Pergamon

Tetrahedron Vol. 51, No. 9, pp. 2679-2688, 1995

0040-4020/95 $9.50+0.00

0040-4020(94)01114-1

Model Studies towards the Applicability of the Readily Available *(S)-N*-Tritylaspartic Anhydride in the Synthesis of Amino Acids and Peptides

Constantinos Athanassopoulos, Chryssoula Tzavara and Dionissios Papaioannou*

Department of Chemistry, University of Patras, 265 00 Patras, Greece

Giovanni Sindona

Department of Chemistry, University of Calabria, I-87030 Arcavacata di Rende (Cs), Italy

Hernâni L. S. Maia

Department of Chemistry, University of Minho, Gualtar, 4700 Braga, Portugal

Abstract: Reactions of *N*-tritylaspartic acid anhydride, readily available through *N,N'*-dicyclohexylcarbodiimide-mediated dehydration of *N*-tritylaspartic acid, with Grignard and Wittig reagents, bulky hydrides, and amines or alcohols of the benzhydryl type, lead regioselectively to products from attack at the β-carbonyl function.

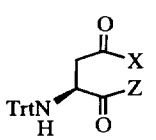
In connection with our studies towards the synthesis of side-chain modified derivatives of glutamic (Glu) and aspartic (Asp) acids suitable for use in peptide synthesis, we projected the anhydrides of *N* - tritylated Glu and Asp as key-intermediates. It was anticipated that nucleophilic attack on the α-carbonyl group of these anhydrides would be unfavoured due to the bulk of the triphenylmethyl (trityl, Trt) group. In addition, the Trt group is compatible with complex metal hydrides hydrides[1,2] and confers excellent configurational stability to chiral α-amino acid derivatives,[3,4] when used for *N* α-protection. Moreover, *N*-tritylated intermediates show increased lipophilicity, which in turn facilitates work-up and purification procedures involving flash column chromatography (FCC). However, attempts to convert Trt-Glu-OH to its corresponding anhydride with *N,N* '- dicyclohexylcarbodiimide (DCC) failed, owing to an unexpectedly facile five-membered ring formation leading to *N* - tritylpyroglutamic acid.[5] We now wish to report our results on the preparation of the anhydride of Trt-Asp-OH and its reactions with a variety of nucleophiles.

RESULTS AND DISCUSSION

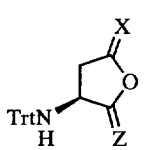
Treatment of Trt-Asp-OH (**1**), readily available from Asp in excellent yield (see experimental), with

DCC in THF/EtOAc (2:1), initially at 0 °C for 2 h and then at room temperature (RT) for 24 h, gave unexceptionally the expected anhydride **2** in 80% yield. Reaction of anhydride **2** with two equivalents of PhMgBr or ArMgBr (Ar=4-methoxyphenyl), initially at -78 °C for 30 min and then at RT for 12 h, followed by routine DCC-mediated lactonization of the thus obtained *N*-tritylhomoserine[6] analogs, gave lactones **3** and **4**, respectively, in 70-75% yields. *N* - Substituted derivatives of similar lactones have been recently shown to exhibit interesting biological properties.[7] Lactones **3** and **4** could be quantitatively transformed to the highly lipophilic benzhydrylalanines **5** and **6** respectively, by catalytic hydrogenolysis in the presence of 10% Pd-C, at RT for 3-5 h, using EtOAc/MeOH (1:1) as the solvent. Amino acid **5** has been earlier prepared in racemic form and its D-enantiomer, obtained by enzymatic resolution of the racemate, was used in the synthesis of luteinizing hormone-releasing hormone analogues.[8]

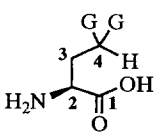
Wittig reaction of anhydride **2** with the stabilized ylide $Ph_3P{=}CHCO_2Me$ in DCM, at RT for 20 h, gave a mere 25% yield of the expected Wittig adduct **7** after routine FCC. The configuration of the double bond represented in structure **7** was assigned on the basis of the relatively large allylic couplings (1.47 and 2.63 Hz) between protons H-4 and H-6. In the alternative configuration, an unfavourable electronic interaction between atom O-1 and the methoxycarbonyl function should be expected. The polar, ninhydrin active, major product of the reaction, having an R_f similar to that of the ylide edduct used, is thought to be the new phosphorane **8**. This derives from a mechanistic study on the reaction of the same phosphorane with phthalic anhydride derivatives.[9] Accordingly, when the same reaction was performed using $Ph_3P{=}C(Me)CO_2Me$ as the Wittig reagent, two non-polar products were isolated by FCC in 60% and 17% yields, respectively, and identified as the Wittig adducts **9** and **10**.



