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T3P (propylphosphonic anhydride) mediated conversion of N^{α}-protected amino/peptide acids into thioacids

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ABSTRACT

A general, mild and an efficient protocol, which makes use of T3P as an acid activator for the synthesis of N^{α}-protected amino/peptide thioacids from corresponding acids in the presence of finely ground Na_2S as hydrosulfide ion donor is described. The protocol employed significantly increases the overall efficiency as the yield, reaction duration and purity of even sterically hindered amino acids.

Thioacids are the vital class of molecules with preferential utility in synthetic organic chemistry particularly in the development of various ligation techniques.[1] α-Amino thioacids have emerged as novel building blocks for the construction of both peptides as well as peptide like adducts.[2] Thioacids react with diverse array of reactants such as isonitriles,[3] azide functions,[4] alkyl halides,[5] and amines.[6] They are easily soluble in organic solvents and the coupling induced by these thioacids proceeds with less epimerization than their (oxygen) acid counterparts. Li et al.,[7] introduced peptidyl thioacids for the first time. The active participation of thioacids in ligation techniques made the synthesis of thioacids crucial. Solid phase peptide synthesis of large peptides is problematic where it suffers from low yields and purification problems. Thus, native chemical ligation strategies have been discovered where peptide thioacid/thioester would be a C-terminal fragment and it connects to the amino component of another peptide fragment to form a polypeptide.[8] Peptide thioacids have been found to be potent acyl donors which readily undergo oxidative activation leading to the insertion of an amide bond upon reaction with N-terminal peptide. Such ligations are conducted with minimal α-epimerization at the C-terminal thioacid. Thus, they allow for the coupling of N-terminal and C-terminal glycopeptides to obtain homogeneous glycoproteins.[3a] Further, thioacids can also be used along with other functionalities such as isonitrile,[3b] azide,[4] acid azide,[9] sulfonyl azides[10] as coupling partners to form amide, imide, or sulfonamide bonds. This application distinguishes the utility of thioacids from thioesters for the preparation of peptides.

Synthesis of thioacids can be generally accomplished by the coupling of an acid with SH-Fmoc, SH-Trt or SH-Tomb,[11] whereas availability of these reagents itself is a limitation. Further, cleavage of such groups involves treatment with acid or base which otherwise is noncompatible to the amine protecting group. Thioacids can also be prepared using NaSH,[12] but yields can be low due to the formation of diacyldisulfide as the major byproduct. However, the most common method for thioacid preparation is the activation of carboxylic acid by *N,N′*-carbonyldimidazole (CDI), followed by the treatment with Na_2S, H_2S, or Li_2S in DMF at 0 °C for an hour or NaSH in H_2O at room temperature.[13] Recently Lawesson's reagent has also been employed under microwave irradiation.[14] Appreciable epimerization was observed for the peptide thioacid prepared using Lawesson reagent at room temperature. The key steps of many ligation techniques in the protein synthesis mainly depend on the accessibility of α-amino thioacids in general and peptide thioacids in particular. Thus synthesis of N-protected amino as well as peptide thioacids through a simple, mild, and economical route is of paramount importance. Thus we directed our attention to develop a viable and mild protocol for the direct access to thioacids employing T3P–Na_2S system.

T3P (propylphosphonicanhydride)[15] is a highly reactive cyclic anhydride. It has been widely employed as coupling agent for the epimerization free synthesis of peptides[16] in the absence of additives 1-hydroxybenzotriazole (HOBt) or 1-hydroxy-7-azabenzotriazole (HOAt) etc. Also it serves as dehydrant in the construction of various heterocycles including oxadiazoles, substituted pyrimidines, and quinolines, indoles, β-lactams.[17] T3P offers several advantages over other reagents in terms of higher yields, shorter reaction duration, ease of isolation of the products, minimal side reactions, inexpensive, and non-toxic nature.

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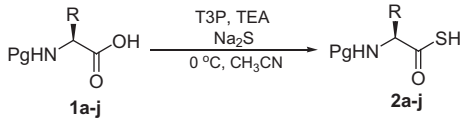


Scheme 1. Synthesis of N^{α}-protected amino thioacids.

Table 1
Screening of solvents for the optimization of reaction

Entry	Solvent	Time (h)	Yield[a] (%)
1	DCM	4	62
2	EtOAc	3	69
3	THF	3	75
4	CH_3CN	2	95

[a] Isolated product yield.

In ongoing studies, our group demonstrated the applicability of T3P for the one pot transformation of an acid to the corresponding ureas and carbamates through Curtius[18] and Lossen rearrangements[19], respectively. Herein we report our results on the direct synthesis of α-amino thioacids from corresponding carboxylic acids in the presence of T3P and finely ground Na_2S as hydrosulfide ion donor.

