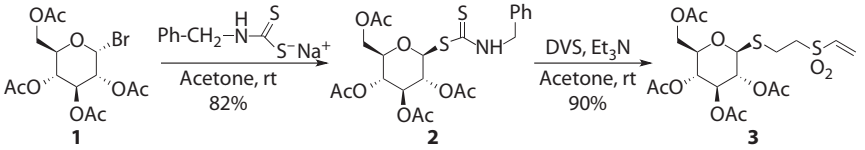
6 Synthesis of Glycosyl Vinyl Sulfones for Bioconjugation

*Francisco Santoyo-Gonzalez,**
Fernando Hernandez-Mateo,
and Omar Boutureira†

CONTENTS



The covalent coupling of two biomolecules to each other (bioconjugation) or to a solid support (immobilization) is one of the cornerstones of *omic* sciences.[1] Among numerous chemical strategies to attain this goal, the versatile Michael-type addition of amine and thiol groups to vinyl sulfones is an attractive methodology.[2] The latter are excellent Michael acceptors because of the electron-poor nature of their double bond, owed to the sulfone's electron-withdrawing capability that makes them good electrophiles.[3,4] All the conjugate additions with vinyl sulfones share a similar reaction pattern, namely, the addition to the β-position of the sulfone. Accordingly, these reactions are well-established methods for creating β-heterosubstituted

* Corresponding author; e-mail: fsantoyo@ugr.es.
† Checker; e-mail: omar.boutureira@urv.cat.

sulfones. Prominent characteristics of this methodology are the water stability of the vinyl sulfone function, the possibility to perform the reactions in physiological conditions (aqueous media, slightly alkaline pH, and room temperature) that preserves the biological function of the biomolecules, the absence of catalysts and by-products, the almost theoretical yields, and the stability of the linkage formed. For these reasons, vinyl sulfones have found application in most of the subdomains of modern proteomics.[5]

Accordingly, and in the context of carbohydrate research, the vinyl sulfone functionalization of the anomeric carbon has proved to be a general strategy for subsequent chemical glycosylation of proteins and for the covalent linkage of a saccharide to amine- and thiol-functionalized supports.[6,7] This strategy has found applications in glycoscience to explore protein–carbohydrate interactions.[6] The authors' group has developed a reliable and simple two-step high-yielding method for the derivatization of saccharides at the anomeric carbon with a vinyl sulfone group spanned by an ethylthio linker.[8] For that purpose, easily accessible or commercially available 1-halo sugars are used as starting materials. The method is based on the preparation of *S*-glycosyl *N*-alkyl dithiocarbamates by treatment of glycosyl halides with salts of alkyl dithiocarbamates[9] in, for example, anhydrous acetone at room temperature. In this way, the formed sugar dithiocarbamates act as masked 1-thiol saccharides. The thiolate sugars are easily generated in a second step by treatment with a common organic base, such as triethylamine, and trapped in situ by commercial divinyl sulfone (DVS) present in the reaction media. The glycosyl vinyl sulfones isolated are ready to be used in any conjugation for preparation of glycosylated materials. The procedure is exemplified by reaction of glucosyl bromide **1** with sodium *N*-benzyldithiocarbamate as a model alkyl dithiocarbamate salt. An improved method for preparation of sodium *N*-benzyl dithiocarbamate compared to that reported in literature[10] is also described. The synthetic approach described herein is generally applicable for the preparation of any glycosyl vinyl sulfone.

EXPERIMENTAL METHODS

General Methods

Commercially available reagents (2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**1**), benzylamine, triethylamine, and divinyl sulfone) and solvents were used without further purification. Thin-layer chromatographies (TLCs) were performed on Merck silica gel 60 F254 aluminum sheets. Detection was effected by charring with sulfuric acid (5% v/v in ethanol), potassium permanganate (1% w/v), and ninhydrin (0.3% w/v) in ethanol and UV light when applicable. Flash column chromatography was performed on Merck silica gel (230–400 mesh, ASTM). Optical rotations were recorded with a PerkinElmer 141 polarimeter at room temperature. IR spectra were recorded with a Satellite Mattson FTIR. ^{1}H and ^{13}C NMR spectra were recorded at room temperature with a Varian DirectDrive (300, 400, and 500 MHz) spectrometer. Chemical shifts are given in ppm and referenced to internal $CDCl_3$. *J* values are given in Hz. Electrospray ionization (ESI) mass spectra were recorded on an LCT premier spectrometer.

