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Carbohydrate Sulfonyl Chlorides for Simple, Convenient Access to Glycoconjugates

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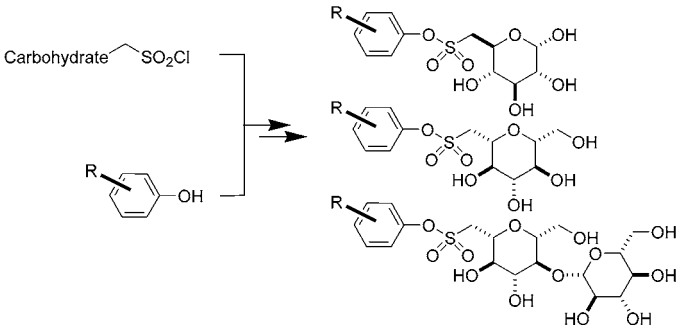
ABSTRACT



The use of carbohydrate sulfonyl chlorides is introduced as a new, facile glycoconjugation method which could find broad applications. We demonstrate the approach by synthesizing a number of glycosylated cholesterol absorption inhibitors which display high inhibitory efficacies in our recently established *in vitro* assay. Furthermore, we highlight an advantage of the electron-withdrawing nature of the sulfonyl linkage which allowed the synthesis of otherwise unstable azetidine conjugates.

The chemical and analytical tools for studying the biological functions of carbohydrates and glycoconjugates have seen explosive development within recent years.[1,2] The structural and synthetic complexity of densely functionalized carbohydrates renders the introduction of carbohydrate domains onto molecules of interest a nontrivial exercise. Moreover, the issues of reactivity and stereoselectivity at the anomeric center during glycosylation adds to the synthetic challenge.[1,3] We have become interested in exploring the effect of glycosylation of small-molecule inhibitors of intestinal cholesterol absorption.[4] In this context, we initiated a study into the development of new strategies for the synthesis of carbohydrate conjugates (Scheme 1).

Examples from the wide range of important applications involving glycoconjugation methods include the synthesis of neoglycoconjugates by Staudinger ligation to cell surfaces,[5] the use of neoglycopeptides as oligosaccharyl transferase inhibitors,[6] and the enhanced biological activities of drugs.[4,7] Thus, for instance, ezetimibe (**10**, Scheme 2)[8]

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(1) (a) *Glycoscience*; Fraser-Reid, B., Tatsuta, K., Thiem, J., Eds.; Springer-Verlag: Berlin, Heidelberg, New York, 2001. (b) *Bioorganic Chemistry: Carbohydrates*; Hecht, S. M., Ed.; Oxford University Press: New York, Oxford, 1999.

(2) (a) *Carbohydrate-based Drug Discovery*; Wong, C.-H., Ed.; Wiley-VCH: Weinheim, 2003. (b) *Glycochemistry. Principles, Synthesis and Applications*; Wang, G., Bertozzi, C. R., Eds.; Marcel Dekker Inc.: New York, Basel, 2001. (c) Palmacci, E. R.; Plante, O. J.; Hewitt, M. C.; Seeberger, P. H. *Helv. Chim. Acta* **2003**, *86*, 3975−3990.

(3) Toshima, K.; Tatsuta, K. *Chem. Rev.* **1993**, *93*, 1503−1531.

(4) Kværnø, L.; Ritter, T.; Werder, M.; Hauser, H.; Carreira, E. M. *Angew. Chem., Int. Ed.* **2004**, *43*, 4653−4656.

(5) Saxon, E.; Bertozzi, C. R. *Science* **2000**, *287*, 2007−2010.

(6) Peluso, S.; Ufret, M. D.; O'Reilly, M. K.; Imperiali, B. *Chem. Biol.* **2002**, *9*, 1323−1328.

(7) Vaccaro, W. D.; Davis, H. R. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 313−318.

