A Facile Synthesis of Thioacids from *N*-Acylbenzotriazoles

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Abstract: Protected amino/peptide thioacids have been synthesized in pure form in excellent yields and with retention of chirality by using protected aminoacyl- and peptidoylbenzotriazoles as active intermediates.

Key words: amino acids, peptides, chirality, thioacids, benzotriazole methodology

Thioacids are one of the most versatile analogues of carboxylic acids. Replacement of carboxyl by thiocarboxyl group provides an important class of organic compounds that have found wide significance. Thus thioacids undergo useful reactions with azides,[1,2] isonitriles,[3,4] amines[5] and alkyl halides.[6] More recently coupling of thioacids and dithiocarbamate terminal amines has been investigated.[7] Thioacids are more acidic and tend to be more soluble than their oxygen analogues. Their increased nucleophilicity makes them attractive for the design of complex molecules particularly for the purpose of peptide ligations.[8]

Previous syntheses of N-protected amino thioacids include reaction of activated derivatives of carboxylic acids such as acid halides,[9] *N*-hydroxysuccinimide esters,[8,10] or mixed anhydrides.[11] Alternatively, propylphosphonic anhydride (T3P)[12] or CDI may be used as a coupling reagent[1,13] with hydrosulfide anion generated from H_2S or the corresponding alkali salt (Na_2S, NaHS or Li_2S). Large peptide thioacids are generally synthesized by solid-phase peptide synthesis (SPPS) on a thioester solid support[14] and Kaiser's oxime resin with hexamethyldisilathiane.[15] Another approach includes prior coupling of the carboxylic acid with either HSFmoc, HSTrt, or HSTmob and subsequent removal of the protecting group with formation of the corresponding thioacid.[16] Lawesson's reagent[17,18] and the carbodiimide[19] method may also be used.

These diverse synthetic approaches have drawbacks. If a carbodiimide is used, *N*-acylureas can be a major by-product, whereas without additives (HOBt, $CuCl_2$) significant racemization may occur.[20] From this point of view a recently published method using EDC/Na_2S in DMF for the synthesis of N-protected amino/peptide thioacids is not promising.[19] Use of Lawesson's reagent required column chromatographic purification of the obtained thioamino acids. It is well known that N-protected thioamino acids tends to be unstable due to their tendency toward oxidation.[10] We found that N-protected amino/peptide thioacids are quite stable in pure solid form at 0 °C for at least several months, but are easily decomposed in solution. Hence prolonged reaction time/purification may result in a decrease of thioacid yield.

Perhaps the most convenient method for the synthesis of amino thioacids is a mixed anhydride activation carboxylic group (i.e. with IBCF). In this case highly reactive mixed anhydride intermediates cannot be isolated, and may cause additional formation of by-products in more complex cases. Thus mixed anhydride activation of the carboxylic group in a guanidine-protected arginine favors lactam by-product formation.[21,22] Herein, we report a novel efficient approach towards the synthesis of N-protected amino/peptide thioacids from N-protected aminoacyl/peptidoyl benzotriazoles in good yield with a simple isolation procedure and retention of chirality.

N-Protected aminoacyl benzotriazoles are stable crystalline compounds and have been extensively used in our group for various nucleophilic acyl substitution reactions under mild conditions.[23,24] Moreover, it was proved earlier that these reactions take place without racemization[23–25] and provide powerful tool for the amide bond construction with retention of chirality at an asymmetric carbon atom.[26–28]

We first developed a standard procedure for the preparation of thioacids from *N*-acylbenzotriazoles. A standard experimental protocol was developed in order to ascertain the scope of the reaction. For this purpose, Cbz-protected L-alanine was chosen as a model acid component, which was easily converted into the corresponding benzotriazole derivative **1**. One of the advantages of *N*-acylbenzotriazoles is good solubility in the wide range of organic solvents. Thus a stirred 0.1 M solution of benzotriazolide (1.0 equiv) in organic solvent in the presence of base (1.0 equiv) was treated with a slow stream of H_2S (bubbled for ca. 5 min) at 0 °C. The reaction mixture was allowed to warm to 20 °C and stirred until the starting material was consumed (TLC control). Bases pyridine, NMM, Et_3N, DIPEA, and DBU were tested in polar (THF, MeCN) and nonpolar (CH_2Cl_2) solvents (Table 1). Pyridine gave unsatisfactory results needing prolonged reaction time, giving low yield and by-products. However strong bases (e.g., DBU or DIPEA) completed the reaction within ten minutes and triethylamine and NMM required 30 minutes at 20 °C (Scheme 1). The reaction was stirred for an addi-

