In Situ Carboxyl Activation using a Silatropic Switch. A New Approach to Amide and Peptide Constructions

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Abstract

The novel reactivity of the *O*-silylthionoesters with amine nucleophiles to generate oxoamides (rather than thioamides) is described. A straightforward first-generation trimethylsilylation protocol using bistrimethylsilylacetamide (BSA) combined with the unique reactivity of the *O*-silylthionoesters toward 1° and 2° amines to generate oxoamides provides the simplest means of activating a thiol acid for peptide bond formation at neutral pH. Excellent stereoretention is observed.

The mild and chemoselective formation of amide bonds, in particular from the context of racemization-free peptide ligations, remains a focus of much modern research activity.[1] In fact, the development of new amide forming reactions tops a recent priority list of process improvements desired by the pharmaceutical industry.[2]

We disclose herein a novel, pH-neutral method for the *in situ* activation of the carboxyl function that underpins a surprisingly simple system for room temperature amide and peptide bond construction. This unique carboxyl activation relies upon the known, spontaneous formation of *O*-silylthionoesters from their *S*-silylthiol ester isomers by a thermodynamically driven tautomerization of the triorganosilicon group from sulfur to oxygen Scheme 1.[3] *Surprisingly, the efficient reaction of O-silylthionoesters with amines to generate amide rather than thioamide linkages is unknown*.[4] The latter are formed exclusively when the analogous *O-alkyl*thionoesters are substrates (Scheme 2).[5] Since thionoesters are significantly more reactive towards nucleophiles than thiol esters,[5c] the *S* to *O* silatropy and the attendant formation of oxoamides serves as a novel and mild *in situ* activation of the carboxyl function for nucleophilic addition.

Our first efforts have focused on the stoichiometric preparation of *O*-silylthionoesters through the direct silylation of thiol acids.[6] The silylation of thiol acids occurs kinetically at sulfur to generate the *S*-trimethylsilylthiol ester, but a rapid silatropic equilibration to the more stable *O*-silylthionoester ensues.[3] Using this simple silylative chemistry, the key reactivity principles underscoring the amidic and peptidic coupling of *O*-silylthionoesters with amines were defined and are described herein.

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With thiobenzoic acid serving as a model, *in situ* silylation with bistrimethylsilylacetamide (BSA) generates the *O*-trimethylsilylthionoester within 4 min at room temperature (Scheme 3).[7] Upon addition of *i*-PrNH$_2$ a reaction ensues at room temperature to provide the corresponding amide in 70% yield after 3 h. The free thiol acid itself is poorly reactive with *i*-PrNH$_2$.[8] Almost identical isolated yields of the amide resulted from a silylative activation reaction run under an argon atmosphere (64%) *versus* one conducted open to air (70%).

The amide yields observed from the silylative activation protocol open to air are a function of the reactivity of the *O*-silylthionoester and are not caused by oxidation of the thiol acid to its corresponding diacyldisulfide, which then reacts with the amine.[9] A variety of observations support this analysis: (1) a CDCl$_3$ solution of thio-benzoic acid (1.0 equiv), BSA (1.1 equiv), and Et$_3$N (1.3 equiv) exposed to air showed no evidence of formation of dibenzoyldisulfide after 2 days at room temperature, and (2) reaction rates are essentially identical for experiments run under argon after freeze-thaw degassing or open to air (1.0 equiv thiobenzoic acid, 1.3 equiv *i*-PrNH$_2$, 1.0 equiv BSA in CDCl$_3$). In other control studies, PhCOSEt was intact after treatment with *i*-PrNH$_2$ in THF for 24 h at room temperature, both in the presence and in the absence of BSA, reaction conditions under which the *O*-silylthionoester is converted completely to the amide. Furthermore, treating 0.5 equiv of PhCOSEt and 0.5 equiv of 4-CH$_3$C$_6$H$_4$COSH with 0.5 equiv of BSA and 1 equiv of *i*-PrNH$_2$ in THF at room temperature provided the "unscrambled" reaction product (4-CH$_3$C$_6$H$_4$CONH*i*-Pr) in 88% isolated yield and recovery of PhCOSEt in 99% yield, further supporting the mechanistic framework suggested within. Of additional mechanistic interest, an authentic sample of bisbenzoyldisulfide does react rapidly with *i*-PrNH$_2$, but the rate of amide formation stalls at 50% conversion (see Supporting Information).

