

# Facile *N*-Urethane-Protected $\alpha$ -Amino/Peptide Thioacid Preparation Using EDC and Na<sub>2</sub>S

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**Abstract:** We report herein an efficient protocol for the synthesis of *N*-urethane-protected  $\alpha$ -amino/peptide thioacids from their corresponding acids mediated by EDC and Na<sub>2</sub>S. The fast reaction under mild conditions enabled the process to be completed in shorter duration with good yield circumventing column purification. The chemistry is compatible with a wide variety of urethane protecting groups, side-chain functionalities, and sterically hindered amino acids.

**Key words:** *N*-urethane-protected  $\alpha$ -amino thioacids, carbodiimides, sodium sulfide

Protected thioacids masked as thioesters have been employed as key components in Native Chemical Ligation (NCL).<sup>1</sup> Thioacids [R(C=O)SH] play a pivotal role in organic synthesis because of their specific reactive profile and chemical behavior.<sup>2,3</sup> They are frequently employed in peptide and peptidomimetic syntheses as well.<sup>4</sup> In particular, thioacids exhibit varied reactivity towards amines,<sup>5</sup> isonitriles,<sup>6</sup> alkyl azides,<sup>7</sup> sulfonyl azides,<sup>8</sup> isocyanates/isothiocyanates,<sup>9</sup> aziridines,<sup>10</sup> electron-deficient sulfonamides,<sup>11</sup> and alkyl halides<sup>12</sup> leading to biologically desirable mimetics.<sup>13,14</sup>

Optically active  $\alpha$ -amino thioacids are generally prepared by the activation of the carboxy group followed by passing H<sub>2</sub>S gas.<sup>15</sup> Alternatively, the *N* <sup>$\alpha$</sup> -protected amino acid is coupled with 9-fluorenylmethane thiol (FmSH), triphenylmethane thiol (TrtSH), or trimethoxybenzyl thiol (TmobSH)<sup>16</sup> to obtain the corresponding thioester which is then deprotected to the thioacid. Recently, Danishefsky's group has described the preparation of thioacids by direct reaction of carboxylic acids with Lawesson's reagent at high temperature.<sup>17</sup> To avoid the use of highly toxic H<sub>2</sub>S gas, solid NaSH and N<sub>2</sub>S have been used along with 1,1'-carbonyldiimidazole (CDI) as coupling reagent.<sup>18</sup> However, long reaction times and slow reactivity can result in low recovery of the product and acyldisulfides, unreacted starting material, and other impurities are often observed as byproducts. Peptide thioacids are generally synthesized by treatment of Kaiser's oxime ester with hexamethyldisilathiane,<sup>19</sup> while processes involving acyl-enzyme thioester intermediates,<sup>20</sup> hydrolysis of resin-bound peptide thioesters,<sup>21</sup> and reaction of NaSH with res-

in-bound peptide are also known.<sup>22</sup> With these promising results and the recent trends in the application of  $\alpha$ -amino thioacids,<sup>23,24</sup> there is a pressing need for a rapid and efficient methodology for the preparation of amino and peptide thioacids.

Owing to its fast reactivity under mild conditions, water-soluble *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide (EDC) is often used for the epimerization-free peptide synthesis.<sup>25</sup> Recently, we had the occasion to explore this reagent for the synthesis of acid azides, ureas, and carbamates.<sup>26</sup> From these initial studies, we initiated our examination on the use of EDC in the synthesis of thioacids. The reaction was fast, and the products were obtained in good yields.

In order to optimize the reaction conditions, we studied the conversion of Fmoc-Ala-OH to its thioacid. Our study initially used *N,N'*-dicyclohexylcarbodiimide (DCC) as coupling agent and Na<sub>2</sub>S as hydrosulfide ion source in various solvents (Table 1, entries 1–6). The first attempt was performed in MeCN by using three equivalents of Na<sub>2</sub>S for three hours which led to thioacid **2b** in 54% yield along with unreacted **1b** (Table 1, entry 1). Based on this preliminary result, the reaction conditions were further fine-tuned using different solvents.