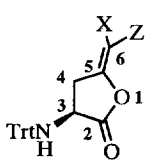
1 : X = Z = OH
14 : X = NH-DMB , Z = Ala-OMe
15 : X = Ala-OMe , Z = NH-DMB
16 : X = O-DMB , Z = Ala-OMe
17 : X = Ala-OMe , Z = O-DMB
18 : X = NH-PB , Z = Ile-OBzl



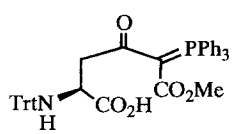
2 : X = Z = O
3 : X = Ph_2 , Z = O
4 : X = Ar_2 , Z = O
11 : X = O , Z = $C(Me)CO_2Me$
12 : X = O , Z = H_2
13 : X = H_2 , Z = O



5 : G = Ph
6 : G = Ar



7 : X = MeO_2C , Z = H
9 : X = MeO_2C , Z = Me
10 : X = Me , Z = CO_2Me



8

The assignment of the configuration of their C-C double bond was also based on the magnitude of the homoallylic couplings observed between the H-4 protons and the protons of the vinylic Me group. Actually, homoallylic couplings of 1.20 and 2.40 Hz were recorded for enollactone **9** and 0 Hz for enollactone **10.** It is worth noticing that the stretching frequency of the carbonyl group of the lactone function in the IR spectra of the enollactones **7**, **9** and **10** is in the region 1814-1822 cm^{-1} . This indicates that these compounds are expected to behave as active esters with comparable hydrolytic stability and acylating potential to the 1-hydroxybenzotriazolyl esters of *N* -tritylamino acids .[10] The alternative structure **11** for compound **10** does not seem possible, because compound **10** is stable to aqueous (aq) $NaHCO_3$ work-up and is finally isolated without problems with FCC.

Reduction of **2** with $NaBH_4$ in THF, for 15 min at -78 °C and for 2 h at RT, followed by DCC-mediated dehydration, gave unexpectedly lactone **12** as the major product and *N* - tritylhomoserine lactone[7] (**13**) as a minor product (ratio **12**:**13** = 5.5:1). The use of diisobutylaluminium hydride (DIBAH) in THF, for 1 h at -78 °C, or the even bulkier K-Selectride® (1M solution in THF), for 2 h at -65 °C, also followed by DCC treatment, converted **2** to the same lactones albeit in the ratios 3:2 and 2:3, respectively. It is thus evident that, even with bulky hydrides and despite the presence of the Trt group, significant amounts of product from α-attack are still formed. The involvement of the amino H-atom and the α-carbonyl O-atom in an inter- or intramolecular hydrogen bond, is suspected to cause preferential activation of the α-carbonyl group towards hydride attack. It should be noticed that *N* - substituted Asp anhydride derivatives with electron-withdrawing protecting groups, such as the benzyloxycarbonyl and the 4-toluenesulphonyl, have been unexceptionally reduced at the α-carbonyl function with $NaBH_4$.[11] For the sake of comparison, lactone **12** was unambiguously obtained, without the involvement of column chromatography purifications, from the commercially available H-Asp(Me)-OH, in 69% total yield, *via* a synthetic protocol earlier developed for the efficient conversion of its homologue H-Glu(Me)-OH to the biologically interesting amino acid hydroxynorvaline.[1]

The applicability of anhydride **2** in solution peptide synthesis was initially tested with its reactions with $TrtNH_2$ and ^tBuOH or ^tBuOK . It was anticipated that these reactions would allow the facile preparation of Trt-Asn(Trt)-OH and Trt-Asp(^{t}Bu)-OH, respectively, useful intermediates for the incorporation of asparagine (Asn) and Asp into peptide chains. Protection of the carboxamide function of glutamine (Gln) and Asn with the Trt group, for peptide synthesis purposes, has been recently disclosed.[12] However, both reactions failed to give the expected products. On the other hand, reaction of anhydride **2** with benzylamine gave (TLC) almost equimolar amounts of the two possible amides from indiscriminate nucleophilic attack at both carbonyl functions. These results prompted us to investigate the use of other suitable amines and alcohols of intermediate bulk as nucleophiles. Thus, one-pot treatment of anhydride **2** with 4,4'-dimethoxybenzhydrylamine (DMB-NH_2) and diisopropylethylamine (DIEA) in DMF for 12 h at 40°C followed by a one-pot benzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophosphate(BOP)-mediated coupling[13] with HCl.H-Ala-OMe in the presence of DIEA, gave a mixture of the two possible dipeptides **14** and **15**, in the ratio 4:1. Pure dipeptide **14** could be obtained directly from the reaction