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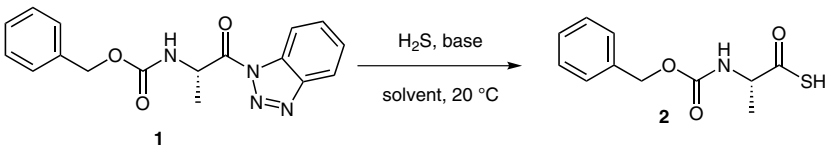


concentration of Cbz-L-Ala-Bt in THF solution: 0.1 M
amount of base: 1 equiv

Scheme 1 Optimization reaction for Cbz-L-Ala-SH **2**

tional 30 minutes at 20 °C then nitrogen was bubbled through the system to remove excess H_2S. The results are summarized in Table 1.

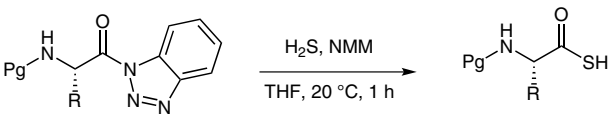
Table 1 Optimization of Reaction Conditions for the Preparation of Cbz-L-Ala-SH **2**

Entry	Base	Solvent	Reaction time[a]	Yield (%)[b]
1	No base	THF	6 h	0
2	Et_3N	THF	30 min	>95
3	NMM	THF	30 min	>95
4	DBU	THF	10 min	>95
5	DIEPA	THF	10 min	>95
6	pyridine	THF	16 h	50
7	NMM	MeCN	30 min	>95
8	NMM	CH_2Cl_2	2 h	85

[a] For the reaction time take time when Bt-compound was completely reacted (TLC).
[b] Calculated by NMR data of reaction mixture after workup.

For the preparation of N-protected α-aminoacyl thioacid, the standard experimental protocol involved bubbling of H_2S through a precooled (0 °C) 0.1 M N-protected α-aminoacylbenzotriazole (1.0 equiv), NMM (1 equiv) in THF for five minutes and then stirring at 20 °C for one hour (Scheme 2, Table 2). The benzotriazole formed during this reaction was easily removed by washing with dilute HCl (1–2 N). In most cases pure thioacids were obtained after evaporation of solvent or crystallization from pentane–Et_2O solution.[29]

This procedure provides pure N-protected amino thioacids (elemental analysis). Moreover Cbz-L-Agr(NO_2)-SH **2d** was obtained in good yield without formation of lactam which occurs with other methods of activation of arginine carboxylic group.[21]



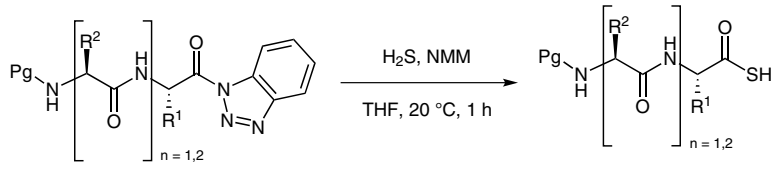
Scheme 2 Preparation of N-protected amino thioacids **2a**–**h**

In order to explore the method further, several peptide thioacids were synthesized. Benzotriazole activated N-protected α-di- and tripeptides were prepared by our previous method.[24] These benzotriazolides were converted into the corresponding thioacids in good yields (Scheme 3, Table 3). The stability of thioacids in solution is poor compared to the N-protected amino acids but both are stable in the solid state. Thus, peptide thioacids **4a**–**d** were kept as crystalline solids which did not change during weeks at 0 °C.[29]

In conclusion, we have reported a novel, facile method for the synthesis of protected amino/peptide thioacids. The reaction conditions were optimized at 20 °C for 30 minutes in the presence of H_2S and NMM.