(8) (a) Clader, J. W. *J. Med. Chem.* **2004**, *47*, 1−9. (b) Rosenblum, S. B.; Huynh, T.; Afonso, A.; Davis, H. R.; Yumibe, N.; Clader, J. W.; Burnett, D. A. *J. Med. Chem.* **1998**, *41*, 973−980.

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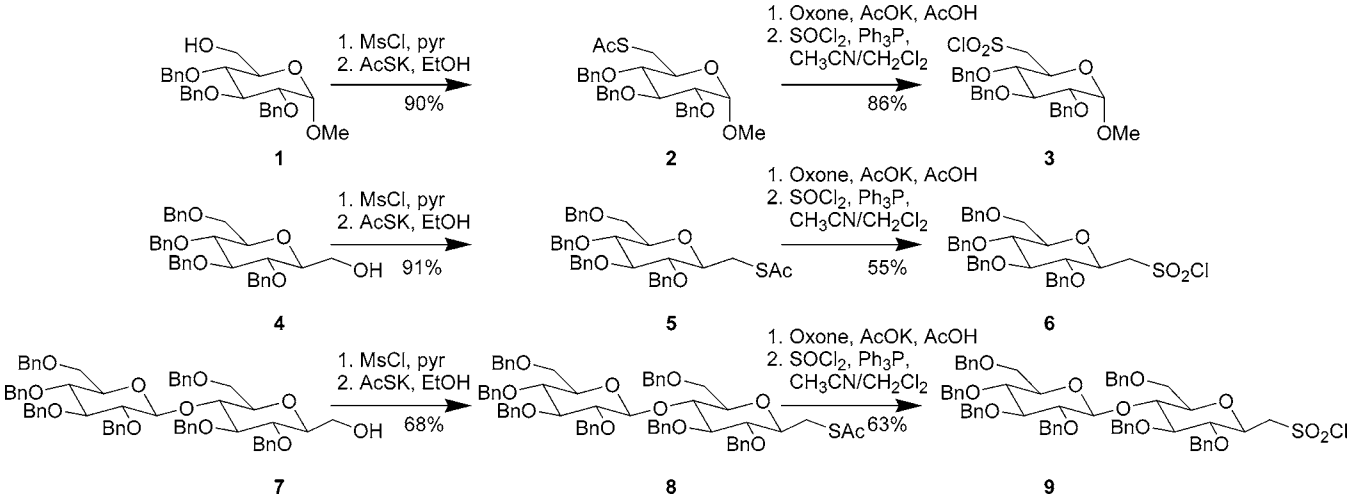
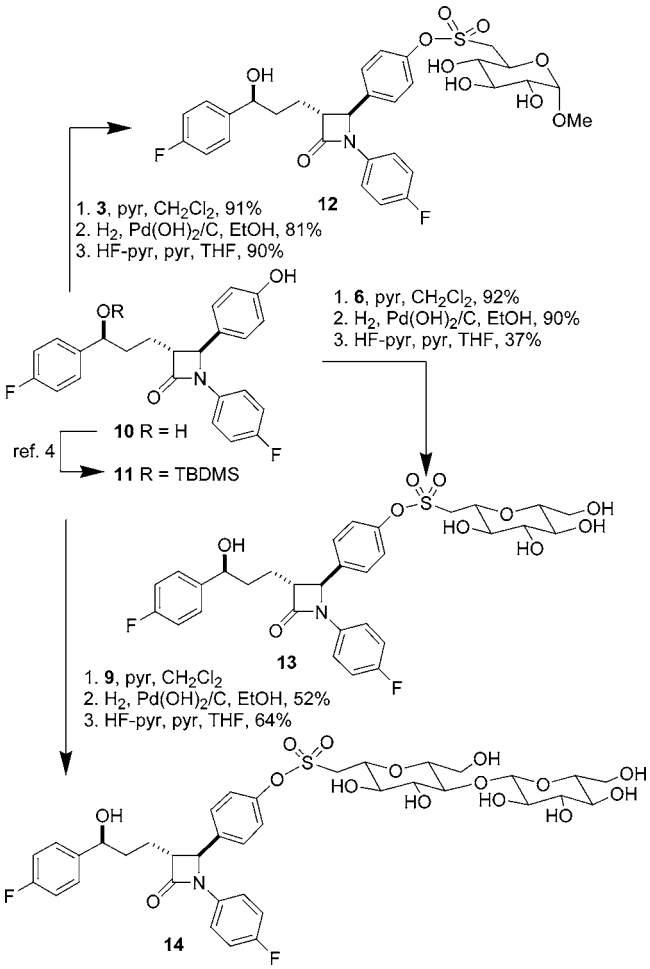
Scheme 1. Synthesis of Carbohydrate Sulfonyl Chlorides



