Activation Method to Prepare a Highly Reactive Acylsulfonamide "Safety-Catch" Linker for Solid-Phase Synthesis[1]

Bradley J. Backes, Alex A. Virgilio, and Jonathan A. Ellman*

Department of Chemistry, University of California Berkeley, California 94720

Received October 19, 1995

Solid-phase synthesis methods are commonly employed for the preparation of oligonucleotides and peptides and are becoming increasingly important for the preparation of small organic molecules, in particular for the preparation of compound libraries for drug development programs.[2] In almost all solid-phase peptide synthesis efforts,[3] and in a majority of small molecule solid-phase synthesis approaches, the compound is attached to the support through a carboxylic acid functionality. Linkage to support is most often accomplished with amide-based linkage elements that provide primary amide products upon cleavage, or with ester-based linkage elements that provide carboxylic acid products upon cleavage. However, for many synthesis efforts it is desirable to cleave the compound from the support by nucleophilic displacement with amines or alcohols to provide the corresponding amide or ester products.[4,5] To achieve this goal, researchers have worked to develop linkers that are stable through a given synthesis sequence, yet can be activated for nucleophilic cleavage upon synthesis completion.[6] Of these, only Kenner's acylsulfonamide safety-catch linker[7] is completely stable to basic or strongly nucleophilic conditions. Activation is accomplished by treatment with diazomethane to provide the *N*-methyl acylsulfonamide, which can then be cleaved with hydroxide or with nucleophilic amines. Kenner initially developed this linker for peptide synthesis and demonstrated the preparation of acid, primary amide, and hydrazide products. We have used an adaptation of the linker for the solid-phase synthesis of the arylacetic acid class of cyclooxygenase inhibitors where basic reaction conditions were employed including acylsulfonamide enolate alkylation reactions.[8] For both peptide and small molecule synthesis, however, the reactivity of the *N*-methyl acylsulfonamide is poor. Non-nucleophilic amines do not react with the *N*-methylated acylsulfonamide, and even for nucleophilic amines, excess reagent is usually employed which can complicate product isolation. Herein, we report on the potential applications of an activation method to prepare a highly reactive acylsulfonamide linkage.

Aminomethylated macroreticular resin is treated with 4-carboxybenzenesulfonamide, *N*,*N*-diisopropylcarbodiimide (DICI), and 1-hydroxybenzotriazole (HOBt) to provide the sulfonamide-derivatized resin **1** (Scheme 1). Acylsulfonamide **2** is then prepared by treating sulfonamide resin **1** with *i*-Pr_2EtN, catalytic DMAP, and the symmetrical anhydride of a carboxylic acid prepared *in situ*.[9] At the end of a given synthesis sequence, we had previously activated the acylsulfonamide **2** for nucleophilic cleavage by treatment with CH_2N_2 according to the procedure of Kenner to provide the *N*-methyl acylsulfonamide **3a**. We hypothesized that alkylation to introduce an electron-withdrawing *N*-alkyl group would provide enhanced reactivity toward nucleophilic displacement. Several alkyl groups were evaluated for activation of acylsulfonamide **2**, with the cyanomethyl group proving to be optimal. Treatment of **2** with bromoacetonitrile or iodoacetonitrile and *i*-Pr_2EtN in DMSO or 1-methyl-2-pyrrolidinone (NMP)[10] provides the *N*-cyanomethyl acylsulfonamide **3b**. The cyanomethyl derivative **3b** (R_1 = $(CH_2)_2$Ph-3,4,5-tri-OMe) is highly labile to nucleophilic displacement, with a $t_{1/2}$ of <5 min for displacement with 0.007 M benzylamine in DMSO. In comparison, the $t_{1/2}$ for the corresponding *N*-methyl derivative **3a** under the same conditions is approximately 790 min. The acylsulfonamide **3b** (R_1 = $(CH_2)_2$Ph-3,4,5-tri-OMe) is rapidly cleaved with a number of amines at room temperature to give the corresponding amide products **4a**−**h** in high yield based upon the initial aminomethyl substitution of the resin (Table 1). This includes both sterically hindered amines and nonbasic amines, as shown by the high yields for cleavage with *tert*-butylamine and aniline, respectively (entries **4f** and **4g**, Table 1). The latter result is noteworthy since no cleavage of the analogous *N*-methyl acylsulfonamide is observed upon treatment with aniline, even under forcing conditions.

