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# TRAM Linker: A Safety-Catch Linker for the Traceless Release of Acrylamides

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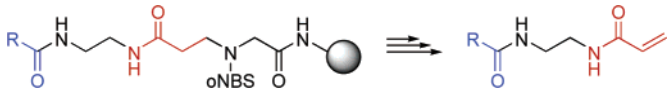
## ABSTRACT




**A novel safety-catch linker for the solid-phase synthesis of small-molecule libraries containing electrophilic reactive groups has been developed. Upon cleavage from solid support, the linker generates a Michael acceptor (an acrylamide) on each library member. Utilization of a two-resin system in the final cleavage step provides crude products in high purity, allowing direct use in biological assays following filtration and evaporation.**

Small molecules that covalently modify proteins have long been of interest as irreversible inhibitors and affinity labeling agents.[1] More recently, electrophilic small molecules have gained promise as tools for proteomic profiling,[2] in new approaches for lead compound identification,[3] selective target inhibition/activation, and active site mapping in engineered systems.[4,5] To facilitate the preparation of electrophilic small molecules, we have prepared a new linker for solid-phase synthesis that releases a Michael acceptor (here an acrylamide) upon cleavage from the resin. We chose acrylamides as electrophiles since they have been shown to be effective for affinity alkylation of cysteines both in vitro[5,6] and in vivo[7] yet are unreactive toward common cellular nucleophiles (e.g., glutathione).

Recently, several reports describing the introduction of potential Michael acceptors onto compounds upon release from solid support have appeared. Vinyl sulfones have been introduced onto peptides during cleavage from Kenner's safety-catch linker.[8] Several groups have utilized oxidative eliminations of selenium or sulfones for the synthesis of acrylamides,[9] α,β-unsaturated esters and ketones,[10] α,β-unsaturated aldehydes,[11] β-dicarbonyl enones,[12] cyclobutenones,[13] cyclopent-2-enones,[14] and α,β-unsaturated γ-butyrolactones.[15] Cyclic acrylamides have also been generated via a ring-closing metathesis cleavage.[16] While many of the aforementioned examples produce compounds in high yields,

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(1) Baker, B. R. *J. Pharm. Sci.* **1964**, *53*, 347−364.
(2) (a) Jessani, N.; Cravatt, B. F. *Curr. Opin. Chem. Biol.* **2004**, *8*, 54−59. (b) Jeffery, D. A.; Bogyo, M. *Curr. Opin. Biotechnol.* **2003**, *14*, 87−95.
(3) Erlanson, D. A.; Braisted, A. C.; Raphael, D. R.; Randal, M.; Stroud, R. M.; Gordon, E. M.; Wells, J. A. *Proc. Nat. Acad. Sci. U.S.A.* **2000**, *97*, 9367−9372.
(4) Foucaud, B.; Perret, P.; Grutter, T.; Goeldner, M. *Trends Pharm. Sci.* **2001**, *22*, 170−173.
(5) Levitsky, K.; Ciolli, C. J.; Belshaw, P. J. *Org. Lett.* **2003**, *5*, 693−696.
(6) Chmura, A. J.; Orton, M. S.; Meares, C. F. *Proc. Nat. Acad. Sci. U.S.A.* **2001**, *98*, 8480−8484.
(7) Fry, D. W.; Bridges, A. J.; Denny, W. A.; Doherty, A.; Greis, K. D.; Hicks, J. L.; Hook, K. E.; Keller, P. R.; Leopold, W. R.; Loo, J. A.; McNamara, D. J.; Nelson, J. M.; Sherwood, V.; Smaill, J. B.; Trumppkallmeyer, S.; Dobrusin, E. M. *Proc. Nat. Acad. Sci. U.S.A.* **1998**, *95*, 12022−12027.
(8) Overkleeft, H. S.; Bos, P. R.; Hekking, B. G.; Gordon, E. J.; Ploegh, H. L.; Kessler, B. M. *Tetrahedron Lett.* **2000**, *41*, 6005−6009.
(9) Sheng, S.-R.; Wang, X.-C.; Liu, X.-L.; Song, C.-S. *Synth. Commun.* **2003**, *33*, 2867−2872.
(10) Sheng, S.-R.; Liu, X.-L.; Wang, X.-C. *J. Chin. Chem. Soc.* **2003**, *50*, 279−281.
(11) Sheng, S.-R.; Wu, L.-L.; Huang, X. *J. Chem. Res., Synop.* **2003**, 258−259.
(12) Sheng, S.-R.; Wei, Z.; Liu, X.-L. *J. Chem. Res., Synop.* **2003**, 552−553.
(13) Cheng, W.-C.; Wong, M.; Olmstead, M. M.; Kurth, M. J. *Org. Lett.* **2002**, *4*, 741−744.
(14) Cheng, W.-C.; Kurth, M. J. *J. Org. Chem.* **2002**, *67*, 4387−4391.
(15) (a) Fujita, K.; Watanabe, K.; Oishi, A.; Ikeda, Y.; Taguchi, Y. *Synlett* **1999**, 1760−1762. (b) Huang, X.; Sheng, S.-R. *J. Comb. Chem.* **2003**, *5*, 273−277.