constitutes a notable recent example of a small-molecule pharmaceutical whose *in vivo* activity can be increased through glycosylation.[7,9] In the context of our ongoing research on cholesterol absorption inhibitors,[4] we became interested in the rapid introduction of a variety of carbohydrate domains onto molecules of interest. Given the inherent complexity that can be associated with traditional glycosylation methods,[1,3] we examined a new conjugation strategy that would obviate issues relating to reactivity and stereochemistry during the introduction of the carbohydrate. In this regard, we focused on carbohydrate sulfonyl chlorides because a conjugation strategy based on sulfonate ester formation would be convenient.[10] The use of a sulfonylation reaction to facilitate the construction of analogues of biopolymers finds some precedence in the preparation of sulfonate-linked oligonucleotides.[11] However, to the best of our knowledge, the use of sulfonylation reactions for the purposes of glycoconjugation has not been previously reported. As a proof-of-concept study, we have prepared three different carbohydrate-derived sulfonyl chlorides and examined their conjugation to ezetimibe, given our expertise with an assay for *in vitro* screening of cholesterol absorption inhibitors.[4]

Scheme 2. Synthesis of Sulfonylated β-Lactam Glycosides



The synthetic phase of our investigations commenced with the synthesis of sulfonyl chlorides **3**, **6**, and **9** from the known *O*-benzylated glucose derivates **1**,[12] **4**,[13] and disaccharide **7**.[14] The sulfonyl chlorides were prepared in high yields through a short sequence of reactions involving oxidation[15] of the intermediate thioacetates **2**,[16] **5**,[17] and **8** (Scheme 1).

(9) van Heek, M.; Farley, C.; Compton, D. S.; Hoos, L.; Alton, K. B.; Sybertz, E. J.; Davis, H. R. *Br. J. Pharmacol.* **2000**, *129*, 1748−1754.

(10) Although there is literature precedence for the synthesis of a sugar sulfonyl chloride, its use was limited to the preparation of simple esters (from 2-propanol, 2-methyl-2-propanol, methanol) and amides (from *N*-butylamine and an amino sugar), see: Ulgar, V.; Maya, I.; Fuentes, J.; Fernandez-Bolanos, J. G. *Tetrahedron* **2002**, *58*, 7967−7973.

(11) (a) Johnson, D. C.; Widlanski, T. S. *Synthesis* **2002**, 809−815. (b) Huang, J. X.; McElroy, E. B.; Widlanski, T. S. *J. Org. Chem.* **1994**, *59*, 3520−3521. (c) Huang, J. X.; Widlanski, T. S. *Tetrahedron Lett.* **1992**, *33*, 2657−2660.

(12) Jaramillo, C.; Chiara, J. L.; Martinlomas, M. *J. Org. Chem.* **1994**, *59*, 3135−3141.

(13) RajanBabu, T. V.; Reddy, G. S. *J. Org. Chem.* **1986**, *51*, 5458−5461.

(14) Spak, S. J.; Martin, O. R. *Tetrahedron* **2000**, *56*, 217−224.

(15) Reddie, R. N. *Synth. Commun.* **1987**, *17*, 1129−1139.