mixture by crystallization. However, when DCM was used as the solvent, this ratio was unexpectedly reversed to 1:9. This result can be also accommodated by the afore mentioned hydrogen bond activating the α-carbonyl group to nucleophilic attack, which in the case of using the hydrogen bond acceptor DMF as solvent is not involved. On the other hand, treating **2** with 4,4'-dimethoxybenzhydrol(DMB-OH) in the presence of DIEA, in DCM at RT for 1 h, also followed by BOP-mediated coupling with HCl.H-Ala-OMe, gave a mixture of the dipeptides **16** and **17** in the ratio 8:2. In this case alcohol itself can act as a better hydrogen bond donnor, and thus, steric effects predominate leading to preferential β-attack. Pure dipeptide **16** could be also obtained through direct crystallization from the reaction mixture. Thus, anhydride **2** can indeed be used for the direct, one pot, preparation of fully protected Asn and Asp peptides. Of course, in the case where direct purification by crystallization is not possible, FCC should be used to separate the product from small amounts of the unwanted side product formed by nucleophilic attack on the α-carbonyl function. Our structure assignment for the two possible products of nucleophilic attack on both carbonyl functions of anhydride **2** was mainly based on the FAB-MS spectra of these compounds and in particular on the fragment lost from the α-carbonyl side of Asn or Asp. Thus, in the case of dipeptides **14** and **15**, the fragments [H-Ala-OMe+CO (or HCO-Ala-OMe)] and [DMB-NH_2+CO (or HCO-NH-DMB)], respectively, were lost from the M+H cations. For dipeptides **16** and **17**, the corresponding fragments were [H-Ala-OMe+CO (or HCO-Ala-OMe)] and [DMB-OH+CO (or HCO-O-DMB)], respectively.

The applicability of **2** in solid phase synthesis of peptides (SPPS) incorporating Asn, by attaching the asparaginyl side chain on solid supports of the benzhydrylamine type, was also examined. Thus, **2** was reacted with the commercially available polymeric benzhydrylamine (PB-NH_2) in DMF for 5 h at RT, followed by BOP-mediated coupling with TosOH.H-Ile-OBzl to give the polymeric dipeptide **18**. Quantitative attachment of the dipeptide on the resin was observed. It was anticipated that the presence of the bulky polymeric backbone, would further improve the selectivity for β-nucleophilic attack. In order to verify this hypothesis, the fully protected dipeptide **18** was cleaved off the resin using the system trimethylsilyl trifluoromethanesulfonate (Tf-OTMS)/trifluoroacetic acid(TFA)/anisole,[14] for 5 h at 0 °C and 20 h at RT, which resulted in complete deprotection. The crude dipeptide thus obtained , without purification, was converted to Boc-Asn-Ile-OBzl (**19**) by N-protection with Boc_2O/N NaOH in dioxane[15] followed by Mitsunobu esterification[16] with benzyl alcohol. The same peptide was unambiguously prepared, in 70% yield, by BOP-mediated coupling of Boc-Asn-OH with TosOH.H-Ile-OBzl. On the other hand, the isomeric dipeptide Boc-Asp(Ile-OBzl)-NH_2 (**20**) was obtained from Boc-Asp-OH, in 75% total yield, through a three-step sequence involving activation with DCC, ammonolysis of the anhydride thus obtained with conc. NH_4OH and finally BOP-mediated coupling with TosOH.H-Ile-OBzl. HPLC examination of the three fully protected dipeptide samples obtained in this way showed that the sample prepared by using the SPPS technique was really a mixture of the two possible isomers **19** and **20** and that α-attack had also taken place at the extent of 12%.

In conclusion, the N- tritylaspartic anhydride is a readily available compound that can be successfully used as a chiral template for the asymmetric synthesis of amino acids and peptides. The usefulness of the present methodology can be easily extended by using (*R*)-Asp as the starting material. Further reactions of

anhydride **2** with other Grignard and Wittig reagents and applications of the derived intermediates in asymmetric synthesis of biologically interesting non-proteinogenic amino acids and peptides is now in progress. Although our preliminary results on the application of **2** in SPPS are not as promising, additional experimental work is now under way to fully examine the possibility of employing anhydride **2** to the SPPS of Asn containing peptides by using mild acid sensitive resins of the benzhydrylamine type.

