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A New Synthetic Method For Proline Diphenyl Phosphonates

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Abstract: The diphenyl ester of the phosphonic acid analogue of proline and related dipeptides were prepared by a reaction of the corresponding *N*-substituted 4-aminobutyraldehyde and triphenyl phosphite. Diastereoisomers were separated and their configuration determined by X-ray crystallography.

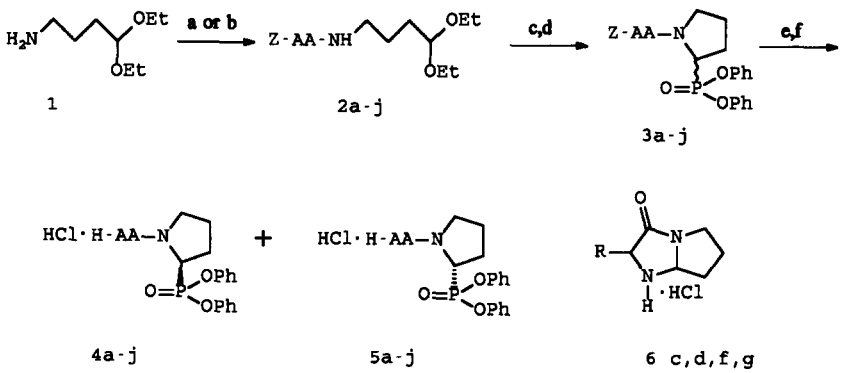
Recently the synthesis of the diphenyl ester of the phosphonic acid analogue of proline (Pro^P) has been described. This compound was prepared as a racemic mixture by the addition of diphenyl phosphite to the pyrrolidine trimer.[1] This report has prompted us to reveal another method for the synthesis of this diphenyl ester and derived AA-Pro^P dipeptides **4,5** (AA=amino acid residue), the isolation of pure diastereoisomers from diastereoisomeric mixtures and the determination of their absolute configuration. These compounds were prepared as potential inhibitors of proline specific serine proteases.

In our approach we used the intermediate aminal-aldehyde mixture, already described earlier in the synthesis of dipeptides of Pro^P diethyl esters from proline.[2] Commercially available 4-aminobutyraldehyde diethyl acetal **1** was coupled to a *Z*-protected amino acid activated as a mixed anhydride with isobutyl chloroformate or as a pentafluorophenyl ester. The *N*-substituted acetals **2a-j** were hydrolysed in acid[3] and the resulting mixture was treated with triphenyl phosphite in acetic acid[4,5] to give diastereoisomeric mixtures of the protected diphenyl phosphonates **3a-j** (see Scheme 1). The latter were purified by vacuum column chromatography. Analytical RP HPLC showed low chiral induction. Diastereoisomeric mixtures of compounds **3b,c,g** could be separated by preparative silica column chromatography. The others were unresolved.

Deprotection of **3a-j** was carried out by hydrogenolysis in the presence of acetic acid.[6] Deprotection of the diastereomeric mixture **3c** and the pure isomers **3c** showed that deprotection didn't change the diastereoisomer ratio. Free diastereoisomers **4** and **5**, with exception of the *L*-Pro, *L*-Ala, *L*-Ile and *L*-Arg derivatives could be separated by vacuum column chromatography on silica. Bicyclic derivatives **6c,d,f,g** were isolated in trace amounts as impurities in the corresponding **4,5** mixture. Careful analysis of the impurity profile of **3** revealed the presence of trace amounts of *Z*-protected **6**. The same kind of cyclization

was reported earlier for benzyl *N*-((*S*)-*Z*-phenylalanyl)-*(S)*-2-amino-5-oxovalerate.[7]

Scheme 1



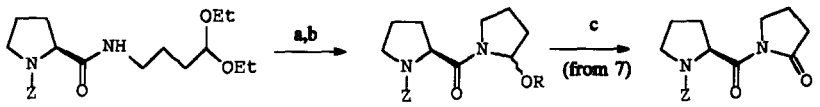
Reagents and conditions: **a)** *i*-BuOCOCl, Et_3N, *L*-*Z*-AA-OH, $CHCl_3$, -10°C - r.t.; **b)** pentafluorophenol, DCC, *L*-*Z*-AA-OH, $CHCl_3$, 0°C - r.t.; **c)** 0.5N HCl/THF, r.t., 1 h; **d)** $P(OPh)_3$, AcOH, 80-85°C, 1 h; **e)** H_2/Pd/C, MeOH/AcOH, r.t., 3-4 h; **f)** vacuum column chromatography, silica, $CHCl_3$-MeOH-AcOH (25:1:1 v:v:v), 0.5N HCl/EtOAc.
L-AA= a: Pro, b: (*S*)-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid (Tic), c:Phe, d: *p*-F-Phe, e: Ala, f: Cyclohexylala, g: 1-Naphthylala, h: Ile, i: Arg, j: Homopro.

