SYNTHESIS OF (1→4)-β-D-GLUCOSAMINE TETRASACCHARIDE

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Abstract

Chemical and enzymatic hydrolysis of chitin and chitosan are the commercial methods for obtaining chitosan oligosaccharide (COS), but these procedures give the mixtures of COS. Herein, we reported a new method for the synthesis of chitotetraose using a convergent ' 2+2 ' strategy. The key step was the preparation of the glycosyl donor, isopropyl 3,4,6-tri-O-acetyl -2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-6-O-tert-butyldimethylsilyl-2-deoxy-2-pht halimido-D-glucopyranoside and the glycosyl acceptor, octyl 6-O-tert-butyldimethylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-6-O-tert-butyldi methylsilyl-2-deoxy-2-phthalimido-Dglucopyranoside. A NIS/TMSOTf-catalysed coupling of the acceptor with the donor afforded two kinds of tetrasaccharide derivatives, which could be separated on a silica gel column. One of two tetrasaccharide derivatives could be transformed to chitotetraose by deprotection.

Introduction

Chitooligosaccharides have many useful biological properties such as antimicrobial, antibacterial, antitumour, and immuno-enhancing effects. Chemical and enzymatic hydrolysis of chitin and chitosan are the commercial methods for obtaining chitooligosaccharides, but these procedures could give the mixtures of chitooligosaccharides[1]. Therefore, we reported a new approach for the synthesis of chitotetraose using a convergent "2+2"strategy. The key step was the preparation of the glycosyl donor, isopropyl 3,4,6-tri-O-acetyl -2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-6-O-tert-butyldimethylsilyl-2-deoxy-2-phthalimido-1-thio-D-Glucopyranosi de and the glycosyl acceptor, octyl 6-O-tert-butyldimethylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-6-O-tert-butyldimethylsilyl-2-deoxy-2- phthalimido-D-glucopyranoside. A NIS/TMSOTf-catalysed coupling of the acceptor with the donor afforded two kinds of tetrasaccharides, which could be separated on a silica gel column. chitotetraose can be obtained by deprotecting the separated compound[2,3].

