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Preparation of Thioacid-Containing Amino Acids and Peptides and Their Application in Ligation Reactions

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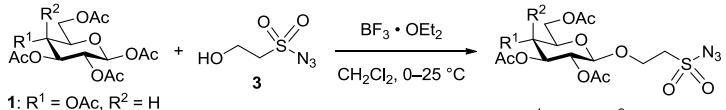
Introduction

Ligation reactions have emerged as an important tool for the chemoselective conjugation of large and complex molecules. One recent example is the reaction of thiocarboxylic acids (thioacids) with electron-deficient organic azides, such as sulfonyl azides [1]. This reaction proceeds at room temperature in different solvents and leads to *N*-acylsulfonamides in excellent yields. In the peptide field, this ligation reaction has been applied for the synthesis of neoglycopeptides [2], peptide mimetics [3], C-terminal labeling of peptide thioacids [4], and labeling of sulfonyl azide-modified peptides [5]. Here we present the preparation of hitherto unknown amino acids and peptides with protected side chain thioacids and their application in ligation reactions with sulfonyl azide-substituted carbohydrates.

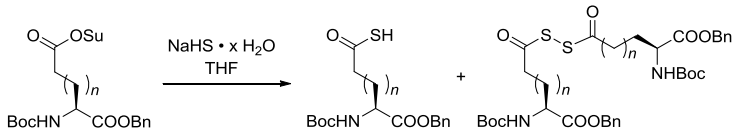
Results and Discussion

Sulfonyl azide-modified carbohydrates **4** and **5** were obtained by Lewis acid-promoted glycosylation of 2-hydroxyethanesulfonyl azide **3** with peracetylated β-D-gluco- (**1**) and β-D-galactopyranose (**2**) (Scheme 1).



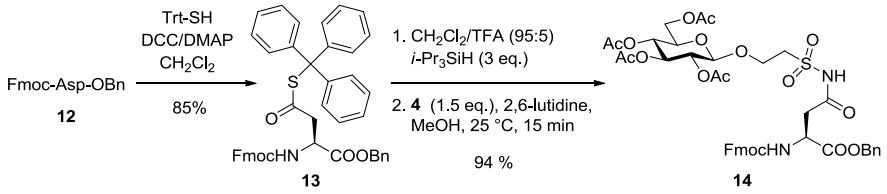
Scheme 1. Preparation of sulfonyl azide-modified carbohydrates.

For the synthesis of thioacid-containing amino acids, we initially reacted Boc-Asp(OSu)-OBn **6** and Boc-Glu(OSu)-OBn **7**, respectively, with sodium hydrogen sulfide to yield the thioacids Boc-Asp(SH)-OBn **8** and Boc-Glu(SH)-OBn **9**, respectively (Scheme 2). These products, however, were accompanied by small amounts (up to 20%) of diacyl disulfides **10** and **11**, respectively, due to oxidation. Fmoc-Asp(SH)-OBn was obtained following an analogous route. The thioacid/diacyl disulfide mixtures could, after work-up, be directly employed in subsequent ligation reactions with sulfonyl azides **4** and **5** giving access to the corresponding *N*-acylsulfonamides in yields between 83% and 94%.



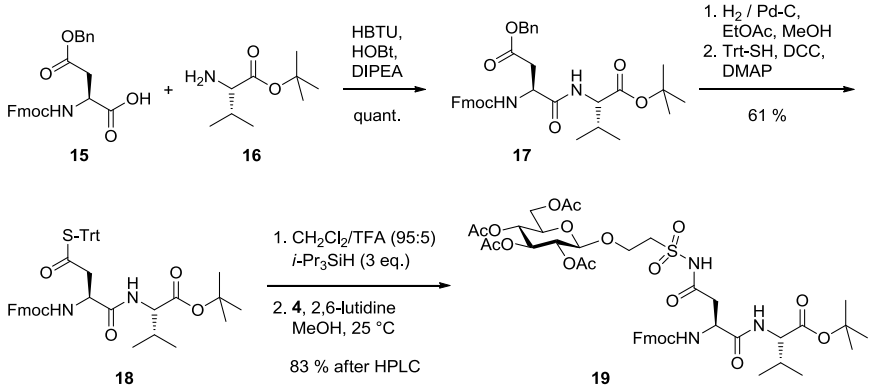
Scheme 2. Synthesis of thioacid-containing amino acids. Su = succinimidyl.

Improved yields of thioacids were obtained from trityl thioesters by treatment with 5% TFA in CH_2Cl_2. As depicted in Scheme 3, Fmoc-Asp-OBn **12** was converted to Fmoc-Asp(STrt)-OBn **13** by activation with DCC/DMAP. Thioacid Fmoc-Asp(SH)-OBn was generated by TFA treatment and, after evaporation, reacted with sulfonyl azide **4** to give ligation product **14** in 94% yield. It is worth mentioning that, under these conditions, the formation of diacyl disulfides was not observed.



*Scheme 3. Synthesis of thioacid from trityl thioester and subsequent ligation reaction with sulfonyl azide-modified glucose **4**. Trt = trityl.*

Scheme 4 shows the preparation of a thioacid-containing dipeptide and its ligation to sulfonyl azide-modified glucose **4**. Fmoc-Asp(OBn)-OH **15** and H-Val-O*t*Bu **16** were coupled to give dipeptide **17**. After side chain deprotection, trityl thioester **18** was obtained via DCC/DMAP activation and reaction with triphenylmethanethiol. Employing the same reaction conditions described in Scheme 3, neoglycopeptide **19** was obtained in 83% yield after purification by RP-HPLC.



*Scheme 4. Synthesis of a thioacid-containing dipeptide and subsequent ligation with sulfonyl azide-modified glucose **4**.*

In summary, we have shown that the thioacid/sulfonyl azide ligation can be efficiently used for the conjugation of sulfonyl azide-modified carbohydrates to amino acids and peptides. Trityl thioesters turned out to be excellent precursors for the preparation of thioacids without concurrent formation of diacyl disulfides. Importantly, the modification of peptides containing thioacid derivatives of aspartic acid occurred without concurrent aspartimide formation.

Acknowledgments
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References

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