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Sulfo-click reaction *via in situ* generated thioacids and its application in kinetic target-guided synthesis†‡

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Herein, we describe a practical, one-pot variant of the sulfo-click reaction, in which 9-fluorenylmethyl-protected thioesters are rapidly deprotected and reacted further with sulfonylazides to give *N*-acyl sulfonamides.

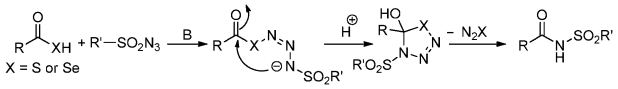
In the last decade, various click chemistry approaches manifested themselves as simple yet reliable tools for material and life sciences.[1] Other bioorthogonal transformations known as the thiol-click reaction or the sulfo-click reaction emerged as alternatives though they are slightly limited in their reactivity profile and substrate scope.[2,3] Herein, we report a rapid, one-pot sulfo-click reaction taking advantage of an *in situ* activation of thioesters into thioacids followed by the amidation step. Adoption of these reaction conditions furthermore eliminated the major limitation of our developed sulfo-click-based kinetic target-guided synthesis approach[4] improving it to fully complement the established *in situ* click chemistry approach.[5]

Though *N*-acyl sulfonamides are known for more than a century, in recent years they gained great attention in medicinal chemistry.[6] Commonly, *N*-acyl sulfonamides are synthesized *via* reactions between sulfonamides and carboxylic acids with carbodiimide reagents or between acid halides and sulfonamides under basic conditions. Recently there have been reports on the preparation of *N*-acyl sulfonamides by a copper catalyzed reaction between sulfonylazides and alkynes.[7] Another modern approach entails the reaction between sulfonylazides and thioacids[3] or sulfonylazides and selenocarboxylates[8] (Scheme 1). Especially, the amidation with thioacids stands out by its ease and bioorthogonality and thus was later named sulfo-click reaction by Liskamp.[3c]

Though the sulfo-click reaction has been proven to be reliable in various applications, it is currently underutilized due to limitations with respect to the preparation and handling of thioacids. In general, thioacids are nucleophilic, readily dimerize, and suffer from storage and stability issues. The synthesis and purification of thioacids are also not always straightforward although various synthetic protocols are available.[9] Reliable protocols have been reported, in which protected thioacids are deprotected prior to the amidation step. For example, 2,4,6-trimethoxybenzyl (Tmob) thiol and trityl thiol were used to prepare the corresponding thioesters from carboxylic acids.[10] These Tmob or trityl thioesters were then selectively deprotected and reacted with sulfonylazides in the presence of 2,6-lutidine to arrive at the *N*-acyl sulfonamides.[10] The generation of the thioacids from these thioesters, however, requires prolonged exposure to an excess of trifluoroacetic acid for complete deprotection and an aqueous workup to remove the residual acid, since the sulfo-click reaction is optimally conducted under slightly basic conditions.

Crich and coworkers recently developed an amidation reaction between 2,4-dinitrobenzenesulfonamides and thioacids, in which the thioacids have been prepared starting from corresponding 9-fluorenylmethyl (Fm) thioesters with 20% piperidine in DMF.[11] Analogously, we envisioned a strategy in which corresponding thioacids are rapidly generated and directly used without any workup and purification in a sulfo-click amidation. However, Williams *et al.* reported that the sulfo-click reaction with piperidine generates piperidine amides of the corresponding thioacids, sulfonamides and only small amounts of targeted *N*-acyl sulfonamides.[3b] Because the nucleophilic piperidine attacks the carbonyl carbon and outcompetes the intramolecular *S*-acyl transfer, the unwanted piperidine amide is generated as one of the two main products.

