ORGANIC LETTERS

2006
Vol. 8, No. 12
2483−2485

N-Acylsulfonamide Linker Activation by Pd-Catalyzed Allylation

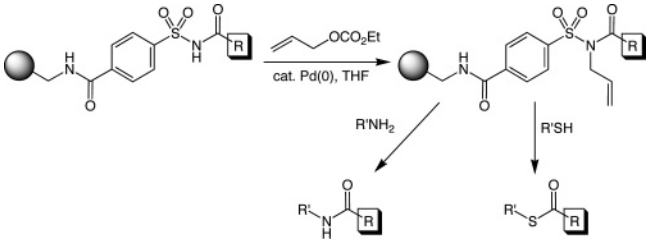
Yi He,[†] Jesse P. Wilkins,[†] and Laura L. Kiessling*[,†,‡]

Departments of Chemistry and Biochemistry, University of Wisconsin—Madison, Madison, Wisconsin 53706

kiessling@chem.wisc.edu

Received February 17, 2006

ABSTRACT



***N*-Acylsulfonamide safety-catch linkers are versatile tools in solid-phase organic synthesis because of their stability. This stability necessitates linker activation prior to compound cleavage. Here, we demonstrate that the *N*-acylsulfonamide group can react with a π-allyl palladium complex and that these mild and neutral conditions can be exploited for linker activation. The advantages of this process are illustrated by its use in the efficient synthesis of thioesters. We anticipate that this activation method will extend the utility of the *N*-acylsulfonamide group.**

A key component in solid-phase synthesis is the linker, which serves as the anchor between the product and the solid support.[1] The utility of a linker depends on the range of conditions it can tolerate. One strategy to achieve high linker stability is to employ a "safety-catch" linker.[2,3] With a safety-catch linker, the release of a compound from the support must be facilitated by linker activation.

Kenner and co-workers introduced the first safety-catch linker.[2,4] Composed of an *N*-acylsulfonamide group, it is stable under either strongly basic or acidic conditions. This stability stems from the low pK_a (ca. 2.5) of the *N*-acylsulfonamide moiety. Linker activation is accomplished via alkylation. When N-alkylated, the acylsulfonamide group becomes susceptible to nucleophilic attack; therefore, it can react to afford carboxylic acids and their derivatives, such as amides, esters, hydrazides, and thioesters.

The *N*-acylsulfonamide safety catch resin is especially valuable for the synthesis of peptide thioesters.[5−7] These species are critical components in the assembly of natural and nonnatural proteins using native-chemical ligation,[8] expressed protein ligation, and Staudinger ligation.[9] Peptide thioesters have been synthesized from the methylated alkylsulfonamide linker.[5] To obtain high yields of the desired thioester products, sodium benzenethiolate is needed. The

[†] Department of Chemistry.
[‡] Department of Biochemistry.

(1) Recent reviews on linkers for SPOS: (a) James, I. W. *Tetrahedron* **1999**, *55*, 4855−4946. (b) Guillier, F.; Orain, D.; Bradley, M. *Chem. Rev.* **2000**, *100*, 2091−2157. (c) Qiang, H.; Quan, L.; Zheng, B. Z. *Prog. Chem.* **2004**, *16*, 236−242.

(2) Kenner, G. W.; McDermott, J. R.; Sheppard, R. C. *Chem. Commun.* **1971**, 636−637.

(3) Review on "safety-catch" linkers: Patek, M.; Lebl, M. *Biopolymers* **1998**, *47*, 353−363.

(4) For a review on the *N*-acylsulfonamide linker, see the following: Heidler, P.; Link, A. *Bioorg. Med. Chem.* **2005**, *13*, 585−599.

(5) Ingenito, R.; Bianchi, E.; Fattori, D.; Pessi, A. *J. Am. Chem. Soc.* **1999**, *121*, 11369−11374.

(6) Shin, Y.; Winans, K. A.; Backes, B. J.; Kent, S. B. H.; Ellman, J. A.; Bertozzi, C. R. *J. Am. Chem. Soc.* **1999**, *121*, 11684−11689.

