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STUDIES ON AMINO ACIDS AND PEPTIDES X[1]

HPLC-MEDIATED TEST OF 2,4-BIS(4-METHOXYPHENYL)-1,3,2,4-DITHIADIPHOSPHETANE 2,4-DISULFIDE (LAWESSON'S REAGENT) AS A RACEMIZATION-FREE COUPLING REAGENT IN PEPTIDE SYNTHESIS

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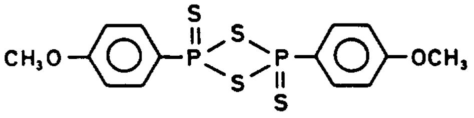
Abstract - The easily available 2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane 2,4-disulfide (Lawesson's Reagent), 1, has been tested as a coupling reagent for racemization in peptide synthesis. The assay procedure is separation of stereomeric products by HPLC. Z-S-Pro-S-Val-S-Pro-O*t*Bu and Z-S-Leu-S-Phe-S-Val-O*t*Bu have been used as test peptides in 2+1 segment couplings, and only a small amount of epimerization (0.5 and <0.1%, respectively) has been observed.

INTRODUCTION

2,4-Bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane 2,4-disulfide, 1, has been introduced as a novel racemization-free coupling reagent in peptide synthesis.[5] This result was based on the 2+1 segment coupling of Z-Gly-S-Ala-OH and Tos-OH•H-S-Leu-OBzl, which showed less than 0.7% racemization. In order to support this preliminary result we decided to extend the racemization test by a study of two test peptides. In studies on the DCC-mediated coupling reactions the chosen peptides Z-S-Pro-S-Val-S-Pro-O*t*Bu and Z-S-Leu-S-Phe-S-Val-O*t*Bu have shown a relatively large tendency to epimerize.[6] The DCC/HOBt-mediated coupling of Z-S-Pro-S-Val-OH with HCl H-S-Pro-O*t*Bu showed 8-9% epimerization,[7] while the DCC/HOSu-mediated coupling of Z-S-Leu-S-Phe-OH with HCl H-Val-O*t*Bu showed 1-2% epimerization.[8]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STARTING MATERIALS

Z-S-Pro-R-Val-OH, 10, Z-S-Leu-S-Phe-OH, 11, and Z-S-Leu-R-Phe-OH, 12, were prepared from the corresponding *tert*-butyl esters, 7-9, by treatment with hydrogen chloride in dioxane. The *tert*-butyl esters were synthesized from the appropriate protected amino acid residues with 1 as coupling reagent. The *tert*-butyl ester hydrochlorides of S-proline, 2, S-valine, 3, R-valine, 4, S-phenylalanine, 5, and R-phenylalanine, 6, were prepared by a modification of a known method.[9]

RESULTS AND DISCUSSION

Z-S-Pro-S-Val-S-Pro-O*t*Bu, 13, Z-S-Pro-R-Val-S-Pro-O*t*Bu, 14, Z-S-Leu-S-Phe-S-Val-O*t*Bu, 15, and Z-S-Leu-R-Phe-S-Val-O*t*Bu, 16, were prepared by 2+1 segment coupling reactions from the appropriate

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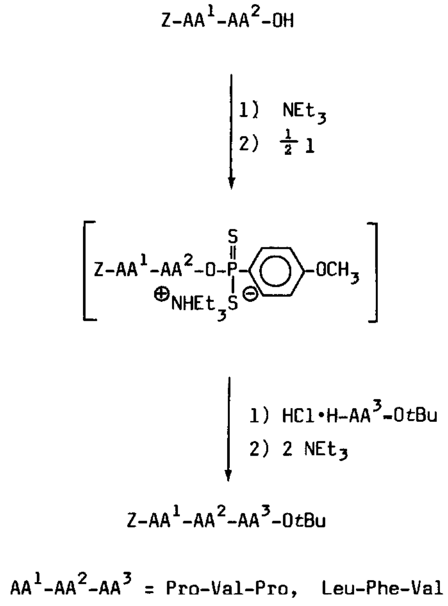
† Deceased (March, 1985)

