Convergent Synthesis of Oligosaccharide Fragments Corresponding to the Cell Wall O-Polysaccharide of Salmonella enterica O53

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Conventional glycoconjugate vaccines are prepared with polysaccharides isolated from bacterial fermentation, an approach with some significant drawbacks such as handling of live bacterial strains, the presence of biological impurities, and interbatch variations in oligosaccharide epitope structure. However, it has been shown in many cases that a synthetic fragment of appropriate structure conjugated to a protein can be an effective vaccine that circumvents the shortcomings of using full-length oligosaccharides. The development of synthetic strategies to prepare glycoconjugate derivatives against pathogenic bacterial strains is therefore of great interest. Oligosaccharide fragments corresponding to the repeat unit of the cell wall O-antigen of Salmonella enterica strain O53 were synthesized in good yield. Sequential and block glycosylation strategies were used for the synthesis of the target compounds. A number of recently developed reaction conditions were used in the synthetic strategy. A one-pot reaction scheme was also developed for the multiple glycosylation steps. The stereoselective outcomes of all glycosylation reactions were very good.

Introduction

Foodborne illness is a serious worldwide health concern[^1]. The increased rate of hospitalizations and deaths due to gastrointestinal disorders caused by bacterial infections has become a significant challenge for medical professionals[^2]. Among the many pathogenic bacteria responsible for enteric diseases, *Salmonella enterica* is predominant[^3]. *Salmonella* species are recognized as a major pathogen of both animals and humans, causing the foodborne illness salmonellosis[^4]. *Salmonella* infections arise from the contamination of poultry, eggs, beef, and other foods, sometimes from unwashed fruits and vegetables[^5]. Several enteric outbreaks caused by *Salmonella* have been witnessed recently in many countries[^6]. There are several strains of *S. enterica*, which are classified by their cell wall O-polysaccharides[^7]. Because these cell wall O-polysaccharides have a direct influence on the pathogenicity of *Salmonella* strains[^8], the structures of several O-polysaccharides from a number of *S. enterica* strains have been elucidated[^9]. Perepelev et al.[^9] reported the structure of the repeating unit of the cell wall O-polysaccharide of *S. enterica* strain O53; it is a tetrasaccharide composed of α-3-galactofuranose, β-1-galactosamine, 2,3-di-O-acetylated α-L-rhamnopyranose and β-1-glucosamine moieties.

Current efforts in medicinal chemistry and drug discovery programs involve the development of alternative approaches for the control of infections by antibiotic-resistant bacterial strains[^10]. Although vaccines based on bacterial cell wall polysaccharides were introduced many years ago, these were ineffective in children owing to the lack of a T-cell-independent immune response. Glycoconjugate vaccines were later developed, and were found to be highly effective in both adults and children[^11]. Conventional glycoconjugate vaccines are prepared by using polysaccharides isolated from bacterial fermentation. This approach has several serious drawbacks, including the handling of live bacterial strains, the presence of biological impurities, and batch-to-batch variations in the exact structure of the oligosaccharide epitope.

A synthetic oligosaccharide fragment, corresponding to the entire polysaccharide with the appropriate structure, in conjunction with a protein could lead to an efficient glycoconjugate vaccine that circumvents the above-mentioned shortcomings. In this context, the development of synthetic strategies for the preparation of glycoconjugate derivatives against *S. enterica* O53 and other related strains would be of great interest. In many cases it has been found that the full-length oligosaccharide repeat unit is not essential for generating a significant immune response; a smaller fragment can act as an immunodominant glycan[^12, 13]. The tetrasaccharide repeat unit of *S. enterica* O53 strain contains two O-acetyl groups that might influence the antigenicity of the molecule. We therefore decided to synthesize di-, tri-, and tetrasaccharide moieties corresponding to the repeat unit of the *S. enterica* O53 strain cell-wall polysaccharide, containing O-acetyl groups at the appropriate posi-

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tions with a 2-aminooethyl linker connected to the reducing end. In addition, we developed a one-pot synthetic strategy for the synthesis of the tetrascarhide derivative. The 2-aminooethyl linker can serve as a readily available amine functionality for connecting the synthetic glycan moieties to a suitable protein through a spacer linkage.