With this background in hand, a study of the BSA activation of thiol acids for amide and peptide bond formation was undertaken. Results for amide formation are gathered in Table 1.

The thiol acids were either available commercially or were prepared from the corresponding 9-fluorenylmethyl thiol esters via a standard piperidine deprotection-acidification protocol[10] and were used without further purification. When the silylation of the thiol acid is carried out at room temperature in the presence of a primary or secondary amine, amide linkages are generated in very good yields within a matter of hours. Both aromatic thiol acids (entries 1–5) and aliphatic thiol acids (entries 6–10) reacted effectively with primary and secondary amines to produce secondary and tertiary amides, respectively. The hydroxyl group was well tolerated under the reaction conditions (entry 4). Even aniline of low nucleophilicity reacted smoothly with *N*-Boc-Glu thiol acid to produce the corresponding anilide (entry 7). Sterically hindered thiol acids and amines also reacted to provide the sterically congested amides in quite good yields (entries 8–10). Remarkably, doubly hindered amides were also obtained in good yields (entries 9, 10), although longer reaction times were required at room temperature. No racemization was observed for entries 7 and 10.

Peptide bond formation was also easily accomplished using the silylative activation of *N*-protected α-amino thiol acids (Table 2). The peptidic thiol acids were generated from the corresponding 9-fluorenylmethyl thiol esters by the method of Crich[10] and used without further purification. As shown in Table 2, Gly, Met, Phe, Glu, and Pro thiol acid residues reacted easily to give the corresponding dipeptides (entries 1–9). It is noteworthy that sterically hindered α-amino thiol acids like Val (entries 10 and 11) and even 2-aminoisobutyric thiol acid (entry 12) are effectively coupled using this method, although longer reaction times were required to achieve acceptable yields at room temperature. The amino acids, Phe, Tyr, Val, Ala, Gly, Met, Trp and Pro were all equally effective as *N*-terminal coupling partners (entries 1–15). The formation of Cbz-Gly-L-Tyr-OMe indicates

that phenolic residues do not interfere with the coupling reaction (entry 2). Cbz-Gly-L-Phe-OMe, which is formed as a single dipeptide in 78% yield from Cbz-Gly-SH and L-Phe-OMe under the general coupling conditions (entry 1), is generated in almost an identical isolated yield (71%) when an equimolar amount of Cbz-L-Arg-OH is added to the reaction mixture. This simple experiment confirms the compatibility of this method with both carboxylic acid and guanidine functionalities.

Even prior to any extensive studies of the influence of electronic and steric effects of the silicon reagent, epimerization of sensitive stereocenters using the pH-neutral "silylative switch" protocol for peptide coupling is competitive with or better than existing technologies. For example, the absence of epimerization at both coupling partners was verified by HPLC analysis for the dipeptides shown in entries 1, 3–5 and for the tripeptide Boc-L-Phe-L-Pro-L-Ala-OEt in entry 13 of Table 2. Furthermore, both the Anderson (entry 14) and the Anteunis (entry 15) tests were conducted to evaluate epimerization-prone linkages during the peptide formation process.[1a] Of significance for future systematic studies of the influence of the silylating agent, PhSiH$_2$Cl gave lower levels of epimerization than BSA in the few cases preliminarily investigated. In the Anteunis test, this method gave less than 5% of epimerization, which is superior to results using DCC (DL% 18.8%) and PyBOP (DL% 6.6%) to facilitate the coupling between Z-Gly-L-Phe-OH and L-Val-OMe·HCl, as reported in the literature.[11]

A comparison of the reaction of Boc-L-Glu(O-tBu)-SH and Gly-OMe using a traditional peptide coupling protocol with the new silylative activation is both illustrative and compelling, demonstrating the unique reactivity of the O-silylthionoester approach to peptide construction. A 1:1.3 mixture of Boc-L-Glu(O-tBu)-SH and Gly-OMe·HCl was first exposed to Et$_3$N to liberate the amine and then to 1 equiv of BSA in THF at room temperature to produce the peptide in 74% yield within 8 h. In contrast, traditional activation of the thiol acid with PyBop and DIEA gave a mixture of the desired peptide (40%) and the thioamide (28%) as depicted in Scheme 4.