(7) (a) Quaderer, R.; Hilvert, D. *Org. Lett.* **2001**, *3*, 3181−3184. (b) Ollivier, N.; Behr, J. B.; El-Mahdi, O.; Blanpain, A.; Melnyk, O. *Org. Lett.* **2005**, *7*, 2647−2650. (c) Zohrabi-Kalantari, V.; Heidler, P.; Larsen, T.; Link, A. *Org. Lett.* **2005**, *7*, 5665−5667.

(8) (a) Hahn, M. E.; Muir, T. W. *Trends Biochem. Sci.* **2005**, *30*, 26−34. (b) Dawson, P. E.; Muir, T. W.; Clarklewis, I.; Kent, S. B. H. *Science* **1994**, *266*, 776−779. (c) Dawson, P. E.; Kent S. B. H. *Annu. Rev. Biochem.* **2000**, *69*, 923−960. (d) Kent, S. B. H. *Annu. Rev. Biochem.* **1988**, *57*, 957−989.

(9) (a) Nilsson, B. L.; Kiessling, L. L.; Raines, R. T. *Org. Lett.* **2001**, *3*, 9−12. (b) Nilsson, B. L.; Kiessling, L. L.; Raines, R. T. *Org. Lett.* **2000**, *2*, 1939−1941. (c) Nilsson, B. L.; Hondal, R. J.; Soellner, M. B.; Raines, R. T. *J. Am. Chem. Soc.* **2003**, *125*, 5268−5269. (d) He, Y.; Hinklin, R. J.; Chang, J.; Kiessling, L. L. *Org. Lett.* **2004**, *6*, 4479−4482.

10.1021/ol060428o CCC: $33.50
Published on Web 05/09/2006

initial cleavage product is presumably a thiophenyl ester, which then undergoes transthioesterification to afford the target thioester. Although the addition of thiophenol improves yields, its inclusion in the cleavage reaction has drawbacks. It is difficult to remove from the thioester products, and its presence can result in mixed disulfide side products.

To avoid these drawbacks, the *N*-acylsulfonamide linker can be converted into a more activated species.[6] Ellman and co-workers demonstrated that the cyanomethylated linker is more electrophilic.[10] Accordingly, when cyanomethylation is used for linker activation, no thiophenol additive is required; the thioester is generated directly.[4] The disadvantage of cyanomethylation is that the conditions are basic and side reactions can occur.[4,11] Moreover, *N*-acylsulfonamide cyanomethylation typically proceeds in lower yield than methylation. Thus, there remains a need for alternative methods for *N*-acylsulfonamide linker activation.

We anticipated that mild and neutral activation conditions could be developed by exploiting the "softness" of the delocalized *N*-acylsulfonamide anion.[12] We further postulated that a π-allyl Pd complex might serve as an appropriate electrophile.[13−15] This metal-catalyzed functionalization reaction would allow *N*-acylsulfonamide activation under neutral conditions.

To test the feasibility of the proposed activation method, we first examined the propensity of tosylsulfonamide **1** to undergo Pd-catalyzed allylation. Sulfonamides have been shown to undergo Pd-catalyzed allylation reactions;[16] the reactivity of acylsulfonamides, however, had not been explored. On the basis of their acidity and delocalization, we anticipated that they would be excellent substrates. Indeed, when **1** was treated with allyl ethyl carbonate in the presence of a catalytic amount of palladium tetrakistriphenylphosphine ($Pd(PPh_3)_4$), it underwent allylation in high yield (95%, Figure 1).

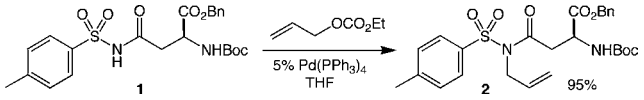
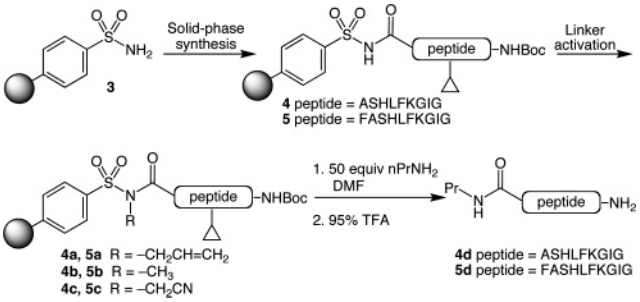


Figure 1. Pd-catalyzed allylation of tosylsulfonamide **1** with allyl ethyl carbonate.