EXPERIMENTAL SECTION

General. Capillary melting points were taken on a Buchi SMP-20 apparatus and are uncorrected. Optical rotations were determined with a Carl-Zeiss precision polarimeter. IR spectra were recorded for KBr pellets or neat samples (for oily compounds) on a Perkin-Elmer 16PC FT-IR spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were obtained at 299.949 and 75.429 MHz, respectively, on a Varian Unity-300 instrument using $CDCl_3$ as the solvent, unless otherwise stated, and tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in δ units, parts per million (ppm) downfield from TMS. FAB-MS spectra were recorded on a Fisons-VG ZAB 2f instrument with *m*-nitrobenzyl alcohol as the matrix. FCC was performed on Merck silica gel 60 (230-400 mesh) and TLC on Merck silica gel 60 F_{254} films (0.2 mm) precoated on aluminium foil. The solvent systems used were : (A) and (B) toluene(Tol)/EtOAc/hexane(Hex) (7:3:1) and (7:3:2), respectively, (C) petroleum ether 40-60° (PE)/EtOAc (8:2), (D) MeCN/H_2O (5:1), (E) Tol/EtOAc (9:1), (F) DCM/MeOH (98:2) and (G) $CHCl_3$/MeOH (95:5). Spots were visualized with UV light at 254 nm, with ninhydrin, and the charring agent $(NH_4)_2SO_4$/conc. H_2SO_4/H_2O (20 g/4 ml/100 ml). In general, experiments involving the use of anhydride **2** were carried out in anhydrous solvents under an atmosphere of argon. THF was dried by distillation from Na-benzophenone prior to use. DCM was distilled from CaH_2 and DMF was also distilled from CaH_2 at *ca.* 14 mm Hg.

The amino acids and amino acid derivatives used in the present work were all of the *S* configuration. All new compounds gave analytical and spectral data in agreement with the proposed structures. For the sake of brevity, selected experimental details and physical data for compounds encountered in this work are only provided below. The amino acid derivatives H-Asp(Me)-OH, HCl.H-Ala-OMe, TosOH.H-Ile-OBzl, Boc-Asp-OH and Boc-Asn-OH were purchased from Sigma. The polymeric benzhydrylamine hydrochloride (H_2N-substitution : 0.36 mmol g^{-1}) was purchased from Protein Research Foundation. Trt-Asp-OH was obtained pure, as a foam, from Asp in 94% yield using the procedure described in ref. 2 for the related preparation of Trt-Glu-OH. The fully protected isomeric dipeptides **19** (m.p. 129-31 °C) and **20** (m.p. 172-74 °C), were routinely prepared by BOP-mediated coupling of TosOH.H-Ile-OBzl with Boc-Asn-OH and Boc-Asp-NH_2 respectively, in the presence of DIEA. The latter Asp derivative was readily obtained from Boc-Asp-OH by an one-pot experiment involving activation with DCC and ammonolysis of the anhydride thus obtained. Dipeptides **19** and **20**, obtained analytically pure (HPLC) by crystallization from DE, had identical R_f values (0.1) in solvent system G. HPLC analysis was performed on an LKB instrument. The analytical column used was a Spherisorb S10 ODS2. The eluted peaks were detected at 254 nm. Elution was carried out at 1.0 ml min^{-1} with a gradient MeCN/H_2O from 35 to 100% within 45 min. The retention times thus

recorded for these dipeptides were 16.32 and 15.33 min, respectively.

