Thioamide Synthesis: Thioacyl-*N*-phthalimides as Thioacylating Agents

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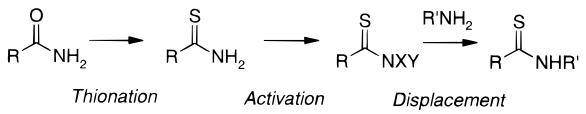
Thiopeptides have attracted interest as synthetic targets owing to their potential as backbone-modified peptide surrogates.[1] The thiopeptide bond is isoelectronic to the parent (oxo)amide bond yet possesses markedly different physical and chemical properties including enhanced stability against enzymatic degradation. Thiopeptides have been hitherto most efficiently prepared by first converting a preformed dipeptide[2] (both termini protected) to the corresponding thiodipeptide, followed by incorporation into the peptide sequence by fragment coupling.[3,4] A significant limitation of this approach is that carboxy-terminus activation of the thiodipeptide unit often leads to epimerization.[5] *N*-Thioacylation using what amounts to be an *activated thiono acid* would avoid this problem and present a means of incorporating the thioamide unit in a stepwise manner directly comparable to *N*-acylation with an activated acid in conventional (oxo)peptide coupling. Thiono analogues of conventional activated acids are, however, unstable and not readily available. In addition, with the advent of combinatorial chemistry, stepwise *N*-thioacylation would become a prerequisite for the construction of thiopeptide libraries.[6]

This need for efficient thioacylating agents has stimulated considerable research interest, and several approaches have been described.[7,8] In particular, thioacyl-*N*-benzimidazolinones,[7a,b] developed by Zacharie *et al.*, and thioacyl-*N*-nitrobenzotriazoles,[7c] reported by Rapoport *et al.*, offer practical solutions for the synthesis of thiopeptides. Our effort in this area has resulted in a novel and efficient procedure for achieving stepwise thioacylation using thioacyl-*N*-phthalimides, which is disclosed herein.

Scheme 1



Scheme 2

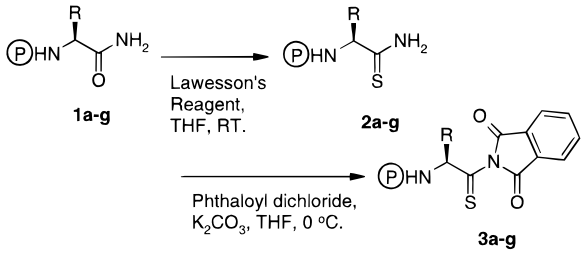


Table 1. Data for the Synthesis of Thioacyl-*N*-phthalimides 3

	1	yield of **2**[a] (%)	yield of **3**[a] (%)	enantiomeric purity of **3**[b] (%)
a	Boc-Phe-NH_2	87	65[c]	99[d]
b	Boc-Leu-NH_2	99	63[c]	>99.5[d]
c	Boc-Phg-NH_2	96	77	>95[d]
d	Boc-Val-NH_2	100	94	97.7[e]
e	Fmoc-Ala-NH_2	77	72[c]	98.4[f]
f	Boc-Ser(Bn)-NH_2	87	75	85.5[g]
g	Boc-Pro-NH_2	82	94	not determined

[a] Isolated products. [b] Determined by HPLC (^{1}H NMR (360 MHz) for compound **3c**) analysis of ratio of diastereoisomers obtained by reaction with a homochiral amine.[d−g] [c] Recrystallized product. [d] (*S*)-α-Methylbenzylamine. [e] D-Alaninol. [f] H-Ala-NHBn. [g] (*R*)-α-Methylbenzylamine.

We envisaged a general reaction scheme for the preparation of a thioacylating agent (Scheme 1). Thus, a primary amide would be first thionated, and subsequently, the $-NH_2$ would be converted to a good leaving group (−NXY). Displacement by an incoming amine would lead to the desired thioamide. Note that the thionation step precedes the activation step in this scheme, which would ensure that sulfur is introduced under mild (nonracemizing) conditions. The starting material, a primary amide, is also the most reactive substrate for Lawesson's reagent,[9] the thionating agent of choice. On account of the ease of preparation, and leaving group ability of the phthalimide anion, *N*-phthalimidation was chosen as the activation method.[10] We explored this general idea with a selection of *N*-protected amino acid amides[11] (Scheme 2 and Table 1).

