Convenient synthesis of glycosylsulforaphane derivatives

Arin Gucchait and Anup Kumar Misra*

Division of Molecular Medicine, Bose Institute, P-1/12, C.I.T. Scheme VII M, Kolkata-700 054, India

E-mail: akmisra69@gmail.com

Preparation of novel glycosylated analogs of sulforaphane, a bioactive natural product has been accomplished using a convenient synthetic strategy. D-Glucosyl and L-rhamnosyl sulforaphane derivatives were obtained in good yield starting from the corresponding glycosylthiols.

Keywords: Sulforaphane, glycosyl, sulfoxide, isothiocyanate, anti-cancer.

Introduction

Over the years, hundreds of studies have examined the relationship between fruit/vegetable intake and cancer risk and incidences[1,2]. The majority of these have concluded that consumption of diet rich in fruits and vegetables offers a significant protective effect against cancer. Thus, it has been strongly associated with reduced risk of cardiovascular disease, cancer, diabetes, Alzheimer disease, cataracts, and age-related functional decline[3–5]. These convincing evidence suggests that a change in dietary behavior, such as increasing consumption of fruit, vegetables, and grains is a practical strategy for significantly reducing the incidence of chronic diseases[6]. Moreover, oxidative stress can also causes oxidative damage to large number of biomolecules, such as proteins, DNA, and lipids which results in an increased risk for cancer and cardiovascular diseases[7,8]. To prevent or slow down the oxidative stress induced by free radicals, sufficient amounts of antioxidants need to be consumed. Epidemiological studies suggest that intake of cruciferous vegetables including broccoli reduces the risks for the induction of certain forms of cancer[9,10]. Sulforaphane (**1**) (Fig. 1), a unique

molecule is present in broccoli, bokchoy and cabbage which possess sulfoxide and isothiocyanate functional group and it influences the process of carcinogenesis during the initiation and promotion phases[11]. Although the potential of sulforaphane in controlling cancer has been documented, enough attention was not given for the development of novel therapeutics based on sulforaphane[12]. It has been envisaged that linking of sulforaphane with a sugar moiety could increase its bioavailability by increasing its solubility in aqueous medium and hence could improve it therapeutic potential. Earlier, Khiar and co-workers reported the synthesis of a series of enantiopure sulforaphane analogs and their biological activities[13]. However, synthesis of the glycosylated derivatives of sulforaphane is not reported earlier. With this assumption, preparation of glycosylated derivatives of sulforaphane has been undertaken and the findings are presented herein.

Results and discussion

D-Glucosylated and L-rhamnosylated sulforaphane derivatives (**4** and **8**) were synthesized starting from the corresponding glycosylthiol derivatives (**2**, **7**). Literature reported 2,3,4,6-
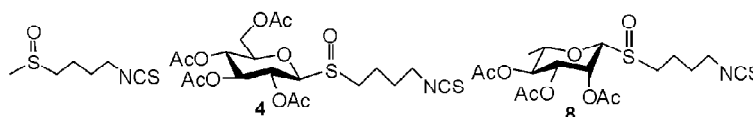

Fig. 1. Structure of sulforaphane and its glycosylated analogs (**6** and **11**).