Due to the high reactivity of **3b**, treatment with *limiting* amounts of an amine nucleophile results in complete consumption of the amine to provide the pure amide product **4**, uncontaminated with excess amine. For example, acylsulfonamide **3b** (R_1 = $(CH_2)_2$Ph-3,4,5-tri-OMe) was treated with a limiting amount of benzylamine at room temperature to provide pure *N*-benzyl amide **4c** in 98% yield based upon the benzylamine reagent. The high efficiency of this process provides the opportunity to apply novel pooling strategies, whereby equimolar quantities of highly pure amide products are obtained by treating support-bound **3b** with a limiting amount of an equimolar mixture of several amines. When support-bound **3b** (R_1 = $(CH_2)_2$Ph-3,4,5-tri-OMe) is treated with a limiting amount (0.5 equiv total amine) of an equimolar mixture of the five amines (4-(3-aminopropyl)morpholine, morpholine, benzylamine, piperidine, cyclohexylamine), a pool of the five amide products **4a**−**e** is obtained (Figure 1). Equal amounts of the five products (±3%) are observed by HPLC analysis, and the free acid (<0.5% of combined amide products) resulting from acylsulfonamide hydrolysis is the only side product observed. When less nucleophilic amines (aniline, *tert*-butylamine) are included in pooling experiments, heating is required and significantly more competitive hydrolysis occurs.[11]

Extension of this activation protocol to carboxylic acids that possess α-electronegative substituents initially proved to be problematic. Acylation of support-bound sulfonamide **1** with

(1) A preliminary account was presented at the 209th American Chemical Society National Meeting, Anaheim, CA, 1995; Abstract ORGN 261.

(2) Reviewed in the following: (a) Gallop, M. A.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gordon, E. M. *J. Med. Chem.* **1994**, *37*, 1233−1251. (b) Gallop, M. A.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gordon, E. M. *J. Med. Chem.* **1994**, *37*, 1385−1401. (c) Terrett, N. K.; Gardner, M.; Gordon, D. W.; Kobylecki, R. J.; Steele, J. *Tetrahedron* **1995**, *51*, 8135−8173. (d) Thompson, L. A.; Ellman, J. A. *Chem. Rev.* **1996**, *96*, 555−600.

(3) (a) Atherton, E.; Sheppard, R. C. *Solid Phase Peptide Synthesis: A Practical Approach*; IRL Press: Oxford, England, 1989. (b) Fields, G. B.; Noble, R. L. *Int. J. Pept. Protein Res.* **1990**, 161−214.

(4) Oxime resin has been used with success for the preparation of *N*-alkyl amides, peptide cyclization, and segment condensation. Selected examples follow: (a) Degrado, W. F.; Kaiser, E. T. *J. Org. Chem.* **1980**, *45*, 1295−1300; **1982**, *47*, 3258−3261. (b) Osapay, G.; Taylor, J. W. *J. Am. Chem. Soc.* **1990**, *112*, 6046−6124. (c) Kaiser, E. T.; Mihara, H.; Laforet, G. A.; Kelly, J. W.; Walters, L.; Findeis, M. A.; Sasaki, T. *Science* **1989**, *243*, 187−192.

(5) For solid-phase strategies to prepare peptide esters, see: Seebach, D.; Thaler, A.; Blaser, D.; Ko, S. Y. *Helv. Chim. Acta* **1991**, *74*, 1102−1119 and references cited therein.

(6) (a) Marshall D. L.; Liener, I. E. *J. Org. Chem.* **1970**, *35*, 867−868. (b) Flanigan, E.; Marshall, G. R. *Tetrahedron Lett.* **1970**, *27*, 2403−2406. (c) Wieland, T.; Lewalter, J.; Burr, C. *Justes Liebigs Ann. Chem.* **1970**, *740*, 31−47.

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

(8) Backes, B. J.; Ellman, J. A. *J. Am. Chem. Soc.* **1994**, *116*, 11171−11172.

