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FACILE REGIOSELECTIVE FORMATION OF THIOPEPTIDE LINKAGES FROM OLIGOPEPTIDES WITH NEW THIONATION REAGENTS

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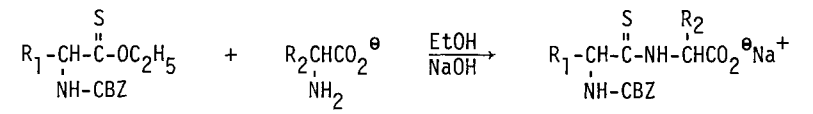
Abstract *The thionation reagent 1 and the new more soluble and readily purified analogue 2 permit, in appropriate solvents, the low temperature backbone thionation of oligopeptides in a regioselective manner and in high yields*

Thiopeptide analogues of oligopeptidic biological substrates and regulators are the focus of increased attention in biochemistry and pharmacology. With thiopeptides, receptor interactions may be altered relative to their parent peptidic effectors In addition, the thioamide bond may be expected to interfere with the hydrolytic mechanism of *in situ* peptidases and this ought to be reflected primarily in the duration of action of the backbone-thionated effector This expectable resistance of the thioamide function to enzymic attack has been recently confirmed in three laboratories[1-3] for the case of simple thioamide analogues of carboxypeptidase A substrates

Systematic studies of the biological effects of regioselectively thionated oligopeptides rely on accessibility to practical and efficient methodology for the synthesis of such analogues Formation of the thiopeptide bond through the reaction of an aminoacid with a thionester of a CBZ-N-protected aminoacid (1) has been reported[4] This approach was successfully



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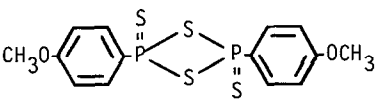
applied to the preparation of di- and tri-peptides incorporating thioamide linkages However, the method appears impractical because the conditions encourage racemization, accentuate solubility problems, and limit the choice of protecting groups. A potentially attractive method to achieve thionation of pre-formed dipeptides without racemization has been promulgated by Lawesson *et al* [5-7] who showed that the exquisite phosphetane 3 first described by Lecher *et al* [8] will effectively transform protected dipeptides into their thioamide analogues While parallel results have been obtained in our laboratories (unpublished), we were forced to the conclusion similarly arrived at by others[9-11] that the very limited solubility of 3 and, especially, the reaction conditions are undesirably restrictive because of the thermal instability of some protecting groups and most importantly the complete lack of regioselectivity with tri- and higher peptides[11] We have overcome these serious limitations by capitalizing on a) an unexpectedly pronounced solvent effect on the course of the reaction, the use of THF as the medium allowing ready thionation at temperatures ranging from 0° to 25° with high regioselectivity as dictated

Table 1 Thionation by 2 of Various Protected Peptides

Substrate	Product[a]	Time (h)	Temp.	Yield (%)[b]
Boc-Phe-C(=O)-NH_2	Boc-Phe-C(=S)-NH_2	0 5	RT	90
Boc-Phe-C(=O)-$NHCH_3$	Boc-Phe-C(=S)-$NHCH_3$	3	RT	85
Boc-Phe-C(=O)-N(pyrrolidine)	Boc-Phe-C(=S)-N(pyrrolidine)	48	50	60[c]
Boc-Gly-$NHCH_3$	Boc-Gly(=S)-$NHCH_3$	0.1	RT	82
Boc-Ala-$NHCH_3$	Boc-Ala(=S)-$NHCH_3$	1	RT	84
Boc-Pro-$NHCH_3$	Boc-Pro(=S)-$NHCH_3$	12	RT	90
Boc-Gly-Gly-OCH_2CH_3	Boc-Gly(=S)-Gly-OCH_2CH_3	0.3	RT	81
Boc-Phe-Leu-OCH_3	Boc-Phe(=S)-Leu-OCH_3	24	40	84
Boc-Gly-Phe-Leu-OCH_3	Boc-Gly(=S)-Phe-Leu-OCH_3	3	RT	88
Boc-Gly-Gly-Phe-Leu-OCH_3	Boc-Gly(=S)-Gly-Phe-Leu-OCH_3	3 5	RT[d]	80
O,N-$(Boc)_2$-Tyr-Gly-Gly-Phe-Leu-OCH_3	O,N-$(Boc)_2$-Tyr-Gly(=S)-Gly-Phe-Leu-OCH_3	3.5	RT[d]	75
Boc-Phe-Gly-Pro-OCH_3	Boc-Phe(=S)-Gly-Pro-OCH_3	48	RT[d]	64
Boc-Phe-Ala-Pro-OCH_3	Boc-Phe(=S)-Ala-Pro-OCH_3	60	RT[d]	85