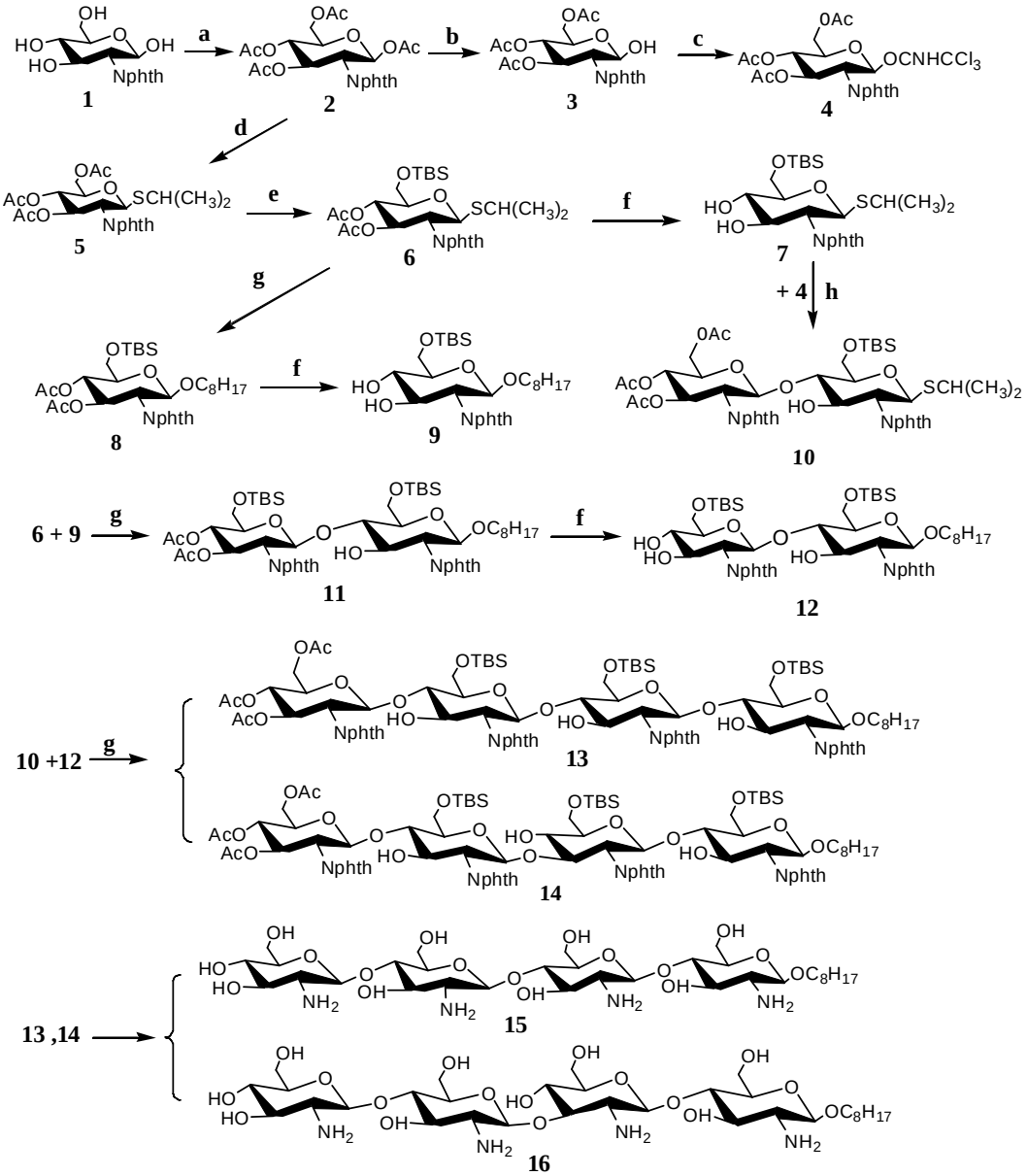
Material and Methods

Material

Silica gel HF254 , silica gel column , sephadex G 25 column , Bruker-ARX-400 spectrometer , Amberlite IR-120 (H^+) resin , filter , UV detector.

Methods

The convergent synthesis of the expected oligosaccharides are described in Scheme 1. ^1H, ^{13}C NMR, ^1H-^1H COSY and ^1H-^{13}C HSQC spectra were recorded with a Bruker-ARX-400 spectrometer for solutions in $CDCl_3$. TLC was performed on silica gel HF254 with detection by spraying with 30% (v/v) H_2SO_4 in MeOH or in some cases by a UV detector.



Scheme1. Conditions and reagents: (a)Ac_2O, pyridine, rt; (b)THF/CH_3OH(V/V):7/3, NH_3, rt; (c)$CNCCl_3$, K_2CO_3, CH_2Cl_2 ,rt ; (d)$BF_3{\cdot}Et_2O$, HSiPr, CH_2Cl_2, reflux; (e)NaOMe, MeOH; TBSCl, Pyr, Ac_2O, rt ; (f)NaOMe, MeOH, rt ; (g)NIS, TMSOTf, CH_2Cl_2 ; (h)TMSOTf, CH_2Cl_2 .

Synthesis of isopropyl 3,4,6-tri-O- acetyl-2-deoxy-2-phthalimido-β-D- glucopyranosyl-(1→4)-6-O-tert-butyldimethylsilyl-2-deoxy-2-phthalimido-1-thio-D-Glucopyranoside(10)

Compound 4(1.62g，2.8mmol) and 7(1.4g，2.9mmol) in anhyd CH2Cl2 at -20℃ was added TMSOTf, under N2 protection. The mixture was stirred under these conditions for 30min, neutralized with Et3N and then concentrated. The residue was subjected to the silica gel column chromatography (1:2 EtOAc-/petroleum ether) to give 10 as a foamy solid; The structure of compound 10 was identified after acetylation. 1H NMR(CDCl3) δ: 0.08, 0.09 (2s, 6H, Si (CH3)2), 0.93 (s, 9 H, t-Bu), 1.14, 1.15 (2s, 6 H, J2.8Hz, SiCH (CH3)2), 1.82, 1.93, 2.01, 2.09(4s, 12 H, COCH3), 3.03(m, 1 H, SCH (CH3)2), 5.50(d，1H，J8.34Hz，H1)，5.42(d, 1H, J10.66Hz,H1'), 4.23(dd, 1H, J1,28.35Hz,J3,210.56Hz,H2), 4.16(t, 1H, J10.35Hz, H2'), 5.77(dd, 1H, J2,310.55Hz, J4,39.05Hz, H3), 5.68(dd, 1H, J2',3'10.09Hz, J4',3'9.16Hz, H3'), 5.12(dd, 1H, J3,49.23Hz, J5,410.04Hz, H4), 4.03(t, 1H, J9.55Hz, H4'), 3.81(m, 1H, H5), 3.37(m, 1H, H5'), 4.08,4.44(m, 2H, H6a, H6b), 3.68,3.43(m, 2H, H6a', H6b'), 7.70-7.87(m, 8H, 2ph).