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each method requires an extractive workup and/or chromatography following cleavage from a solid phase. In this paper, we demonstrate a cleavage strategy that directly yields acrylamides in high purity (>95%) following simple filtration and evaporation allowing direct use of released compounds in biological screens.[17] The linker was designed such that the Michael acceptor is generated from the linker attachment site, maximizing the potential for scaffold diversification.

Our linker cleavage strategy is shown in Figure 1. During synthesis of a library, the safety-catch exists as a protected secondary amine. Upon completion of library synthesis, the secondary amine is deprotected and alkylated to give the quaternary ammonium salt. Library members are then cleaved from resin with a volatile base, generating the acrylamide electrophile. The cleavage chemistry of our safety-catch linker for the *t*raceless *r*elease of *a*crylam*i*des (TRAM linker) is similar to the REM resins,[18] generating a Michael acceptor via a Hofmann elimination. While the REM resins release tertiary amines from the resin, the TRAM linker releases the Michael acceptor.

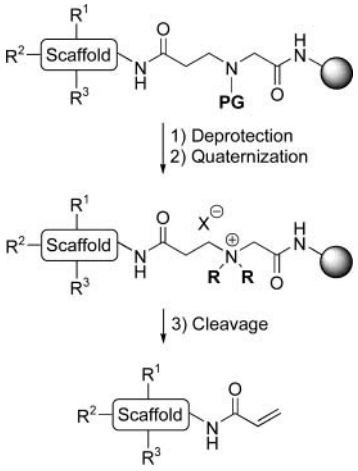
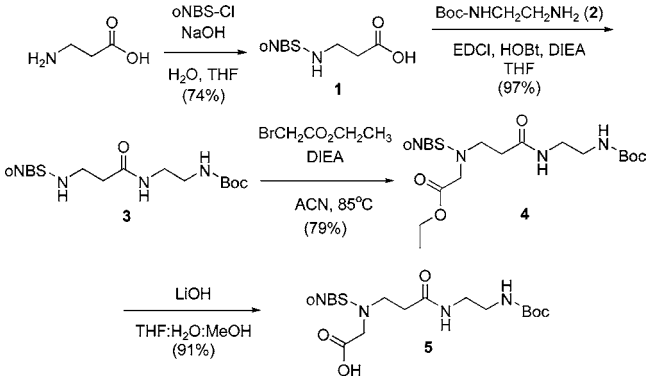



**Figure 1.** Cleavage strategy for the TRAM linker.

For ease of synthesis, we chose the *ortho*-nitrobenzene sulfonyl (oNBS)[19] group for protection of the secondary amine. Synthesis of the linker (Scheme 1) began with oNBS protection of $\beta$-alanine followed by EDCI-mediated coupling with *N*-Boc-ethylenediamine (**2**)[20] to yield sulfonamide **3**. We selected an ethylenediamine spacer because this linker

**Scheme 1.** Synthesis of the TRAM Linker




length was shown to be sufficient for exo-mechanism affinity alkylation of cysteines.[5] This spacer could possibly be substituted as required for other applications. Alkylation of the sulfonamide nitrogen of **3** with ethyl bromoacetate yielded the protected linker **4**. Hydrolysis of the ethyl ester **4** gave linker **5**, suitable for loading onto solid supports. Although the sulfonamide moiety present in compound **3** is necessary for the alkylation step, it should be possible to substitute the secondary amine protecting group of **4** to accommodate other library chemistries.

