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Highly Efficient Coupling of β-Substituted Aminoethane Sulfonyl Azides with Thio Acids, toward a New Chemical Ligation Reaction

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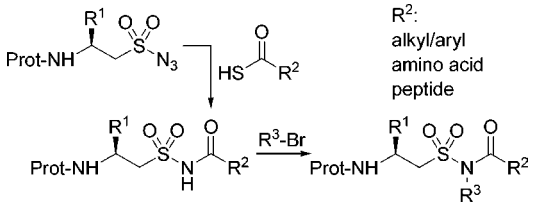
ABSTRACT



A highly efficient coupling of protected β-substituted aminoethane sulfonyl azides with thio acids is reported. In the case of peptide thio acids, this method encompasses a new chemoselective ligation method. Furthermore, the resulting α-amino acyl sulfonamides can be alkylated with suitable electrophiles to obtain densely functionalized sulfonamide scaffolds.

Today, there is tremendous interest in the development of new chemoselective (bio)conjugation or ligation reactions for coupling of large and complex molecules.[1] Among these are the reaction of an aldehyde/ketone with a hydrazide to yield a hydrazone,[2] an aldehyde/ketone with an amino-oxy moiety to yield an oxime,[3] a (peptide) thio ester with a cysteine to yield a native amide bond,[4] a triphenylphosphine (thio) ester with an azide to yield an amide bond,[5] and the reaction between an acetylene with organic azides to yield the corresponding 1,4-disubstituted 1,2,3-triazoles. The latter reaction is also known from click chemistry, as was recently independently developed by Meldal[6] and Sharpless.[7]

The reaction of an azide with a thio acid has been described in the literature.[8] Recently, the reaction between a thio acid and a sulfonyl azide has been reinvestigated by Williams et al.[9] It was found that thio acids react chemoselectively with azides to α-amino acyl sulfonamides via a concerted [2 + 3] cycloaddition and formation of a thiatriazoline intermediate.

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(3) Lelièvre, D.; Turpin, O.; El Kazzouli, S.; Delmas, A. *Tetrahedron* **2002**, *58*, 5525.

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The application of peptide-based thio acids and amino acid-derived sulfonyl azides in this chemoselective coupling reaction would provide an entry toward large peptide mimic systems with a high degree of chemical diversity because of the presence a β-substituted aminoethane sulfonamide residue in the ligated product (Figure 1). We have previously

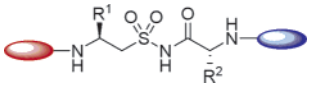
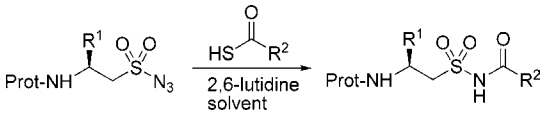


Figure 1. General structure of the α-amino acyl/peptidyl sulfonamides.

reported the synthesis of N-protected β-aminoethane sulfonyl chlorides.[10] Recently, we also found that the conversion of the chlorides into the corresponding azides proceeded with good to excellent yields.

First, the coupling of either Cbz- or Fmoc-protected β-aminoethane sulfonyl azides was investigated, which were derived from the amino acids glycine (entries 1−4), phenylalanine (entries 5−8), valine (entries 9 and 10), and (protected) serine (entries 11 and 12) (Scheme 1, Table 1).[11]

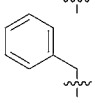
Scheme 1. Synthesis of N-β-Protected Acyl Sulfonamides



These sulfonyl azides reacted smoothly with thioacetic acid or thiobenzoic acid at room temperature: the reaction was complete in 15 min, and the resulting acyl sulfonamides (**1**−**12**) could be isolated in good to excellent yields (Table 1). As a solvent, $CHCl_3$ was used in entries 1−4, but because of the low solubility of the other sulfonyl azides, DMF was a good alternative for reactions in entries 5−12.

Then, as a first step toward chemical ligation of amino acids and peptides, the thio acid derived from Boc-leucine

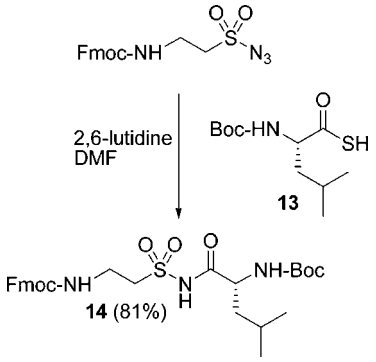
Table 1. Synthesis of N-β-Protected Acyl Sulfonamides

entry	Prot	R^1	R^2	sulfonamide[a,b]	solvent
1	Fmoc	H	Me	**1** (90%)	$CHCl_3$
2	Fmoc	H	Ph	**2** (quant.)	$CHCl_3$
3	Cbz	H	Me	**3** (quant.)	$CHCl_3$
4	Cbz	H	Ph	**4** (quant.)	$CHCl_3$
5	Fmoc		Me	**5** (91%)	DMF
6	Fmoc		Ph	**6** (87%)	DMF
7	Cbz		Me	**7** (95%)	DMF
8	Cbz		Ph	**8** (96%)	DMF
9	Fmoc		Me	**9** (95%)	DMF
10	Fmoc		Ph	**10** (96%)	DMF
11	Fmoc		Me	**11** (93%)	DMF
12	Fmoc		Ph	**12** (94%)	DMF