The origin of the different reactivity of <u>O-alkyl</u>thionoesters and <u>O-silyl</u>thionoesters towards amines is suggested in Scheme 5. The mechanism of the known reaction of O-alkylthionoesters with amines to give thioamides is straightforward.[5c] Attack by the nucleophilic amine at the C=S bond of the thionoester generates tetra-hedral intermediate **A** that can only collapse to create a thioamide or revert back to starting materials. Without cleavage of the strong R-O bond of **A** in Scheme 5, oxoamides cannot be formed from the tetrahedral intermediate generated from the <u>O-alkyl</u>thionoesters. In contrast, however, the tetrahedral intermediate formed by attack of an amine on an <u>O-silyl</u>thionoester can participate in a tautomeric migration of silicon from oxygen to sulfur, possibly via the pentavalent Si intermediate **B**.

In conclusion, O-silylthionoesters, generated *in situ* from thiol acids via a straightforward and simple sequence of S-silylation followed by S-to-O silatropy, were shown to react with 1° and 2° amines in a mild, ambient temperature construction of amides and mono-, di-, and tripeptides. Both unhindered and highly hindered substrates participated in room temperature amide and peptide bond formation, although longer reaction times were required for the sterically encumbered entities. Stereocenter epimerization was absent for most substrates, and very low in substrates known to be problematic in traditional peptide coupling protocols. Although a thorough study of the influence of the nature of the silane on the reaction rate and epimerization level of the amide/peptide coupling has yet to be undertaken, a single example probing variation of the silane structure suggests that unwanted epimerization can be positively influenced by the nature of the silylating agent. At present, this straightforward first-generation trimethylsilylation protocol combined with the

unique reactivity of the *O*-silylthionoesters toward 1° and 2° amines to generate oxoamides provides the simplest means of activating a thiol acid for peptide bond formation at neutral pH.[12] The tolerance of hydroxylic and phenolic groups to the reaction conditions holds promise for extension of the silylative protocol to glycopeptide synthesis. This and experiments to understand how the nature of the triorganosilyl moiety influences the reactivity of the *O*-triorganosilylthionoester toward various nucleophiles are planned. Finally, in its most compelling conception, the formation of amides would take place directly from carboxylic acids and amines via *in situ* generated *O*-silylthionoesters using only catalytic quantities of a thiosilylating agent. Experiments to achieve this forward-looking goal are in progress.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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4. As a control reaction to assess the mechanism of the reaction of thiol acids with azides to generate amides, Williams and coworkers investigated the reaction of *O*-trimethylsilylthionobenzoate with benzyl azide, which generated only trace amounts of corresponding amide (see reference 13h). In a footnote within that manuscript (#24), false coupling results obtained with unpurified and unsparged thiol acid were assumed to be caused by impurities and/or incomplete formation of the thionoester.

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6. Tautomerization of an *S*-silylthiolester to an *O*-silylthionoester functions as a novel *in situ* activation of the carboxyl function for reaction with nucleophiles. Therefore, any synthetic method that can directly generate an *S*-silylthiol ester under mild reaction conditions can serve as an entry to the *O*-silylthionoester.