For optimization of our linker cleavage strategy (Figure 1, Scheme 2), we prepared model derivatives **9a** and **10a** of the TRAM linker to readily evaluate conditions for linker quaternization and cleavage. We incorporated the naphthylacetamide moiety into our model derivative to facilitate quantitation of experimental yields by HPLC−UV.

Optimization of the cleavage sequence began with the final cleavage step. We used a volatile tertiary amine base (DIEA) along with an acid scavenger resin[21] to allow the direct isolation of TRAM-derived acrylamides free from contaminating tertiary ammonium salts produced in the cleavage reaction. Four variables were considered: equivalents of DIEA, solvent, and both the identity and equivalents of the supported acid scavenger. For our experiments varying DIEA and solvent, we used 10 equiv of silica−TBD (1,5,7-triazabicyclo[4.4.0]dec-5-ene) as an acid scavenger in the cleavage reaction. Simultaneous variation of equivalents of DIEA and solvent (Table 1) indicated that 2 equiv of DIEA in DMF were the optimal conditions.

We next turned our attention to the supported acid scavenger. Two previous reports[21] describe the use of two-resin systems for directly isolating REM resin products in high purity. We chose to evaluate the previously reported acid scavenger resins as well as a silica-bound derivative in our cleavage reaction (Table 2). Use of 10 equiv of silica−TBD gave the highest yields, although polystyrene−TBD (PS−TBD) was comparable. When assessing the purity of

(16) Veerman, J. J. N.; Van Maarseveen, J. H.; Visser, G. M.; Kruse, C. G.; Schoemaker, H. E.; Hiemstra, H.; Rutjes, F. P. J. T. *Eur. J. Org. Chem.* **1998**, 2583−2589.

(17) Zaragoza, F. *Angew. Chem., Int. Ed.* **2000**, *39*, 2077−2079.

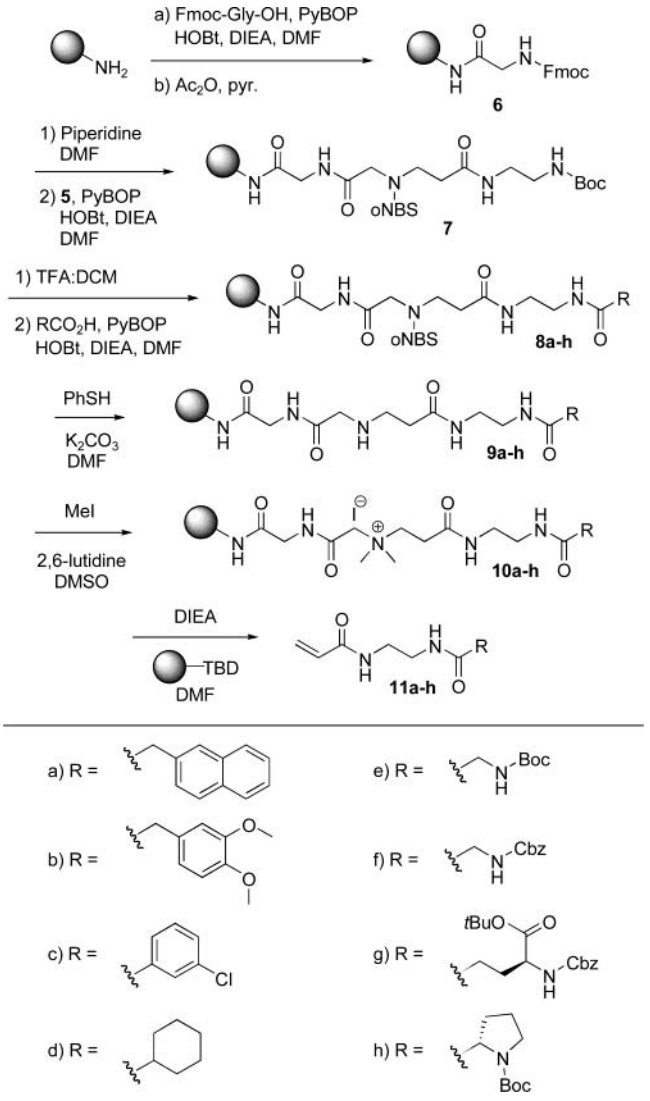
(18) (a) Morphy, J. R.; Rankovic, Z.; Rees, D. C. *Tetrahedron Lett.* **1996**, *37*, 3209−3212. (b) Heinonen, P.; Lonnberg, H. *Tetrahedron Lett* **1997**, *38*, 8569−8572. (c) Kroll, F. E. K.; Morphy, R.; Rees, D.; Gani, D. *Tetrahedron Lett.* **1997**, *38*, 8573−8576. (d) Plater, M. J.; Murdoch, A. M.; Morphy, J. R.; Rankovic, Z.; Rees, D. C. *J. Comb. Chem.* **2000**, *2*, 508−512.