Table 2 Preparation of N-Protected Amino Thioacids **2a**–**h**

Entry	Thioacid, **2**	Yield (%)	Mp (°C)	Lit. mp	$[\alpha]^{20}_D$
1	Cbz-L-Ala-SH, **2a**	92	77–78	74–7610	–11.1 (*c* = 1.0, MeOH)
2	Cbz-DL-Ala-SH, **2a** + **2a′**	93	65–66	data not provided	racemic
3	Cbz-Gly-SH, **2b**	91	97–98.5	data not provided	–
4	Cbz-L-Val-SH, **2c**	78	gum	gum13	–13.1 (*c* = 2.1, MeOH)
5	Cbz-L-Agr(NO_2)-SH, **2d**	84	153–155	Novel	–9.0 (*c* = 0.5, MeOH)
6	Cbz-L-Trp-SH, **2e**	86	114–115	Novel	–44.5 (*c* = 0.2, MeOH)
7	Cbz-L-Phe-SH, **2f**	91	92.5–93.5	91–9319	–36.8 (*c* = 0.5, MeOH)
8	Fmoc-L-Ala-SH, **2g**	88	118–120	78–7919	–6.1 (*c* = 0.2, MeOH)
9	Fmoc-L-Phe-SH, **2h**	85	88.5–90	Data not provided	–40.9 (*c* = 1.0, MeOH)



3a–d **4a–d**

Scheme 3 Preparation of N-protected di- and tripeptide thioacids **4a**–**d**

Table 3 Preparation of N-Protected Dipeptide Thioacids **4a**–**c** and Tripeptide Thioacid **4d**

Entry	Thioacid **4**	Yield (%)	Mp (°C)	$[\alpha]^{20}_D$
1	Cbz-L-Ala-Gly-SH, **4a**	87	100–102	nd
2	Cbz-L-Val-Gly-SH, **4b**	73	159–161	–25.2 (*c* = 0.5, MeOH)
3	Cbz-L-Ala-Phe-SH, **4c**	79	107–109	–17.9 (*c* = 0.14, MeOH)
4	Cbz-L-Ala-L-Phe-Gly-SH, **4d**	69	162–163	–31.4 (*c* = 0.5, MeOH)

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(29) **General Procedure**: H_2S was bubbled through a precooled (0 °C) solution of N-protected α-aminoacylbenzotriazole (1.0 mmol), NMM (1 mmol) in THF (10 mL) for 5 min. The reaction mixture was allowed to warm at r.t. and stirred for an additional 1 h, diluted with Et_2O (25 mL) and washed several times with 2 N HCl. The organic layer was dried ($MgSO_4$), the solvent was removed, and the residue was crystallized from pentane–Et_2O to give the desired thioacids. **(*S*)-2-{[(Benzyloxy)carbonyl]amino}-5-(2-nitroguanidino)pentanethioic *S*-Acid [Cbz-L-Agr(NO_2)-SH, 2d; Table 2, Entry 5]**: white microcrystals (yield: 84%); mp 153–155 °C; $[\alpha]^{20}_D$ –9.0 (*c* = 0.5 in MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.47 (s, 1 H), 7.79–8.14 (m, 3 H), 7.30–7.42 (m, 5 H), 5.04–5.21 (m, 2 H), 4.28–4.41 (m, 1 H), 3.07–3.22 (m, 2 H), 1.51–1.82 (m, 4 H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 196.3, 159.4, 156.3, 136.7, 128.5, 128.0, 127.8, 66.1, 63.6, 60.9, 32.5, 28.0. Anal. Calcd for $C_{14}H_{19}N_5O_5S$: C, 45.52; H, 5.18; N, 18.96. Found: C, 45.85; H, 5.15; N, 18.62.
2-{[(Benzyloxy)carbonyl]amino}propanethioic *S*-Acid (Cbz-DL-Ala-SH, 2a + 2a′; Table 2, Entry 2): white