It was observed that the use of THF, dioxane, and DMSO resulted in poor yields and the formation of unidentified byproducts (Table 1, entries 2–4), but a yield of **2b** of 61% was observed when DMF was used (Table 1, entry 5). Further, we tested the reaction in the presence of HOBt as an additive in DMF and, although product yield was increased to 69%, 22% of unreacted **1b** was still observed (Table 1, entry 6). To explore the scope and limitations of carbodiimides, EDC was then chosen. The initial experiments using **1b** with EDC/HOBt and Na<sub>2</sub>S in DMF gave **2b** in an acceptable yield of 82% (Table 1, entry 7).

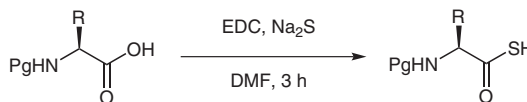
With this combination 11% of **1b** was also detected. At this stage we attempted to isolate pure **2b** through column purification, but this was not satisfactory due to the almost identical *R<sub>f</sub>* values of both **1b** and **2b**.<sup>15b</sup> Subsequently, EDC in DMF turned out to give satisfactory results in the model reaction (Scheme 1). To our delight, the desired product was obtained in 92% yield. Interestingly, no unreacted **1b** was found which enabled simple recrystallization to be used for the isolation of the pure product. Another test case was conducted with diisopropylcarbodiimide (DIC) and DIC/HOBt (Table 1, entries 10 and 11),

**Table 1** Optimization of Reaction Conditions for the Synthesis of **2b**

Entry	Coupling reagent	Additive	Solvent	Time (h)	Yield of <b>2a</b> (%) <sup>a</sup>	Yield of <b>1a</b> (%) <sup>a</sup>
1	DCC–NMM	–	MeCN	8	54	25
2	DCC–NMM	–	THF <sup>b</sup>	8	48	35
3	DCC–NMM	–	dioxane	8	57	32
4	DCC–NMM	–	DMSO	6	51	31
5	DCC–NMM	–	DMF	5	61	25
6	DCC–NMM	HOBt	DMF	4	69	22
7	EDC	HOBt	DMF	3	82	11
8	EDC	–	DMF	3	92	–
9	EDC	–	DMSO	3	89	6
10	DIC	–	DMF	5	48	51
11	DIC–NMM	HOBt	DMF	4	52	38

<sup>a</sup> Yields are given according to the mass analysis of the crude reaction mixture measured by LC-MS.<sup>b</sup> An aliquot of H<sub>2</sub>O was added to dissolve N<sub>2</sub>S.

and the results were more or less similar to DCC/HOBt. Thus, optimal conditions for the synthesis of thioacids are as follows: under argon atmosphere, acid (**1** equiv) was dissolved in DMF (5 mL), EDC (1.1 equiv) was added at 0 °C, after five minutes of stirring a solution of Na<sub>2</sub>S (3 equiv) in DMF (10 mL) was added, and the reaction mixture was stirred for three hours.

**Scheme 1** Synthesis of *N*-urethane-protected  $\alpha$ -amino thioacids

To illustrate the generality and scope of the optimized protocol, a range of *N*-urethane protecting groups and various side-chain-functionalized amino acids were converted to the corresponding thioacids, and the results are summarized in Table 2. In general, good yields are obtained and no significant difference in the reactivity was observed for all the *N*-protecting groups. The effect of side-chain functionalities was also observed by synthesizing thioacids from the sterically hindered amino acids Aib, Val, and Ile where a reaction time of up to eight hours was required to complete the conversion. Our study was then extended to prepare some peptide thioacids. For this, a series of di- and tripeptide acids were synthesized using the EDC method.<sup>27</sup> Thus, *N*-protected  $\alpha$ -amino acids were activated by EDC/HOBt. A fresh solution of *O,N*-bis/tris-trimethylsilyl amino acid was added directly in one portion and stirring was continued until completion of the reaction.