(19) Fukuyama, T.; Jow, C.-K.; Cheung, M. *Tetrahedron Lett.* **1995**, *36*, 6373−6374.

(20) Pons, J.-F.; Fauchere, J.-L.; Lamaty, F.; Molla, A.; Lazaro, R. *Eur. J. Org. Chem.* **1998**, 853−859.

(21) (a) Ouyang, X.; Armstrong, R. W.; Murphy, M. M. *J. Org. Chem.* **1998**, *63*, 1027−1032. (b) Alhambra, C.; Castro, J.; Chiara, J. L.; Fernandez, E.; Fernandez-Mayoralas, A.; Fiandor, J. M.; Garcia-Ochoa, S.; Martin-Ortega, M. D. *Tetrahedron Lett.* **2001**, *42*, 6675−6678.

**Scheme 2.** Solid-Phase Synthesis of Acrylamides




isolated products by $^1$H NMR, we found batch-dependent impurities arising from the silica−TBD scavenger contaminated our products and thus chose 10 equiv of PS−TBD as our optimal scavenger.

Previous investigations of the quaternization reaction had found that 20 equiv of alkylating reagent in DMSO[22] were optimal for amine quaternization in the related REM resin systems. Both DMSO and NMP[23] have been shown to be superior to DMF as solvents for REM resin quaternization; however, a side-by-side comparison has not been reported. We therefore compared cleavage yields in these two solvents with varying equivalents of MeI/2,6-lutidine (Table 3). These experiments indicated that 40 equiv MeI/2,6-lutidine in NMP gave the highest yields for quaternization, although yields with 10 and 20 equiv of MeI/2,6-lutidine were comparable to those using 40 equiv. It is noteworthy that the inclusion of 2,6-lutidine in the quaternization reaction roughly doubled the final cleavage yields under otherwise identical experimental conditions (data not shown). Previous reports of similar transformations[24] may similarly benefit by including this acid scavenger. Although NMP gave the highest yields in the quaternization reaction, colored impurities generated in this reaction contaminated the isolated products. On the basis of these observations, we chose DMSO with 40 equiv of MeI/2,6-lutidine as our optimal condition, giving the highest purities with minor decreases in yield.

Having determined optimal quaternization and cleavage conditions[25] in our model system, we next sought to demonstrate the applicability of the TRAM linker for a variety of substrates (Scheme 2). Loading of Fmoc-Gly-OH

**Table 1.** Evaluation of Base and Solvent in Cleavage Reaction

| equiv[a] of DIEA | yield (%)[b] DCM | DMF | DMSO | THF |
|---|---|---|---|---|
| 1 | 57 | 62 | 43 | 53 |
| 2 | 58 | 65 | 45 | 56 |
| 5 | 58 | 65 | 47 | 55 |
| 10 | 59 | 64 | 44 | 57 |

[a] Molar equivalents relative to loading of resin **6**. [b] Percent yield determined via HPLC−UV using (±)-γ-(2-naphthyl)-γ-butyrolactone as an internal standard (see Supporting Information).