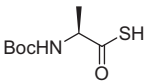
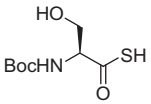
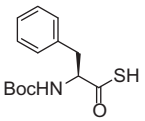
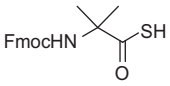
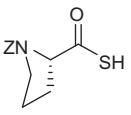
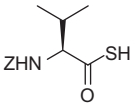
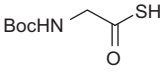
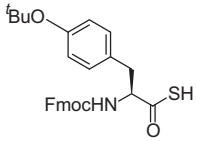
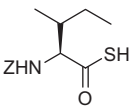
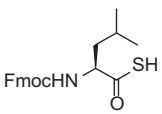
Initially the thioacid preparation was carried out using Fmoc-Leu-OH as an acid component in the presence of T3P as an acid activator, TEA as base to assist T3P activation and Na_2S being the hydrosulfide anion donor (Scheme 1). The reaction conditions were then optimized by fine tuning the equivalents of T3P, TEA, and Na_2S.

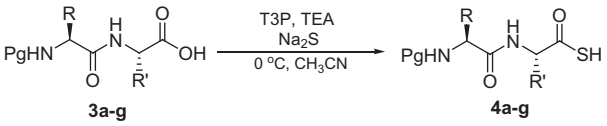
At first, the reaction was examined in different solvents such as DCM, EtOAc, THF, and CH_3CN. The results are summarized in Table 1. In the reaction of Fmoc-Leu-OH, **1j** with Na_2S (3.0 equiv) in the presence of T3P (1.5 equiv) and TEA (2.0 equiv) in dry DCM, transformation of the acid to thioacid was moderate at 0 °C to rt. In THF and EtOAc the yields were satisfactory. In contrast, when the reaction was performed under the similar conditions in CH_3CN, complete consumption of acid was observed after 2 h and thioacid, **2j** was obtained in a 95% yield.[20]

The reaction was monitored by HPLC analysis of different aliquots of crude reaction mixture at different intervals of time. In the presence of a base such as TEA, the carboxylate ion attacks the cyclic anhydride ring of T3P. Meanwhile, the hydrosulfide anion generated by Na_2S in the solution adds to the more electrophilic carbonyl carbon of mixed anhydride and results in the formation of thioacid. After evaporation of the solvent, simple work-up is sufficient to remove the byproducts liberated during the reaction process and affords the resultant thioacid in good yields. Table 2 summarizes the results. In all these preparations, no further column purification was needed as the protocol resulted in water soluble byproducts and thioacids could be isolated through a simple work-up.

The generality of the protocol was demonstrated using simple as well as bifunctional amino acids which also resulted in thioacids with good to excellent yields (Table 2). The protocol can be successfully applied even to the sterically hindered amino acids Aib, **1g,** and Pro, **1c**. Interestingly it is to note that Boc-Ser-OH also furnished the corresponding thioacid **2f** in a good yield despite the fact that the oxidation of –OH group of Ser-OH in the presence of T3P is known.[21] This can be due to the absence of DMSO which is known to assist the oxidation. The reaction takes place under

Table 2
List of N^{α}-protected amino thioacids

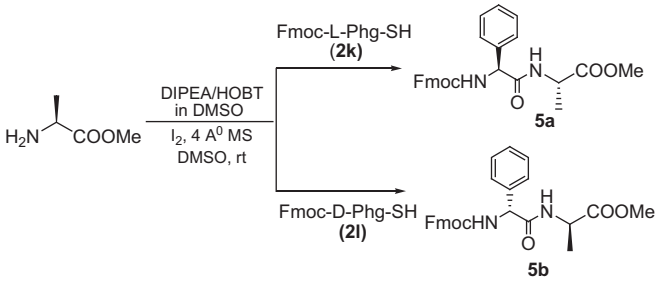
Entry	Thioacid (2)	M.P.	Yield (%)	Entry	Thioacid (2)	M.P.	Yield (%)
1	 **2a**	Gum	93	6	 **2f**	83–84	74
2	 **2b**	Gum	89	7	 **2g**	70–72	82
3	 **2c**	Gum	88	8	 **2h**	Gum	87
4	 **2d**	Gum	91	9	 **2i**	91–92	84
5	 **2e**	82–83	90	10	 **2j**	86–88	95



Scheme 2. Synthesis of peptide thioacids.