7. Thiobenzoic acid: ^{1}H NMR: 7.91 (H$_{ortho}$, dd, $J = 1.2, 8.4$ Hz, 2H), 7.61 (H$_{para}$, dt, $J = 1.2, 8.0$ Hz, 1H), 7.47 (H$_{meta}$, t, $J = 7.8$ Hz, 2H), 4.58 (S-H, br s, 1H). IR: 2567(sh), 1660, 1594, 1580. *O*-Trimethylsilylthionoester (see reference 14h) ^{1}H NMR: 8.21 (H$_{ortho}$, d, $J = 8.4$ Hz, 2H), 7.54 (H$_{para}$, t, $J = 7.2$ Hz, 1H), 7.37 (H$_{meta}$, t, $J = 7.8$ Hz, 2H), 0.5 (Me$_3$Si, s, 9H). ^{13}C NMR: 212.6 (S=C-O), 139.6, 133.0, 128.9, 128.1, 0.3.

8. From the recent focus on reagent-based methods to activate thiol acids for reaction with amines to give amides (see reference 10 and 13 of this manuscript), one might infer that thiol acids are unable to directly acylate amines. Examples of thiol acids acylating amines without special activation have appeared in the literature for decades (Cronyn MW, Jiu J. J Am Chem Soc. 1952; 74:4726–4726.Sheehan JC, Johnson DA. J Am Chem Soc. 1952; 74:4726–4727.Hawkins PJ, Tarbell DS, Noble P. J Am Chem Soc. 1953; 75:4462–4465.Hirabayashi Y, Mizuta M, Kojima M, Horio Y, Ishihara H. Bull Chem Soc Jpn. 1971; 44:791–793.However, some caution in interpreting published results is appropriate, since the direct reaction of thioacids with amines is highly dependent on the purity the thioacid.

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12. In the search for effective new peptide ligations, thiol acids and their derivatives have attracted much interest and recently resulted in impressive synthetic advances, most notably from the laboratories of Crich (a) Sasaki K, Crich D. Org Lett. 2010; 12:3254–3257. [PubMed: 20568826] (b) Crich D, Sharma I. Angew Chem, Int Ed. 2009; 48:2355–2358.(c) Crich D, Sasaki K. Org Lett. 2009; 11:3514–3517. [PubMed: 19719195] (d) reference #12, Danishefsky (e) Wu X, Stockdill JL, Wang P, Danishefsky SJ. J Am Chem Soc. 2010; 132:4098–4100. [PubMed: 20199071] (f) Wang P, Danishefsky SJ. J Am Chem Soc. 2010; 132:17045–17051. [PubMed: 21049949] (g) Rao Y, Li X, Danishefsky SJ. J Am Chem Soc. 2009; 131:12924–12926. [PubMed: 19697932] and Williams (h) Kolakowski RV, Shangguan N, Sauers RR, Williams LJ. J Am Chem Soc. 2006; 128:5695–5702. [PubMed: 16637636] (i) Barlett KN, Kolakowski RV, Katukojvala S, Williams LJ. Org Lett. 2006; 8:823–826. [PubMed: 16494450] (j) Shangguan N, Katukojvala S, Greenberg R, Williams LJ. J Am Chem Soc. 2003; 125:7754–7755. [PubMed: 12822965] among others (k) Pan J, Devarie-Baez NO, Xian M. Org Lett. 2011; 13:1092–1094. [PubMed: 21271725] (l) Zhang X, Li F, Lu XW, Liu CF. Bioconjugate Chem. 2009; 20:197–200.(m) Hackenberger CPR, Schwarzer D. Angew Chem, Int Ed. 2008; 47:10030–10074.

Scheme 1.
In Situ Carboxyl Activation using a Silicon Switch

Scheme 2.
Differential Reactivity of Thionoesters

Scheme 3.
Exploratory Reactions using Thiobenzoic Acid

Scheme 4.
Silylative *vs* Traditional Activation of a Thiol Acid

Scheme 5.
S-to-*O* Silylative Switch Amidation Mechanism

Table 1

S-to-*O* Silylative Switch-Induced Amide Formation

entry	amide	hr *yld*[a] (%)
1		3 91
2		3 89
3		3 76
4		5 74
5		24 62
6		3 82
7[b]		24 69
8		5 65
9		23 81
10[b]		24 71

[a] General procedure: 1 equiv of thiol acid, 1.3 equiv of amine and 1 equiv of BSA stirred at room temperature in THF. Isolated yields.

[b] No racemization was observed.

