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Resin-Bound Sulfonyl Azides: Efficient Loading and Activation Strategy for the Preparation of the *N*-Acyl Sulfonamide Linker

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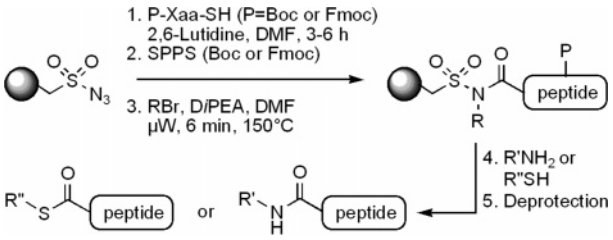
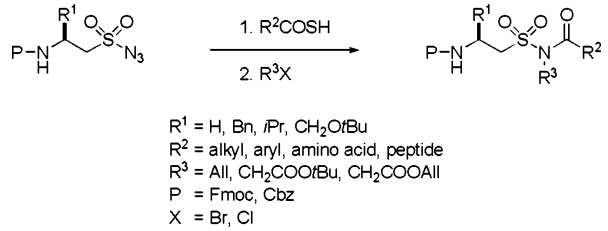


This paper describes an optimized protocol for the efficient loading of resin-bound aminoethane sulfonyl azides by either Boc- or Fmoc-protected amino thioacids. The resulting *N*-acyl sulfonamide is a convenient linker for use in Boc- or Fmoc-based solid-phase peptide synthesis. Activation of the *N*-acyl sulfonamide via a microwave-assisted alkylation procedure and subsequent treatment with functionalized nucleophiles yields C-terminally modified peptides that can be applied in chemoselective (bio)conjugation or ligation reactions.

The reaction between a sulfonyl azide and a thio acid proceeds in a very efficient and chemoselective way to give *N*-acyl sulfonamides.[1−4] These *N*-acyl sulfonamides find applications in medicinal chemistry,[2] as a linker for anchoring to the solid support,[3] and in chemoselective ligation and (bio)conjugation reactions.[1,4] Recently, we reported the highly efficient coupling of β-substituted aminoethane sulfonyl azides with thio acids derived from amino acids and peptides.[4] The resulting α-amino acyl sulfonamides could be efficiently and chemoselectively N-alkylated in the presence of base and an electrophilic halide (Scheme 1). Here, we report our results with resin-bound sulfonyl azides as a novel approach to obtain the *N*-acyl sulfonamide linker.

SCHEME 1. General Structure of the N-Alkylated *N*-Acyl Sulfonamides



The *N*-acyl sulfonamide linker, as originally introduced by Kenner,[5] is stable toward acidic and basic conditions and thus compatible with both Boc and Fmoc SPPS methods. After N-alkylation, this linker becomes sensitive toward cleavage by nucleophiles in inter- or intramolecular reactions.

The original Kenner linker suffered from poor loading efficiencies, racemization in the loading step, and a low reactivity of the alkylated (activated) linker. Backes and Ellman[6] addressed these problems by the introduction of a more nucleophilic aliphatic *N*-acyl sulfonamide linker that could be loaded with minimal racemization by lowering the temperature. The susceptibility for cleavage from this linker was increased by alkylation with an electron-withdrawing haloacetonitrile rather than diazomethane. Although these modifications allowed for the synthesis of cyclic and C-terminally modified peptides,[3] the loading of the first amino acid onto the sulfonamide linker is still of major concern with respect to both efficiency and degree of epimerization. Moreover, alkylation with a haloacetonitrile is not always efficient.[3d] These key steps still require improvement.[7] We have addressed these issues by using a resin-bound sulfonyl azide/thioacid amidation as the loading step, followed by microwave-assisted N-alkylation of the peptidyl *N*-acyl sulfonamide. (Scheme 2).