Preparation of the thioacylating agents **3** turned out to be an experimentally facile process. Thus, thionation of the amino acid amides **1** with Lawesson's reagent proceeded smoothly at room temperature and provided the thioamides **2** in excellent yields. These were easily converted to the thioacyl-*N*-phthalimides **3** in good to excellent yields by treatment with phthaloyl dichloride at 0 °C. The products were stable toward purification on a silica column and prolonged storage at 4 °C. Compounds **3a**, **3b**, and **3e** were crystalline.

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(1) Review: Spatola, A. F. In *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins;* Weinstein, B., Ed.; Marcel Dekker: New York, 1983; Vol. 7, Chapter 5, pp 267−357.

(2) Synthesis of a more complex multithiopeptide incorporating *n* adjacent thioamide linkages would require thionation of a preformed peptide of $(n + 1)$ residues.

(3) (a) Clausen, K.; Thorsen, M.; Lawesson, S.-O.; Spatola, A. F. *J. Chem. Soc., Perkin Trans. 1* **1984**, 785−798. (b) Thorsen, M.; Yde, B.; Pedersen, U.; Clausen, K.; Lawesson, S.-O. *Tetrahedron* **1983**, *39*, 3429−3435.

(4) Direct thionation of a peptide usually leads to a mixture of regioisomeric thiopeptides unless a strong steric/conformational bias is present; for example, see: Seebach, D.; Ko, S. Y.; Kessler, H.; Köck, M.; Reggelin, M.; Schmieder, P.; Walkinshaw, M. D.; Bölsterli, J. J.; Bevec, D. *Helv. Chim. Acta* **1991**, *74*, 1953−1990.

(5) Unverzagt, C.; Geyer, A.; Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1229−1230.

(6) This aspect will be discussed fully in a later publication.

(7) (a) Zacharie, B.; Sauvé, G.; Penney, C. *Tetrahedron* **1993**, *49*, 10489−10500. (b) Belleau, B.; Brillon, D.; Sauvé, G.; Zacharie, B. U.S. Patent No. 5,138,061, Aug 11, 1992. (c) Reported during the preparation of this paper: Shalaby, M. A.; Grote, C. W.; Rapoport, H. *J. Org. Chem.* **1996**, *61*, 9045−9048.

(8) (a) Høeg-Jensen, T.; Olsen, C. E.; Holm, A. *J. Org. Chem.* **1994**, *59*, 1257−1263. (b) Katritzky, A. R.; Moutou, J.-L.; Yang, Z. *Synthesis* **1995**, 1497−1505. (c) Katritzky, A. R.; Moutou, J.-L.; Yang, Z. *Synlett* **1995**, 99−100. (d) Le, H.-T.; Mayer, M.; Thoret, S.; Michelot, R. *Int. J. Peptide Protein Res.* **1995**, *45*, 138−144. (e) DeBruin, K. E.; Boros, E. E. *J. Org. Chem.* **1990**, *55*, 6091−6098. (f) Elmore, D. T.; Guthrie, D. J. S.; Kay, G.; Williams, C. H. *J. Chem. Soc., Perkin Trans. 1* **1988**, 1051−1055.

(9) For a review of Lawesson's reagent see: Cava, M. P.; Levinson, M. I. *Tetrahedron* **1985**, *41*, 5061−5087.

(10) A literature survey revealed the following references to (thioacyl)-*N*-phthalimides: (a) Goerdeler, J.; Stadelbauer, K. *Chem. Ber.* **1965**, *98*, 1556−1561. (b) Goerdeler, J.; Horstmenn, H. *Chem. Ber.* **1960**, *93*, 670−678; (c) **1960**, *93*, 663−670.

(11) Unless indicated otherwise, amino acid symbols represent the L-enantiomers.

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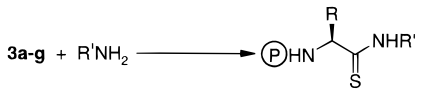
The enantiomeric purities of the thioacyl-*N*-phthalimides **3** were determined by reaction with the two enantiomers of a chiral amine: α-methylbenzylamine for **3a,b**,**c**,**f**, alaninol for **3d**, or alanine benzylamide for **3e**. These reactions were carried out in dichloromethane (in the α-methylbenzylamine cases) or chloroform and proceeded in high yields (0 °C, 10 min in all cases). HPLC and ^{1}H NMR analysis of the diastereomeric pairs of thioamide products indicated that the compounds **3a**−**e** were highly enantiopure (Table 1). In particular, the result for the phenylglycine derivative **3c** demonstrates the compatibility of our synthetic procedure with an amino acid that is especially prone to racemization. The compound **3f**, derived from *N*-Boc-*O*-Bn-serine, presented an anomalous case since the α-methylbenzylthioamides were obtained in *ca.* 85% diastereomeric purity. It is suspected that some epimerization, presumably *via* β-elimination/1,4-addition of benzyl alcohol, accompanied the thioacylation step.