(16) Paterson, D. E.; Griffin, F. K.; Alcaraz, M. L.; Taylor, R. J. K. *Eur. J. Org. Chem.* **2002**, 1323−1336

Previous glycosylations of ezetimibe (**10**) have been carried out on the phenol and furnished conjugates with increased activity profiles *in vivo*[7,9] and *in vitro*.[4] As a means of testing our premise involving conjugation of carbohydrates via sulfonate ester formation, we prepared sulfonylated derivatives **12**−**14** as outlined in Scheme 2.[18]

Conjugates **12**−**14** were subsequently evaluated for inhibition of intestinal cholesterol uptake at fixed substrate concentrations of 6 μM using the brush border membrane vesicle assay (Figure 1).[4] Compared to ezetimibe (**10**) (16% inhibition), the sulfonylated β-lactam glycosides displayed interesting activities as cholesterol absorption inhibitors. The highest efficacy was observed for the cellobiose derivative **14** (41% inhibition) compared to the monosaccharide derivatives **12** (20% inhibition) and **13** (15% inhibition).[19]

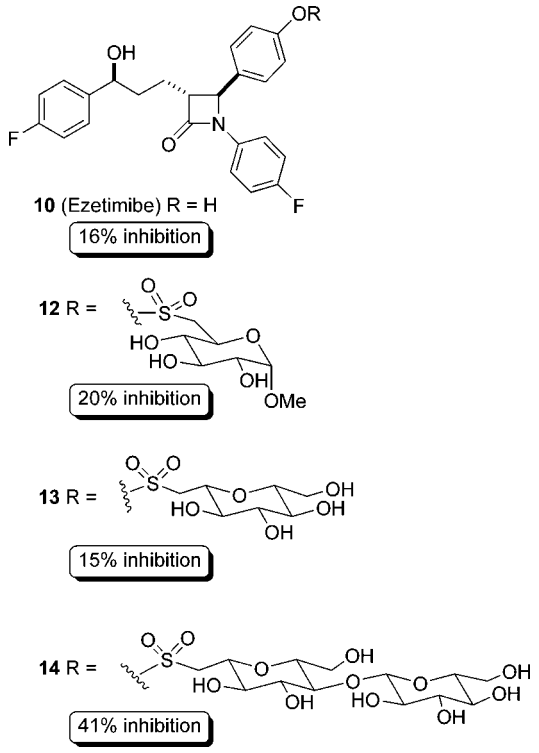


Figure 1. Inhibition of intestinal cholesterol absorption in the brush border membrane vesicle assay.[4]

We have observed an additional important advantage to the use of sulfonyl glyconjugation in the context of a structure−activity relationship study of ezetimibe. Specifically, we sought to address whether replacement of the β-lactam ring by an azetidine (see **15**, Figure 2) would furnish structures retaining activity as cholesterol absorption inhibitors. In this respect, previous studies had suggested that the β-lactam ring is critical for activity,[20,21] with a related open β-amino acid being inactive.[21] As shown in an overlay of the geometry optimized structures (*ab initio* minimization, B3LYP/6-31G*)[22] of the β-lactam in **10** with azetidine **15**, the puckered azetidine ring results in only minor perturbations of the positioning of the side chains compared to the flatter β-lactam. On the basis of this analysis it might be anticipated that the azetidine ring could serve as an appropriate replacement of the β-lactam.