residues with 1 as the coupling reagent (Scheme 1). The S,R,S-isomers were synthesized with the surmise that a diastereomeric mixture of the protected tripeptides might be efficiently separated on an HPLC chromatograph under appropriate conditions. We observed, however, that it was difficult to obtain complete base-line separation of the two isomers which, in turn, meant a relatively high limit of detection. After tedious work with Z-Leu-Phe-Val-O*t*Bu we finally found that it was possible to obtain a limit of detection of 0.1% on a 4.6 mm • 250 mm Spherisorb S5 ODS column. In fact no S,R,S-isomer was detectable in the pure S,S,S-isomer, which meant that less than 0.1% S,R,S-isomer was present. The synthetic mixture of the two isomers of Z-S-Pro-Val-S-Pro-O*t*Bu was separated on a 8 mm • 250 mm Nucleosil C_{18} column. However, the chromatogram of the pure S,S,S-isomer showed the presence of 0.5% of the S,R,S-isomer. The fact that no epimerization (0.1%) has occurred in the test of Z-Leu-Phe-Val-O*t*Bu and that the extent of epimerization of Z-Pro-Val-Pro-O*t*Bu was as low as 0.5% proves the efficiency of 1 as a racemization-free coupling reagent. An epimerization of 0.5% may seem to be high, but when compared to other coupling reagents it must be considered as a quite low epimerization (cfr. ref. in note 6).



Scheme 1

EXPERIMENTAL

HPLC spectra were recorded on a Hewlett-Packard 1084 liquid chromatograph and the columns were a Sperisorb S5 ODS 4.6 mm • 250 mm (Z-Leu-Phe-Val-O*t*Bu) and a Nucleosil C_{18} 8 mm • 250 mm (Z-Pro-Val-Pro-O*t*Bu). ^1H and ^{13}C NMR spectra were obtained for the compounds 7-16, and the expected signals were identified. IR spectra were recorded on a Beckman IR-18 spectrophotometer. The spectra showed that all carbonyl functions absorbed in one broad band, 1660-1740 cm^{-1}. The C-O stretching of the esters all absorbed at 1150 cm^{-1}. Mass spectra and precise mass measurements were recorded on a Micromass 7070F spectrometer operating at 70 eV with direct inlet. All the mass spectra showed $[M]^+$. All the *tert*-butyl esters showed $[M-56]^+$, $[M-73]^+$, and $[M-101]^+$ corresponding to loss of 2-methylpropene, followed by loss of OH and CO. Microanalyses were carried out by Løvens Kemiske Fabrik, DK-2750 Ballerup (Microanalytical Laboratory). Optical rotations were measured in a 1 dm cell in a Perkin-Elmer 241 polarimeter. Silica gel 60 (Merck) was used for chromatography. Mp.'s are uncorrected. 1 (available from Fluka AG, Merck-Schuchardt, Aldrich Chemical Co., and Riedel de Haen) was prepared as described earlier.[13] Z-S-Pro-OH, Z-S-Leu-OH, and Z-S-Pro-S-Val-OH were purchased from Fluka AG. The petroleum ether (PE) has bp. 45 °C.

General procedure for the preparation of the tert-*butyl ester hydrochlorides of amino acids*

To a stirred suspension of 0.025 mol amino acid in 50 ml anhydrous dioxane 5 ml of concentrated sulfuric acid was added slowly. The two-necked round-bottomed flask was equipped with a specially designed reflux condenser (Figure 1), and approx. 30 g of gaseous 2-methylpropene was added. The condenser caused the gas to liquefy, and the reaction mixture started to reflux. The reaction mixture was stirred overnight and then poured into a chilled mixture of 400 ml ether and 250 ml 1 M NaOH. The phases were separated, and the aqueous phase was washed well with ether. The organic phases were collected, dried with Na_2SO_4, and concentrated under reduced pressure to approx. 10 ml. Addition of dry hydrogen chloride gave the crystalline hydrochloride.

General procedure for cleavage of the tert-*butyl esters*

0.010 mol of the *tert*-butyl ester was stirred with 60 ml of 8 M HCl in dioxane for 12 h. The solvent was evaporated under reduced pressure, and the residue recrystallized.