The sulfonyl azide linker was efficiently synthesized on a multigram scale starting from taurine.[8] Attachment to the resin was achieved via BOP coupling with a carboxylic acid func-

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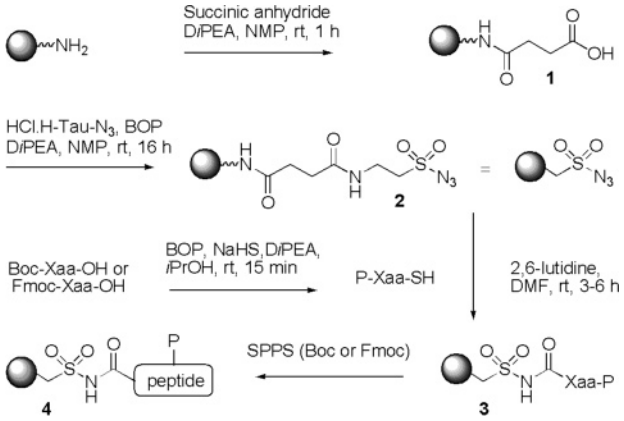
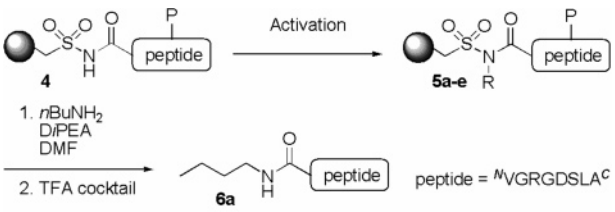
SCHEME 2. Preparation of Resin-Bound Sulfonyl Azide

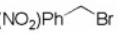
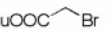
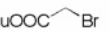
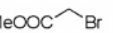


tionalized resin **1**. The coupling reactions were monitored by the Kaiser test[9] (free amines) and a Malachite green test[10] (free carboxylates), respectively. Then, the resin-bound sulfonyl azide was treated with Boc-Phe-SH[11] in the presence of 2,6-lutidine as base in DMF for 3 h at room temperature. The thioacid/azide coupling has been shown to be effective for Boc- and Fmoc-protected amino acids,[1,4] and under these mild reaction conditions, amino thio acids are configurationally stable as shown earlier by Williams et al.[1a] After removal of the Boc group with TFA, Fmoc-Gly-OH was coupled with BOP/D*i*PEA, and the loading yield was calculated via an Fmoc determination[12] and indicative of a yield of 73% over five reaction steps. Alternatively, Fmoc-Ser(*t*Bu)-OH was preactivated with BOP/D*i*PEA and converted into the corresponding thio acid by the addition of a solution of NaHS in *i*PrOH.[13] Fmoc-Ser(*t*Bu)-SH was used without further purification in the coupling reaction with the resin-bound sulfonyl azide. In this case, a high loading efficiency of 85% was obtained after 6 h. Similarly, coupling of Boc-Phe-SH—which was synthesized via the in situ BOP/D*i*PEA/NaHS approach—followed by TFA treatment and a BOP/D*i*PEA-mediated coupling of Fmoc-Gly-OH resulted in an excellent loading yield of 94%. Thus, the BOP/D*i*PEA/NaHS approach enables the direct synthesis and loading to the sulfonyl azide resin of Boc- or Fmoc-protected amino thioacids.[14] As a model peptide, an octameric RGD sequence, Boc-Val-Gly-Arg-(Pbf)-Gly-Asp(O*t*Bu)-Ser(*t*Bu)-Leu-Ala∼, was synthesized (vide infra).

Following the loading, we studied the microwave-assisted alkylation reaction of the sulfonamide Cbz-Tau-NHAc (P = Cbz, R^1 = H, R^2 = CH_3, and R^3 = H, Scheme 1). This reaction was optimized in solution (Table S1, Supporting Information), and the best results were obtained with allylbromide, 4-nitrobenzyl bromide, and *tert*-butyl bromoacetate rather than haloacetonitrils, with yields ranging from 75 to 94%. These optimized reaction conditions were used to alkylate the resin-bound *N*-peptidyl sulfonamide as is shown in Table 1.

