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Short Communication

Ultrasound accelerated conversion of N^{α}-urethane protected peptide esters to their thiopeptides using P_2S_5

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ABSTRACT

A fast and efficient synthesis of N^{α}-protected thiopeptide esters from the corresponding peptide esters using P_2S_5 as thionating agent assisted by ultrasonication has been described. The conversion of peptide bond into thioamide was complete in 20–40 min at rt. The reaction was accomplished without using any base. The products isolated were characterized using ^{1}H NMR, ^{13}C NMR and mass spectroscopy.

1. Introduction

Peptides are involved in many important physiological processes like pain transmission, blood pressure regulation, food intake, *etc.* They act as neurotransmitters, neuromodulators or hormones and evaluate their action by activating specific peptide receptors.

Backbone modification of peptides by the insertion of different peptide bond surrogates has lead to the development of peptidomimetics. The peptide bond surrogates have been found to be more potent and stable with increased biological potency [1]. Their utility in medicinal, biological, pharmaceutical, drug development and other fields of protein chemistry has been well established.

Thiopeptide bond (–C(=S)–NH–) is isoelectronic to the parent amide (–C(=O)–NH–) bond yet possesses markedly different physical and chemical properties. The receptor activity is varied when thio analogues are present in the peptide chain. These modified peptides are more active *in vivo* as bioresponse modifiers and immunomodulators due to the stability of the thioamide bonds towards enzymatic degradation when compared with that of the oxygenated analogues [2]. They are the starting compounds for the preparation of various heterocycles like thiazoles and isothiazoles which are abundantly present in natural products.

Chemical transformation of carbonyl oxygen to sulphur using P_2S_5 as sulphonating agent was reported by Wislicenus [3] and Henry [4]. The reaction involves refluxing the carbonyl compound and thionating agent in toluene, xylene or pyridine. The necessity of using a large excess of reagent and prolonged reaction durations are the main disadvantages in such transformations [5]. A number of other reagents such as R_3BF_4/NaSH [6], R_2PSX [7], $(Et_2Al)_2S$ [8], P_4S_{10}/Al_2O_3 [9] and P_2S_5/Na_2CO_3 [10] are in use as thionating agents for the conversion of amide into thioamides. In addition, Lawesson's reagent (LR) [11] and a combination of P_4S_{10}/HMDO [12] are also employed for thionation but the reaction conditions are quite harsh. They need higher temperatures and longer duration for the completion which are generally not affordable in peptide chemistry. Also, the high cost of LR and formation of byproducts limit its use. In an earlier report, Rapoport et al., [13] synthesized thiopeptide esters using a lengthy protocol *via*, benzotriazole route. This involved the thionation of the amide bond between a Boc-amino acid and 4-nitro-1,2-phenylene diamine using P_2S_5/Na_2CO_3 and cyclization to benzotriazole. Further displacement of the benzotriazole group with amino acid methyl ester yielded thiopeptide ester. In our initial attempt using a similar protocol, the thionation of the amide bond between Fmoc-amino acid and *o*-phenylenediamine was not satisfactory. The insolubility of the amide posed difficulty for thionation and also the use of Na_2CO_3 was not compatible with Fmoc chemistry. Therefore we focused on developing an easy and rapid method for thionation of the peptide carbonyl. Stanley Raucher had reported a procedure for the conversion of amide to thioamide by using P_2S_5 under ultrasound irradiation [14]. We envisaged an analogous ultrasound mediated mild protocol for the conversion of peptide esters into thiopeptide esters at rt under neutral medium. In the present work, we describe an efficient direct conversion of peptide esters into thiopeptide esters at rt assisted by ultrasonication under neutral medium.

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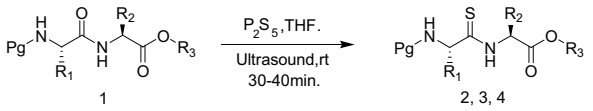


1 2, 3, 4

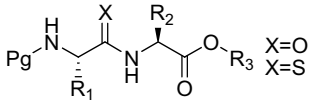
R_1=H, CH_3, $CH(CH_3)_2$, $CH_2C_6H_5$, $CH_2CH(CH_3)_2$, $CH(CH_3)(C_2H_5)$, CH_2SBzl, $CH_2COOBzl$ *etc*.,
R_2= H, CH_3, $CH(CH_3)_2$, $CH_2C_6H_5$, $CH_2CH(CH_3)_2$, $CH(CH_3)(C_2H_5)$, CH_2SBzl, $CH_2COOBzl$ *etc*.,
Pg=Fmoc, Cbz, Boc.
R_3=Me, Et, Bzl *etc*.,

Scheme 1. Synthesis of thiopeptides.