The efficiency of the Pd-catalyzed allylation of tosylsulfonamide **1** prompted us to investigate its utility for activating *N*-acylsulfonamide linkers derived from arylsulfonamide **3** (Table 1). Arylsulfonamides tend to be better leaving groups than alkylsulfonamides, but their use is limited by difficulties in alkylating the acylsulfonamide products.[10] Thus, we reasoned that derivatives of **3** would serve as challenging substrates for activation via allylation.

Table 1. Activation of the Arylsulfonamide Linker and Cleavage with *n*-Propylamine[19]



activation conditions	**4d**: % yield[a] (% purity)	**5d**: % yield[a] (% purity)
1% $Pd(OAc)_2$[b]	13 (75)	11 (75)
5% $Pd(OAc)_2$[b]	46 (84)	36 (80)
10% $Pd(OAc)_2$[b]	54 (85)	40 (78)
20% $Pd(OAc)_2$[b]	62 (80)	38 (78)
$TMSCHN_2$[c]	90 (75)	78 (83)
ICH_2CN[c]	29 (74)	16 (79)

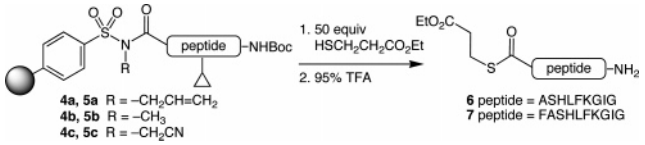
[a] Yields are crude and calculated using the experimentally determined amino acid loadings; the % purities are calculated from the LC traces. The triangle in compounds **4** and **5** denotes that the immobilized peptides are protected using the groups employed in standard Fmoc synthesis. [b] R = allyl, 200 equiv of allyl ethyl carbonate, 50% PPh_3, THF. [c] R = methyl, 300 equiv of $TMSCHN_2$, THF. [c] R = CH_2CN, 300 equiv of ICH_2N_2, 60 equiv of DIPEA, NMP.

Fragments of the peptide antibiotic Magainin (compounds **4** and **5**, Table 1) were synthesized to address the utility of the activation strategy.[17] Prior to peptide synthesis, we optimized the loading conditions for the arylsulfonamide resin **3**. Specifically, we employed *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU)[18] to obtain excellent loading yields. Immobilized peptides **4** and **5** were then assembled by automated peptide synthesis using Fmoc-protected amino acids.

We tested whether the compounds **4** and **5** could be activated for cleavage using a Pd-catalyzed allylation reaction. We employed an alternative to the air-sensitive $Pd(PPh_3)_4$, the catalyst used in our test reaction. We reasoned that the combination of the more robust palladium(II) acetate

(10) Backes, B. J.; Virgilio, A. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1996**, *118*, 3055−3056.
(11) Zohrabi-Kalantari, V.; Heidler, P.; Larsen, T.; Link, A. *Org. Lett.* **2005**, *7*, 5665−5667.
(12) (a) Pearson, R. G.; Songstad, J. *J. Am. Chem. Soc.* **1967**, *89*, 1827−1836. (b) Ho, T.-L. *Chem. Rev.* **1975**, *75*, 1−20.
(13) Tsuji, J.; Takahashi, H.; Morikawa, M. *Tetrahedron Lett.* **1965**, 4387−4388.
(14) Reviews on Pd-catalyzed allylation: (a) Tsuji, J. *Acc. Chem. Res.* **1969**, *2*, 144−152. (b) Godleski, S. A. In *Comprehensive Organic Synthesis*; Barton, S. D., Ollis, D. W., Eds.; Pergamon Press: New York, 1991; pp 585−661.
(15) For a review on Pd-catalyzed allylation using allyl carbonates under neutral conditions: Tsuji, J.; Minami, I. *Acc. Chem. Res.* **1987**, *20*, 140−145.
(16) (a) Bystrom, S. E.; Aslanian, R.; Backvall, J. E. *Tetrahedron Lett.* **1985**, *26*, 1749−1752. (b) Cerezo, S.; Cortes, J.; Moreno-Manas, M.; Pleixats, R.; Roglans, A. *Tetrahedron* **1998**, *54*, 14869−14884.
(17) (a) Zasloff, M. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 5449−5493. (b) Zasloff, M.; Martin, B.; Chen, H. C. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 910−913.
(18) (a) Dourtoglou, V.; Ziegler, J. C.; Gross, B. *Tetrahedron Lett.* **1978**, 1269−1272. (b) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397−4398. (c) Lew, A.; Chamberlin, A. R. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3267−3272.