microcrystals (yield: 93%); mp 65–66 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.28–7.40 (m, 5 H), 5.15 (br s, 2 H), 5.30–5.44 (m, 1 H), 4.36–4.50 (m, 1 H), 1.42 (d, *J* = 7.1 Hz, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 200.2, 155.7, 136.1, 128.7, 128.4, 128.3, 77.2, 67.4, 57.7, 18.2. Anal. Calcd for $C_{10}H_{13}NO_3S$: C, 55.21; H, 5.48; N, 5.85. Found: C, 55.58; H, 5.11; N, 5.62.

2-{[(Benzyloxy)carbonyl]amino}ethanethioic *S*-Acid (Cbz-Gly-SH, 2b; Table 2, Entry 3): colorless needles (yield: 91%); mp 97–98.5 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.29–7.40 (m, 5 H), 5.32–5.48 (m, 1 H), 5.15 (br s, 2 H), 4.12 (d, *J* = 5.9 Hz, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 196.5, 136.5, 129.2, 128.92, 128.7, 77.6, 68.1, 52.4. Anal. Calcd for $C_{10}H_{11}NO_3S$: C, 53.32; H, 4.92; N, 6.22. Found: C, 53.32; H, 4.98; N, 6.24.

(*S*)-2-{[(Benzyloxy)carbonyl]amino}-3-(1*H*-indol-3-yl)propanethioic *S*-Acid (Cbz-L-Trp-SH, 2e; Table 2, Entry 6): white microcrystals (yield: 86%); mp 114–115 °C; $[\alpha]^{20}_D$ –44.5 (*c* = 0.2 in MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.09 (br s, 1 H), 7.57 (d, *J* = 7.7 Hz, 1 H), 7.29–7.39 (m, 6 H), 7.19–7.25 (m, 1 H), 7.01–7.06 (m, 1 H), 5.21–5.31 (m, 1 H), 5.11 (br s, 2 H), 4.67–4.79 (m, 1 H), 3.31 (d, *J* = 5.6 Hz, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 200.5, 156.0, 136.3, 128.6, 128.4, 128.2, 123.4, 122.5, 120.0, 118.7, 114.6, 111.5, 109.1, 68.1, 67.4, 62.2, 27.7, 25.7. Anal. Calcd for $C_{19}H_{18}N_2O_3S$: C, 64.39; H, 5.12; N, 7.90. Found: C, 64.62; H, 5.23; N, 7.70.

(*S*)-2-[{[(9*H*-Fluoren-9-yl)methoxy]carbonyl}amino]-3-phenylpropanethioic *S*-Acid (Fmoc-L-Phe-SH, 2h; Table 2, Entry 9): white microcrystals (yield: 85%); mp 88.5–90 °C; $[\alpha]^{20}_D$ –40.9 (*c* = 1.0 in MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.73–7.78 (m, 2 H), 7.47–7.56 (m, 2 H), 7.35–7.43 (m, 2 H), 7.25–7.34 (m, 5 H), 7.12–7.19 (m, 2 H), 5.14–5.25 (m, 1 H), 4.59–4.72 (m, 1 H), 4.35–4.49 (m, 2 H), 4.18 (t, *J* = 6.8 Hz, 1 H), 2.96–3.21 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 199.4, 155.7, 143.7, 141.4, 135.2, 129.4, 129.0, 127.9, 127.6, 127.2, 125.1, 120.1, 77.2, 67.3, 62.5, 47.2, 37.7. Anal. Calcd for $C_{24}H_{21}NO_3S$: C, 71.44; H, 5.25; N, 3.47. Found: C, 71.73; H, 5.12; N, 3.90.