N-Tritylaspartic anhydride (2). Trt-Asp-OH (15.02 g, 40 mmol) was dissolved in 300 ml EtOAc/THF (2:1) and treated with DCC (8.25 g, 40 mmol) at 0 °C for 2 h and at RT for 24 h. *N,N'*-Dicyclohexylurea (DCU) was filtered off, washed with EtOAc, and the filtrate was concentrated to 100 ml under reduced pressure and refrigerated overnight. Additional DCU precipitated was filtered off and the filtrate evaporated to dryness. The residue was dissolved into EtOAc and the resulting solution triturated with PE and refrigerated overnight to give 8.9 g of product. The filtrate was concentrated and the residue crystallized from diethyl ether (DE) to provide additional 2.5 g of **2**. This way, a 80% total yield of product, as white crystals, was secured. Anhydride **2** had m.p. 175-77 °C, $[\alpha]_D^{25}$ - 60.3° (c1, $CHCl_3$), IR : 1864 and 1783 cm^{-1}, ^1H NMR : 4.020 (1H, dd, *J* 8.25 and 9.15 Hz, H-3), 2.083 (1H, dd, *J* 8.25 and 18.60 Hz, H- 4a), 1.901 (1H, dd, *J* 9.15 and 18.60 Hz, H-4b), 1.640 (1H, br.,NH), ^{13}C NMR : 173.20 and 168.00 (C-2 and C-5), 71.00 (Ph_3C), 54.19 (C-3), 37.28 (C-4) ppm, FAB-MS : z/e 357 (*M*), 280 (*M*-Ph). Anal. Calcd for $C_{23}H_{19}NO_3$: C, 77.29; H, 5.36; N, 3.92. Found: C, 76.98; H, 5.43; N, 4.05.

Reactions of anhydride 2 with Grignard reagents. To a cooled (-78 °C) solution of the Grignard reagent made up from 7 mmol each of Mg turnings and aryl bromide in THF (20 ml), anhydride **2** (3 mmol) was added in three equal portions within 30 min. The resulting solution was allowed to attain RT and thus kept overnight. The resulting greenish solution was quenched with saturated aq. NH_4Cl, diluted with water and exctracted into DE. The organic layer was extracted several times alternatively with NaOH and H_2O and the combined aq. layers were acidified to pH 5 with 5% aq citric acid. Extraction into DE, drying and evaporation under reduced pressure, left a residue that was dissolved in THF (50 ml), cooled to 0 °C, and treated overnight with DCC (3 mmol). Excess DCC was destroyed with a few drops of gl. AcOH and H_2O and the precipitated DCU was filtered off. Concentration to a small volume, addition of EtOAc and cooling overnight, followed by new filtration and evaporation of the filtrate gave an oily residue that was crystallized with DE to give a 50-55% yield of product. An additional 20% yield can be secured on FCC of the residual material using solvent system C as eluant.

Lactone **3** had m.p. 215 °C, $[\alpha]_D^{25}$ -100.3° (c1, $CHCl_3$), R_f(Tol) 0.39, IR : 3332, 1786, 1770 cm^{-1}, ^1H NMR : 7.24-7.18 and 7.13-7.05 (10H, two m, Ph-H), 3.378 (1H, dd, *J* 8.70 and 10.50 Hz, H-3), 2.765 (1H, br., NH), 1.915 (1H, dd, *J* 8.70 and 12.90 Hz, H-4a), 1.902 (1H, dd, *J* 10.50 and 12.90 Hz, H-4b) , ^{13}C NMR : 173.84 (C-2), 83.20 (C-5), 71.40 (Ph_3C), 51.40 (C-3), 41.48 (C-4), FAB-MS : m/z 495 (*M*), 451(*M*-CO_2), 418 (*M*-Ph). Anal. Calcd for $C_{35}H_{29}NO_2$: C, 84.82; H, 5.90; N, 2.83. Found: C, 84.94; H, 5.87; N, 2.75.

Lactone **4** had m.p. 206-207 °C, $[\alpha]_D^{25}$ -43.6° (c1, $CHCl_3$) , R_f(Tol) 0.22, IR : 3368, 1782 cm^{-1}, ^1H NMR : 7.01-6.93 and 6.78-6.72 (8H, two m, Ar-H), 3.762 and 3.729 (6H, two s, OMe), 3.377 (1H, dd, *J* 7.65 and 11.55 Hz, H-3), 2.174 (1H, s, NH), 1.856 (1H, dd, *J* 11.55 and 12.90 Hz, H-4a), 1.766 (1H, dd, *J* 7.65 and 12.90 Hz, H-4b), ^{13}C NMR : δ 177.01 (C-2), 158.89 and 158.76 (ArC-1), 136.12 and 133.04 (ArC-4), 113.50 and 113.39 (ArC-2 and ArC-3), 86.31 (C-5), 70.97 (Ph_3C), 55.09 (two OCH_3), 54.59 (C-3), 44.72 (C-4). Anal.

Calcd for $C_{37}H_{33}NO_4$: C, 79.97; H, 5.99; N, 2.52. Found: C, 79.82; H, 5.87; N, 2.74.