In the case of the *L*-Ile derivatives **4h** and **5h**, one isomer easily crystallized from an EtOAc-MeOH (10:1) solution of the racemic mixture. X-ray analysis[8] showed this isomer to be the *(S)*-Ile-*(R)*-Pro^P diastereoisomer **4h**. The other **4** and **5** could not be obtained ion crystalline form. Chromatographic behaviour of the isomers obtained was as already described in the literature for the corresponding diethyl esters: heterochiral (*D,L*) forms migrate faster on silica than the corresponding homochiral isomers (*L,L*)[2]. From these data, together with a comparison of the ^1H NMR spectrum pattern in $CDCl_3$ and the sign of the optical rotation[9] of compounds **4** and **5** with the data of **4h**, we could assign the *(S,R)*- and *(S,S)*-configuration to **4** and **5**, respectively. The ^1H NMR spectra of the *(S,R)* isomers show a single set for the CH-P proton. The *(S,S)* isomers on the contrary have a double set, the latter due to the occurrence of the cis- and trans isomers.[10] The same double set is seen for the NH_3^+ protons.

The real intermediate reacting with $P(OPh)_3$ remains unknown. Hydrolysis of acetal **2a** (Scheme 2) followed by heating of the product in CCl_4 under reflux leads to formation of a separable (silicagel) mixture of hemiaminal **7** and ethoxy derivative **8**. The structure of **7** was confirmed by oxidation to 2-pyrrolidinone **9** by means of potassium permanganate in acetic acid.

The diphenyl esters of these AA-Pro^P dipeptides were tested as inhibitors of dipeptidyl peptidase IV (DPP IV, EC 3.4.14.5., CD 26). The Pro-Pro^P and Cyclohexylala-Pro^P were the most active ones. Only the *(S)*-AA-*(R)*-Pro^P diastereoisomers were inhibitors.

Scheme 2



2a 7: R=H 8: R=Et 9

Reagents and conditions: **a)** 0.5N HCl/THF, r.t., 1 h; **b)** CCl_4, reflux, 1 h; **c)** $KMnO_4$, $AcOH/H_2O$, r.t., 3 h.

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5. Diphenyl *(R,S)*-1-[*(S)*-2-benzyloxycarbonylaminoacyl]-pyrrolidine-2-phosphonates (**3a-j**). (General procedure). Aqueous HCl solution (0.5N - 30 ml, 15 mmol) was added at r.t. to a solution of diethylacetal **2** (10 mmol) in THF (60 mL) and the mixture was stirred for 1 h. Et_2O (85 mL) and a NaOH solution (1N) was added until pH 7-8. The organic layer was separated, the aqueous layer washed with ether (60 mL), the combined organic layers dried ($MgSO_4$) and evaporated under reduced pressure. The resulting oil was dissolved in AcOH (30 mL), $P(OPh)_3$ (20 mmol) was added and the solution stirred at 80-85°C for 1 h. The mixture was cooled and evaporated under reduced pressure at 45-50°C. The residue was dissolved in $CHCl_3$ (50 mL), the solution was washed with water, aqueous $NaHCO_3$, dried ($MgSO_4$) and evaporated. The residue was purified on silica using EtOAc-petroleum ether (1:1 or 1:2).
6. Diphenyl *(R)-* and *(S)*-1-[*(S)*-aminoacyl]pyrrolidine-2-phosphonate hydrochlorides (**4a-j, 5a-j**) (General procedure). *Z*-Protected phosphonate **3** (1 mmol) was dissolved in a mixture of MeOH (20 mL) and AcOH (0.5 mL). The solution was hydrogenated over 10% Pd/C at r.t. for 3-6 h. The catalyst was filtered off on celite and the filtrate was evaporated under reduced pressure. The residue was dissolved in dry $CHCl_3$, 0.5N HCl in EtOAc (2.5 mL) was added and the product was precipitated with Et_2O or hexane-Et_2O. The solid was collected and dried in a vacuum desiccator over NaOH. This procedure has been used when there was no separation of diastereoisomers on TLC. In case separation was observed, the *Z*-protected phosphonate **3** was deprotected as above. After removal of the catalyst and evaporation of the filtrate the residue was separated on silica with $CHCl_3$-MeOH-AcOH (25:1:1) as eluent. Fractions containing pure diastereoisomers were evaporated under reduced pressure and treated as above.
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8. X-ray data will be published elsewhere.