Table 3
List of N^{α}-protected peptide thioacids

Entry	Pg	R	R′	Yield (%)
4a	Fmoc	$CH_2C_6H_5$	CH_3	92
4b	Fmoc	$CH_2COOC_6H_5$	$CH(CH_3)_2$	85
4c	Boc	$CH(CH_3)CH_2CH_3$	$CH_2COOC_6H_5$	82
4d	Boc	H	$-(CH2)_3-$	84
4e	Z	$CH(CH_3)_2$	$CH_2CH(CH_3)_2$	88
4f	Z	$CH(CH_3)OC_6H_5$	$CH_2C_6H_5$	82



Scheme 3. Racemization study of Fmoc protected L and D-Phg-SH.

mild conditions and the protocol is compatible with different amino protecting groups Boc, Z, and Fmoc. In the case of Boc-protected amino acids, an additional equivalent of base was used to avoid decrease in the yield which was possibly due to the acidic byproduct liberated during the reaction.

Encouraged by the above results, we further extended the protocol for the synthesis of peptidyl thioacids (Scheme 2). The peptide Fmoc-Phe-Ala-OH was chosen as an acid component and under the optimized condition, as monitored through the HPLC it showed a gradual disappearance of an acid peak R_t 14.25 and appearance of a peak correspond to thioacid R_t 18.28, with an incomplete consumption of acid was observed. Later, fine tuning the equivalence of T3P from 1.5 to 2.5 equiv served best in enhancing the yield of thioacid from good to excellent (Table 3).[22] The use of T3P for the activation leads to the formation of water soluble byproducts. After a simple work-up, the products can be isolated in quantitative yield with high purity. The protocol is completely devoid of any column purification.

The possibility of racemization during the synthesis of N^{α}-protected amino/peptide thioacids from corresponding acids via the present protocol was assessed following the HPLC analysis of the thioacids derived from the Fmoc protected L- and D-Phg employing the present protocol and also the HPLC analyses of the diastereomeric dipeptides obtained by these derivatives with NH_2-Ala-OMe following known coupling method (Scheme 3).[23,24]

Racemization study was carried out through the HPLC analyses of the thioacids, **2k,** and **2l** synthesized by the present protocol. These thioacids appear at different R_t values, that is, R_t = 14.2 min. (**2k**, L-isomer) and R_t = 15.1 min. (**2l**, D-isomer), also the equimolar mixture of **2k** and **2l** appears as distinct peaks at R_t = 14.62 and 15.37 min.

When the optically pure **2k** and **2l**, as outlined in Scheme 3, were coupled separately with L-Ala-OMe they yielded peptides **5a** and **5b**. The HPLC analysis of the pure peptides **5a** and **5b** showed single peaks at different R_t values, that is, at R_t = 18.17 and 18.96 min, respectively. These studies showed that the prepared peptides, **5a** and **5b** contain a single optically pure isomer and consequently it was proved that the present protocol was free from racemization. HOBt has been employed in carbodiimide mediated coupling reactions to suppress the racemization.[25] Similarly, the studies carried out by Danishefsky et al.,[23] revealed that the thioacid in the presence of HOBt, forms an –OBt ester which on acylation with an amino component leads to amide bond formation. In such couplings also, HOBt markedly reduces the oxazolone promoted racemization at the C-terminus. The application of T3P as an activator can be extended to the preparation of peptide thioacids without causing racemization.

In conclusion, herein we have described a simple, mild, and an alternative protocol for the direct conversion of N^{α}-protected amino/peptide acids into thioacids by employing T3P/Na_2S system. [26] A series of carboxylic acids including N^{α}-Fmoc/Z/Boc amino acids have been converted into corresponding thioacids in excellent yields. Also the protocol can further be extended even to the large scale preparations as the byproducts released were innocuous, water soluble and the protocol is operationally simple.