Catalytic hydrogenolysis of lactones 3 and 4. To a solution of lactone **3** or **4** (1 mmol) in 20 ml of EtOAc/MeOH (1:1) 10% Pd-C was suspended and H_2 bubbled through for 3-5 h at RT. The reaction mixture was then passed through a celite pad and the filtered catalyst was washed with MeOH. Pure amino acids **5** and **6** respectively were obtained in quantitative yields by evaporating the filtrate and leaching the crystalline residue with DE to remove TrtH.

Amino acid **5** had m.p. 204-205 °C, $[\alpha]_D^{25}$ +13.7° (c1, AcOH), R_f(D) 0.44, IR : 3220-2300, 1626, 1600, 1540, 1494 cm^{-1}, ^1H NMR (d^6- Me_2CO) : 7.45-7.15 (10H, m, Ph-H), 4.330 (1H, dd, *J* 5.10 and 10.95 Hz, H-4), 3.440 (1H, dd, *J* 4.35 and 10.05 Hz, H-2), 2.710 (1H, ddd, *J* 4.35, 10.95 and 15.30 Hz, H-3a), 2.277(1H, ddd, *J* 5.10, 10.05 and 15.30 Hz, H-3b), FAB-MS : m/z 256 (*M*+H). Anal. Calcd for $C_{16}H_{17}NO_2$: C, 75.27; H, 6.71; N, 5.49. Found: C, 75.02; H, 6.85; N, 5.63.

Amino acid **6** had m.p. 189-190 °C, $[\alpha]_D^{25}$ +13.7° (c1, AcOH), R_f(D) 0.41, IR : 3328, 3220-2300,1628,1613, 1536, 1512 cm^{-1}. Anal. Calcd for $C_{18}H_{21}NO_4$: C, 68.55; H, 6.71; N, 4.44 Found: C, 68.75; H, 6.77; N, 4.25.

Reactions of anhydride 2 with stabilized Wittig reagents. To a solution of anhydride **2** (1.0 g, 2.8 mmol) in DCM (10 ml) the Wittig reagent $Ph_3P{=}C(R)CO_2Me$ (3.2 mmol) was added and the resulting solution kept at RT for 12-20 h for completion of reaction (TLC). The solvent was then evaporated and the resulting oily residue taken-up in EtOAc and washed with 5% aq $NaHCO_3$ and water. The organic phase was dried and evaporated to leave a residue that was subjected to FCC using as eluant the solvent system indicated below.

Enollactone **7**, obtained pure by using Tol as eluant, was recrystallized from Tol and had m.p 114-115 °C, $[\alpha]_D^{25}$ -28.4° (c0.5, $CHCl_3$), R_f(E) 0.36, IR : 1817, 1723 and 1665 cm^{-1}, ^1H NMR: 5.540 (1H,dd, *J* 1.47 and 2.63 Hz, H-6), 3.705 (1H, t, *J* 9.14 Hz, H-3), 3.615 (3H, s, OMe), 2.673 (1H, ddd, *J* 1.47, 9.14 and 18.78 Hz, H-4a), 2.277 (1H, ddd, *J* 2.63, 9.14 and 18.78 Hz, H-4b). Anal. Calcd for $C_{26}H_{23}NO_4$: C, 75.53; H, 5.61; N, 3.39. Found: C, 75.60; H, 5.57; N, 3.44.

Enollactones **9** and **10**, obtained pure by using solvent system C as the eluant, had R_f (C) 0.49 and 0.60 respectively, and were recrystallized from DE/PE. Enollactone **9** had m.p. 132-133 °C, $[\alpha]_D^{25}$ -36.7° (c1, $CHCl_3$), IR : 3344, 1822, 1702, 1684 cm^{-1}, ^1H NMR : 3.691 (1H, t, *J* 9.30 Hz, H-3), 3.607(3H, s, OMe), 2.520 (1H, ddq, *J* 1.20, 9.30 and 18.30 Hz, H-4a), 2.216 (1H, ddq, *J* 2.40, 9.30 and 18.30 Hz, H-4b), 1.794 (3H, q, *J* 1.20 and 2.40 Hz, C-Me), ^{13}C NMR : 178,40 and 172.00 (C-2 and CO_2Me), 154.98 (C-5), 103.30 (C-6), 68.28 (Ph_3C), 49.66 (OCH_3), 48.31 (C-3), 33.57 (C-$\underline{C}H_3$), 33.56 (C-4). Anal. Calcd for $C_{27}H_{25}NO_4$: C, 75.86; H, 5.90; N, 3.28. Found: C, 75.99; H, 5.85; N, 3.12.