**Table 2** Synthesized *N*-Urethane-Protected  $\alpha$ -Amino Thioacids

Entry	Compd <b>2</b>	Thioacid	$[\alpha]_D^{20}$ (c, 1.2, CHCl <sub>3</sub> )	Yield (%) <sup>a</sup>	Mp (°C)
1	<b>2a</b>			89	107–109
2	<b>2b</b>		–11.7	91	78–79
3	<b>2c</b>		+12.9	86	76–78
4	<b>2d</b>		–58.2	90	101–103
5	<b>2e</b>		–39.6	88	81–83
6	<b>2f</b>		–41.0	91	87–88
7	<b>2g</b>		–49.8	85	gum
8	<b>2h</b>		–88.1	86	gum

**Table 2** Synthesized N-Urethane-Protected  $\alpha$ -Amino Thioacids (continued)

Entry	Compd 2	Thioacid	$[\alpha]_D^{20}$ (c, 1.2, CHCl <sub>3</sub> )	Yield (%) <sup>a</sup>	Mp (°C)
9	<b>2i</b>		-28.6	79	121–123
10	<b>2j</b>		-66.7	94	91–93
11	<b>2k</b>		-55.0	91	65–67
12	<b>2l</b>			86	gum
13	<b>2m</b>		-22.7	90	gum
14	<b>2n</b>		-10.2	90	113–115

<sup>a</sup> Isolated yield based on **1**.

Acidification followed by recrystallization of the crude product led to peptide acids in good yield. The resulting peptide acids were then converted to their thioacids under conditions similar to those employed for the preparation of  $\alpha$ -amino thioacids (Table 3).<sup>28–30</sup> To determine whether the reaction conditions lead to any detectable amount of epimerization in the present protocol, Fmoc-Ala-SH enantiomers were synthesized, and the optical purity was measured by chiral HPLC. The D- and L-Fmoc-Ala-SH were baseline separated with a retention time of 8.4 and 13.5 minutes, respectively.<sup>31</sup> In addition, specific rotations of **2b** and **2c** were recorded as -11.7 and +12.9, respectively. These results indicate that the protocol is free from epimerization.

In conclusion, we have demonstrated that N-urethane-protected  $\alpha$ -amino/peptide oxoacids can be readily converted into their corresponding thioacids in high yields under mild conditions. The reaction of N-protected  $\alpha$ -amino acids, EDC, and Na<sub>2</sub>S in DMF led to the desired products in good yields. Notably, this procedure has been shown to be fully compatible with all urethane protections as well as a range of side-chain functionalities. The protocol is operationally simple, effective, and minimizes side products so that the crude products can be used directly for further ap-

**Table 3** Synthesized Peptide Thioacids

Entry	Compd 3	Peptide thioacid	Yield (%) <sup>a</sup>
1	<b>3a</b>		78
2	<b>3b</b>		89
3	<b>3c</b>		78
4	<b>3d</b>		91
5	<b>3f</b>		88

<sup>a</sup> Yields are given after recrystallization of the crude product from THF-H<sub>2</sub>O.

plications. No epimerization in the peptide thioacids has been detected by chiral HPLC.

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## References and Notes

- (a) Kent, S. B. H. *Chem. Soc. Rev.* **2009**, 38, 338. (b) Muir, T. W. *Annu. Rev. Biochem.* **2003**, 72, 249.
- Crich, D.; Sasaki, K.; Rahaman, Md. Y.; Bowers, A. A. *J. Org. Chem.* **2009**, 74, 3886.
- (a) Haug, H.; Carey, R. I. *J. Peptide Res.* **1998**, 51, 290. (b) Hadad, C. M.; Rablen, P. R.; Wiberg, K. B. *J. Org. Chem.* **1998**, 63, 8668.
- Barlett, K. N.; Kolakowski, R. V.; Katukojvala, S.; Williams, L. *J. Org. Lett.* **2006**, 8, 823.
- (a) Blake, J. *Int. J. Pept. Protein Res.* **1981**, 17, 273. (b) Yamashiro, D.; Blake, J. *Int. J. Pept. Protein Res.* **1981**, 18, 383. (c) Mitin, Y. V.; Zapevalova, N. P. *Int. J. Pept. Protein Res.* **1990**, 35, 352.
- (a) Wu, X.; Stockdill, J. L.; Wang, P.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2010**, 132, 4098. (b) Rao, Yu.; Li, X.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2009**, 131, 12924.
- (a) Park, S.-D.; Oh, J.-H.; Lim, D. *Tetrahedron Lett.* **2002**, 43, 6309. (b) Fazio, F.; Wong, C.-H. *Tetrahedron Lett.* **2003**, 44, 9083. (c) Mckerverey, M. A.; O'Sullivan, M. B.; Mayers, P. L.; Green, R. H. *J. Chem. Soc., Chem. Commun.* **1993**, 94.