(*S*)-2-[2-{[(Benzyloxy)carbonyl]amino}propanamido]-ethanethioic *S*-Acid (Cbz-L-Ala-Gly-SH, 4a; Table 3, Entry 1): white microcrystals (yield: 87%); mp 100–102 °C. ^{1}H NMR (300 MHz, CD_3OD): δ = 7.23–7.40 (s, 5 H), 5.01–5.16 (m, 2 H), 4.80 (m, 1 H), 4.14–4.25 (m, 1 H), 3.99–4.13 (m, 1 H), 1.37 (d, *J* = 7.2 Hz, 3 H). ^{13}C NMR (75 MHz, CD_3OD): δ = 176.1, 171.6, 138.1, 129.4, 129.0, 128.9, 111.4, 67.7, 51.9, 41.8, 18.3. HRMS (DART): *m*/*z* $[M + H]^+$ calcd for $C_{13}H_{17}N_2O_4S$: 297.0904; found: 297.0891.

(*S*)-2-[2-{[(Benzyloxy)carbonyl]amino}-3-methylbutan-amido]ethanethioic *S*-Acid (Cbz-L-Val-Gly-SH, 4b; Table 3, Entry 2): white microcrystals (yield: 73%); mp 159–161 °C; $[\alpha]^{20}_D$ –25.2 (*c* = 0.5 in MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.27–7.45 (m, 5 H), 6.80–6.90 (m, 1 H), 5.35–5.50 (m, 1 H), 5.05–5.16 (m, 2 H), 4.11–4.24 (m, 2 H), 4.04–4.10 (m, 1 H), 2.11–2.25 (m, 1 H), 0.99 (d, *J* = 6.8 Hz, 3 H), 0.95 (d, *J* = 6.8 Hz, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 195.1, 172.0, 156.8, 136.2, 128.7, 128.4, 128.2, 67.4, 60.5, 50.4, 30.8, 19.5, 17.9. HRMS (ESI^-): *m*/*z* $[M - H]^-$ calcd for $C_{15}H_{19}N_2O_4S$: 323.1086; found: 323.1071.

(*S*)-2-[(*S*)-2-{[(Benzyloxy)carbonyl]amino}propan-amido]-3-phenylpropanethioic *S*-Acid (Cbz-L-Ala-L-Phe-SH, 4c; Table 3, Entry 3): white microcrystals (yield: 79%); mp 107–109 °C; $[\alpha]^{20}_D$ –17.9 (*c* = 0.14 in MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.20–7.41 (m, 10 H), 7.10–7.19 (m, 2 H), 6.42–6.61 (m, 1 H), 5.04–5.18 (m, 2 H), 4.81–4.93 (m, 1 H), 4.10–4.27 (m, 1 H), 3.12–3.24 (m, 1 H), 2.97–3.17 (m, 1 H), 1.31 (d, *J* = 7.1 Hz, 3 H). HRMS (ESI^-): *m*/*z* $[M - H]^-$ calcd for $C_{20}H_{21}N_2O_4S$: 385.1228; found: 385.1247.

(5*S*,8*S*)-8-Benzyl-5-methyl-3,6,9-trioxo-1-phenyl-2-oxa-4,7,10-triazadodecane-12-thioic *S*-Acid (Cbz-L-Ala-L-Phe-Gly-SH, 4d; Table 3. Entry 4): white microcrystals (yield: 69%); mp 162–163 °C; $[\alpha]^{20}_D$ –31.4 (*c* = 0.5 in MeOH). ^{1}H NMR (300 MHz, CD_3OD): δ = 7.13–7.42 (m, 10 H), 5.00–5.14 (m, 5 H), 4.61–4.70 (m, 1 H), 4.00–4.13 (m, 3 H), 3.17–3.29 (m, 1 H), 2.91–3.03 (m, 1 H), 1.21 (d, *J* = 7.2 Hz, 3 H). ^{13}C NMR (75 MHz, CD_3OD): δ = 175.5, 173.7, 138.4, 138.0, 130.3, 129.5, 129.4, 129.0, 128.9, 127.7, 67.9, 55.8, 52.3, 51.4, 49.0, 38.3, 17.9. HRMS (ESI^-): *m*/*z* $[M - H]^-$ calcd for $C_{22}H_{24}N_3O_5S$: 442.1442; found: 442.1455.