Sodium *N*-Benzyldithiocarbamate

An aqueous 1M solution of NaOH (20 mL, 20 mmol) was cooled by means of an ice bath. Benzylamine (2.2 mL, 20 mmol) and carbon disulfide (1.2 mL, 20 mmol) were then added and the reaction mixture stirred vigorously for 2 h. After this time, the majority of the solvent was removed under reduced pressure. Isopropanol/diethyl ether (1:5 v/v, 60 mL) was added to the residue giving a white amorphous precipitate that was collected by filtration. After drying in a desiccator under vacuum over anhydrous phosphorous pentoxide at room temperature for 18 h, 3.2 g (78%) of the sodium salt of *N*-benzyldithiocarbamate was obtained. The compound showed spectroscopic data identical to those reported by Zhang et al.[10]

S-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-*N*-benzyldithiocarbamate (2)

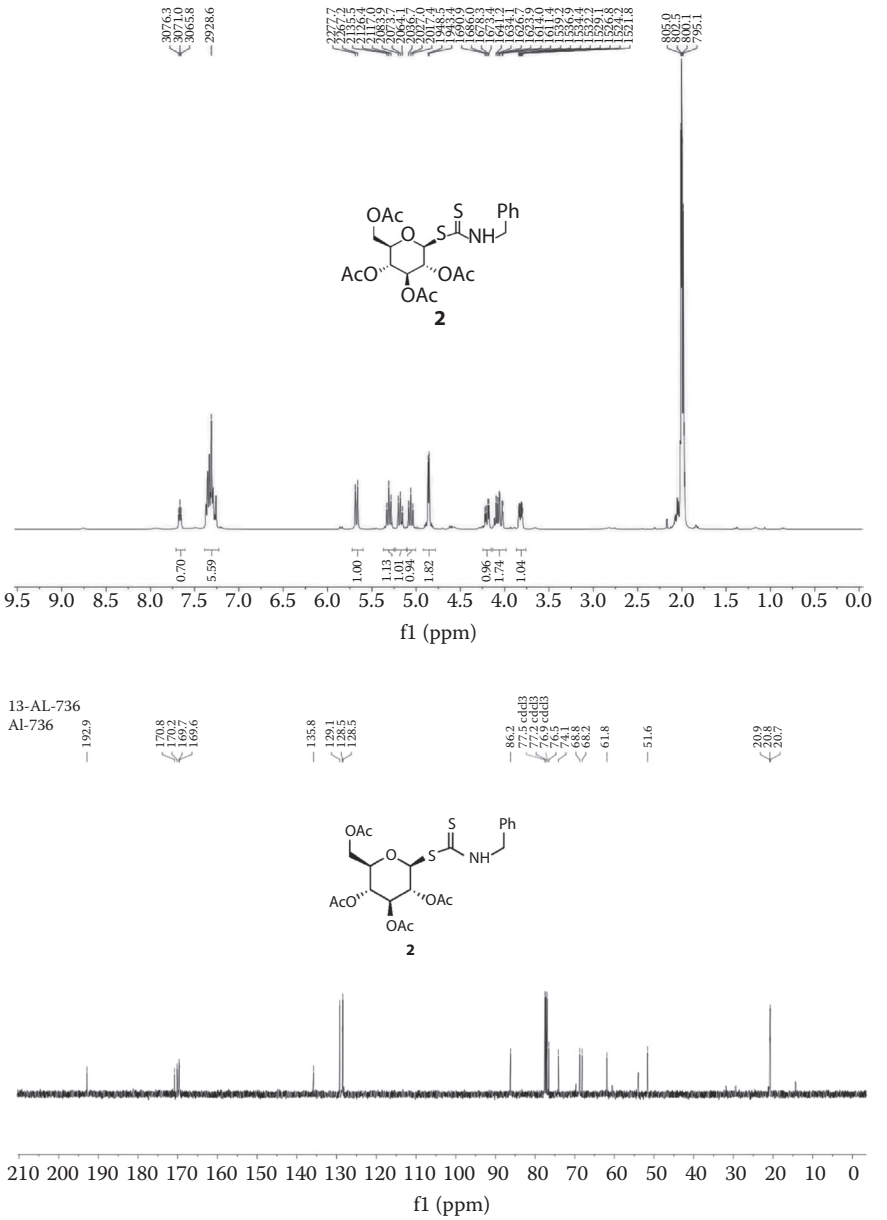
A mixture of glucopyranosyl bromide **1** (0.411 g, 1 mmol) and sodium *N*-benzyldithiocarbamate (0.410 g, 2.0 mmol) in anhydrous acetone (30 mL) was magnetically stirred at room temperature until TLC (1:1 EtOAc–hexane) showed complete conversion of the starting material (2–4 h). The mixture was neutralized with a few drops of aqueous 5% HCl and the organic solvent was removed under reduced pressure. Water (40 mL) was added and the mixture was extracted with dichloromethane (2×60 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated and the residue was chromatographed (1:2 EtOAc–hexane) yielding the glucopyranosyl dithiocarbamate derivative **2** as syrup (0.42 g, 82%). R_f (1:1 EtOAc–hexane) 0.49; $[\alpha]_D$ +12.2° (*c* 1, $CHCl_3$); ν_{max}(film)/cm^{-1} 3291, 2942, 1753, 1512, 1372, 1227, 1046, and 914. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.67 (t, 1H, *J* 5.1 Hz, NH), 7.39–7.26 (m, 5H), 5.67 (d, 1H, *J* 10.5 Hz), 5.31 (t, 1H, *J* 9.3 Hz), 5.18 (t, 1H, *J* 10.1 Hz), 5.06 (t, 1H, *J* 9.7 Hz), 4.86 (d, 2H, *J* 5.0 Hz), 4.20 (dd, 1H, *J* 12.5, 4.8 Hz), 4.04 (dd, 1H, *J* 12.7 and 2.3 Hz), 3.82 (ddd, 1H, *J* 10.1, 4.7 and 2.2 Hz), 2.01, 2.00, 2.00, and 1.99 (4s, 12H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 192.9, 170.8, 170.2, 169.7, 169.6, 135.8, 129.1, 128.5, 128.5, 86.2, 76.5, 74.1, 68.8, 68.2, 61.8, 51.6, 20.9, 20.8, 20.7. HRMS (*m*/*z*) (ESI) calcd for $C_{22}H_{27}NO_9S_2Na$ $[M+Na]^+$: 536.1025. Found: 536.1019.