Enollactone **10** had m.p. 145-148 °C, $[\alpha]_D^{25}$ +87.9° (c1, $CHCl_3$), IR : 3330, 1814, 1698, 1654 cm^{-1}, ^1H NMR : 3.801 (1H, d, *J* 7.35 Hz, H-3), 3.625 (3H, s, OMe), 2.676 (1H, d, *J* 18.30 Hz, H-4a), 2.016 (1H,dd, *J* 2.40, 7.35 and 18.30 Hz, H-4b), 1.931 (3H, s, C-Me). Anal. Calcd for $C_{27}H_{25}NO_4$: C, 75.86; H, 5.90; N, 3.28 Found: C, 75.80; H, 5.93; N, 3.25.

Reduction of anhydride 2 with $NaBH_4$. Anhydride **2** (7.2 g; 20 mmol) was dissolved in THF (50 ml) and cooled to -78 °C whereby $NaBH_4$ (1.53 g; 40 mmol) was added in one portion with good stirring. The reaction mixture was slowly left to attain RT whereby it was further stirred for 2 h. Excess hydride was then destroyed by dropwise addition of gl. AcOH with ice-cooling. The reaction mixture was diluted with 150 ml 5% aq citric acid and exracted twice with EtOAc. The combined organic layers were washed with water, dried and evaporated. The residue was treated with DCC, as described for lactones **3** and **4** The crude product was finally crystallized DE to give 2.74 g (40%) of lactone **12**. Concentration of the mother liquid and FCC of the residue, with solvent system A as eluant, gave a further 35% yield of lactone **12**. The isomeric lactone **13** had R_f(A) 0.52. For the sake of comparison, lactone **12** was unambiguously obtained from H-Asp(Me)-OH, in 69% total yield, using the following synthetic protocol. *N* - tritylation of H-Asp(Me)-OH, in an identical manner to that described for H-Glu(Me)-OH,[1] gave Trt-Asp(Me)-OH as a foam in 94% yield. The latter compound was converted to its corresponding 1-hydroxybenzotriazolyl active ester with DCC/1-hydroxybenzotriazole(HOBt) followed by one-pot $NaBH_4$ reduction. Routine saponification of the crude hydroxyester thus obtained, followed by DCC treatment, gave crude lactone **12** that was purified by crystallization from DE.

Lactone **12** had m.p 153-54 °C, $[\alpha]_D^{25}$ +4.5° (c1, $CHCl_3$), R_f (A) 0.44, IR : 3328, 1786 cm^{-1}, ^1H NMR : 3.931(1H, dd, *J* 6.7 and 8.7 Hz, H-2a), 3.588 (2H, m, H-2b and H-3), 2.030 (1H, dd, *J* 7.7 and 17.6 Hz, H-4a), 1.898 (1H, dd, *J* 7.9 and 17.6 Hz,H-4b), 1.898 (1H, br., NH), FAB-MS : m/z 344 (*M*+H), 266 (*M*-Ph). Anal. Calcd for $C_{23}H_{21}NO_2$: C, 80.48; H, 6.16; N, 4.08. Found: C, 80.29; H, 6.02; N, 4.32.

Synthesis of asparaginyl dipeptides. To a solution of DMB-NH_2 (2 mmol) and DIEA (1.0 ml, 6 mmol) in DMF, held at 40 °C, anhydride **2** (0.72 g, 2 mmol) was added in one portion. The reaction mixture was kept at this temperature fo 12 h and then brought to RT. To the resulting solution DIEA (0.33 ml, 2 mmol), HCl.H-Ala-OMe (0.30 g, 2.2 mmol) and BOP (0.88 g, 2 mmol) were added sequentially. The resulting solution was kept at RT for 2 hrs and then diluted with 5% aq. citric acid and extracted into EtOAc. The organic layer was sequentially washed with H_2O, 5% aq. $NaHCO_3$ and H_2O, dried and evaporated to leave an oily residue. Crystallization from DE/PE provided pure dipeptide **14** in 65% yield. In a separate small scale experiment both isomeric dipeptides **14** and **15** were isolated pure through FCC of the above mentioned oily residue by using solvent system F for elution. Thus, the ratio of the two isomers prepared was determined as 4:1. When the same experiment was repeated using DCM as the reaction solvent and the reaction of anhydride **2** with DMB-NH_2 was performed for 12 h at RT, the expected dipeptides **14** and **15** were isolated, in the ratio 1:9.