TABLE 1. Activation and Cleavage Efficiency of Resin-Bound *N*-Peptidyl Sulfonamide



Entry	Electrophile	5	% Yield (% Purity)[a]
1[b]		a	52 (72)
2[b]		b	68 (86)
3[b]		c	48 (84)
4[c]		c	37 (100)
5[b]		d	66 (84)
6[d]	$TMSCHN_2$	e	89 (74)

[a] Yields are based on the crude cleavage product; purities are assessed by analytical HPLC. [b] Reaction conditions: alkylbromide (20 equiv), D*i*PEA (10 equiv) in DMF, 6 min, 150 °C μW; then, *n*$BuNH_2$ (10 equiv), D*i*PEA (10 equiv) in DMF, rt, 24 h. [c] Reaction conditions: alkylbromide (20 equiv), D*i*PEA (10 equiv) in DMF, 16 h, rt, then *n*$BuNH_2$ (10 equiv), D*i*PEA (10 equiv) in DMF, rt, 24 h. [d] Reaction conditions: $TMSCHN_2$ (2 M in hexane/THF, 350 equiv), 2 h, rt,[3b] then, *n*$BuNH_2$ (10 equiv), D*i*PEA (10 equiv) in DMF, rt, 24 h.

Among the bromide series, alkylation with 4-nitrobenzyl bromide (entry 2) followed by cleavage with *n*butylamine proved to be the most efficient. The combination of isolated yield and purity of the C-terminally modified RGD peptide was comparable to alkylation with trimethylsilyl diazomethane and subsequent cleavage with *n*butylamine (entry 6). Apparently, efficient alkylation and activation resulting from its electron-withdrawing properties make 4-nitrobenzyl bromide[7a,c] a promising alternative for iodoacetonitrile, bromoacetonitrile, or trimethylsilyl diazomethane.[15] The conditions of entry 2 were ultimately used to evaluate the possibility of C-terminal modification of an RGD peptide by functionalized nucleophiles (vide infra).

For synthesis of the RGD-containing peptide, resin-bound sulfonyl azide **2** was first treated with Boc-Ala-SH as described (vide supra). Then, the Boc group was removed by acidolysis with TFA, and the α-amino group was acylated with Fmoc-Leu-OH in the presence of BOP/D*i*PEA as the condensing agent. The remaining amino acid residues were coupled via Fmoc/*t*Bu solid-phase peptide synthesis protocols, and the final amino acid (valine) was introduced as the corresponding Boc derivative. Then, resin **4** was subjected to a microwave-assisted alkylation with 4-nitrobenzyl bromide as the electrophile in the

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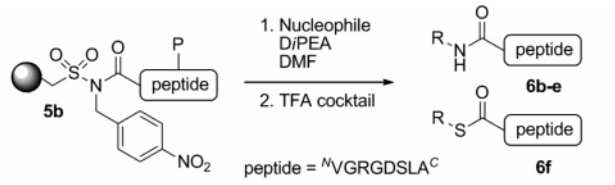
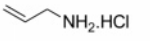
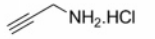
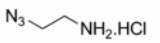
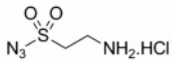
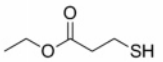
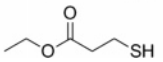
(15) Trimethylsilyl diazomethane provides a more efficient alkylation (see ref 3c), while haloacetonitrile provides an enhanced reactivity toward nucleophilic displacement (see ref 6).