Acknowledgments

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20. *General for the synthesis of amino thioacid* **2a–j**: To a solution of acid (1.0 mmol) and T3P (1.2 mmol) in dry CH_3CN (8.0 mL) was added Et3 N (1.5 mmol) at 0 °C followed by the finely ground Na_2S (3.0 mmol) and the reaction mixture was stirred for 2 to 3 h till the completion of reaction as monitored by TLC. Then the reaction mixture was concentrated and then diluted with water. The resultant solution was then acidified with citric acid solution (10%) and the product was extracted into EtOAc (10 mL). The organic layer was washed with water (2×10 mL), brine (10 mL) and dried over anhydrous Na_2SO_4. Solvent was evaporated in vacuo to obtain aminothioacid in a quantitative yield.
Method for HPLC analysis: Gradient 0.1% TFA, water/acetonitrile (30:70) in 30 min with a flow rate of 0.5 ml/min. λ = 254 nm (Fmoc, Z), 211 nm (Boc).
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22. *General procedure for the synthesis of peptide thioacids* **4a–f**: Peptide thioacids were prepared using similar experimental conditions as for the preparation of amino thioacids. The only difference involves the usage of 2.5 mmol of T3P.
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24. *Coupling method for the preparation of peptide ester using N-protected amino thioacids* **2a–j**: To a solution of thioacid (1.2 equiv) in DMSO was added amino acid ester (1.0 equiv) and 4 Å MS (2 mg). To the resultant reaction mixture, a stock solution of DIPEA (1.5 equiv)/HOBT (2.0 equiv) in DMSO and I_2 (0.6 equiv) in DMSO was added. The reaction mixture was then allowed to stir at room temperature for 40–50 min till the completion of the reaction as monitored through TLC. The crude product is then purified through column chromatography.
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26. *Characterization data for selected compounds*
Fmoc-Leu-COSH (2j): White solid, mp 88–90 °C. IR (neat, υ_{max}) 1659, 1766, 3243 cm^{-1}.R_f 0.4 (EtOAc/*n*-hexane, 60:40).R*t* 20.41 (30–100% CH_3CN, 30 min).^1H NMR ($CDCl_3$, 400 MHz) δ ppm 1.05 (d, *J* = 6.2 Hz, 6H), 1.68 (m, 2H), 1.82 (m, 1H), 3.92 (t, *J* = 4.8 Hz, 1H), 4.3 (t, *J* = 4.1 Hz, 1H) 4.56 (d, *J* = 5.9 Hz), 4.9 (br, 1H), 6.9 (br, 1H), 7.2–7.6 (m, 8H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 21.8, 22.4, 39.5, 45.1, 64.3, 66.9, 125.9, 126.5, 127.3, 127.8, 142.6, 143.2, 156.9, 198.7.HRMS (ESI) calcd for $C_{21}H_{23}NO_3S$ *m*/*z* 392.1296 $[M+Na]^+$, Found 392.1285. C, 68.25; H, 6.26; N, 3.80; O, 12.98; S, 8.71.
Boc-Phe-COSH (2b): gum, IR (neat, υ_{max}) 1723, 1765, 3349 cm^{-1}.R_f 0.32 (EtOAc/*n*-hexane, 60:40).R_t 16.76 (30–100% CH_3CN, 30 min).^1H NMR (CDCl3, 400 MHz) δ ppm 1.39 (s, 9H), 3.1 (d, *J* = 6.5 Hz, 2H), 3.85 (t, *J* = 5.2 Hz), 4.63 (br, 1H), 7.08–7.26 (m, 5H), 7.9 (br, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 29.3, 36.6, 68.2, 7(8.7, 126.5, 128.4, 128.9, 140.5, 157.1, 198.5.HRMS (ESI) calcd for $C_{14}H_{19}NO_3S$ *m*/*z* 304.0983 $[M+Na]^+$, Found 304.0968. C, 59.78; H, 6.80; N, 4.96; O, 17.03; S, 11.43.
Z-Pro-COSH (2c): gum, IR (neat, υ_{max}) 1747, 1762, 3259 cm^{-1}. R_f 0.35 (EtOAc/*n*-hexane, 60:40).R_t 14.33 (30–100% CH_3CN, 30 min). ^1H NMR ($CDCl_3$, 400 MHz) δ ppm 1.53–1.56 (m, 2H), 1.93–2.01 (m, 2H), 3.35 (t, *J* = 4.8 Hz, 2H), 4.31 (t, *J* = 5.4 Hz, 1H), 4.72 (br, 1H), 5.39 (s, 2H), 7.23 (s, 5H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 21.3, 28.5, 48.1, 65.9, 73.5,128.3, 128.7, 129.5, 140.7, 156.9, 197.9. HRMS (ESI) calcd for $C_{13}H_{15}NO_3S$ *m*/*z* 288.0671 [M+Na], Found 288.0677.C, 58.88; H, 5.73; N, 5.25; O, 18.06; S, 12.08.
Z-Val-Leu-COSH (4e): White solid, mp 115–117 °C. IR (KBr, υ_{max}) 1747, 1762, 3259 cm^{-1}. R_f 0.25 (EtOAc/*n*-hexane, 60:40). R_t 20.45 (30–100% CH_3CN, 30 min) ^1H NMR ($CDCl_3$, 400 MHz) δ ppm 0.95 (d, *J* = 6.1 Hz, 6H), 1.05 (d, *J* = 6.4 Hz, 6H), 1.8–1.84 (m, 3H), 2.59 (m, 1H), 4.2 (t, *J* = 5.2 Hz, 1H), 4.44 (d, *J* = 6.9 Hz, 1H), 4.9 (br, 1H), 5.29 (s, 2H), 6.9 (br, 1H), 7.29 (s, 5H), 7.4 (br, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 16.8, 22.3, 23.1, 31.8, 42.2, 59.5, 64.9, 65.2, 127.8, 128.1, 129.3, 140.3, 156.7, 170.8, 198.7. HRMS (ESI) calcd for $C_{19}H_{28}N_2O_4S$ *m*/*z* 403.1667 [M+Na], Found 403.1669.C, 59.96; H, 7.44; N, 7.39; O, 16.80; S, 8.41.