Z-S-Pro-R-Val-OH, 10: 10 was prepared from 7. Recrystallized from AcOEt/PE.

Z-S-Leu-S-Phe-OH, 11: 11 was prepared from 8. Recrystallized from $CH_2Cl_2/Et_2O/PE$.

Z-S-Leu-R-Phe-OH, 12: 12 was prepared from 9. Precise mass measurement: Found (calc.): 412.2026 (412.1998).

General procedure for the coupling reaction with 1 *as coupling reagent*

0.010 mol N-protected amino acid or peptide and 0.010 mol triethylamine (TEA) were mixed in 10 ml anhydrous CH_2Cl_2 and stirred until a clear solution was obtained. 0.005 mol 1 was added in small portions (exothermic reaction) and stirred until the suspension turned clear. The mixture was cooled to approx. -15 °C and 0.010 mol amino acid ester hydrochloride added. Finally, 0.020 mol TEA was added dropwise to the mixture (exothermic reaction). Stirring was continued overnight, while the mixture attained room temperature. Work-up was performed in two ways: a) The reaction mixture was transferred directly to a silica gel column and chromatographed with a suitable eluent. b) The solvent was evaporated under reduced pressure, AcOEt added to the residue, and the organic phase washed with 2 • 25 ml water, 2 • 25 ml 0.5 M NaHCO$_3$, 2 • 25 ml 0.5 M citric acid and 2 • 25 ml water. The aqueous phases were washed with a small amount of AcOEt. The combined organic phases were dried with Na$_2$SO$_4$ and evaporated under reduced pressure. The residue was subjected to column chromatography.

Z-S-Pro-R-Val-OtBu, 7: 7 was prepared from Z-S-Pro-OH and 4. Work-up b). Eluent: Et$_2$O. Precise mass measurement: Found (calc.): 404.2310 (404.2311).

Z-S-Leu-S-Phe-OtBu, 8, 8 was prepared from Z-S-Leu-OH and 5. Work-up a). Eluent: 50% Et$_2$O/ 50% PE. Microanalysis: Found (calc.): C 68.55 (69.21), H 7.88 (7.74), N 5.81% (5.98%).

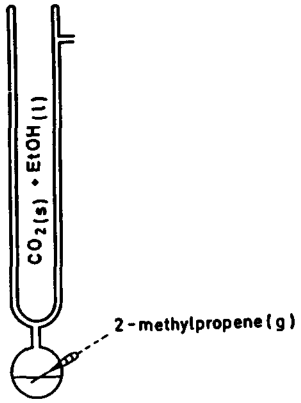


Figure 1

Table 1. Experimental and physical data

		Mp. [°C]	$[\alpha]_D$[a]	Yield[f]
2	HCl•H-S-Pro-OtBu	109-10 (110-12[9])	-26.4 (-30.5[b][9])	48
3	HCl•H-S-Val-OtBu	151 (147-49[9])	+19.4; +22.0[b] (+20.5[b][9])	50
4	HCl•H-R-Val-OtBu	151	-22.0[b]	52
5	HCl•H-S-Phe-OtBu	235-37(dec.) (dec.[9])	+25.5 (+44.2[b][9])	71
6	HCl•H-R-Phe-OtBu	235-7(dec.)	-25.5; -46.1[c]	77
7	Z-S-Pro-R-Val-OtBu	oil	-18.3	81
8	Z-S-Leu-S-Phe-OtBu	oil	-21.4	68
9	Z-S-Leu-R-Phe-OtBu	94-6	-9.8	60
10	Z-S-Pro-R-Val-OH	87-9 (89-91[10])	-37.9 (-38.6[d][10])	80
11	Z-S-Leu-S-Phe-OH	118-19 (120-23[11]) (119-21[12])	-8.4 (+5.8[e][11])	89
12	Z-S-Leu-R-Phe-OH	amorph	-20.7	88
13	Z-S-Pro-S-Val-S-Pro-OtBu	amorph	-118.6	27
14	Z-S-Pro-R-Val-S-Pro-OtBu	amorph	-47.9	23
15	Z-S-Leu-S-Phe-S-Val-OtBu	111 (oil[8])	-44.6	39
16	Z-S-Leu-R-Phe-S-Val-OtBu	amorph	-4.0	35

[a] c = 1.0, MeOH; [b] c = 2, EtOH; [c] c = 1, EtOH; [d] c = 2.0, MeOH; [e] c = 1.0, pyridine; [f] yields are not optimized.