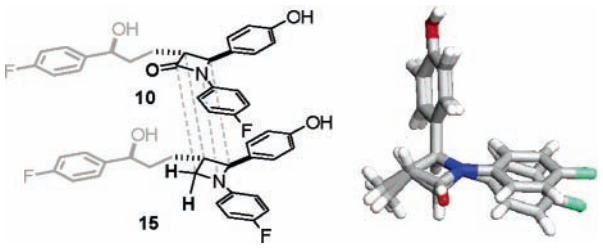
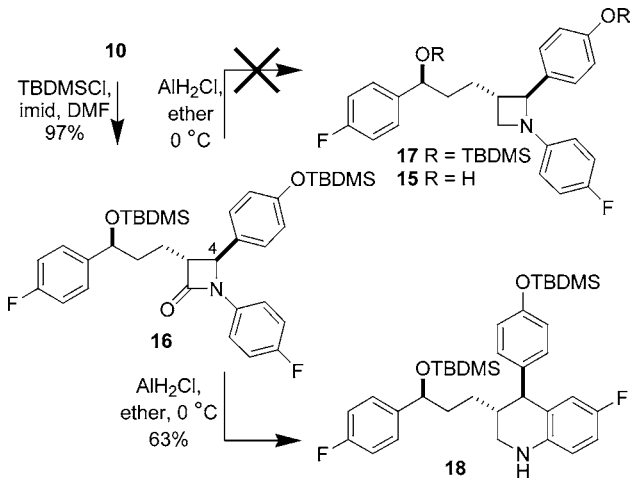


Figure 2. Pictorial representation of the overlay of ezetimibe (**10**) and the azetidine **15**. Geometry-optimized overlay at right with the flexible C3 side chain omitted for clarity.[22]

In our initial attempts to prepare an azetidine possessing the side chains found optimal in ezetimibe, we observed that the electron-rich phenol at C-4 renders the azetidine unstable. Attempts at reduction of the β-lactam with AlH_2Cl[23] led to the isolation of rearranged products including predominantly **18**[24] (Scheme 3). Reduction of **16** to the corresponding 1,3-amino alcohol and closure to the azetidine **17** ($LiAlH_4$, then CBr_4, Ph_3P) permitted its isolation. However, upon deprotection azetidine **15** proved unstable.

Scheme 3. Attempted Synthesis of Azetidine **15**



(17) Gervay, J.; Flaherty, T. M.; Holmes, D. *Tetrahedron* **1997**, *53*, 16355−16364.

(18) For the synthesis of ezetimibe (**10**), see: Wu, G. Z.; Wong, Y. S.; Chen, X.; Ding, Z. X. *J. Org. Chem.* **1999**, *64*, 3714−3718.

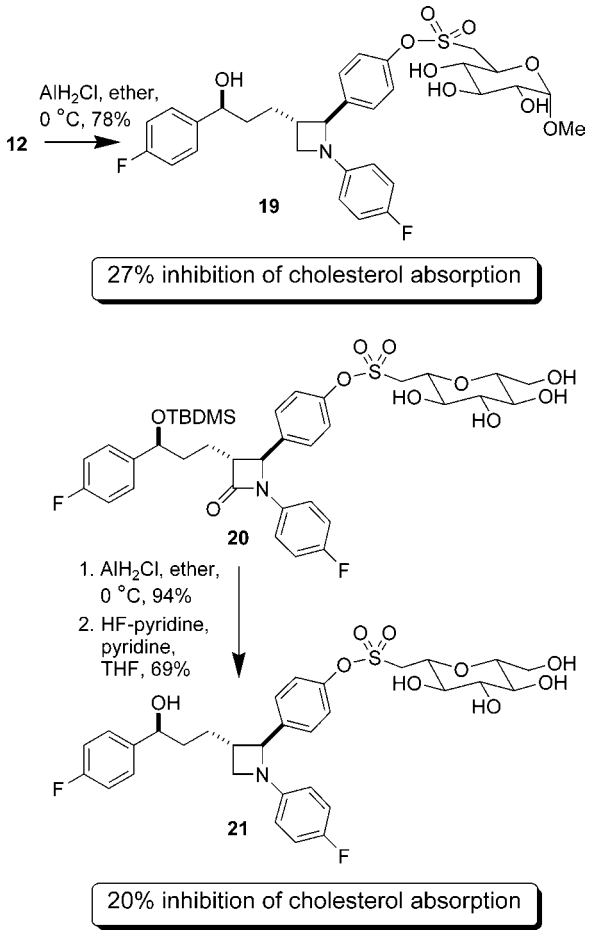
(19) Comparable *in vitro* results were obtained for the corresponding *O*-glycosides lacking the sulfonyl linkage, see ref 4.