Table 1
Thionation of various protected dipeptides using P_2S_5 under ultrasonication

Entry	PG	R_1	R_2	R_3	Time (min)	Conversion (%)
2a	Fmoc	$CH(CH_3)_2$	$CH_2C_6H_5$	CH_3	30	80
2b	Fmoc	$CH(CH_3)_2$	$CH(CH_3)_2$	CH_3	30	85
2c	Fmoc	$(CH_2)_2SCH_3$	H	CH_3	35	100
2d	Fmoc	CH_3	$CH_2CH(CH_3)_2$	CH_3	25	100
2e	Fmoc	$CH(CH_3)_2$	$CH_2CH(CH_3)_2$	$CH_2C_6H_5$	45	80
2f	Fmoc	$CH_2C_6H_5$	CH_2OH	CH_3	25	90
2g	Fmoc	C_6H_5	$CH_2C_6H_5$	CH_3	30	90
2h	Fmoc	C_6H_5	$CH_2C_6H_5$	CH_3	30	90
3a	Cbz	$CH(CH_3)(C_2H_5)$	H	CH_3	15	95
3b	Cbz	$(CH_2)_2COOCH_2C_6H_5$	$CH_2CH(CH_3)_2$	CH_3	25	88
3c	Cbz	$(CH_2)_2$ $COOCH_2C_6H_5$	$CH_2SCH_2C_6H_5$	CH_3	35	90
4a	Boc	$-(CH_2)_3-$	$CH(CH_3)_2$	CH_3	15	95
4b	Boc	CH_3	$-(CH_2)_3-$	$CH_2C_6H_5$	45	50

Table 2
C^{13} NMR and mass data of the dipeptides



Entry	^{13}C (ppm)		Mass	
	X = O	X = S	Calculated	Observed [M+1]
2a	169.73	197.26	516.65	517.66
2b	169.98	202.96	468.61	469.62
2c	173.23	205.40	458.59	459.60
2d	172.07	203.89	454.58	455.59
2e	170.76	204.59	558.73	559.74
2f	170.97	210.10	504.60	505.61
2g	168.05	192.18	550.67	551.68
2h	168.05	192.18	550.67	551.68
3a	170.73	205.14	352.45	353.46
3b	172.49	206.71	514.63	515.64
3c	173.59	208.72	594.74	595.75
4a	167.02	207.90	344.47	345.48
4b	172.03	199.99	392.51	393.52

2. Experimental

2.1. General

All solvents were freshly distilled before use. Amino acids were used as received from Sigma–Aldrich Company, USA. Mass spectra were also recorded on MALDI-TOF (KRATOS). ^1H and ^{13}C NMR spectra were recorded on a Bruker AMX 300 MHz spectrometer. The ultrasound bath (Elma, T 310/H) was German made and operated at 35 kHz.

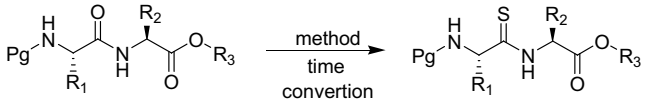
2.2. Typical procedure for the conversion of C=O to C=S

To a solution of N^{α}-protected dipeptide ester (10 mmol) in THF (10 ml), P_2S_5 (12 mmol, 2.66 g) was added and the reaction mixture was subjected to ultrasound at rt. The course of the reaction was monitored by TLC. After the completion of the reaction, the solvent was evaporated *in vacuo* and the crude thiopeptide was purified through column chromatography (silica gel 100–200 mesh) using EtOAc–hexane (1:9) as an eluant.