Table 2

Activation of Thiol Acids for Peptide Synthesis via the "Silylative Switch".

entry	amino thiol acid	amino ester	di or tripeptide	time (hr	yld^a (%)	epimerization
1	Cbz-Gly-SH	L-Phe-OMe · HCl	Cbz-Gly-L-Phe-OMe	10 10	78 71^b	L/D = 100: 0
2	Cbz-Gly-SH	L-Tyr-OMe · HCl	Cbz-Gly-L-Tyr-OMe	10	69	
3	Boc-L-Met-SH	L-Val-OEt · HCl	Boc-L-Met-L-Val-OEt	10	83	LL/DL > 99: 1
4	Boc-L-Phe-SH	L-Ala-OEt · HCl	Boc-L-Phe-L-Ala-OEt	12	72	LL/DL > 99: 1
5	Boc-L-Glu(OrBu)-SH	L-Val-OMe · HCl	Boc-L-Glu(OrBu)-L-Val-OMe	10	71	LL/DL > 99: 1
6	Boc-L-Glu(OBn)-SH	Gly-OEt · HCl	Boc-L-Glu(OBn)-Gly-OEt	8	76	
7	Boc-L-Glu(OBn)-SH	L-Met-OMe · HCl	Boc-L-Glu(OBn)-L-Met-OMe	10	68	
8	Boc-L-Glu(OBn)-SH	L-Trp-OMe · HCl	Boc-L-Glu(OBn)-L-Trp-OMe	8	74	
9	Boc-L-Pro-SH	L-Val-OMe · HCl	Boc-L-Pro-L-Val-OMe	10	65	
10	Boc-L-Val-SH	L-Pro-OMe · HCl	Boc-L-Val-L-Pro-OMe	48	70	
11	Boc-L-Val-SH	L-Phe-OMe · HCl	Boc-L-Val-L-Phe-OMe	63	67	
12	Boc-Aib-SH	L-Trp-OMe · HCl	Boc-Aib-L-Trp-OMe	54	74	
13	Boc-L-Phe-L-Pro-SH	L-Ala-OEt · HCl	Boc-L-Phe-L-Pro-L-Ala-OEt	8	65^c	LLL/LDL > 99: 1
14	Cbz-Gly-L-Phe-SH	Gly-OEt · HCl	Cbz-Gly-L-Phe-Gly-OEt	15	80^c 60^c 73^d	L/D > 96: 4 L/D > 96: 4 L/D = 97: 3
15	Cbz-Gly-L-Phe-SH	L-Val-OMe · HCl	Cbz-Gly-L-Phe-L-Val-OMe	15	74 50^c 55^d	LL/DL > 95: 5 LL/DL > 96: 4 LL/DL = 97: 3

General procedure: A solution of 1 equiv of the *N*-protected α-amino thiol acid (generated from the corresponding 9-fluorenylmethyl thiol esters via piperidine deprotection-HCl acidification) and 1 equiv of BSA in THF was added to a solution of 1.3 equiv of the amino acid hydrochloride salt and 1.3 equiv of triethylamine or diisopropylethylamine (DIEA) in THF. The mixture was then stirred at room temperature for 8–63 h.

^{a}Isolated yield; the epimerization ratio was determined by HPLC.

^{b}A solution of 1 equiv of Cbz-Gly-SH and 1 equiv of BSA in THF was added to a solution of 1 equiv of Cbz-L-Arg-OH, 1.3 equiv of L-Phe-OMe · HCl and 1.3 equiv of triethylamine. The reaction mixture was stirred at room temperature for 10 h.

[c] To a THF solution of 1 equiv dipeptidic thiol acid, 1.1 equiv PhSiH2Cl, 1.3 equiv amino ester hydrochloride salt was added 2.3 equiv DIEA stirred at room temperature for 8 or 15 h.

[d] To a THF solution of 1 equiv of dipeptidic thiol acid, 1.1 equiv PhSiH2Cl, 2 equiv amino acid ester hydrochloride salt was added 3.0 equiv DIEA stirred at room temperature for 15 h.