Results and Discussion

Because two O-acetyl groups are present in the native structure of the O-polysaccharide, this synthetic strategy was designed for the synthesis of the target molecules with O-acetyl groups present at the appropriate positions. The oligosaccharide fragments 1, 2, and 3 were synthesized via stereoselective glycosylation of the suitably functionalized monosaccharide intermediates 4,[14] 5,[15] 6,[16] 7,[17,18] and 8 by applying recently developed glycosylation conditions (Figure 1). Compound 3 was synthesized using [2+2] block glycosylation approach. The monosaccharide derivatives were prepared from the commercially available reducing sugars using published reaction conditions. To prepare the 1,2-cis α-galactofuranosyl linkage in compound 12, per-O-benzylated α-galactofuranosyl trichloroacetimidate derivative 7 (α/β 1:9) was used as the glycosyl donor,[18] which was prepared from 2,3,5,6-tetra-O-benzyl-α/β-β-galactofuranose[19] by treatment with trichloroacetonitrile in the presence of DBU, following reaction conditions similar to those reported by Gandolfi-Donadio et al.[17]

Disaccharide thioglycoside donor 12 was prepared by glycosylation of compound 7 with thioglycoside acceptor 6 in the presence of nitroaryl tetrafluoroborate (NOBF₄) as the glycosylation activator,[20] exploiting the orthogonal properties[21] of compound 6.

Ethyl 3,4,6-tri-O-benzyl-2-deoxy-2-N-phthalimido-1-thio-β-d-galactopyranoside (8) was prepared in 81% yield from the previously reported compound 6,[16] by benzylzation using benzyloxide and sodium hydride.[22] Iodionium-ion-promoted stereoselective glycosylation of compound 4 with thioglycoside derivative 5 in the presence of a combination of N-iodosuccinimide (NIS) and perchloric acid supported over silica (HClO₄–SiO₂) furnished disaccharide derivative 9 in 77% yield. NMR spectral analysis of compound 9 confirmed its formation (signals at δ = 5.19 (d, J = 8.5 Hz, H1₆), 4.44 (br s, H1₃) in 1H NMR, and at δ = 98.9 (C1₆), 97.4 (C1₃) in 13C NMR spectra). Compound 9 was subjected to a series of functional group transformations, which include: a) treatment with hydrazine monohydrate at elevated temperature,[23] b) acetylation using acetic anhydride and pyridine, c) removal of the allyl ether by treatment with palladium chloride,[24] and d) catalytic transfer hydrogenation[25] using triethylsilane in the presence of Pearlman’s catalyst to furnish compound 10 in 57% overall yield. NMR spectral analysis established the structure of compound 10[2] (signals at δ = 4.77 (br s, H1₃), 4.44 (d, J = 8.0 Hz, H1₆) in 1H NMR, and at δ = 100.2 (C1₆), 98.4 (C1₃) in 13C NMR spectra). Removal of the allyl ether from compound 9 with palladium chloride[26] gave the disaccharide acceptor 10 in 67% yield. Compound 10 was allowed to couple stereoselectively with thioglycoside derivative 8 in the presence of a combination of NIS and HClO₄–SiO₂[23,24] to furnish the trisaccharide derivative 11 in 70% yield. Formation of compound 11 was confirmed by its spectral analysis (signals at δ = 5.18 (d, J = 8.5 Hz, H1₆), 5.10 (d, J = 8.0 Hz, H1₃), 4.33 (br s, H1₃) in 1H NMR, and at δ = 98.9 (C1₆), 97.6 (C1₃), 97.0 (C1₄) in 13C NMR spectra). Compound 11 was subjected to a similar set of reactions used for the preparation of compound 1 from compound 9, to furnish compound 2 in overall 55% yield. NMR spectral analysis established the structure of compound 2[2] (signals at δ = 4.95 (br s, H1₃), 4.64 (d, J = 8.5 Hz, H1₆), 4.53 (d, J = 7.5 Hz, H1₃) in 1H NMR, and at δ = 101.9 (C1₆), 100.2 (C1₃), 98.2 (C1₄) in 13C NMR spectra) (Scheme 1).