TABLE 2. Synthesis of C-Terminally Modified RGD Peptides



Entry‡	Nucleophile	6	% Yield (% Purity)[a]
1[b]		b	57 (82)
2[b]		c	59 (81)
3[b]		d	96 (83)
4[b]		e	intractable material
5[c]		f	46 (92)
6[d]		f	52 (92)

[a] Yields are crude based on the crude cleavage product; purities are assessed by analytical HPLC. [b] Reaction conditions: R-NH_2 (10 equiv), D*i*PEA (10 equiv) in DMF, rt, 24 h. [c] Reaction conditions: R-SH (50 equiv), D*i*PEA (10 equiv), sodium-thiophenolate (0.5 equiv) in DMF, rt, 24 h. [d] Reaction conditions: R-SH (50 equiv), D*i*PEA (10 equiv) in DMF, rt, 24 h. ‡ Ref 22.

presence of D*i*PEA as the base in DMF for 6 min at 150 °C. The activated peptidyl sulfonamide resin **5b** was treated with a suitable nucleophile with D*i*PEA as base in DMF for 24 h at room temperature. After this, the DMF solution was evaporated in vacuo, and the residue was treated with a mixture of TFA and scavengers to remove all protecting groups. The TFA solution was diluted with MTBE/hexane, and the precipitated peptide was collected by centrifugation and subsequently lyophilized. The yield was based on the weight of the crude peptide, and the purity and identity were assessed by HPLC and LC-MS, respectively (Table 2).

As the RGD motif plays an important role in cell−cell adhesion processes,[16] the introduction of functional groups in the nucleophile facilitates the chemoselective coupling of this peptide to, among others, fluorophores, affinity tags, proteins, and dendrimer scaffolds.[17]

Suitable nucleophiles were therefore selected to obtain C-terminally modified peptides that can be used in (ring-closing) metathesis or Diels−Alder reactions (entry 1),[18] Cu(I)-catalyzed click reactions (entries 2 and 3),[19] Staudinger ligations (entry 3),[20] sulfonyl azide-based ligation reactions (entry 4),[1,4] or native chemical ligations (entries 5 and 6).[21]

The nitrogen-based nucleophiles, allylamine (entry 1), propargylamine (entry 2), and 2-azidoethylamine (entry 3), resulted in good to excellent overall cleavage yields with purities of approximately 80%. However, nucleophilic displacement with tauryl sulfonyl azide (entry 4) resulted in a mixture of compounds that was difficult to characterize, probably due to sensitivity of the sulfonyl azide moiety toward nucleophiles.[8] The peptide thio ester C-terminally modified with ethyl 3-mercaptopropionate was isolated in a fair to good yield with an excellent purity (entry 5 and 6). Interestingly, this peptide thio ester could also be isolated in the absence of sodium-thiophenolate (entry 6), indicating that the 4-nitrobenzyl moiety is a good activator. This result was an indication that alkylation with 4-nitrophenyl bromide resulted in a linker that was more reactive than the methylated congener as obtained with trimethylsilyl diazomethane in line with the data reported by Link et al.[7c] and Kiessling et al.[7e] Kiessling and co-workers also found that activation by allylation was efficient to obtain peptide thio esters without the addition of sodium-thiophenolate. If we compare resin **5a** and **5b** (Table 1), it is clear that **5b** is more susceptible toward nucleophilic displacement since a better yield and higher purity of the C-terminally modified peptide **6a** is obtained.

In conclusion, this paper describes an optimized protocol for the loading efficiency of the first amino acid building block on the *N*-acyl sulfonamide linker by reacting a resin-bound sulfonyl azide and a thio acid. These amino acid-based thio acids have been prepared in situ by a preactivation with BOP/D*i*PEA in the presence of NaHS. The mild reaction conditions allow the efficient synthesis of Boc- as well as Fmoc-protected thio acids. Moreover, the activation of the *N*-peptidyl sulfonamide resin has been optimized and relies on a microwave-assisted alkylation with 4-nitrobenzylbromide. This activated *N*-peptidyl sulfonamide linker is more active toward nucleophiles as compared to activation by methylation and allylation. In this way, efficient alkylation could be combined with a high linker reactivity in a straightforward procedure. Thus, these results contribute to a better access toward C-terminally modified peptides with respect to overall yield, purity, and diversity.