tetra-O-acetyl-1-thio-β-D-glucopyranose (**2**)[14] was treated with 1,4-dibromobutane in the presence of DBU[15] to give the corresponding thioalkylated product (**3**) in 72% yield, which upon further treated with sodium azide to furnish azidobutyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside (**4**) in 78% yield. Compound **4** was allowed to react with a combination of triphenylphosphine and carbondisulfide (CS$_2$)[13,16] to give the corresponding isothiocyanate derivative **5** in 72% yield. Finally, controlled oxidation of sulfide moiety using an equimolar quantity of 3-chloroperbenzoic acid (m-CPBA)[17] at a lower temperature –20°C resulted in the formation of expected (4-isothiocyanatobutylsulfinyl)-2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside (**6**) in 70% yield as a mixture of regio-isomers (R:S = 1:4) (Calculated from NMR spectra). In order to optimize the selective oxidation of sulfide into sulfoxide a varied quantity of m-CPBA has been used in CH$_2$Cl$_2$ solvent. Reaction condition was optimized using different catalysts and solvents. Use of 1.0 equiv. of m-CPBA in CH$_2$Cl$_2$ at –20°C can furnish satisfactory yield (70%) of sulfoxide derivatives in 1 h. However, use of other oxidizing agents, such as H$_2$O$_2$, oxone, $tert$-butylhydroperoxide did not give satisfactory yield of the controlled oxidation product. The formation of intermediates after each step was confirmed by the NMR and mass spectral analysis of the products (Scheme 1). Similar procedure was adopted for the synthesis of L-rhamnosylated sulforaphane derivative (**11**). Literature reported 2,3,4-tri-O-acetyl-1-thio-β-L-rhamnopyranose (**7**)[14] was treated with 1,4-dibromobutane in the presence of DBU followed by NaN$_3$ to give azidobutylthioglycoside (**9**) in 76% over all yield, which on treatment with a combination[13] of PPh$_3$ and CS$_2$ furnished 4-isothiocyanatobutylthioglycoside (**10**) in 70% yield. Controlled oxidation of compound **10** using m-CPBA[15] resulted the formation of (4-isothiocyanato-butylsulfinyl)-2,3,4-tri-O-acetyl-β-L-rhamnopyranoside (**11**) in 72% yield as a mixture of the regio-isomers (Scheme 1).

Conclusion

In summary, a convenient synthetic strategy has been developed for the preparation of glycosylated analogs of sulforaphane in good yield. The synthetic strategy involves minimum number of steps with satisfactory yield. By adopting similar synthetic strategy one can synthesize a variety of D- and L-glycosylated sulforaphane derivatives with good yield.

Experimental

Azidobutyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside (4): To a solution of the 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosylthiol (**2**; 300 mg, 0.82 mmol) in dry CH$_2$Cl$_2$ (6 mL) was added 1,4-dibromobutane (200 μL, 1.64 mmol) followed by DBU (250 μL, 1.64 mmol) at 0°C. The resulting mixture was stirred at room temperature for 30 min. After complete disappearance of the starting materials, the solvents were removed under reduced pressure. To a solution of the crude product (**3**) in DMF (5 mL) was added NaN$_3$ (160 mg, 2.47 mmol) and tetrabutyl ammonium bromide (530 mg, 1.65 mmol) and the mixture was stirred at 60°C for 3 h. The solvents were removed under reduced pressure and co-evaporated with toluene (2×15 mL) and the crude product was purified by flash chromatography on SiO$_2$ using hexane-EtOAc (4:1) as eluant to give pure compound **4** (300 mg, 78%). Yellow oil; ^{1}H NMR (500 MHz, CDCl$_3$): δ 5.21 (t, J 9.0 Hz, 1H, H-3), 5.17–5.00 (m, 2H, H-2, H-4), 4.46 (d, J 10.0 Hz, 1H, H-1), 4.26 (dd, J 12.5, 5.0 Hz, 1H, H-6$_a$,) 4.14
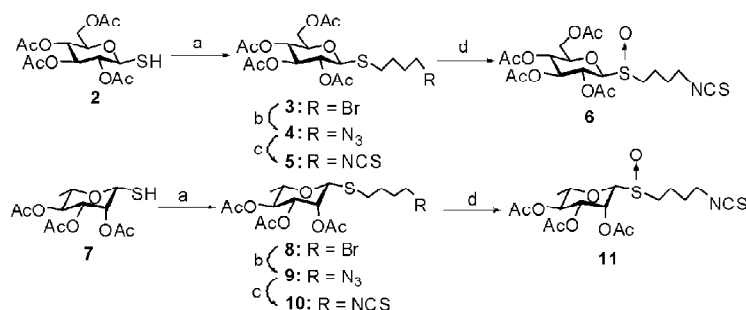
Scheme 1. (a) 1,4-Dibromobutane, DBU, CH$_2$Cl$_2$, room temperature, 72%; (b) NaN$_3$, DMF, 60°C, 3 h, 78% for **4** and over all 76% for **9**; (c) PPh$_3$, CS$_2$, THF, room temperature, 3 h, 72% for **5** and 70% for **10**; (d) m-CPBA, CH$_2$Cl$_2$, –20°C, 1 h, 70% for **6** (R:S = 1:4) and 72% for **11** (R:S = 1:4).