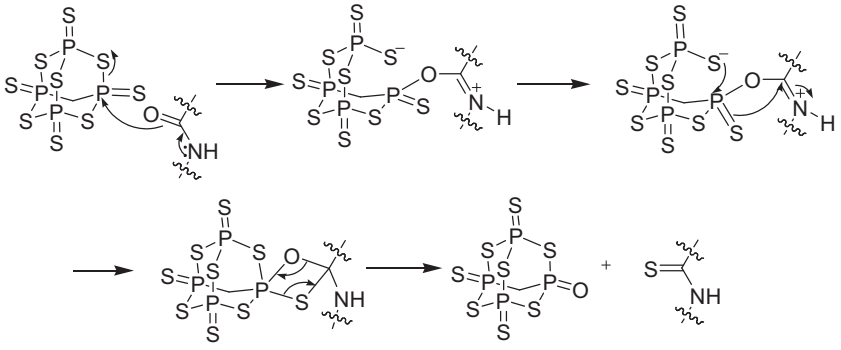
3. Results and discussion

Ultrasonication has acquired a place of its own in organic synthesis as a tool to accelerate the rate of a chemical reaction even at ambient temperature. Our study began with the preparation of N^{α}-protected peptide esters [15] under standard peptide coupling conditions. Conversion of the amide carbonyl to thiocarbonyl was accomplished using P_2S_5 under ultrasonication (Scheme 1). For this, a solution of peptide ester and P_2S_5 in THF was subjected to ultrasonication at rt and no base was used for the reaction. However, the addition of base for example Na_2CO_3 did not contribute much either to the rate or the yield of the reaction. The reaction proceeded with almost complete conversion and no side products

Table 3
Comparative study of the methods applied for the synthesis of thiopeptides



Reagents	Temperature (condition)	Time	Yield (%)
Lawesson's	80 °C	30–60 min	50–60
Na_2CO_3/P_2S_5	rt	4–6 h	60
Na_2CO_3/P_2S_5	80°c	3 h	70
P_2S_5	rt	4 h	40–50
P_2S_5	80°c	30–60 min	65–75
P_2S_5	·)))	10–30 min	80–100



Scheme 2. Mechanism for the thionation of an amide bond into thioamide.

were observed. The resulting crude product was subjected directly to column purification. All the thiopeptides were obtained in excellent yields. The Fmoc, Boc and Cbz protected thiopeptide esters could be easily and selectively prepared without affecting either the N^{α}-carbamate carbonyl or the ester carbonyl. The results obtained for various N^{α}-protected dipeptide esters are furnished in Table 1. The products were confirmed by ^{1}H NMR, ^{13}C NMR and mass spectral techniques (Table 2). A comparative study for making thiopeptides by various methods for the synthesis is given in Table 3.

The mechanism of the conversion of amide to thioamide proceeds through the transfer of sulphur from P_4S_{10} to the oxygen of carbonyl amide. Transfer occurs *via* four membered cyclic transition state (Scheme 2). The ultrasound produces a shock wave in the form of pressure which collapses the so formed cavitation in the reaction liquid. It influences the rapid bond breaking and making process. This accounts for the increased chemical reactivity due to increased molecular collisions and hence accelerates the rate of the reaction even at rt.

4. Conclusion

In conclusion, we have reported the ultrasound promoted efficient conversion of N^{α}-protected dipeptide esters to their corresponding thiopeptide analogues employing P_2S_5 as thionating agent at rt. The conversion was carried out without the use of any base. All the thiopeptides made were fully characterized by ^{1}H NMR, ^{13}C NMR and mass spectroscopy. This route enables an easy access to thiopeptides in good yields.

Acknowledgement

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[15] General procedure for the synthesis of N^{α}-protected peptide esters: To a solution of N^{α}-protected amino acid (10 mmol) in THF were added, *N*-methylmorpholine (11 mmol, 1.21 mL) and ethyl chloroformate (11 mmol, 1.05 mL) at 0 °C and stirred for 15 min. Then, an ester of amino acid (12 mmol) in DCM was added, and stirring continued for further 2–3 h and TLC analysis was carried to determine the completion of the coupling. After a simple acid base workup, N^{α}-protected peptide esters were isolated in above 85% yield. As and when necessary, the crude products were subjected to column chromatography using varying concentrations of EtOAc:hexane as an eluant. The pure products were obtained after the evaporation of solvent under *vacuo*.