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STUDIES ON AMINO ACIDS AND PEPTIDES-III.* 2,4-BIS(4-METHOXYPHENYL)-1,3,2,4-DITHIADIPHOSPHETANE 2,4-DISULFIDE, LR, AS A NEW RACEMIZATION FREE COUPLING REAGENT IN PEPTIDE SYNTHESIS

U. PEDERSEN, M. THORSEN, E.-E.A.M. EL-KHRISY‡, K. CLAUSEN and S.-O. LAWESSON

Department of Organic Chemistry, Chemical Institute, University of Aarhus, DK-8000 Aarhus C, Denmark

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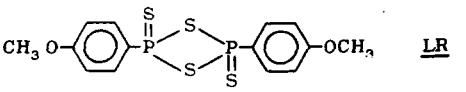
Abstract - The easily accessible 2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane 2,4-disulfide, LR, has been reacted with salts of N-protected amino acids 1 (Z-Gly-OH, Boc-Gly-OH, Boc-S-Ser(Bzl)-OH, Boc-S-Tyr(Bzl)-OH, Z-S-Arg(Z_2)-OH, and Z-S-Pro-OH), at room temperature in CH_2Cl_2 to give the intermediates 2, mixed anhydrides. When 2 is treated with two moles of a base and one mole of the salt of an amino acid ester 3 (TosOH·H-Gly-OBzl, HCl·H-Gly-OBzl, HCl·H-Gly-OEt, and HCl·H-S-Phe-OtBu) at 0°C, the expected peptide 4 is isolated in high yields. LR is also found to be a useful reagent in a fragment coupling between Z-Gly-S-Ala-OH and TosOH·H-S-Leu-OBzl). This tripeptide was tested by means of HPLC (deprotection and amino acid analysis according to Izumiya was not necessary), and no epimerization (<0.7 %) was observed.

INTRODUCTION

In the last few years several coupling reagents have been developed for peptide synthesis and especially phosphorous reagents have been of great interest.[1-3] In the formation of the amide bond in peptide synthesis it is not surprising that organophosphorous reagents have received much attention. Mixed carboxylic-phosphorous anhydrides are very reactive compounds which on nucleophilic attack by an amine give amides 4. The present paper reports on a new coupling reagent, 2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane 2,4-disulfide, LR, for use in peptide synthesis.

RESULTS AND DISCUSSION

A series of N-protected amino acids were reacted with triethylamine (TEA) in CH_2Cl_2 at room temperature to give the salts 1. LR was added under mild conditions and the intermediate 2 was formed. At 0°C addition of two moles of TEA and one mole of the salt of an amino acid ester 3 to 2 gave the expected protected dipeptide 4 in high yield. Based on optical rotation data (Table 1) no racemization was observed.



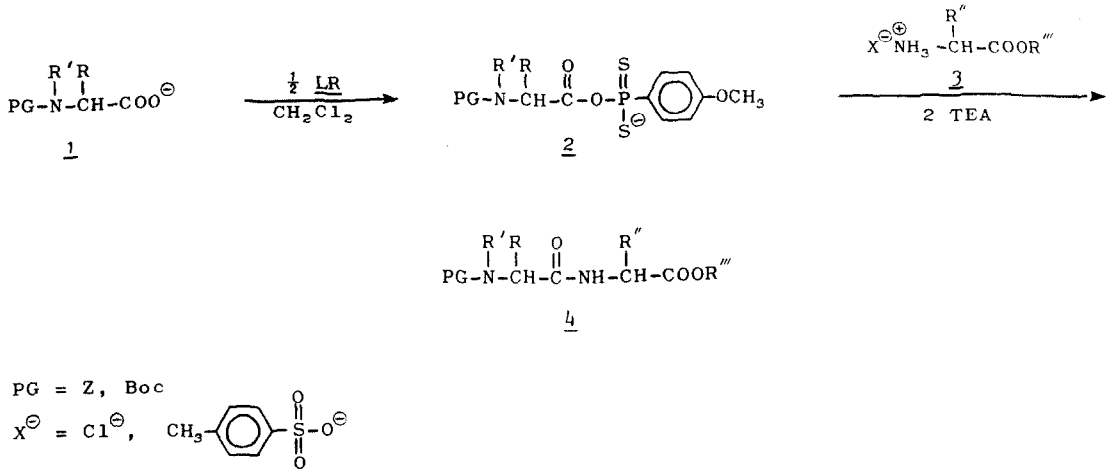
LR

In the same way the fully protected tripeptide Z-Gly-S-Ala-S-Leu-OBzl was prepared by fragment coupling of Z-Gly-S-Ala-OH with TosOH·H-S-Leu-OBzl. Testing by means of HPLC showed that no epimerization had occurred (<0.7%).

These results show that LR compares favourably with established reagents including DCC. By coupling with LR no additives are necessary to suppress racemization (epimerization).

*Part II, see ref.6.