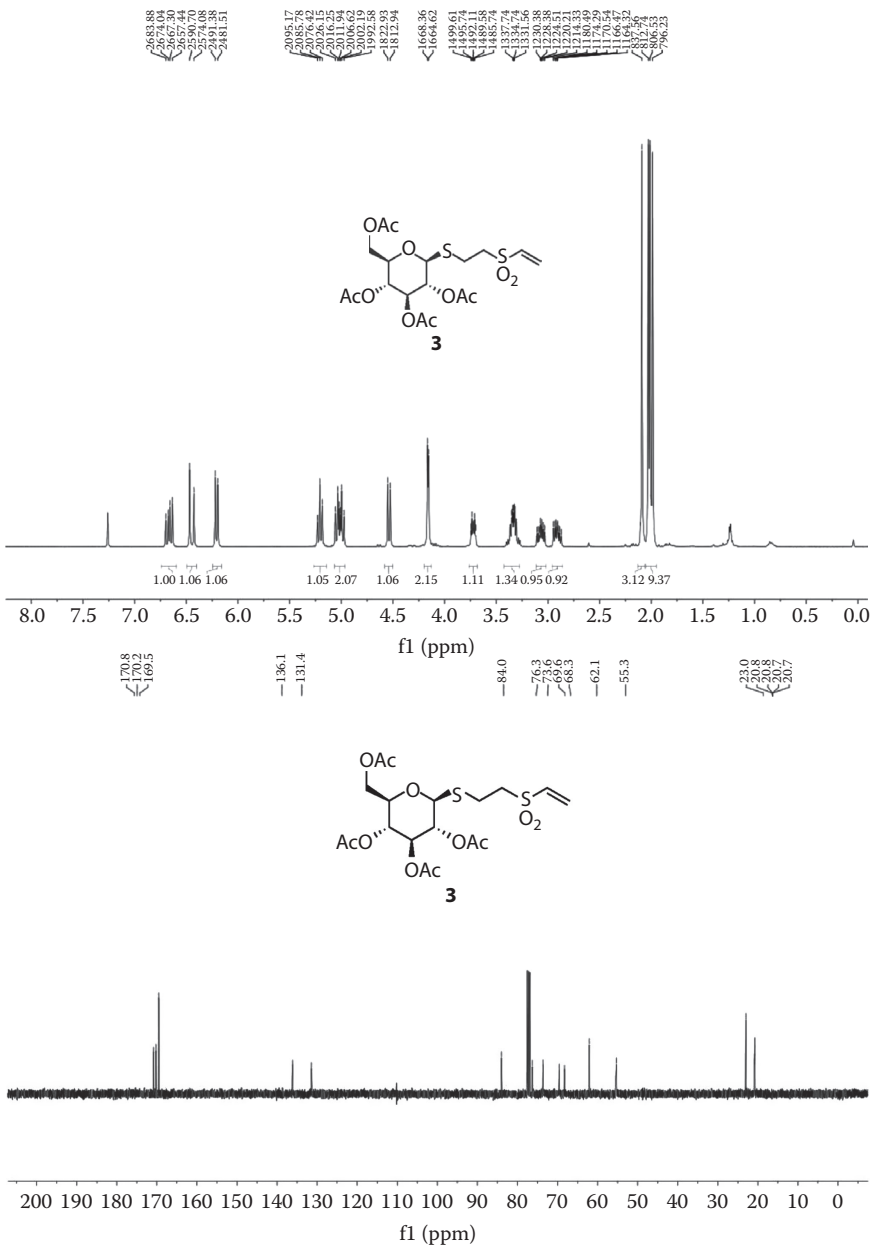
[2-(Ethenesulfonyl)ethyl] 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (3)