In another approach the tetrascarhide 3 as its 2-aminooethyl glycoside was synthesized by applying a [2+2] block glycosylation strategy. Stereoselective glycosylation of the thioglycoside acceptor 6 with trichloroacetimidate derivative 7 in the presence of NOBF₄[20] furnished the disaccharide thioglycoside derivative 12 in 65% yield, together with a minor quantity (~5%) of the other isomeric product, which was separated by column chromatography. The stereochemistry of the glycosyl linkage was confirmed by its spectral analysis (signals at δ = 5.21 (d, J = 10.5 Hz, H1₆), 5.17 (d, J = 3.5 Hz, H1₃) in 1H NMR, and at δ = 102.5 (C1₆), 81.2 (C1₃) in 13C NMR spectra). The coupling constant (J = 3.5 Hz) for the α-galactofuranosyl linkage in the 1H NMR spectrum of compound 12 confirmed formation of the 1,2-cis α-galactofuranosyl linkage.[28] Stereoselective glycosylation of disaccharide thioglycoside donor 12 with disacchar-

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**Figure 1.** Structures of the synthesized oligosaccharide fragments 1, 2, and 3 as their 2-aminooethyl glycosides and their synthetic intermediates.
ide acceptor 10 in the presence of a combination of NIS and HClO₄/SiO₂ furnished tetrasaccharide derivative 13 in 66 % yield, which was confirmed from its spectral analysis [signals at δ = 5.38 (s, PhCH₃), 5.17 (d, J = 8.5 Hz, H1ₐ), 5.08 (d, J = 4.0 Hz, H1ₐ), 5.06 (d, J = 10.0 Hz, H1ₐ), 4.40 (brs, H1ₐ), 102.9 (C1ₐ), 101.6 (PhCH₃), 98.9 (C1ₐ), 97.6 (C1ₐ), 96.3 (C1ₐ) in ¹³C NMR spectra (Scheme 2)]. In a parallel set of experiments, tetrasaccharide derivative 13 was synthesized under one-step one-pot reaction conditions. In this strategy, thioglycoside 6 was allowed to react with β-galactofuranosyl trichlorooacetimidate donor 7 in the presence of NOBF₄, until the starting material was consumed and a new spot appeared in the TLC plate. Compound 10 was then added to the reaction mixture followed by NIS and HClO₄/SiO₂, and the reaction was continued to furnish compound 13 in 46 % overall yield in one pot. Compound 13 was subjected to a similar set of reactions used in the preparation of compound 1 from compound 9, to furnish compound 3 in 46 % overall yield (Scheme 3). The NMR spectral analysis of compound 3 confirmed its formation [signals at δ = 5.00 (d, J = 8.5 Hz, H1ₐ), 4.81 (d, J = 3.5 Hz, H1ₐ), 4.79 (brs, H1ₐ), 4.48 (d, J = 7.0 Hz, H1ₐ) in ¹H NMR, and at δ = 102.6 (C1ₐ), 100.2 (C1ₐ), 98.1 (C1ₐ), 97.6 (C1ₐ) in ¹³C NMR spectra].

Conclusions

In summary, oligosaccharides corresponding to the cell wall O-polysaccharide of Salmonella enterica strain O53 were synthesized, maintaining the exact structural components present in the native polysaccharide. The tetrasaccharide derivative was also synthesized under one-pot reaction conditions. The stereo-selective outcome and yields were satisfactory in all glycosylation steps.

Experimental Section

General methods: All reactions were monitored by thin-layer chromatography over silica-gel-coated TLC plates. The spots on TLC were visualized by warming ceric sulfate (2% Ce(SO₄)₃ in 2n...
H$_2$SO$_4$) sprayed plates on a hot plate. Silica gel (230–400 mesh) was used for column chromatography. NMR spectra were recorded on a Bruker Avance 500 MHz instrument, using CDCl$_3$ as solvent and TMS as internal reference unless stated otherwise. Chemical shifts (δ) are expressed in ppm. The complete assignment of proton and carbon spectra was carried out by using a standard set of NMR experiments: 1H NMR, 13C NMR, 1H DEPT 135, 2D COSY, 2D HSQC, etc. MALDI-MS data were recorded on a Bruker Daltonics mass spectrometer. Optical rotations were recorded with a Jasco P-2000 polarimeter. Microanalysis was carried out on Carlo Erba analyzer. Commercially available grades of organic solvents of adequate purity were used in all reactions. HClO$_4$–SiO$_2$ was prepared by following experimental conditions similar to those reported by Chakraborti et al.$^{[24]}