9. All compounds were fully characterized by spectroscopic means. Some OR and ^{1}H-NMR data:
Diphenyl *(S,R)*-1-[*(S)*-prolyl]pyrrolidine-2-phosphonate hydrochloride: (**4,5a**)
^{1}H NMR ($CDCl_3$/TMS): 1.5-2.7 (m, 8H), 3.0-4.0 (m, 4H), 6.3 (m, 1H), 7.9-8.5 (m, 1H).
Diphenyl *(S)*-1-[*(S)*-phenylalanyl]pyrrolidine-2-phosphonate hydrochloride (**5c**):
$[\alpha]_D^{20}$= 103.3° (C1, MeOH).
^{1}H NMR ($CDCl_3$/TMS): 0.7-2.6 (m, 4H), 2.9-3.7 (m, 4H), 3.9 (m, 0.2H), 4.5 (m, 0.8H), 4.7 (m, 0.8H), 5.25 (m, 0.2H), 6.9-7.5 (m, 15H), 8.2 (br s, 0.6H), 8.8 (br s, 2.4H).
Diphenyl *(R)*-1-[*(S)*-phenylalanyl]pyrrolidine-2-phosphonate hydrochloride (**4c**):
$[\alpha]_D^{20}$= 5.5° (C1, MeOH).
^{1}H NMR ($CDCl_3$/TMS): 1.7-2.6 (m, 4H), 3.1-3.8 (m, 4H), 4.4 (m, 1H), 5.05 (m, 1H), 6.8-7.5 (m, 15H), 8.7 (br s, 3H).
Diphenyl *(S)*-1-[*(S)*-p-fluorophenylalanyl]pyrrolidine-2-phosphonate hydrochloride (**5d**):
$[\alpha]_D^{20}$= 86.6° (C1, MeOH).
^{1}H NMR ($CDCl_3$/TMS): 0.8-2.7 (m, 4H), 2.8-3.7 (m, 4H), 3.9 (m, 0.25H), 4.55 (m, 0.75H), 4.7 (m, 0.75H), 5.25 (m, 0.25H), 6.5-7.5 (m, 14H), 8.3 (br s, 0.75H), 8.85 (br s, 2.25H).
Diphenyl *(R)*-1-[*(S)*-p-fluorophenylalanyl]pyrrolidine-2-phosphonate hydrochloride (**4d**):
$[\alpha]_D^{20}$= -2.2° (C1, MeOH).
^{1}H NMR ($CDCl_3$-CD_3OD /TMS): 1.9-2.6 (m, 4H), 2.8-3.2 (m, 2H), 3.4-4.0 (m, 2H), 4.5 (m, 1H), 5.0 (m, 1H), 6.7-7.6 (m, 14H).
Diphenyl *(S)*-1-[*(S)*-cyclohexylalanyl]pyrrolidine-2-phosphonate hydrochloride (**5f**):
$[\alpha]_D^{20}$= 61.5° (C1, $CHCl_3$).
^{1}H-NMR ($CDCl_3$/TMS): 0.5-2.7 (m, 17H), 3.2-4.1 (m, 2H), 4.1-4.6 (m, 1H), 4.9 (m, 0.6H)-5.2 (m, 0.4H), 6.7-7.5 (m, 10H), 8.36 (br s, 1.2H), 8.55 (br s, 1.8H).
Diphenyl *(R)*-1-[*(S)*-cyclohexylalanyl]pyrrolidine-2-phosphonate hydrochloride (**4f**):
$[\alpha]_D^{20}$= -43.5° (C1, $CHCl_3$).
^{1}H NMR ($CDCl_3$/TMS): 0.5-3.0 (m, 17H), 3.1-4.1 (m, 2H), 4.3 (m, 1H), 5.35 (m, 1H), 6.7-7.5 (m, 10H), 8.5 (br s, 3H).
Diphenyl *(R)*-1-[*(S)*-isoleucyl]pyrrolidine-2-phosphonate hydrochloride (**4h**):
$[\alpha]_D^{20}$= -26.0° (C1, MeOH).
^{1}H NMR ($CDCl_3$/TMS): 0.75 (t, 3H), 1.0 (d, 3H), 1.25 (m, 1H), 1.55 (m, 1H), 1.8-2.7 (m, 5H), 3.3-4.0 (m, 2H), 4.05 (m, 1H), 5.05 (m, 1H), 7.0-7.4 (m, 10H), 8.35 (br s, 3H).
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