To a solution of the glucosyl dithiocarbamate **2** (0.513 g, 1 mmol) in anhydrous acetone (30 mL) was added divinyl sulfone (0.3 mL, 3 mmol) and triethylamine (0.28 mL, 2 mmol). The reaction mixture was magnetically stirred at room temperature until TLC (1:1 EtOAc–hexane) showed complete disappearance of the starting material (1 h). After concentration, chromatography (1:1 EtOAc–hexane → EtOAc) yielded the glycosyl vinyl sulfone **3** as syrup. Total yield: 0.43 g (90%). R_f (1:1 EtOAc–hexane) 0.23; $[\alpha]_D$ −22.5° (*c* 1, $CHCl_3$). ν_{max}(film)/cm^{-1}: 2943, 1752, 1431, 1374, 1226, 1136, 1039, and 914. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.67 (dd, 1H, *J* 16.6, 9.8 Hz), 6.45 (d, 1H, *J* 16.6 Hz), 6.21 (d, 1H, *J* 9.9 Hz), 5.21 (t, 1H, *J* 9.4 Hz), 5.03 (t, 1H, *J* 9.8 Hz), 5.00 (t, 1H, *J* 9.7 Hz), 4.54 (d, 1H, *J* 10.0 Hz), 4.16 (d, 2H, *J* 3.7 Hz), 3.72 (dt, 1H, *J* 10.1, 3.8 Hz), 3.40–3.27 (m, 2H), 3.07 (m, 1H), 2.91 (m, 1H), 2.09, 2.03, 2.01, and 1.99 (4s, 12H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 170.8, 170.2, 169.5, 136.1, 131.4, 84.0, 76.3, 73.6, 69.6, 68.3, 62.1, 55.3, 23.0, 20.8, 20.8, 20.7, 20.7. HRMS (*m*/*z*) (ESI) calcd for $C_{18}H_{26}O_{11}S_2Na$ $[M+Na]^+$: 505.0814. Found: 505.0819.

ACKNOWLEDGMENTS

This chapter was funded by Ministerio de Ciencia e Innovación of the Government of Spain (CTQ2011-29299-CO2-01). O.B. thanks the Ministerio de Ciencia e Innovación, Spain (Juan de la Cierva Fellowship) and the European Commission (Marie Curie Career Integration Grant).





REFERENCES

1. Hermanson, G. T., Ed. *Bioconjugate Techniques*, 2nd edn. Academic Press, San Diego, CA, 2008.
2. Meadows, D. C.; Gervay-Hague, J. *Med. Res. Rev*. **2006**, *26*, 793–814.
3. Forristal, I. *J. Sulfur Chem*. **2005**, *26*, 163–195.
4. Simpkins, N. S. *Tetrahedron* **1990**, *46*, 6951–6984.
5. Lopez-Jaramillo, F. J.; Hernandez-Mateo, F.; Santoyo-Gonzalez, F. Vinyl sulfone: A multi-purpose function for protemics, In *Integrative Proteomics*, Eastwood Leung, H.-C., Ed. InTech, Rijeka, Croatia, 2012, pp. 301–326.
6. Lopez-Jaramillo, F. J.; Ortega-Munoz, M.; Megia-Fernandez, A.; Hernandez-Mateo, F.; Santoyo-Gonzalez, F. *Bioconjug. Chem*. **2012**, *23*, 846–855.
7. Morales-Sanfrutos, J.; Lopez-Jaramillo, J.; Ortega-Muñoz, M.; Megia-Fernandez, A.; Perez-Balderas, F.; Hernandez-Mateo, F.; Santoyo-Gonzalez, F. *Org. Biomol. Chem*. **2010**, *8*, 667–675.
8. Megia-Fernandez, A.; de la Torre-Gonzalez, D.; Parada-Aliste, J.; Lopez-Jaramillo, F. J.; Hernandez-Mateo, F. Santoyo-Gonzalez, F. *Chem. Asian J*. **2014**, 9, 620–631.
9. Fernandez, J. M. G.; Mellet, C. O. *Adv. Carbohydr. Chem. Biochem*. **2000**, *55*, 35–135.
10. Zhang, J.; Lin, X.; Ren, J.; Liu, J.; Wang, X. *Appl. Radiat. Isot*. **2009**, *68*, 101–104.