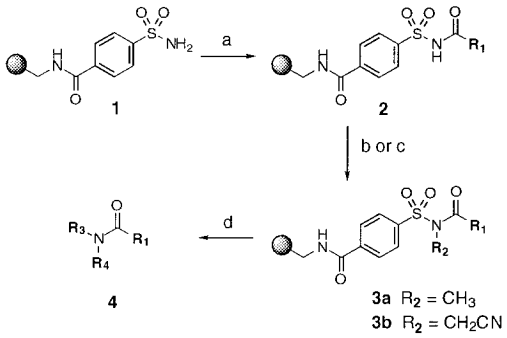
(9)) This method has proven to be successful with a number of carboxylic acids and represents an improvement over the method reported previously employing pentafluorophenyl esters.

(10) NMP is employed rather than DMSO when using gel-form resin to better solvate the resin.

(11) In initial amine pooling experiments where aniline was employed, only a 70−80% yield of the anilide product was observed due to competitive hydrolysis or slow reaction rates.

0002-7863/96/1518-3055$12.00/0

Scheme 1[a]



[a] (a) $(\mathbf{R_1}CO)_2O$, catalytic DMAP, *i*-Pr_2EtN; (b) CH_2N_2; (c) XCH_2CN, *i*-Pr_2EtN; (d) $\mathbf{R_3R_4}NH$.

Table 1. Synthesis of Amide Products **4** (Scheme 1)

cmpd	R_1	amine	% yield[a]
4a	$(CH_2)_2$Ph-3,4,5-tri-OMe	4-(3-$NH_2(CH_2)_3$)morpholine	98
4b	$(CH_2)_2$Ph-3,4,5-tri-OMe	morpholine	98
4c	$(CH_2)_2$Ph-3,4,5-tri-OMe	benzylamine	98
4d	$(CH_2)_2$Ph-3,4,5-tri-OMe	piperidine	98
4e	$(CH_2)_2$Ph-3,4,5-tri-OMe	cyclohexylamine	98
4f	$(CH_2)_2$Ph-3,4,5-tri-OMe	*tert*-butylamine	92
4g	$(CH_2)_2$Ph-3,4,5-tri-OMe	aniline	96[b]
4h	$(CH_2)_2$Ph-3,4,5-tri-OMe	benzylamine	97[c]

[a] Yields of analytically pure material based upon the initial aminomethyl substitution level of the resin. [b] Elevated temperature (65 °C) and 1 M aniline were employed. [c] Yield for the four-step synthesis sequence including the aminolysis step, as based upon the original loading level of 4-bromophenylacetic acid (see ref 8).

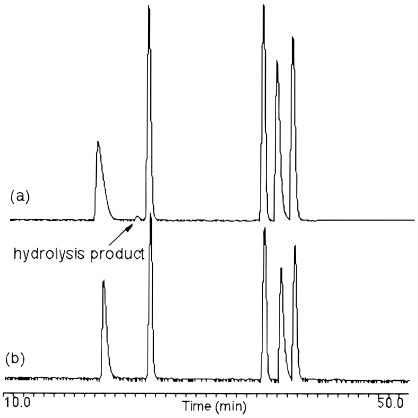
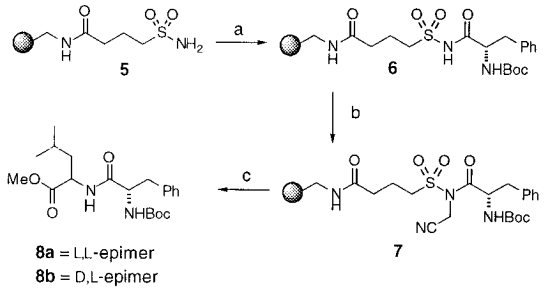


Figure 1. (a) HPLC trace of addition of limiting amounts of five amines to acylsulfonamide resin **3b** (R_1 = $(CH_2)_2$Ph-3,4,5-tri-OMe) resulted in equimolar amounts (±3%) of the five amide products listed in order of elution (relative peak area): **4a** 4-(3-aminopropyl)morpholine (0.97), **4b** morpholine (1.00), **4c** benzylamine (1.00), **4d** piperidine (1.01), **4e** cyclohexylamine (1.01). (b) HPLC trace of standard solution containing an equimolar mixture of the five amide products.