Ethyl 3,4,6-tri-O-benzyl-2-deoxy-2-N-phthalimido-1-thio-β-D-galactopyranoside (8): To a solution of compound 6 (1.87 mmol) in anhydrous THF (15 mL) was added benzyl bromide (0.5 mL, 4.20 mmol) and the reaction mixture was cooled to 0°C. To the cooled reaction mixture was added sodium hydride (60% oil coated, 225 mg, 5.61 mmol) and it was stirred at 0°C for 2 h. The reaction was quenched by the addition of saturated aqueous NH$_4$Cl (50 mL), and then was extracted with CH$_2$Cl$_2$ (50 mL). The organic layer was washed with H$_2$O (50 mL), dried (Na$_2$SO$_4$) and concentrated under reduced pressure. The crude product was purified over SiO$_2$ using hexane/EtOAc (9:1) as eluent to give pure compound 8 (945 mg, 81%). Yellow oil; $[α]$_D$^{25}$ = +66 (c = 1.2, CHCl$_3$); IR (neat): 3021, 2934, 1699, 1534, 1454, 1262, 1091, 1027, 751 cm$^{-1}$; 1H NMR (CDCl$_3$, 500 MHz): δ = 7.85–6.98 (m, 19 H, Ar–H); 5.26 (d, J = 10.5 Hz, 1H, H-1); 4.99 (d, J = 12.5 Hz, 1H, PhCH$_2$), 4.81 (t, J = 10.5 Hz each, 1H, H-2), 4.62 (2d, J = 11.0 Hz each, 2H, 2PhCH$_2$), 4.51 (ABq, J = 12.0 Hz each, 2H, 2PhCH$_3$), 4.37 (dd, J = 10.5, 2.5 Hz, 1H, H-3); 4.32 (d, J = 12.0 Hz, 1H, PhCH$_3$), 4.09 (brs, 1H, H-4), 3.81–3.78 (m, 1H, H-6) 3.67–3.63 (m, 2H, H-$	ext{CH}_2$), 2.71–2.61 (m, 2H, SCH$_2$H); 13C NMR (CDCl$_3$, 125 MHz): δ = 168.3, 167.6 (PhCH$_2$), 138.7–123.1 (Ar–C), 81.2 (C1), 77.5 (C3), 77.4 (C5), 74.5 (PhCH$_3$), 73.5 (PhCH$_2$), 72.2 (C4), 71.4 (PhCH$_2$), 68.6 (C6), 51.6 (C2), 23.6 (SCH$_2$CH$_3$), 14.8 (SCH$_2$CH$_3$); ESI-MS: 664.2 [M + Na$^+$]; Anal. calc. for C$_{26}$H$_{35}$NO$_{13}$ (623.23): C 71.24, H 6.98%; found: C 71.10, H 6.16%.