Synthesis of octyl 6-O-tert-butyldimethylsilyl-2-deoxy-2-phthalimido-β-D- glucopyranosyl-(1→4)-6-O- tert-butyldimethylsilyl-2-deoxy-2- phthalimido-D-glucopyranoside(12)

Compound **6**(3.87g，6.86mmol) and **9**(3.67g，6.86mmol) in anhyd CH_2Cl_2 at -20℃ was added NIS and TMSOTf, respectively, under N_2 protection. The mixture was stirred under these conditions for 30min, neutralized with Et_3N and then concentrated. The residue was subjected to the silica gel column chromatography (1:4 EtOAc- petroleum ether) to give 11 as a foamy solid. Compound 11 (1.65g) in MeOH (17 mL)was treated with 1 M NaOMe at room temperature (rt)for 2 h (maintained pH at 9-10) and then neutralized with Amberlite IR-120 (H^+) resin. After filtration, the filtrate was concentrated and subjected to the column chromatography (1:1 EtOAc-petroleum ether) to give 12 as a foamy solid. The structure of compound 12 was identified after acetylation. ^{1}H NMR（$CDCl_3$） δ: 0.04, 0.07 (2s, 12H, 2Si (CH_3)$_2$), 0.92 (s, 18H, 2t-Bu), 0.8（t, 3H, J7.2Hz, CH_3）, 1.83, 1.95, 2.01, (3s, 9H, 3COCH_3), 0.9-1.2(m, 12H, $CH_2(CH_2)_4CH_2$), 3.32(m, 2H, OCH_2), 5.45(d，1H，J8.34Hz，H_1), 5.19(d, 1H, J8.45Hz,H_1'), 4.19(dd, 1H, $J_{1,2}$8.36Hz, $J_{3,2}$10.6Hz,H_2), 4.10(dd, 1H, $J_{1',2'}$8.46Hz, $J_{3',2'}$10.69Hz, H_2'), 5.78(dd, 1H, $J_{2,3}$10.54Hz, $J_{4,3}$9.03Hz, H_3), 5.67(dd, 1H, $J_{2',3'}$10.55Hz, $J_{4',3'}$ 9.2Hz, H_3'), 5.11(t, 1H, J9.5Hz, H_4), 4.01（t, 1H, J9.43Hz, H_4'）,3.74(m, 1H, H_5), 3.46(m, 1H, H_5'), 3.63-3.67(m, 3H, H_{6a}, H_{6b}, H_{6a}'), 3.79 (m, 1H, , H_{6b}), 7.69-7.85(m, 8H, 2ph).

Synthesis of (1→4)-β-D-glucosamine tetrasaccharide(15)

Coupling of 10 and 12 as described in the preparation of 11 gave 13 and 14 as foamy solid, which cound be separated by the silica gel column chromatography (1:1 EtOAc- petroleum ether). Compound 13 was treated with $BF_3{\cdot}Et_2O$ in CH_2Cl_2 for 60min at rt, then was concentrated and added to NH_3-saturated MeOH. The mixture was stirred at rt for 7days and then concentrated. The residue was dissolved in H_2O and then passed through a sephadex G25 column using water as eluent to give foamy 15 after lyophilization. The structure of compound 15 was identified. ^{1}H-NMR($CDCl_3$） δ: -0.68, -0.63, -0.55, -0.54, -0.36, -0.27(6S, 18H, 3Si (CH_3)$_2$), 0.36, 0.37, 0.66 (3s, 27H, 3t-Bu), 0.77（t, 3H, J7.2Hz, CH_3）, 0.9-1.2(m, 12H, $CH_2(CH_2)_4CH_2$), 1.72, 1.79, 1.99, (3s, 9H, 3COCH_3), 7.65-7.83(m, 16H, 4ph), 5.07,5.30,5.38,5.52(4d, 4H, J8.6Hz,4H_1), 5.62(dd, 1H, J9.14Hz, 10.42Hz, H_3), 5.00(t, 1H, J9.5Hz, H_4).

^{13}C-NMR($CDCl_3$） δ: 97.9(C-1), 98.7(C-1), 98.9(2C, C-1), 54.9(C-2), 55.9(2C, C-2), 56.1(C-2), 70.3(C-3), 70.6(2C, C-3), 70.7(C-3), 69.2(C-4), 82.5(C-4), 82.9(C-4), 83.5(C-4), 74.5(C-5), 74.7(C-5), 74.8(C-5), 72.2(C-5), 62.1(C-6), 62.4(C-6), 62.5(C-6), 63.8(C-6), 122-134(ph-C).

Results and Discussion

The β-(1–4) linked *N*-acetylglucosamine moiety is a frequently occurring structural unit in various naturally and biologically important oligosaccharides and their conjugates. Of these biologically active chitooligosaccharides, chitotetraose has the highest affinity among chitooligosaccharides to bind to rat NKR-PL antigen, a carbohydrate-binding protein in rat natural killer(NK) cells. Mohamed R.E. Aly have reported about the synthesis of chitotetraose and chitohexaose. Herein,we have shown a highly efficient and practical method for the preparation of (1→4)-β-D-glucosamine tetrasaccharide, which provides the pure material for the research of chitotetraose[4].

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