[a] Reagents and conditions: 1 equiv of sulfonyl azide, 1.3 equiv of thio acid, 1.3 equiv of 2,6-lutidine, solvent, rt, 15 min. [b] Yield of isolated product.

was used (Scheme 2). Boc-Leu-SH (**13**) was synthesized from Boc-protected leucine hydroxysuccinimide ester (Boc-Leu-ONSu) by treatment with sodium hydrogensulfide (NaHS) as was essentially described by Goldstein and Gelb.[12] In our hands, the reaction workup and purification of **13** was

Scheme 2. Synthesis of (α-Amino) Acyl Sulfonamide **14**[a]



[a] Reagents and conditions: 1 equiv of sulfonyl azide, 1.3 equiv of dipeptide thio acid, 1.3 equiv of 2,6-lutidine, DMF, rt, 15 min. Yield is based on isolated product.

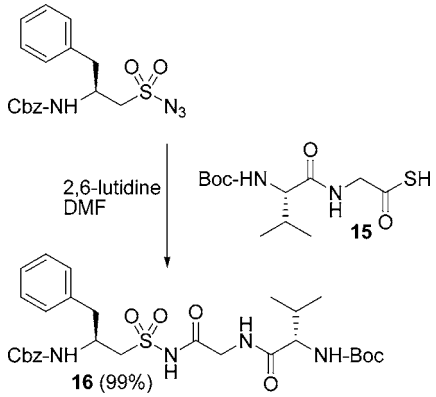
(10) Brouwer, A. J.; Monnee, M. C. F.; Liskamp, R. M. J. *Synthesis* **2000**, 1579.

(11) **General Procedure for the Synthesis of N-β-Protected Acyl Sulfonamides.** To a mixture of Fmoc-Phe-Ψ[CH_2SO_2]-N_3 (248 mg, 0.54 mmol) and 2,6-lutidine (81 μL, 0.70 mmol, 1.3 equiv) in DMF (2.5 mL) was added dropwise thiobenzoic acid (82 μL, 0.70 mmol, 1.3 equiv). After the addition of each drop, the evolution of N_2 could be observed and the color of the reaction mixture turned to pale yellow. After completion of the addition, the reaction mixture was allowed to react for 15 min at room temperature. Then, the reaction mixture was evaporated to dryness, and the residue was crystallized from EtOAc/hexane. Compound **6** was obtained as a white solid in 87% yield. R_f = 0.61 (EtOAc/hexane, 4:1 v/v). Mp: 234 °C (dec). $[\alpha]^{23}_D$ −21.8 (*c* 0.1 DMF). ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.18 (1H, broad s), 7.91 (4H, m), 7.58−7.19 (15H, broad m), 4.19−4.03 (4H, m), 3.82 (1H, m), 3.63 (1H, m), 2.85 (2H, m). ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.8, 155.6, 144.3, 141.1, 138.1, 133.6, 132.1, 129.7, 129.0, 128.7, 128.1, 127.5, 126.8, 125.6, 120.6, 65.7, 56.2, 48.9, 47.0. ESMS: calcd for $C_{31}H_{28}N_2O_5S$ 540.17, found 541.55 $[M + H]^+$, 563.30 $[M + Na]^+$, 539.45 $[M - H]^-$. Anal. Calcd for $C_{31}H_{28}N_2O_5S$: C, 68.87; H, 5.22; N, 5.18. Found: C, 68.97; H, 5.16; N, 5.06.

very troublesome, and it was decided to use the crude Boc-Leu-SH. Nevertheless, its coupling to sulfonyl azide Fmoc-Gly-Ψ[CH_2SO_2]-N_3 proceeded very well, and α-(amino) acyl sulfonamide **14** was obtained in 81% yield.

Next, we attempted the reaction of a dipeptide thio acid **15**[12] with Cbz-Phe-Ψ[CH_2SO_2]-N_3 to obtain tripeptide mimic **16** (Scheme 3). Under identical conditions, the azide reacted

Scheme 3. Synthesis of Peptidyl Sulfonamide **16**[a]



[a] Reagents and conditions: 1 equiv of sulfonyl azide, 1.3 equiv of dipeptide thio acid, 1.3 equiv of 2,6-lutidine, DMF, rt, 15 min. Yield is based on isolated product.

smoothly with the dipeptide thio acid to give the orthogonally protected tripeptide mimic **16** containing a sulfonamide in 99% yield. Both amino groups can be selectively deprotected, thereby allowing also a selective elongation of the peptide chain in either direction.