‡On leave from National Research Centre, Dokki, Cairo, Egypt



PG = Z, Boc

$X^{\ominus}$ = $Cl^{\ominus}$,

Table 1. Experimental and physical data

Starting materials		Reaction time/hr	Yields (%)	Mp.°C	$[\alpha]_D$
Z-Gly-OH	TosOH·H-Gly-OBzl	16	96	109 (110[b])	-
Z-Gly-OH	TosOH·H-Gly-OBzl	4	68	109 (110[b])	-
Z-Gly-OH	HCl·H-Gly-OEt	16	82	80 (82-4[a])	-
Boc-Gly-OH	HCl·H-Gly-OBzl	4	93	84 (84-5[b])	-
Boc-S-Ser(Bzl)-OH	HCl·H-Gly-OBzl	4	83	52 (52-4[b])	-7.1 (-7.1[b])[a]
Boc-S-Tyr(Bel)-OH	TosOH·H-Gly-OBzl	4	83	103-4 (103-5[b])	-3.4 (-3.4[b])[a]
Z-S-Arg(Z_2)-OH	HCl·H-Gly-OBzl	16	47	136-8 (136-7[b])	+16.3 (+16.6[b])[b]
Z-S-Pro-OH	HCl·H-S-Phe-OtBu	16	92	72-3 (74-5[b])	-27.5 (-27.4[b])[a]
Z-S-Pro-OH	HCl·H-Gly-OEt	16	95	oil	-60.4[c]
Z-Gly-S-Ala-OH	TosOH·H-S-Ley-OBzl	16	84	101-2 (102[d])	-26.9[d] (-31.6[d])[e]

[a] c 2.00, AcOEt, 22°C. [b] c 2.00, CH_2Cl_2, 22°C. [c] c 1.67, AcOEt, 22°C. [d] Coupling between dipeptide Z-Gly-S-Ala-OH and TosOH·H-S-Leu-OBzl according to DCC/HOBT method gives $[\alpha]_D$ -25.4. c 1.00, AcOEt, 22°C. [e] c 1.00, AcOEt, 22°C.

EXPERIMENTAL

^1H NMR spectra were recorded at 60 MHz on a Varian EM-360 spectrometer. ^{13}C NMR spectra were recorded at 20 MHz on a Varian CFT-20 spectrometer. TMS was used as internal standard and chemical shifts are expressed in δ-values. $CDCl_3$ was used as solvent. Mass spectra were recorded on a Micromass 7070 F spectrometer operating at 70 eV using direct inlet. Microanalyses were carried out by Løvens Kemiske Fabrik, DK-2750 Ballerup (Microanalytical Laboratory). Optical rotations were measured in a 1 dm cell on a Perkin-Elmer 241 polarimeter. Silica gel 60 (Merck) was used for chromatography. M.ps are uncorrected.

Z-Pro-Gly-OEt. Microanal.: C 60.41, H 6.78, N 8.12. Calc.: C 61.07, H 6.63, N 8.38%. ^1H NMR ($CDCl_3$): δ 1.25 (3H,t, 7 Hz), 2.00 (4H,m), 3.45(2H,m), 3.90 (2H,d,5 Hz), 4.12(2H,q,6 Hz), 4.30(1H, m), 5.05(2H,s), ~6.8(1H,br), 7.25(5H, s). ^{13}C NMR ($CDCl_3$): $\delta C_0(1)$ = 154.8, $\delta C_1^{\alpha}(1)$ = 60.0, $\delta C_1(1)$ = 172.1, $\delta C_2^{\alpha}(2)$ = 40.6, $\delta C_2(2)$ = 169.2. Mass spectra show peaks for $[M]^+$ and $[M+1]^+$ with $[C_7H_7]^+$ as base peak.

Starting materials

The used N-protected amino acids and HCl- or *p*-toluenesulphonic acid salts of amino acid esters were purchased from Fluka or prepared by known methods. LR can easily be prepared[7] and is commercially available from Aldrich, Fluka, and Merck-Schuchardt.

General procedure for the preparation of a protected peptide with LR as coupling reagent. 0.01 mole of the N-protected amino acid was dissolved in 10 ml CH_2Cl_2 and 1.01 g (0.01 mole) TEA were added and stirred for 15 min at room temperature. 2.02 g (0.005 mole) LR and 10 ml CH_2Cl_2 were added to the mixture and was stirred for 20 min (until the mixture became clear), and then cooled to 0°C in an ice-bath. 2.02 g (0.02 mole) TEA and 0.01 mole of the salt of an amino acid ester were added to the cooled mixture and stirred for 1 h at 0°C and 3-15 hrs at room temperature. The mixture was applicated directly on to a silica gel column and the protected peptide was eluated with CH_2Cl_2/AcOEt (ratio 3:1).

Epimerization test of Z-Gly-S-Ala-S-Leu-OBzl by HPLC. By running high performance liquid chromatography on a Hewlett-Packard HP 1048 B Liquid Chromatograph with Nucleosil 10 C_{18} as a stationary phase, no epimerization (if any, limits of detection 0.7%) could be observed under the following conditions: Column 250 × 7.8 mm, flow: 3 ml/min, temperature: 50°C, wavelength: 220 nm, mobil phase: buffer A: 0.1% TFA, buffer B: CH_3CN, start: 35% B, end 70% B.

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