[a]Structures confirmed by U.V 200 MHz ^1H NMR and MS. [b]Isolated yields of chromatographically pure products [c]Incomplete by TLC after this time [d]Reaction started at 0° and allowed to warm to RT



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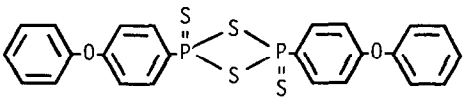

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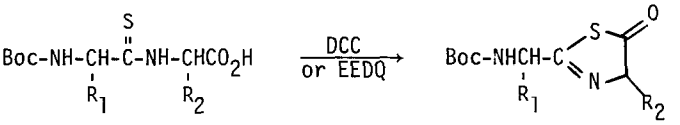
by the steric environment of the individual amide function, b) the use of a modified reagent (4) with good solubility in compatible organic solvents such as THF, and c) the amenability of thiodipeptides to engage readily into elongation exclusively from the amino-terminal end In this regard, attempted elongation of thiodipeptides from the carboxyl end yielded thiaaz-lactones (5 → 6) which were resistant to subsequent aminolysis under standard coupling conditions.



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The modified reagent, 2,4-*bis*(4-phenoxyphenyl)-1,3,2,4-dithiaphosphetane 2,4-disulfide (4) was prepared from phenyl ether following the procedure of Lecher *et al* [8] as applied to anisole. Crude 4 was precipitated by hexane (1 ml) and easily recrystallized from toluene to give pure yellow crystals, mp 187-190° (50-55% yield), $C_{24}H_{18}P_2S_4$, Anal. C, H, S It has good solubility in $CHCl_3$, THF, toluene, acetonitrile at r t and lower

Using dry THF as solvent and 0.6 eq of 4 per mole of peptide, thionation was allowed to proceed at 0° → 23 ± 2° (occasionally at 40-50° for "hindered" peptide linkages) and reaction progress monitored by TLC. Depending on the substrate, the time for mono-thionation ranged from

Table 2 Elongation Products for Monothiopeptides with Free NH_2-Terminal

Peptidic Substrates	Coupling Conditions	Products[a]	Yield[b]
Boc-Tyr-Gly-OH + Gly(S)-Phe-Leu-OCH_3	EEDQ, THF, RT, 24 h	Boc-Tyr-Gly-Gly(S)-Phe-Leu-OCH_3	80%
O,N-$(Boc)_2$-Tyr-OH + Gly(S)-Gly-Phe-Leu-OCH_3	DCC-HOBT, DMF, 0° → RT, 24 h	O,N-$(Boc)_2$-Tyr-Gly(S)-Gly-Phe-Leu-OCH_3	65%
O,N-$(Boc)_2$-Tyr-Gly-Gly-OH + NH_2Phe(S)-Leu-OCH_3	DCC,HOBT, DMF, 0° → RT, 24 h	O,N-$(Boc)_2$-Tyr-Gly-Gly-Phe(S)-Leu-OCH_3	66%

[a]Structures confirmed by U V. 200 MHz ^1H NMR and MS

[b]Isolated yields of chromatographically pure products.

0 5 to 60 h The results for mono-amides, di-, tri- and tetra-peptides are summarized in Table 1 Included is the protected penta-peptide leu-enkephalin which was regioselectively

transformed in high yield into a single mono-thionated product. Under other conditions such as noted above[5-7,11] non-selective polythionation is the only result in marked contrast to the observations assembled in Table 1 Protecting groups were untouched and linkages involving glycine reacted fastest and selectively. When relatively large side chains flank the amide group or when proline is involved, the rates were much slower under our conditions.

Elongation of mono-thiopeptides was accomplished by first removing the t-Bocs (HCO_2H or HCl/AcOH) followed by coupling (EEDQ[12] or DCC-HOBT[13]) with appropriate t-Boc-protected peptide acids under standard conditions (Table 2)

Clearly, and contrary to pervasive impressions, our results demonstrate that regio-selective mono thionation of oligopeptides can be readily accomplished especially at sites where glycine residues are involved.

The pharmacological properties of the corresponding deprotected thiopeptides will be described elsewhere shortly.

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