To start the optimization of a one-pot deprotection/amidation reaction, various thioesters were prepared from corresponding carboxylic acids and FmSH following the reported procedures.[12] In search of a non-nucleophilic base which would rapidly deprotect the Fm thioester and promote the amidation, 1,8-diazabicycloundec-7-ene (DBU) has served the purpose. A convenient procedure was attained, which performs well with diverse thioacids and sulfonylazides after screening the different proportions of DBU from 5% to 3% DBU in DMF. Optimal deprotection was achieved in one minute at room temperature by a mixture of 3.5% DBU/DMF and subsequent



Scheme 1 Reaction between sulfonylazides and thioacids or sulfonylazides and selenocarboxylates to yield *N*-acyl sulfonamides.

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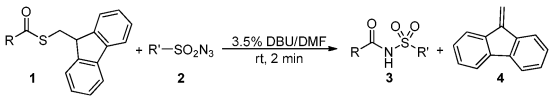
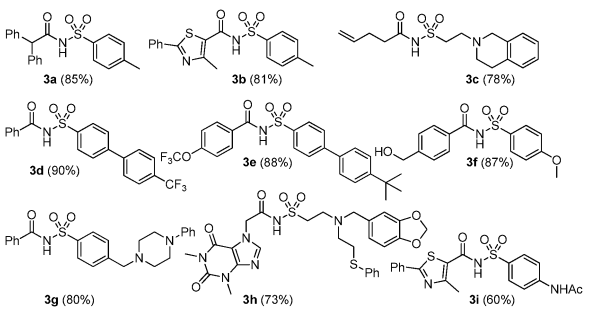


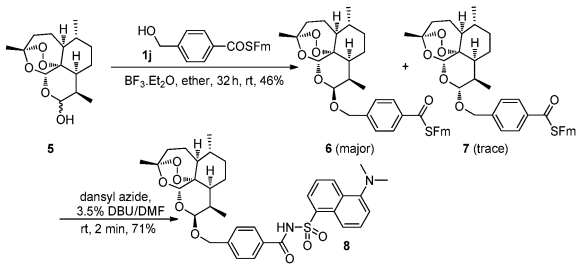
Scheme 2 Sulfo-click reaction between sulfonylazides and thioacids generated from 9-fluorenylmethyl-protected thioesters.

amidation with 1 equivalent of sulfonylazide was completed in another minute to obtain *N*-acyl sulfonamide along with DBF **4** (Scheme 2). All products were purified and isolated in good to excellent yields. Representative examples of this rapid deprotection/amidation reaction are shown in Table 1. Reactions between aromatic or aliphatic azides and aromatic or aliphatic thioacids performed well in the one-pot two-reaction sequence. Functional groups like an acetamide **3i**, a hydroxyl **3f** and an ether **3e** and **3f** were tolerated in the reaction. Heterocycles like caffeine **3h**, thiazole **3b** and **3i**, piperazine **3g**, and tetrahydroisoquinoline **3c** were also compatible with the reaction conditions. This reaction was also examined in high dilutions of thioacids and sulfonylazides. At 20 μM concentration of thioester and sulfonylazide, the reaction attained completion at room temperature in 12 hours. It is noteworthy that the deprotection/amidation reaction worked similarly well with Cs_2CO_3 with good yields. First the deprotection completed with 5 equivalents of Cs_2CO_3 in DMF in 90 minutes and after the addition of the sulfonylazide, the *N*-acyl sulfonamide was formed cleanly in 30 minutes at room temperature.

The good yields and the high chemoselectivity of the amidation reaction are especially useful for the preparation of highly complex biomolecules. First, artelinic acid, a semi-synthetic derivative of the natural product artemisinin, has been successfully derivatized with a fluorescent dansyl moiety using the new amidation reaction conditions. Commercially available dihydroartemisinin **5** was reacted under known Lewis acid conditions with the thioester of the *p*-hydroxymethyl benzoic acid **1j** resulting in the thioester of artelinic acid (Scheme 3).[13] The major product obtained was the 10β isomer **6** along with trace amounts of the 10α isomer **7**. Next, the sulfo-click reaction of the isomeric mixture containing **6** and **7** was deprotected with 3.5% DBU/DMF and reacted with dansylazide to provide the dansyl analogue **8** of artelinic acid in excellent yields after the trace amounts of the 10α isomer has been removed by preparative HPLC. By ^{1}H NMR spectroscopy, the structure of the 10β isomer **8** was

