b) To the NH_3 saturated MeOH (200 mL) was added **23** (1 g). The mixture was stirred at rt for 7 days, then concentrated. The residue was dissolved in H_2O (5 mL) and then purified by charcoal chromatography (H_2O) to give **24** as a white solid. Selected ^{13}C NMR (100 MHz, D_2O) for **24**: 103.05, 103.51, 103.59, 103.63, 103.67, 103.7 (6 C-1). MALDI TOF-MS calcd for $C_{44}H_{84}N_6O_{25}$: 1096.55 [M]. Found: 1119.2 $[M+Na]^+$.