2-(N-Benzoylxyr carbonyl)aminomethyl O-(2,3-di-O-acetyl-4-O-allyl-α-L-rhamnopyranosyl)-(1→3)-4,6-benzylidene-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (11): To a solution of compound 10 (300 mg, 0.37 mmol) and compound 8 (260 mg, 0.42 mmol) in dry CH$_2$Cl$_2$ (8 mL) was added MS 4 A (0.5 g) and the reaction mixture was cooled to −40°C under argon. To the cooled reaction mixture were added Na$_2$CO$_3$ (110 mg, 0.49 mmol) and HClO$_4$–SiO$_2$ (10 mg) and it was allowed to stir at same temperature for 1 h. The reaction mixture was filtered and washed with CH$_2$Cl$_2$ (50 mL). The combined organic layer was successively washed with 5% Na$_2$SO$_4$ (100 mL), saturated NaHCO$_3$ (100 mL) and H$_2$O (100 mL), dried (Na$_2$SO$_4$) and concentrated. The crude product was purified over SiO$_2$ using hexane/EtOAc (5:1) as eluent to give pure compound 9 (1.7 g, 77%). White solid; mp: 117−118°C (EtOH/ $[α]$_D$^{25}$ = +31 (c = 1.2, CHCl$_3$); IR (KBr): 3033, 2927, 1775, 1715, 1650, 1445, 1230, 1273, 1088, 1017, 712 cm$^{-1}$; 1H NMR (CDCl$_3$, 500 MHz): δ = 7.77–7.25 (m, 14 H, Ar=H), 5.76–5.70 (m, 1H, CH$_2$CH$_2$), 5.52 (s, 1H, PhCH$_3$), 5.19 (d, J = 8.5 Hz, 1H, H-1); 5.15–5.06 (m, 3H, 3H-3, CH$_2$CH$_2$), 4.98–4.87 (m, 3H, NH, 2PhCH$_3$), 4.66 (brs, 1H, H-2), 4.50 (J = 9.5 Hz each, 1H, H-3); 4.44 (brs, 1H, H-4), 4.36 (dd, J = 10.0, 4.5 Hz, 1H, H-5); 4.28 (t, J = 9.0 Hz each, 1H, H-2); 3.93–3.88 (m, 3H, H-$	ext{CH}_2$OCH$_2$(CH$_2$)), 3.79–3.75 (m, 2H, H-$	ext{CH}_2$OCH$_3$), 3.70 (t, J = 9.0 Hz each, 1H, H-4); 3.65–3.56 (m, 2H, H-$	ext{CH}_2$OCH$_3$), 3.26–3.21 (m, 2H, NCH$_2$), 3.12 (t, J = 9.5 Hz each, H-4a), 1.93, 1.74 (2 s, COCH$_3$), 0.70 (d, J = 6.5 Hz, 3H, CH$_3$); 13C NMR (CDCl$_3$, 125 MHz): δ = −169.2, 161.9 (2COCH$_3$), 156.1 (C(BOz)), 137.0−132.6 (−Ar–C), 139.9 (CH$_2$CH$_2$), 116.3 (CH$_2$), 101.9 (PhCH$_3$), 98.9 (C1), 97.4 (C1), 80.2 (C4), 78.4 (C4), 74.4 (C3), 73.1 (OCH$_2$CH$_2$), 70.8 (C2), 70.3 (C3), 69.1 (C6), 68.5 (OCH), 67.8 (C5), 66.5 (2C, C$_{5}$PhCH$_3$), 56.2 (C2), 40.7 (NCH$_2$), 20.8, 20.5 (2C, 2COCH$_3$), 17.0 (CH$_3$); MALDI-MS: 867.3 [M + Na$^+$]; Anal. calc. for C$_{26}$H$_{34}$O$_{14}$ (844.31): C 62.55, H 5.73%; found: C 62.40, H 6.20%.
The reaction mixture was filtered and washed with CH\textsubscript{2}Cl\textsubscript{2} (50 mL), dried (Na\textsubscript{2}SO\textsubscript{4}) and concentrated. The crude product was purified over SiO\textsubscript{2} using hexane/EtOAc (8:1) as eluent to give pure compound 13 (50 mg, 0.31 mmol) in CH\textsubscript{3}Cl (3 mL) was added to the reaction mixture followed by NIS (80 mg, 0.36 mmol) and HClO\textsubscript{2} (10 mg) and it was allowed to stir at –20 °C for 1 h. The reaction mixture was filtered and washed with CH\textsubscript{2}Cl\textsubscript{2} (25 mL). The combined organic layer was successively washed with 5% Na\textsubscript{2}SO\textsubscript{4} (25 mL), saturated NaHCO\textsubscript{3} (25 mL) and H\textsubscript{2}O (25 mL), dried (Na\textsubscript{2}SO\textsubscript{4}) and concentrated. The crude product was purified over SiO\textsubscript{2} using hexane/EtOAc (5:1) as eluent to give pure compound 13 (260 mg, 46% in two steps).

2-Aminoethyl O-(2,3-di-O-acetyl-\(\beta\)-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-\(\beta\)-D-glucopyranosyl (1): To a solution of compound 9 (500 mg, 0.59 mmol) in EtOH (10 mL) was added NH\textsubscript{2}NH\textsubscript{2}•H\textsubscript{2}O (0.2 mL) and the mixture was stirred at 80°C for 4 h. The solvents were removed under reduced pressure and a solution of the crude mass in acetic anhydride (2 mL) and pyridine (2 mL) was kept at room temperature for 1 h. The solvents were removed under reduced pressure and the crude mass was passed through a short pad of SiO\textsubscript{2} using EtOAc as eluent. To a solution of the acetylated product in CH\textsubscript{2}Cl\textsubscript{2} (10 mL) was added PdCl\textsubscript{2} (80 mg, 0.45 mmol) and the reaction mixture was allowed to stir at room temperature for 1.5 h. The reaction mixture was filtered through a Celite bed, washed with CH\textsubscript{2}Cl\textsubscript{2} (25 mL) and concentrated to give the crude product, which was passed through a short pad of SiO\textsubscript{2} using EtOAc as eluent. To a solution of the de-O-allylated product in CH\textsubscript{2}Cl\textsubscript{2} (10 mL) were added 20% Pd(OH)\textsubscript{2}•C\textsubscript{2}H\textsubscript{5}OH (80 mg) and Et\textsubscript{3}SiH (0.4 mL, 2.5 mmol) and the reaction mixture was allowed to stir at room temperature for 12 h. The reaction mixture was filtered through a Celite bed and the filtering bed was washed with CH\textsubscript{2}OH/H\textsubscript{2}O (10 mL, 2:1 v/v). The combined filtrate was concentrated under reduced pressure and passed through a Sephadex LH-20 column using CH\textsubscript{2}OH/H\textsubscript{2}O (2:1) as eluent to give pure compound 1 (165 mg, 57%). White powder; [\(\alpha\]\textsubscript{D}\textsubscript{25}]: +12 (c = 1.2, H\textsubscript{2}O); IR (KBr): 3448, 2928, 1630, 1376, 1235, 1071 cm\textsuperscript{-1} \textsuperscript{1}H NMR (D\textsubscript{2}O, 500 MHz): \(\delta\) = 1.89 (3 H, H\textsubscript{a}), 4.03 (3 H, H\textsubscript{b}), 2.10 (3 H, H\textsubscript{c}) ppm; \textsuperscript{13}C NMR (CDCl\textsubscript{3}, 125 MHz): \(\delta\) = 62.2, 76.9, 74.2 ppm. Anal. calcd for C\textsubscript{12}H\textsubscript{22}O\textsubscript{6}N\textsubscript{3}S\textsubscript{2} (252.3): C 46.76, H 6.39 %; found: C 46.78, H 6.32 %.