- (8) (a) Shangguna, N.; Katukojvala, S.; Greenberg, R.; Williams, L. J. *J. Am. Chem. Soc.* **2003**, *125*, 7754. (b) Merckx, R.; van Haren, M. J.; Rijkers Drik, T. S.; Liskamp, R. M. J. *J. Org. Chem.* **2007**, *72*, 4574.
- (9) Crich, D.; Sasaki, K. *Org. Lett.* **2009**, *11*, 3514.
- (10) Assem, N.; Natarajan, A.; Yudin, A. K. *J. Am. Chem. Soc.* **2010**, *132*, 10986.
- (11) Crich, D.; Sharma, I. *Angew. Chem. Int. Ed.* **2009**, *48*, 2355.
- (12) Fu, X.; Jiang, S.; Li, C.; Xin, J.; Yang, Y.; Ji, R. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 465.
- (13) Kolb, J.; Beck, B.; Almstetter, M.; Heck, S.; Herdtweck, E.; Dömling, A. *Mol. Diversity* **2003**, *6*, 297.
- (14) Yazmin, T.; Rosa-Bauza Berst, F.; Ellman, J. A. *ChemBioChem.* **2007**, *8*, 981.
- (15) (a) Le, H.-T.; Gallard, J.-F.; Mayer, M.; Guittet, E.; Michelot, R. *Bioorg. Med. Chem.* **1996**, *4*, 2201. (b) Hoeg-Jensen, T.; Jakobsen, H.; Olsen, C. E.; Holm, A. *Tetrahedron Lett.* **1991**, *32*, 7617. (c) Hoeg-Jensen, T.; Holm, A.; Sorensen, H. *Synthesis* **1994**, 383.
- (16) (a) Crich, D.; Sana, K.; Guo, S. *Org. Lett.* **2007**, *9*, 4423. (b) Crich, D.; Sharma, I. *Angew. Chem. Int. Ed.* **2009**, *48*, 2355.
- (17) Rao, Y.; Li, X.; Nagorny, P.; Hayashida, J.; Danishefsky, S. J. *Tetrahedron Lett.* **2009**, *50*, 6684.
- (18) Monfardini, I.; Huang, J.-W.; Beck, B.; Cellitti, J. F.; Pellicchai, M.; Dömling, A. *J. Med. Chem.* **2011**, *54*, 890.
- (19) Schwabacher, A. W.; Maynard, T. L. *Tetrahedron Lett.* **1993**, *34*, 1269.
- (20) Tan, X.-H.; Yang, R.; Wirjo, A.; Liu, C.-F. *Tetrahedron Lett.* **2008**, *49*, 2891.
- (21) Zhang, X.; Lu, X.-W.; Liu, C.-F. *Tetrahedron Lett.* **2008**, *49*, 6122.
- (22) Shigenaga, A.; Sumikawa, Y.; Tsuda, S.; Sato, S.; Otaka, A. *Tetrahedron* **2010**, *66*, 3290.
- (23) (a) Wang, P.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2010**, *132*, 17045. (b) Wang, P.; Li, X.; Zhu, J.; Chen, J.; Yuan, Y.; Wu, X.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2011**, *133*, 1597.
- (24) Pan, J.; Nelmi, O.; Devarie, B.; Xian, M. *Org. Lett.* **2011**, *13*, 1092.
- (25) Goyal, N. *Synlett* **2010**, 335; and references cited therein.
- (26) Sureshbabu, V. V.; Lalithamba, H. S.; Narandra, N.; Hemantha, H. P. *Org. Biomol. Chem.* **2010**, *8*, 835.
- (27) (a) **General Procedure for the Preparation of Peptide Acids**  
A solution of N<sup>α</sup>-protected amino acid (1 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (5 mL) was cooled to 0 °C, EDC (1 mmol), HOBt (1.2 mmol), and O,N-bis-TMS-amino acid (1.5 mmol) were added. The reaction mixture was stirred for 3–4 h (TLC analysis), and then evaporation of the solvent and acidification with 1 M HCl furnished pure peptide acid.  