(dd, J 12.5, 2.5 Hz, 1H, H-6$_b$), 3.71–3.67 (m, 1H, H-5), 3.31 (d, J 6.0 Hz, 2H, NCH_2), 2.75–2.65 (m, 2H, SCH_2), 2.10 (s, 3H, COCH_3), 2.09 (s, 3H, COCH_3), 2.08 (s, 3H, COCH_3), 2.06 (s, 3H, COCH_3), 1.71–1.68 (m, 4H, 2CH_2); ^{13}C NMR (125 MHz, CDCl$_3$): δ 170.4–169.2 (4COCH$_3$). 83.3 (C-1), 76.0 (C-5), 73.8 (C-3), 69.6 (C-4), 68.1 (C-2), 61.9 (C-6), 50.8 (NCH$_2$), 28.9 (CH$_2$), 27.8 (CH$_2$), 26.6 (CH$_2$), 20.7–20.5 (4COCH$_3$); HRMS [M+Na]$^+$: Calcd. 484.1366; Found: 484.1375.

(4-Isothiocyanatobutyl) 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside (5): To a solution of compound **4** (250 mg, 0.54 mmol) in anhydrous THF (8 mL) was added PPh$_3$ (170 mg, 0.65 mmol) and the mixture was stirred at room temperature for 3 h. After complete disappearance of starting materials, CS$_2$ (330 μL, 5.14 mmol) was added to the reaction mixture and stirred at room temperature for another 3 h. The organic solvent was removed under reduced pressure and the residue was purified by flash column chromatography using hexane-EtOAc (5:1) as eluant to give the pure product **5** (190 mg, 72%). Yellow oil; ^{1}H NMR (500 MHz, CDCl$_3$): δ 5.21 (t, J 9.0 Hz, 1H, H-3), 5.08–5.00 (m, 2H, H-2, H-4), 4.47 (d, J 10.0 Hz, 1H, H-1), 4.27 (dd, J 12.5, 5.0 Hz,1H, H-6$_a$) 4.15 (dd, J 12.5, 2.5 Hz, 1H, H-6$_b$), 3.72–3.69 (m, 1H, H-5), 3.57 (t, J 6.0 Hz, 2H, NCH_2), 2.77–2.67 (m, 2H, SCH_2), 2.09 (s, 3H, COCH_3), 2.06 (s, 3H, COCH_3), 2.02 (s, 3H, COCH_3), 2.00 (s, 3H, COCH_3), 1.83–1.73 (m, 4H, 2CH_2); ^{13}C NMR (125 MHz, CDCl$_3$): δ 170.3–169.2 (4COCH$_3$), 83.2 (C-1), 76.0 (C-5), 73.8 (C-3), 69.6 (C-4), 68.1 (C-2), 61.9 (C-6), 44.5 (NCH$_2$), 28.8 (CH$_2$), 28.5 (CH$_2$), 26.4 (CH$_2$), 20.7–20.5 (4COCH$_3$); HRMS [M+Na]$^+$: Calcd. 500.1025; Found: 500.1033.

(4-Isothiocyanatobutylsulfinyl) 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside (6): To a solution of compound **5** (150 mg, 0.31 mmol) in dry CH$_2$Cl$_2$ (5 mL) was added *m*-CPBA (55 mg, 0.31 mmol) and the mixture was stirred at –20°C for 1 h. After complete conversion of the starting the organic layer was diluted with CH$_2$Cl$_2$ (20 mL) and the organic layer was washed with saturated NaHCO$_3$ solution (2×10 mL). The organic layer was separated, dried over Na$_2$SO$_4$, evaporated to dryness and the residue was purified by flash column chromatography using CH$_2$Cl$_2$-CH$_3$OH (50:1) to give pure compound **6** as a mixture of regio-isomers (110 mg, 70%). Yellow oil; ^{1}H NMR (500 MHz, CDCl$_3$): δ 5.45 (t, J 9.0 Hz, 0.25H), 5.35 (t, J 9.0 Hz, 0.25H), 5.29 (t,