Table 1 *N*-Acyl sulfonamides prepared from sulfonylazides and thioacids generated from 9-fluorenylmethyl-protected thioesters with 3.5% DBU/DMF. The yields are given in parentheses





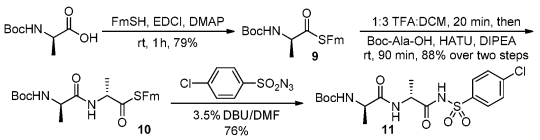
Scheme 3 Preparation of a semi-synthetic derivative of the natural product artemisinin.

confirmed by the doublet at δ 4.8 ppm with a vicinal equatorial-axial coupling constant of H-10 with H-9 and only one singlet at δ 5.38 ppm, which corresponds to H-12.

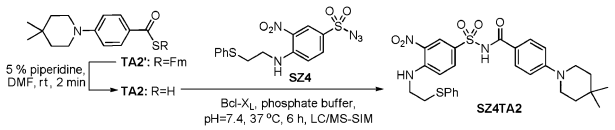
As a second example, a peptidic *N*-acyl sulfonamide has been synthesized. After Boc deprotection of Fm thioester **9**, the resulting amine was reacted with Boc-Ala-OH under standard peptide coupling conditions providing the dipeptidic Fm thioester **10** (Scheme 4). The sulfo-click reaction between the Fm thioester **10** and *p*-chlorobenzenesulfonyl azide with 3.5% DBU/DMF resulted in two minutes in the *N*-acyl sulfonamide **11** in good yields without detectable epimerization.

The use of Fm thioesters in the sulfo-click reaction is also compatible with applications requiring bioorthogonal conditions. Previously, we developed similar to *in situ* click chemistry a kinetic target-guided synthesis (TGS) approach, in which the sulfo-click reaction between thioacids and sulfonylazides was used for the screening of compounds with biological activity.[4] In kinetic TGS, the biological target is engaged in the assembly of its own inhibitory bidentate ligand from a pool of complementary reacting fragments. In proof-of-concept studies, we demonstrated that incubation of the protein target Bcl-X_L with a library of thioacids and sulfonylazides leads to the selective formation of four hit *N*-acyl sulfonamides displaying inhibitory activity towards Bcl-X_L.[4b] Although the initial proof-of-concept study underlines the potential of kinetic TGS, the issues related to the preparation and handling of the thioacid fragments restrict the kinetic TGS approach to a minor degree.

Inspired from the success of the rapid one-pot *in situ* deprotection/amidation reaction sequence, known thioacid fragments **TA1–TA3** were prepared as Fm thioesters **TA1′–TA3′** and then applied in a kinetic TGS approach to sulfonylazides **SZ1–SZ6** in the presence of Bcl-X_L after the thioesters have been deprotected.[4] Not surprisingly, deprotection of the three Fm thioesters **TA1′–TA3′** with 3.5% DBU/DMF cleanly furnished thioacids **TA1–TA3** in one minute. The resulting thioacids **TA1–TA3** were immediately diluted with methanol to 2 mM stock solutions (about 85 fold dilution) and then directly used for the kinetic TGS screening of Bcl-X_L. Sulfonylazides **SZ1–SZ6** and thioacids **TA1–TA3** derived



Scheme 4 Preparation of a peptidic *N*-acyl sulfonamide.