(b) For the preparation of O,N-bis-TMS-amino acids, see: Tantry, S. J.; Vasanthakumar, G.-R.; Sureshbabu, V. V. *Lett. Pept. Sci.* **2003**, *10*, 51.
- (28) **General Procedure for the Synthesis of Amino/Peptide Thioacids**  
To a DMF solution of an acid (1.0 mmol), EDC (1.1 equiv) was added at 0 °C under a nitrogen atmosphere. After stirring for 10 min, finely ground Na<sub>2</sub>S (3 equiv) was added to the reaction mixture which was allowed for stir for 3–4 h until the disappearance of the starting material (TLC analysis). The residue was dissolved in EtOAc (15 mL), and the solution was then carefully acidified at 0 °C to a pH of 3 by using 1 M KHSO<sub>4</sub>. The organic layer was then immediately separated and removed under reduced pressure. The crude product was triturated with Et<sub>2</sub>O or recrystallized with THF–H<sub>2</sub>O to obtain pure thioacid.
- (29) **Fmoc-Ile-COSH**  
Yellow solid; mp 81–83 °C. IR (KBr): ν<sub>max</sub> = 1689, 1739, 2550, 3342 cm<sup>-1</sup>. R<sub>f</sub> = 0.39 (EtOAc–n-hexane = 60:40). RP-HPLC: t<sub>R</sub> = 15.2 (60–100% MeCN, 30 min). ESI-HRMS: m/z calcd for C<sub>21</sub>H<sub>23</sub>NO<sub>3</sub>S: 392.1296 [M + Na]<sup>+</sup>; found: 392.1290. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ = 0.88 (t, J = 5.6 Hz, 3 H), 0.98 (d, J = 3.8 Hz, 3 H), 1.12–1.24 (m, 2 H), 2.38–2.47 (m, 1 H), 4.28 (d, J = 6.7 Hz, 1 H), 4.37 (d, J = 7.1 Hz, 1 H), 4.61 (d, J = 4.4 Hz, 2 H), 5.91 (br s, 1 H), 7.43 (br s, 1 H), 7.26–7.84 (m, 8 H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ = 10.8, 14.3, 24.1, 37.0, 46.4, 65.9, 72.8, 125.9, 127.3, 128.6, 128.9, 139.2, 142.6, 155.2, 197.2.
- (30) **Fmoc-Ala-Phe-COSH**  
White solid; mp 126–128 °C. IR (KBr): ν<sub>max</sub> = 1681, 1748, 1768, 2549, 3328 cm<sup>-1</sup>. R<sub>f</sub> = 0.53 (CHCl<sub>3</sub>–MeOH = 80:20). RP-HPLC: t<sub>R</sub> = 11.4 (60–100% MeCN, 30 min). ESI-HRMS: m/z calcd for C<sub>27</sub>H<sub>26</sub>N<sub>2</sub>O<sub>4</sub>S: 497.1511 [M + Na]<sup>+</sup>; found: 497.1501. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ = 1.2 (d, J = 4.8 Hz, 3 H), 2.6 (d, J = 5.6 Hz, 2 H), 2.8 (br s, 1 H), 3.38 (t, J = 7.4 Hz, 1 H), 3.6 (br s, 1 H), 3.9 (t, J = 6.9 Hz, 2 H), 4.1 (m, 1 H), 4.3 (m, 1 H), 6.32 (br s, 1 H), 7.1 (br s, 1 H), 7.2–7.9 (m, 13 H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ = 17.2, 37.4, 46.8, 51.2, 67.9, 69.7, 125.7, 126.8, 127.2, 127.9, 128.4, 128.9, 131.2, 139.1, 141.4, 143.2, 155.7, 172.1, 197.2.
- (31) Chiral-HPLC analyses were carried out employing Chiralpak IA, 250 × 4.6 mm; solvent: hexane–EtOH (7:3); flow rate: 1.0 mL/min.

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