J 9.0 Hz, 1H), 5.14 (t, J 9.0 Hz, 1H), 5.12 (t, J 9.0 Hz, 0.25H), 5.11 (t, J 9.0 Hz, 1H), 4.38 (d, J 10.0 Hz, 1H), 4.31 (dd, J 12.5, 5.0 Hz, 1H), 4.25–4.20 (m, 1.82H), 3.83 (m, 1.30H), 3.63 (m, 2.6H), 3.20 (m, 0.25H), 3.04 (m, 1H), 2.88 (m, 1H), 2.74 (m, 0.25H), 2.10 (s, 3.75H), 2.09 (s, 3.85H), 2.07 (s, 3.80H), 2.05 (s, 3.75H), 2.03–1.89 (m, 5.15H); ^{13}C NMR (125 MHz, CDCl$_3$): δ 170.2–168.6 (8COCH$_3$), 90.4 (C-1), 87.0 (C-1′), 77.1 (C-5′), 77.0 (C-5), 73.6 (C-3′), 73.0 (C-3), 68.4 (C-4, C-4′), 67.6 (C-2), 66.8 (C-2′), 61.5 (C-6′), 61.2 (C-6), 46.0 (NCH$_2$), 45.7 (NCH$_2$′), 44.5 (SOCH$_2$, SOCH$_2$′), 29.2 (CH$_2$′), 28.9 (CH$_2$), 22.0 (CH$_2$′), 20.7–20.3 (8COCH$_3$), 19.5 (CH$_2$); HRMS [M+Na]$^+$: Calcd. 516.0974; Found: 516.0980.

Azidobutyl 2,3,4-tri-O-acetyl-1-thio-β-L-rhamnopyranoside (6): To a solution of the 2,3,4-tri-*O*-acetyl-β-L-rhamnopyranosylthiol (**7**; 300 mg, 0.98 mmol) in dry CH$_2$Cl$_2$ (5 mL) was added 1,4-dibromobutane (235 μL, 1.96 mmol) followed by DBU (290 μL, 1.96 mmol) at 0°C. The resulting mixture was stirred at room temperature for 30 min. After complete disappearance of the starting materials, the solvents were removed under reduced pressure. To a solution of the crude product (**8**) in DMF (5 mL) was added NaN$_3$ (190 mg, 2.94 mmol) and tetrabutyl ammonium bromide (630 mg, 1.97 mmol) and the mixture was stirred at 60°C for 3 h. The solvents were removed under reduced pressure and co-evaporated with toluene (2×15 mL) and the crude product was purified by flash chromatography on SiO$_2$ using hexane-EtOAc (5:1) as eluant to give pure compound **9** (300 mg, 76%); colorless oil; ^{1}H NMR (500 MHz, CDCl$_3$): δ 5.46 (d, J 3.5 Hz, 1H, H-2), 5.05 (t, J 10.0 Hz, 1H, H-4), 5.00 (dd, J 10.0, 3.5 Hz, 1H, H-3), 4.70 (br s, 1H, H-1), 3.54–3.48 (m, 1H, H-5), 3.30 (br s, 2H, NCH_2), 2.72 (br s, 2H, SCH_2), 2.19 (s, 3H, COCH_3), 2.05 (s, 3H, COCH_3), 1.97 (s, 3H, COCH_3), 1.71 (br s, 4H, 2 CH_2), 1.29 (d, J 6.0 Hz, 3H, CH_3); ^{13}C NMR (125 MHz, CDCl$_3$): δ 170.0–169.5 (3COCH$_3$), 82.3 (C-1), 75.0 (C-5), 71.8 (C-3), 70.7 (C-4), 70.3 (C-2), 50.8 (NCH$_2$), 31.0 (CH$_2$), 27.8 (CH$_2$), 26.8 (CH$_2$), 20.7 (COCH$_3$), 20.6 (COCH$_3$), 20.5 (COCH$_3$), 17.7 (CH$_3$); HRMS [M+Na]$^+$: Calcd. 426.1311; Found: 426.1304.