Table 2. Synthesis of Peptide Thioesters **6** and **7** from Activated *N*-Acylsulfonamide Linkers **4a** and **4b** (Table 1)[a]



peptide/additive	**4a** (allyl) **6**: % yield (% purity)	**5a** (allyl) **7**: % yield (% purity)	**4b** ($-CH_3$) **6**: % yield (% purity)
none	21 (57)	12 (nd)[b]	<1
0.5 equiv of DIPEA	44 (54)	38 (56)	<1
1.0 equiv of DIPEA	52 (76)	41 (67)	<1

[a] Methylated sulfonamide **5b** did not produce any detectable level of thioester **7** (<1%). [b] Not determined.

and triphenylphosphine would be more useful in solid-phase synthesis. The utility of the activation conditions was evaluated by treatment with excess *n*-propylamine; amines will cleave only the *N*-alkyl acylsulfonamide linker. For evaluation of the activation efficiency, we removed the peptide protecting groups and analyzed the products using liquid chromatography−mass spectrometry (LCMS) (Table 1).

From the allylated substrates **4a** and **5a**, crude peptides **4d** and **5d** were generated in high purities (>75%, Table 1). These results indicate that the Pd-catalyzed allylation is efficient. From a comparison of the results obtained using different catalyst loadings, there is little influence on yield as long as ≥5% $Pd(OAc)_2$ is employed. We conducted subsequent allylation experiments using 10% $Pd(OAc)_2$ because these conditions afforded the products in good yields and with high purities. A comparison of our optimized activation conditions and cyanomethylation indicates that the yields from the former are superior.[10] Given these results, our next goal was to determine whether this allylation could be used in thioester synthesis in the absence of any thiophenol additive.

To evaluate the allylation activation method in thioester synthesis, we compared the ability of ethyl 3-mercaptopropionate to effect the release of peptides anchored through methylated or allylated linkers (Table 2). We obtained peptide thioesters **6** or **7** from **4a** or **5a**, and no additive was required. The addition of the nonnucleophilic base diisopropylethylamine further improved the reaction efficiency; the highest thioester yields and purities were obtained when one equivalent of this volatile amine was added. In contrast, none of the desired product was obtained from methylated linkers **4b** and **5b** under these conditions. These data suggest that the allylated linker is more reactive than its methylated counterpart.[20] These advantageous reactivity differences facilitate thioester synthesis.

In conclusion, new conditions for *N*-acylsulfonamide safety-catch linker activation have been developed that rely on Pd-catalyzed allylation. Our findings should expedite the synthesis of thioester building blocks for protein assembly. Moreover, these mild and neutral activation conditions provide an alternative to methylation; our data suggest that the resulting allylated linker is more reactive. Thus, our results should broaden the substrate compatibility of the versatile *N*-acylsulfonamide linker in solid-phase organic synthesis. We anticipate that our findings may provide new avenues for the synthesis and release of libraries of small, nonpeptidic molecules in solid-phase organic synthesis.

Acknowledgment. This research was supported by the NIH (GM49975). We thank Dr. M. B. Soellner, A. Tam, and L. Li for helpful conversations and Dr. G. Case for assistance with peptide synthesis.

Supporting Information Available: Experimental protocols for loading amino acids on aryl sulfonamide resin **3**, experimental procedures for the synthesis of compounds **2** and **4**−**7** and relevant characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

OL060428O

(19) The amino acids used for the synthesis of peptides **4** and **5** were Fmoc-Ala-OH, Fmoc-Phe-OH, Fmoc-Gly-OH, Boc-Gly-OH (terminal Gly), Fmoc-His(Trt)-OH, Fmoc-Ile-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, and Fmoc-Ser(tBu)-OH.

(20) The reactivity difference may result from differences in electronegativity of the allyl and methyl groups. pK_a values of allylammonium and methylammonium are 9.47 and 10.67, respectively, from: Hall, H. K. *J. Am. Chem. Soc.* **1957**, *79*, 5441−5444. Differences in conformation or strain may also contribute to the observed reactivity differences.