In order to assess their utility in thiopeptide synthesis, the thioacyl-*N*-phthalimides were treated with a selection of amino acid amides, including a dipeptide. The results are summarized in Table 2. As we anticipated, the thioacyl-*N*-phthalimides reacted smoothly with these nucleophiles in good to quantitative yields under very mild conditions (chloroform, 0 °C, 10 min). The high reactivity of our new thioacylating agents was noteworthy: thus, sterically demanding couplings as exemplified by entries 4 and 8 (hindered valine nucleophiles) and entry 10 (hindered thioacylating agent **3d**, derived from *N*-Boc-valine) proceeded efficiently. Some explanation for this high reactivity may be found in the crystalline conformations of the compounds **3a** and **3b**, as determined by X-ray crystallography.[12]

The selectivity of these reagents was illustrated by the reaction of **3a** with serine benzylamide (Table 2, entry 2) which provided exclusively the *N*-thioacylated product without need for protection of the hydroxyl group (note also that reaction of **3d** with alaninol (Table 1) provided only the *N*-thioacylated product). The high diastereomeric purities observed for the coupling products of compound **3e** with the two enantiomers of alanine benzylamide (Table 2, entries 12 and 13) demonstrated a negligible degree of epimerization during the thioacylation step.[13]

Table 2. Reactions of Thioacyl-*N*-phthalimides 3 with Amino Acid Amides



entry	thioacyl-*N*-phthalimide **3**[a]	R′NH$_2$	yield[b] (%)
1	**a**	H-Ala-NHBn[c]	98
2	**a**	H-Ser-NHBn[c]	81
3	**a**	H-Phe-Gly-NH$_2$[d]	73
4	**a**	H-Val-NHBn[c]	69
5	**a**	H-Phe-NHBn[c]	68
6	**b**	H-Gly-NHBn[c]	100
7	**b**	H-Phe-Gly-NH$_2$[d]	79
8	**b**	H-Val-NH$_2$[c]	72
9	**b**	H-Phe-NHBn[c]	71
10	**d**	H-Phe-NHBn[d]	77
11	**e**	H-Gly-NHBn[d]	100
12	**e**	H-Ala-NHBn[c]	99
13	**e**	H-D-Ala-NHBn[c]	76
14	**e**	H-Phe-NHBn[c]	68
15	**e**	H-Phe-Gly-NH$_2$[d]	55
16	**f**	H-Gly-NHBn[d]	60
17	**g**	H-Gly-NHBn[d]	74
18	**g**	H-Ala-NHBn[d]	50

[a] See Table 1 for structures. [b] Isolated products. [c] Reaction performed with the HCl salt in the presence of 1.0 equiv of triethylamine. [d] Reaction performed with the free base.

In summary, we have developed a new thioacylating procedure based on thioacyl-*N*-phthalimides. The reagents are very easily prepared in high enantiomeric purity from readily available starting materials, and the *N*-thioacylation step proceeds smoothly in high yields under mild conditions. This method presents an efficient means of achieving a stepwise thiocoupling reaction. Present efforts are directed toward application of this thioacylation methodology to solid-phase synthesis.

Acknowledgment. Technical support from Dr. I. Linney is gratefully acknowledged.

Supporting Information Available: Representative experimental procedures and characterization data for compounds **2** and **3** and the thioacylation products (9 pages).

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(12) (a) The phthalimide ring is completely planar, while the thioacyl group lies *ca.* 36° out of the plane and, therefore, is not stabilized by conjugation. (b) The authors have deposited atomic coordinates for compounds **3a** and **3b** with the Cambridge Crystallographic Data Centre. The coordinates may be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K.

(13) Diastereoisomeric purity of the coupling product of **3e** and D-alanine benzylamide: 98.9%. See also reaction of **3e** with L-alanine benzylamide (Table 1).