Experimental Section

Synthesis of the Resin-Bound β-Aminoethane Sulfonyl Azide Linker 2. A portion of resin (3 g; ~0.8 mmol) was allowed to swell in NMP for 1 h. Subsequently, the resin was washed with 50% HOAc in NMP (10 mL; 2 × 10 min), NMP (10 mL; 2 × 2 min), *i*PrOH (10 mL; 1 × 2 min), NMP (10 mL; 2 × 2 min), 20% D*i*PEA in NMP (10 mL; 1 × 2 min), NMP (10 mL; 3 × 2 min), *i*PrOH (10 mL; 1 × 2 min), and finally NMP (10 mL; 3 × 2 min). A solution of succinic anhydride (801 mg; 8 mmol) and D*i*PEA (1.4 mL; 8 mmol) in NMP (10 mL) was added to the resin, and the resulting mixture was shaken for 1 h before the resin was filtered and washed with NMP (10 mL; 3 × 2 min), *i*PrOH (10 mL; 1 × 2 min), and NMP (10 mL; 3 × 2 min). Completion of the reaction was confirmed by a negative Kaiser test[9] and a positive Malachite

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(21) (a) Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. H. *Science* **1994**, *266*, 776. (b) Dawson, P. E.; Kent, S. B. H. *Annu. Rev. Biochem.* **2000**, *69*, 923.

(22) H_2S was also used in this procedure to obtain the peptide thio acid. However, the isolated peptide was characterized as a mixture of the thio and oxy acids due to hydrolysis of the thio acid (compound **6g**, Supporting Information). Alternatively, the approach of Vetter[14]—cleavage by 2,4,6-trimethoxy benzyl mercaptan—to obtain peptide thio acids did not lead to the desired compound.

green test[10] for acid functionalized resin **1**. Then, a solution of HCl·H-Tau-N_3 (597 mg; 3.2 mmol), BOP (1.4 g; 3.2 mmol), and D*i*PEA (1.1 mL; 6.4 mmol) in NMP (10 mL) was added to the resin, and the obtained reaction mixture was shaken for 16 h. Finally, the resin was filtered and washed with NMP (10 mL; 3 × 2 min), *i*PrOH (10 mL; 1 × 2 min), and DCM (10 mL; 3 × 2 min) and dried under vacuum. The sulfonyl azide functionalized resin **2** was stored for further use.

General Procedure for the Synthesis of N^{α}-Protected Amino Thioacids. An N^{α}-Fmoc- or N^{α}-Boc-protected amino acid (1 equiv; 0.20 mmol), BOP (1.1 equiv), and D*i*PEA (1.5 equiv) in *i*PrOH (10 mL) was stirred for 10 min at room temperature before a solution of NaHS·*x*H_2O (4 equiv) in *i*PrOH (10 mL) was added. The progress of the reaction was monitored by TLC analysis (eluent: EtOAc/hexane/HOAc 9:1:0.1 v/v/v). After 15 min of stirring, the reaction was quenched with 1 N $KHSO_4$ (20 mL), and the reaction mixture was concentrated to a volume of 20 mL. (*Caution: on acidification of the reaction mixture, highly toxic H_2S evolves; this procedure should be performed in a well-ventilated hood.*) The residue was extracted with EtOAc (1 × 40 mL) and subsequently washed with 1 N $KHSO_4$ (2 × 20 mL) and brine (1 × 20 mL) and dried (Na_2SO_4). After removal of the solvent, the crude reaction product was immediately used for coupling to the resin-bound sulfonyl azide **2**.

General Procedure for Loading N^{α}-Protected Amino Thioacids to the Resin-Bound Sulfonyl Azide Linker 2. A portion of dried sulfonyl azide functionalized resin **2** (1 equiv; ~50 μmol) was allowed to swell in DMF (1 mL) for 1 h before the solvent was drained. To the swollen resin, 2,6-lutidine (80 equiv) and the N^{α}-protected amino thioacid (4 equiv) in DMF (800 μL) were added, and the obtained reaction mixture was gently shaken for 3−6 h. The resin was subsequently washed with DMF (1 mL; 3 × 2 min), *i*PrOH (1 mL; 1 × 2 min), and DCM (1 mL; 4 × 2 min). In the case of loading with an N^{α}-Fmoc-protected amino thio acid, the resin was dried under high vacuum overnight before determination of the amino acid loading. In case of loading with an N^{α}-Boc-protected amino thio acid, the Boc group was cleaved to enable coupling of the next amino acid residue.