Furthermore, to investigate the potential of this reaction as a novel chemoselective (bio)conjugation or chemical ligation reaction, the reaction of entry 2 (Table 1) was also carried out in a more aqueous system, that is, THF/H_2O 2:1 v/v. Successfully, acyl sulfonamide **2** was obtained in a quantitative yield. Moreover, neither Cbz-GlyΨ[CH_2SO_2)-NH_2 nor Cbz-GlyΨ[CH_2SO_2)-N_3 reacted (after 3 days of stirring) with thioacetic acid and acetic acid, respectively, to give acyl sulfonamide **3**. These experiments underline the chemoselective character of the sulfonyl azide/thio acid reaction pair.

We took advantage of the increased acidity of the sulfonamide NH[13] in a chemoselective N-alkylation reaction (Scheme 4).[14] First, alkylation of **3** was attempted with allylbromide in the presence of 1 equiv of K_2CO_3 in DMF.

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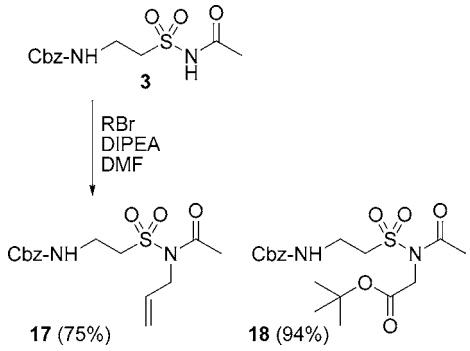
Scheme 4. N-Alkylation of Acyl Sulfonamide **3**[a]

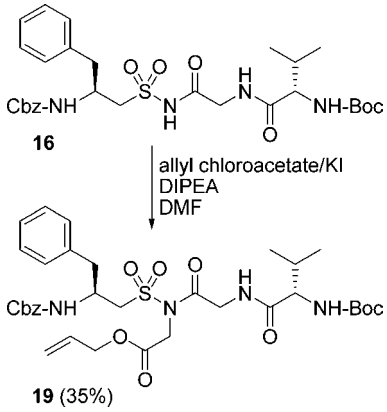


[a] Reagents and conditions: 1 equiv of **3**, 1.05 equiv of bromide, 2 equiv of DIPEA, DMF, rt, 16 h. Yields are based on isolated product.

However, the yield of **17** was only 17%. Increasing the amount of K_2CO_3 to 3 equiv, in the presence of 2 equiv of allylbromide, also resulted in alkylation of the urethane NH, and the dialkylated product was isolated in 15% yield. These conditions clearly eradicated any chemoselectivity. Fortunately, when DIPEA (2 equiv) was used, the chemoselective alkylation product **17** was obtained in 75% yield. Employing this chemoselective alkylation protocol, we employed *tert*-butyl bromoacetate as an electrophile, and alkylation product **18** was obtained in 94% yield (Scheme 4).

Finally, when we tried to alkylate tripeptide mimic **16** with allyl chloroacetate, thus introducing a third orthogonality in a protecting group strategy (Scheme 5), alkylation product

Scheme 5. N-Alkylation of Tripeptide Mimic **16**[a]



[a] Reagents and conditions: 1 equiv of **16**, 2 equiv of allyl chloroacetate, 1 equiv of KI, 4 equiv of DIPEA, DMF, 40 °C, 16 h. Yield is based on isolated product.

19 could only be isolated in a very small amount. However, the addition of KI (1 equiv) and increasing the amount of electrophile (2 equiv) and base (4 equiv) at a reaction temperature of 40 °C allowed the alkylation product **19** to be isolated in 35% yield. Mass spectrometric analysis showed that only monoalkylated product was present, and

NMR analysis (500 MHz, ^{1}H, COSY, TOCSY, HSQC, and HMBC) verified the correct position of the *N*-alkyl group.

In conclusion, we have developed a high-yielding synthesis of (α-amino) acyl sulfonamides and peptidyl sulfonamides featuring the use of N-protected *β*-aminoethane sulfonyl azides and (amino) thio acids or peptide thio acids. The high yields, also in aqueous solvents, points to the possibility of using this reaction in bioconjugation and chemical ligation protocols. Furthermore, the efficient chemoselective N-alkylation procedure may provide an entry into attachment of an additional peptide chain and thereby knotted peptide systems.

The chemical ligation of larger peptide thio acids and peptido-peptidosulfonamide azides is currently under investigation.

Acknowledgment. We thank Dr. Johan Kemmink for measuring the 500 MHz NMR spectra and Hans Jacobs for assistance with the HPLC analyses.

Supporting Information Available: Experimental procedures, spectroscopic data (^{1}H and ^{13}C), and HPLC data for compounds **1**−**19**. This material is available free of charge via the Internet at http://pubs.acs.org.

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