2-Aminoethyl O-(2-acetamido-2-deoxy-\(\beta\)-D-glucopyranosyl)-(1→3)-2-acetamido-2-deoxy-\(\beta\)-D-glucopyranosyl (1): A solution of compound 6 (500 mg, 0.94 mmol) and compound 7 (800 mg, 1.17 mmol) in dry CH\textsubscript{2}Cl\textsubscript{2} (20 mL) was cooled to –20°C under argon. To the cooled reaction mixture was added NOBF\textsubscript{4} (80 mg, 0.68 mmol) and it was allowed to stir at same temperature for 1 h. After consumption of the starting materials ( TLC: hexane/EtOAc (6:1) ), a solution of compound 10 (250 mg, 0.31 mmol) in CH\textsubscript{3}Cl (3 mL) was added to the reaction mixture followed by NIS (80 mg, 0.36 mmol) and HClO\textsubscript{2} (10 mg) and it was allowed to stir at –20 °C for 1 h. The reaction mixture was filtered and washed with CH\textsubscript{2}Cl\textsubscript{2} (25 mL). The combined organic layer was successively washed with 5% Na\textsubscript{2}SO\textsubscript{4} (25 mL), saturated NaHCO\textsubscript{3} (25 mL) and H\textsubscript{2}O (25 mL), dried (Na\textsubscript{2}SO\textsubscript{4}) and concentrated. The crude product was purified over SiO\textsubscript{2} using hexane/EtOAc (5:1) as eluent to give pure compound 13 (260 mg, 46% in two steps).

Ethyl O-(2,3,5,6-tetra-O-benzyl-\(\alpha\)-D-galactofuranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-2-N-phthalimido-\(\beta\)-D-galactopyranosyl (12): A solution of compound 6 (500 mg, 0.94 mmol) and compound 7 (800 mg, 1.17 mmol) in dry CH\textsubscript{2}Cl\textsubscript{2} (20 mL) was cooled to –20°C under argon. To the cooled reaction mixture was added NOBF\textsubscript{4} (80 mg, 0.68 mmol) and it was allowed to stir at same temperature for 1 h. After consumption of the starting materials ( TLC: hexane/EtOAc (6:1) ), a solution of compound 10 (250 mg, 0.31 mmol) in CH\textsubscript{3}Cl (3 mL) was added to the reaction mixture followed by NIS (80 mg, 0.36 mmol) and HClO\textsubscript{2} (10 mg) and it was allowed to stir at –20 °C for 1 h. The reaction mixture was filtered and washed with CH\textsubscript{2}Cl\textsubscript{2} (25 mL). The combined organic layer was successively washed with 5% Na\textsubscript{2}SO\textsubscript{4} (25 mL), saturated NaHCO\textsubscript{3} (25 mL) and H\textsubscript{2}O (25 mL), dried (Na\textsubscript{2}SO\textsubscript{4}) and concentrated. The crude product was purified over SiO\textsubscript{2} using hexane/EtOAc (5:1) as eluent to give pure compound 13 (260 mg, 46% in two steps).
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