Typical Procedure for the Alkylation of *N*-Acyl Sulfonamide Resin 4 with 4-Nitrobenzylbromide. A total of 100 mg (15 μmol; 1 equiv) of *N*-acyl sulfonamide resin **4** was transferred to a microwave vessel and swollen in DMF by gentle agitation (1 mL) for 1 h. To this suspension, 4-nitrobenzyl bromide (65 mg; 0.30 mmol; 20 equiv) and D*i*PEA (26 μL; 0.15 mmol; 10 equiv) were added. The vessel was capped and heated for 6 min at 150 °C by microwave irradiation. The solvent was removed by filtration, and the resin was washed with DMF (1 mL; 3 × 2 min), Et_2O (1 mL; 1 × 2 min), and DMF (1 mL; 3 × 2 min). The alkylated resin **5b** was either used immediately or washed with DCM (1 mL; 3 × 2 min), dried, and stored for further use.

Typical Procedure for the Cleavage of Alkylated *N*-Acyl Sulfonamide Resin 5b with an Amine Nucleophile (Entry 3, Table 2). A total of 150 mg (22.5 μmol; 1 equiv) of *N*-acyl sulfonamide resin **5b** was allowed to swell in DMF (0.8 mL) for 1 h. To this suspension, 2-azidoethylamine hydrochloride (28 mg; 225 μmol; 10 equiv) and D*i*PEA (39 μL, 225 μmol; 10 equiv) were added. The mixture was shaken for 24 h at room temperature before the resin was filtered and washed with DMF (5 × 1 mL). The combined DMF washings were evaporated under high vacuum. The crude peptide was dissolved in a mixture of TFA, H_2O, and T*i*S (9.5:0.25:0.25 v/v/v; 2 mL) and stirred for 3 h. The TFA mixture was diluted with MTBE/hexane (1:1 v/v, 2 × 10 mL), and the crude product was collected by centrifugation. Subsequently, the product was redissolved in CH_3CN/H_2O (1:1 v/v) and lyophilized. Finally, the product (18 mg, 96%) was obtained as a white powder. R_t 10.71 min (83% purity); ESMS calcd. for $C_{33}H_{59}N_{15}O_{11}$ 841.45, found 842.70 $[M + H]^+$.

Typical Procedure for the Cleavage of Alkylated *N*-Acyl Sulfonamide Resin 5b with a Thiol Nucleophile (Entry 6, Table 2). A total of 150 mg (22.5 μmol; 1 equiv) of *N*-acyl sulfonamide resin **5b** was allowed to swell in DMF (0.8 mL) for 1 h. To this suspension, ethyl 3-mercaptopropionate (147 μL; 1.13 mmol; 50 equiv) and D*i*PEA (39 μL; 225 μmol; 10 equiv) were added. The mixture was shaken for 24 h at room temperature before the resin was filtered and washed with DMF (5 × 1 mL). The combined DMF washings were evaporated under high vacuum. The crude peptide was dissolved in a mixture of TFA, H_2O, and T*i*S (9.5:0.25:0.25 v/v/v; 2 mL) and stirred for 3 h. The TFA mixture was diluted with MTBE/hexane (1:1 v/v, 2 × 10 mL) to precipitate the product. Subsequently, the product was redissolved in CH_3CN/H_2O (1:1 v/v) and lyophilized. Finally, the product (11 mg; 52%) was obtained as a white powder. R_t 12.63 min (92% purity); ESMS calcd. for $C_{36}H_{63}N_{11}O_{13}S$ 889.43, found 889.85 $[M + H]^+$.

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Supporting Information Available: Details of experimental procedures, $^1H/^{13}C$ NMR spectral data, and LC-MS spectra for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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