Scheme 5 Kinetic TGS incubations with **TA2** generated from **TA2′**.

from the corresponding thioesters **TA1′–TA3′** were incubated as binary mixtures at 20 μM concentration each in the presence and absence of Bcl-X_L in the appropriate phosphate buffer at 37 °C for 6 hours. At the same time as a control experiment, all binary building block combinations with sulfonylazides **SZ1–SZ6** and the thioacids **TA1–TA3**, which were synthesized and purified as thioacids, were incubated with and without Bcl-X_L. Contrary to the previous results,[4] the amplification factor for hit combination **SZ4TA2**, the ratio between **SZ4TA2** in the Bcl-X_L-templated reaction and **SZ4TA2** in the non-templated reaction, was not detected in the incubation samples containing the fragments **SZ4** and **TA2**, which derived from the thioester **TA2′**. The strong basicity of DBU was considered to change the pH of the Bcl-X_L-containing incubation sample and thereby possibly affect the protein conformation or even denaturing it.

Therefore, the kinetic TGS experiments were repeated with a weaker base (Scheme 5). Piperidine was considered to be a good candidate, even though it has been proven to undergo a side reaction yielding piperidine amides. After the deprotection of the Fm thioesters, it was assumed that the dilution and the buffering effect by the phosphate buffer of the incubation samples may significantly decrease the nucleophilicity of piperidine and thus suppress the formation of unwanted piperidine amides. The thioacids **TA1–TA3** were rapidly generated by the treatment of thioesters **TA1′–TA3′** with a mixture of 5% piperidine/DMF and immediately diluted with methanol to 2 mM stock solutions. Kinetic TGS incubation samples were prepared with and without Bcl-X_L in all possible fragment combinations and then analyzed by LC/MS-SIM. Gratifyingly, an amplification factor for hit combination **SZ4TA2** in the incubation samples containing the thioacid **TA2** derived from the thioester **TA2′** (see Fig. S1, traces C and D in ESI‡) was calculated to be in the same range as the amplification for the Bcl-X_L-templated incubations containing the purified thioacid **TA2** (see Fig. S1, traces A and B in ESI‡).

Next, studies were conducted to check whether the unwanted piperidine amide was formed during the kinetic TGS incubations with thioacid **TA2** derived from thioester **TA2′**. LC/MS-SIM analysis set to simultaneously detect ions with masses corresponding to **SZ4TA2** and piperidine amide identified the formation of both *N*-acyl sulfonamide **SZ4TA2** and piperidine amide in both the non-templated reaction and the Bcl-X_L-templated reactions (Fig. S2 in ESI‡). As expected, the amount of **SZ4TA2** was increased in the Bcl-X_L-containing incubation sample, while the piperidine amide was detected at approximately the same amounts in the Bcl-X_L-templated and non-templated incubations. Further attempts were made to estimate the amounts of **SZ4TA2** and piperidine amide formed during the kinetic TGS. Both compounds were synthesized and mixtures containing **SZ4TA2** and piperidine amide at equimolar concentrations were analyzed by LC/MS-SIM. In this comparison, the peak area of the piperidine amide was significantly larger than the peak area of the *N*-acyl sulfonamide **SZ4TA2**, which clearly indicates that the electrospray ionization of the piperidine amide is better than that of **SZ4TA2**. Thus, it can be concluded that though the piperidine amide can be identified by LC/MS-SIM, it has been formed in the incubation sample only to minor amounts in comparison to the *N*-acyl sulfonamide **SZ4TA2**. These results suggest that the unwanted byproduct piperidine amide did not have an unfavorable impact on the kinetic TGS incubations and the hit compound **SZ4TA2** is easily detectable by LC/MS-SIM analysis.

In conclusion, we have developed a practical, one-pot variant of the sulfo-click reaction, in which Fm thioesters are rapidly deprotected and further reacted with sulfonylazides to give *N*-acyl sulfonamides in high yields. This variant of the sulfo-click reaction has also been shown to be applicable for kinetic TGS screening. We are currently using this kinetic TGS approach for the screening of various protein–protein interaction targets.

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