Z-S-Leu-R-Phe-OtBu, 9: 9 was prepared from Z-S-Leu-OH and 6. Work-up a). Eluent: 50% Et$_2$O/50% PE. Microanalysis: Found (calc.): C 69.05 (69.21), H 7.76 (7.74), N 5.71% (5.98%).

Z-S-Pro-S-Val-S-Pro-OtBu, 13: 13 was prepared from Z-S-Pro-S-Val-OH and 2. Work-up b). Eluent: Et$_2$O. Precise mass measurement: Found (calc.): 501.2843 (501.2839).

Z-S-Pro-R-Val-S-Pro-OtBu, 14: 14 was prepared from 10 and 2. Work-up b). Eluent: Et$_2$O. Precise mass measurement: Found (calc.): 501.2841 (501.2839).

Z-S-Leu-S-Phe-S-Val-OtBu, 15: 15 was prepared from 11 and 3. Work-up a). Eluent: 20% Et$_2$O/80% CH$_2$Cl$_2$. Precise mass measurement: Found (calc.): 567.3308 (567.3308).

Z-S-Leu-R-Phe-S-Val-OtBu, 16: 16 was prepared from 12 and 3. Work-up a). Eluent: 20% Et_2O/80% CH_2Cl_2. Precise mass measurement: Found (calc.): 567.3308 (567.3308).

Procedure for the determination of the extent of epimerization by means of HPLC

The appropriate conditions for the separation of a stereomeric mixture were determined by applying a "racemic" mixture of the test peptide to the chromatograph. After optimizing the conditions the limit of detection of S,R,S in S,S,S was found with synthetic mixtures of both isomers.

HPLC test of 13. Running pure S,S,S-isomer showed 0.5% epimerization. Flow 1.0 ml/min. Temperature 50 °C. Wavelength 220 nm. Buffer system EtOH/H_2O (start 25% EtOH, stop (120 min) 55% EtOH).

HPLC test of 15. Running pure S,S,S-isomer showed <0.1% epimerization. Flow 1.0 ml/min. Temperature 30 °C. Wavelength 220 nm. Buffer system: Isocratic (60% EtOH/40% H_2O).

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REFERENCES AND NOTES

1) Part of this work has been presented at the Eleventh International Symposium on the Organic Chemistry of Sulphur, September 1984, in Lindau, FRG. For part IX in this series, see T.P.Andersen *et al., Liebigs Ann.Chem.* (submitted).

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5) U.Pedersen, M.Thorsen, E.-E.A.M.El-Khrisy, K.Clausen, and S.-O.Lawesson, *Tetrahedron* 38, 3267 (1982).

6) The N-acetyl-, N-trifluoroacetyl- and N-benzoyl-protected analogs of the mentioned tripeptides have not been considered (even though they generally show greater epimerization (D.S.Kemp in *The Peptides*, Vol.1, edited by E.Gross and J.Meienhofer, Academic Press 1979, 315)), since the use of these N-protecting groups in peptide synthesis is minimal.

7) W.König and R.Geiger, *Chem.Ber.* 103, 2024 (1970).

8) F.Weygand, D.Hoffmann, and E.Wünsch, *Z.Naturforsch.* 21B, 426 (1966).

9) R.Roeske, *J.Org.Chem.* 28, 1251 (1963).

10) I.M.Shaw and A.Taylor, *J.Chem.Soc.Perkin Trans.I* 1979, 1866.

11) J.H.Jones and G.T.Young, *J.Chem.Soc.C* 1968, 53.

12) E.L.Smith, D.H., Spackman, and W.J.Polglase, *J.Biol.Chem.* 199, 801 (1952).

13) I.Thomsen, K.Clausen, S.Scheibye, and S.-O.Lawesson, *Org.Synth.* 62, 158 (1984).