the symmetrical anhydride of Boc-L-phenylalanine employing catalytic DMAP and *i*-Pr_2EtN, followed by alkylation with iodoacetonitrile and *i*-Pr_2EtN in NMP, and displacement with excess benzylamine resulted in only a 29% yield of the *N*-benzyl amide product. In contrast, alkylation of the Boc-L-phenylalanine acylsulfonamide resin with CH_2N_2 followed by benzylamine treatment resulted in a 94% yield of the *N*-benzyl amide product, suggesting that incomplete cyanomethylation of the acylsulfonamide had occurred. Presumably, the electronegative α-protected amine attenuates the nucleophilicity of the acylsulfonamide anion. Similarly, low yields were also observed for cyanomethylation followed by benzylamine cleavage of acylsulfonamide **1** (R_1 = Ph) that was prepared by acylation of **1** with benzoic acid. Again, this is due to incomplete alkylation

Scheme 2[a]



[a] (a) Boc-L-Phe-OH, PyBOP, *i*-Pr_2EtN (2x); (b) ICH_2CN, *i*-Pr_2EtN; (c) Leu-OMe.

of acylsulfonamide **2** (R_1 = Ph) in the cyanomethylation step.[12] However, efficient activation and displacement with both of these substrates are accomplished by employing an aliphatic sulfonamide linker rather than the aryl sulfonamide linker **1** (Scheme 2), apparently due to the increased basicity and resulting enhanced nucleophilicity of an aliphatic acylsulfonamide anion.[13] Accordingly, 3-carboxypropanesulfonamide[14] is coupled to aminomethyl Merrifield resin employing DICI and HOBt in DMF. The support-bound aliphatic sulfonamide **5** is then submitted twice to coupling conditions employing Boc-L-phenylalanine, (1*H*-benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP), and *i*-Pr_2EtN in DMF to provide acylsulfonamide **6**.[15] Alkylation with iodoacetonitrile and *i*-Pr_2EtN in NMP at ambient temperature to give **7**, followed by cleavage with L-leucine methyl ester, provides the dipeptide product **8a** in 77% yield with 1.2−1.5% of the L,D-epimer being observed.[16] Displacement of **7** with D-leucine methyl ester gives **8b** in 81% yield (1.3% D,D-epimer).[17,18]

We are currently optimizing the acylation of sulfonamide **5** with protected amino acids to eliminate epimerization for peptide segment condensation and cyclization strategies. In addition, using the *N*-cyanomethyl activation method, the acylsulfonamide linker is being utilized in the synthesis of other compound classes.

Acknowledgment. We gratefully acknowledge NSF, the Beckman Foundation, the Burroughs Wellcome Fund, the A.P. Sloan Foundation, Affymax, Tularik, and Hoffman-La Roche for support of this work. A.A.V. also thanks Berlex for a Graduate Fellowship.

Supporting Information Available: Experimental details, including analytical data for all compounds described in the article (6 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

JA9535165

(12) Acylsulfonamide **2** (R_1 = Ph) is likely to be more acidic than acylsulfonamide **2** (R_1 = alkyl) since the pK_a values of benzamide and acetamide in DMSO solution are 23.35 and 25.5, respectively: Bordwell, F. G. *Acc. Chem. Res.* **1988**, *21*, 456−463.

(13) The pK_a values for methanesulfonamide and benzenesulfonamide in DMSO solution are 17.5 and 16.1, respectively: Bordwell, F. G. *Acc. Chem. Res.* **1988**, *21*, 456−463.

(14) 3-Carboxypropanesulfonamide was prepared from commercially avaliable 4,4′-dithiobutyric acid. See supporting information for details.

(15) Treatment of **5** with the symmetrical anhydride of Boc-L-phenylalanine, using catalytic DMAP, and *i*-Pr_2EtN followed by cyanomethylation and cleavage with L-leucine methyl ester resulted in good yields of **8** (80−86%) but high levels of racemization (6−7% L,D-epimer).

(16) Although a number of coupling reagents and conditions were surveyed, PyBoP with *i*-Pr_2EtN in DMF provided the best combination of efficient loading with reduced epimerization.

(17) Increased levels of epimerization are not observed when submitting **7** to the alkylation conditions a second time before cleavage with L-leucine methyl ester, indicating that racemization does not occur in the activation step.

(18) Complete cyanomethylation and benzylamine cleavage is also observed with the acylsulfonamide prepared from sulfonamide **5** and benzoic acid.