**Table 2.** Evaluation of Solid-supported Acid Scavengers in Cleavage Reaction

| | yield (%)[b] | | |
|---|---|---|---|
| equiv[a] | IRA-95 | PS−TBD | silica−TBD |
| 5 | 31 | 55 | 53 |
| 10 | 47 | 61 | 65 |

[a] Molar equivalents of scavenger amine relative to loading of resin **6**. [b] Percent yield determined via HPLC−UV using (±)-γ-(2-naphthyl)-γ-butyrolactone as an internal standard (see Supporting Information).

**Table 3.** Evaluation of Solvent and MeI/2,6-Lutidine in Quaternary Amine Formation

| equiv[a] of MeI/2,6-lutidine | yield (%)[b] DMSO | NMP |
|---|---|---|
| 10 | 59 | 68 |
| 20 | 59 | 69 |
| 40 | 63 | 71 |

[a] Molar equivalents relative to loading of resin **6**. [b] Percent yield determined via HPLC−UV using (±)-γ-(2-naphthyl)-γ-butyrolactone as an internal standard (see Supporting Information).

(22) Cameron, K. S.; Morphy, J. R.; Rankovic, Z.; York, M. *J. Comb. Chem.* **2002**, *4*, 199−203.
(23) Yamamoto, Y.; Tanabe, K.; Okonogi, T. *Chem. Lett.* **1999**, 103−104.
(24) Wu, T. Y. H.; Schultz, P. G. *Org. Lett.* **2002**, *4*, 4033−4036.

onto aminomethyl polystyrene resin and capping with acetic anhydride/pyridine afforded resin **6**. Fmoc quantitation of **6** established an experimental loading level for all subsequent experiments. Removal of the Fmoc protecting group and coupling with linker **5** yielded resin **7**. Following removal of the Boc protecting group, a variety of carboxylic acids were coupled onto the linker-functionalized resin to give **8a**−**h**. The oNBS protecting groups of **8a**−**h** were removed with the potassium salt of thiophenol to give secondary amine resins **9a**−**h**. Quaternization of the secondary amine was achieved with 40 equiv of MeI/2,6-lutidine in DMSO to afford quaternary ammonium salt resins **10a**−**h**. Last, the product acrylamides were cleaved from solid support with 2 equiv of DIEA and 10 equiv of PS−TBD scavenger resin in DMF to yield compounds **11a**−**h**.

All eight TRAM-derived acrylamides were isolated in good yields (Table 4) after filtration and evaporation. More importantly, acrylamides **11a**−**h** were all isolated in greater than 95% purity as judged by $^1$H NMR. Common protecting groups such as Boc (**11e**,**h**), Cbz (**11f**,**g**), and *tert*-butyl ester (**11g**) were stable to the linker cleavage chemistries.

In summary, we have developed a general linkage strategy for the introduction of acrylamide electrophiles onto small-molecule library members. Our strategy should be generalizable to other Michael acceptors such as acrylates, enones, and $\alpha,\beta$-unsaturated sulfones/sulfonamides. This linker should facilitate the synthesis and identification of small molecules that covalently modify protein targets via proximity-accelerated alkylation.[5] Such compounds may find utility in proteomic profiling, as isoform-selective modulators of protein function in engineered systems, and possibly in disrupting protein−protein interactions.

(25) Optimal protocol: alkylation with 40 equiv of MeI/2,6-lutidine in DMSO and cleavage with 2 equiv of DIEA in DMF in the presence of 10 equiv of PS−TBD scavenger.

**Table 4.** Yields and Purities of TRAM-derived Acrylamides

| model compound | isolated yield (%) | purity (%)[a] |
|---|---|---|
| **11a** | 63 | >95 |
| **11b** | 64 | >95 |
| **11c** | 69 | >95 |
| **11d** | 69 | >95 |
| **11e** | 73 | >95 |
| **11f** | 64 | >95 |
| **11g** | 67 | >95 |
| **11h** | 70 | >95 |

[a] Determined via integration of $^1$H NMR spectra.

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**Supporting Information Available:** Experimental procedures and structural characterization for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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