(4-Isothiocyanatobutyl) 2,3,4-tri-O-acetyl-1-thio-β-L-rhamnopyranoside (10): To a solution of compound **9** (250 mg, 0.62 mmol) in anhydrous THF (8 mL) was added PPh$_3$ (195 mg, 0.74 mmol) and the mixture was stirred at room temperature for 3 h. After complete disappearance of starting materials, CS$_2$ (375 μL, 6.19 mmol) was added to the

reaction mixture and stirred at room temperature for another 3 h. The organic solvent was removed under reduced pressure and the residue was purified by flash column chromatography using hexane-EtOAc (6:1) as eluant to give the pure product **10** (180 mg, 70%). Yellow oil; ^{1}H NMR (500 MHz, CDCl$_3$): δ 5.44 (d, *J* 3.0 Hz, 1H, H-2), 5.01 (t, 1H, *J* 10.0 Hz, H-4), 4.98 (dd, *J* 10.0, 3.5 Hz, 1H, H-3), 4.71 (br s, 1H, H-1), 3.53 (m, 2H, H-5, NC*H*$_2$), 2.75–2.70 (m, 2H, SC*H*$_2$), 2.17 (s, 3H, COC*H*$_3$), 2.03 (s, 3H, COC*H*$_3$), 1.95 (s, 3H, COC*H*$_3$), 1.82–1.74 (br s, 4H, 2 C*H*$_2$), 1.27 (d, *J* 6.0 Hz, 3H, C*H*$_3$); ^{13}C NMR (125 MHz, CDCl$_3$): δ 169.9–169.5 (3COC*H*$_3$), 82.3 (C-1), 75.0 (C-5), 71.8 (C-3), 70.7 (C-4), 70.3 (C-2), 44.5 (NCH$_2$), 30.7 (*C*H$_2$), 28.7 (*C*H$_2$), 26.6 (*C*H$_2$), 20.7 (CO*C*H$_3$), 20.6 (CO*C*H$_3$), 20.5 (CO*C*H$_3$), 17.7 (*C*H$_3$); HRMS [M+Na]$^+$: Calcd. 442.0970; Found: 442.0977.

(4-Isothiocyanatobutylsulfinyl) 2,3,4-tri-O-acetyl-β-ʟ-rhamnopyranoside (11): To a solution of compound **10** (150 mg, 0.30 mmol) in dry CH$_2$Cl$_2$ (5 mL) was added *m*-CPBA (55 mg, 0.30 mmol) and the mixture was stirred at –20°C for 1 h. After complete conversion of the starting the organic layer was diluted with CH$_2$Cl$_2$ (20 mL) and the organic layer was washed with saturated NaHCO$_3$ solution (2×10 mL). The organic layer was separated, dried over Na$_2$SO$_4$, evaporated to dryness and the residue was purified by flash column chromatography using CH$_2$Cl$_2$-CH$_3$OH (50:1) to give pure compound **11** as a mixture of regio-isomers (116 mg, 72%); ^{1}H NMR (500 MHz, CDCl$_3$): δ 5.80 (d, *J* 3.0 Hz, 1H), 5.67 (d, *J* 3.0 Hz, 0.26H), 5.18–4.99 (m, 2.5H), 4.37 (br s, 1H), 4.31 (br s, 0.27H), 3.66–3.60 (m, 3.91H), 2.98–2.93 (m, 1.31H), 2.84–2.78 (m, 1.40H), 2.20 (s, 3H), 2.19 (s, 0.80H), 2.07 (s, 4.12H), 2.05 (s, 3.85H), 2.04–1.90 (m, 5.40H); ^{13}C NMR (125 MHz, CDCl$_3$): δ 169.7–169.1 (m, 6COC*H*$_3$), 90.6 (C-1), 89.9 (C-1′), 76.4 (C-5′), 76.2 (C-5), 71.5 (C-3′), 71.2 (C-3), 70.0 (C-4′), 69.9 (C-4), 65.9 (C-2, C-2′), 49.5 (NCH$_2$′), 49.0 (NCH$_2$), 44.6 (SOCH$_2$), 44.5 (SOCH$_2$′), 29.1 (*C*H$_2$′), 28.9 (*C*H$_2$), 20.8–20.5 (6COC*H*$_3$), 19.7 (*C*H$_2$′), 19.6 (*C*H$_2$), 17.6 (*C*H$_3$), 17.5 (*C*H$_3$′); HRMS [M+Na]$^+$: Calcd. 458.0920; Found: 458.0926.

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