(20) Dugar, S.; Kirkup, M. P.; Clader, J. W.; Lin, S. I.; Rizvi, R.; Snow, M. E.; Davis, H. R.; McCombie, S. W. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2947−2952.

(21) Clader, J. W.; Burnett, D. A.; Caplen, M. A.; Domalski, M. S.; Dugar, S.; Vaccaro, W.; Sher, R.; Browne, M. E.; Zhao, H. R.; Burrier, R. E.; Salisbury, B.; Davis, H. R. *J. Med. Chem.* **1996**, *39*, 3684−3693.

We reasoned that substitution of the electron-rich aromatic group by an electron-deficient sulfonylated aryl would lead to a stable azetidine analogue.[25] As outlined in Scheme 4,

Scheme 4. Sulfonylated Azetidine Glycoconjugates



reduction of the β-lactam ring could be carried out using AlH_2Cl[22] to give azetidine sulfonyl conjugates **19** and **21** in 78 and 94% yield, respectively, following deprotection. Interestingly, the azetidine glycoconjugates **19** and **21** displayed activities as cholesterol absorption inhibitors in the brush border membrane vesicle assay (27% and 20% inhibition, respectively) similar to that observed for the β-lactams counterparts **12** and **13** (20% and 15% inhibition, respectively). Thus, the use of a sulfonylated sugar permits the synthesis of stable glycosylated azetidine analogues of ezetimibe.

In summary, we have documented the synthesis of readily available carbohydrate sulfonyl chlorides. We specifically demonstrated the use of these by conjugation to the phenol of ezetimibe, a potent cholesterol absorption inhibitor. Furthermore, we highlight an advantage of the electron-withdrawing nature of the sulfonyl linkage which allowed the synthesis of otherwise unstable azetidine conjugates. The novel sulfonylated glycosides all displayed high *in vitro* efficacies as cholesterol absorption inhibitors. The use of a sulfonylation reaction for the synthesis of carbohydrate conjugates has potentially wider applications which includes sulfonamide linkages.[26] Carbohydrate sulfonyl chlorides form the basis of a versatile method for conjugation which could find broader use in the syntheses of glycoconjugates.

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Supporting Information Available: Detailed descriptions of experimental procedures and analytical data for compounds **1**−**9**, **11**−**14**, and **19**−**21**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(22) *Ab initio* calculations: Initial energy-minimization by molecular mechanics using MacroModel v. 7.0 [pseudosystematical Monte Carlo conformational search (100 steps, limit 30 kJ/mol) with $CHCl_3$ as solvent, MMFFs force field] was followed by a quantum-mechanical geometry optimization using Jaguar v 4.2 (B3LYP/6-31G*) in Maestro v 4.1.

(23) (a) Yamashita, M.; Ojima, I. *J. Am. Chem. Soc.* **1983**, *105*, 6339−6342. (b) Ojima, I.; Zhao, M. Z.; Yamato, T.; Nakahashi, K.; Yamashita, M.; Abe, R. *J. Org. Chem.* **1991**, *56*, 5263−5277. (c) Ojima, I.; Yamato, T.; Nakahashi, K. *Tetrahedron Lett.* **1985**, *26*, 2035−2038.

(24) As a working hypothesis, we believe that azetidine **17** could open up to an intermediate benzylic carbocation greatly facilitated by electron-donating substituents. This carbocation could subsequently undergo Friedel−Crafts reaction with the adjacent aniline and furnish **18**.

(25) Ritter, T.; Stanek, K.; Larrosa, I.; Carreira, E. M. *Org. Lett.* **2004**, *6*, 1513−1514.

(26) In contrast to phenols, conjugation via primary and secondary alcohols may be problematic, as the sulfonate esters could suffer a number of fates including substitution and elimination. However, in the context of oligonucleotide analogue synthesis the work of Widlanski (ref 11) demonstrates the stability of sulfonate-linked oligonucleotides derived from secondary alcohols.