Dipeptide **14** had m.p. 185-188 °C, $[\alpha]_D^{25}$ -24.4° (c1,$CHCl_3$), R_f(F) 0.26, IR : 3354, 3294, 1736, 1657, 1646, 1632 cm^{-1}, ^1H NMR : 8.340 [1H, d, *J* 7.39 Hz, NH(Ala)], 5.941 (2H, s, NH(DMB) and $CHAr_2$), 4.456 {1H, q, *J* 7.39,CH(Ala)}, 3.940 (1H, unresolved d, H-2), 3.780, 3.775 and 3.762 (9H, three s, OMe), 2.426 (1H, dd, *J* 2.97 and 15.21 Hz, H-3a), 1.317 (3H, d, *J* 7.39 Hz, H-3a), 0.978 (1H, dd, *J* 5.54 and 15.21 Hz, H-3b), FAB-MS : m/z 686 (*M*+H), 608 [(*M*+H)-PhH], 555 [(*M*+H)-(CO+Ala-OMe)]. Anal. Calcd for $C_{42}H_{43}N_3O_6$: C, 73.55; H, 6.32; N, 6.13. Found: C, 73.62; H, 6.35; N, 5.97. For comparison dipeptide **15** (an

oil) had R_f (F) 0.20, whereas in its FAB-MS spectrum, the characteristic peak for α-amide bond cleavage was at m/z 415 [(*M*+H)-(CO+DMB-NH_2)].

Synthesis of aspartyl dipeptides. To an ice-cold solution of anhydride **2** (0.36 g, 1 mmol) and DMB-OH (0.27 g, 1.1 mmol) in DCM (0.5 ml), DIEA (0.5 ml, 3 mmol) was added. The resulting solution was kept at RT for 1 h and then HCl.H-Ala-OMe (0.15 g, 1.1 mmol) and BOP (0.44 g, 1 mmol) were added. After 2 h at RT, the solvent evaporated and the residue was worked-up as described above for dipeptide **14**. The desired dipeptide **16** was obtained pure in a 70% yield by crystallization from DE/PE. In a separate experiment, the two possible isomeric dipeptides **16** and **17** were isolated through FCC of the crude reaction mixture by using the solvent system B for elution. This way, the ratio of the two dipeptides synthesized was 4:1.

Dipeptide **16** had had m.p. 85-87 °C, $[\alpha]_D^{25}$ +2.0° (c1, $CHCl_3$), R_f (B) 0.29, IR : 3312, 1740, 1662 cm^{-1}, FAB-MS : m/z 687 (*M*+H), 556 [(*M*+H)-(CO+Ala-OMe)]. Anal. Calcd for $C_{42}H_{42}N_2O_7$: C, 73.45; H, 6.17; N, 4.08. Found: C, 73.58; H, 6.02; N, 3.93. For comparison, dipeptide **16** (an oil) had R_f(B) and FAB-MS : m/z 687 (*M*+H), 415 [(*M*+H)-(CO+DMB-OH)].

HPLC analysis of the reaction of anhydride 2 with PB-NH_2.

Synthesis of polymer-anchored dipeptide 18. A suspension of 0.33 g of the resin PB-NH_2 in anhydrous DMF (1 ml) was treated sequentially with DIEA (0.1 ml, 0.6 mmol) and anhydride **2** (0.14 g, 0.4 mmol) and stirred at RT for 5 h. The resin was then filtered and sequentially washed, twice each time, with DMF, MeOH and DE. Then it was resuspended in DMF (1 ml) and treated sequentially with TosOH.H-Ile-OBzl (0.12 g, 0.3 mmol), DIEA (0.1 ml, 0.6 mmol) and BOP (0.11 g, 0.24 mmol) and the resulting mixture was stirred at RT for 12 h. The resulting resin was then filtered and washed as above to give 0.395 g of resin **18** (100% substitution by increase of weight) after drying under high vacuo.

Deprotection and derivatization. To an ice-cold suspension of resin **18** (0.35 g) in TFA (2 ml) and anisole (0.2 ml), Tf-OTMS (0.4 ml) was added syringwise, under argon, . This combination of reagents corresponds to Sigma's DEPRO^{TM}-200 peptide deprotection kit. The resulting mixture was stirred at 0 °C for 5 h and at RT for 20 h and then cooled to 0 °C and brought to pH 8 by the dropwise addition of conc. NH_4OH. The resin was filtered off and washed with 50% aq AcOH. The filtrate was concentrated under vacuo and the residue taken-up in water and washed twice with DE. The aq layer was freeze-dried and the residue was taken-up in H_2O (1.5 ml) and dioxane (2 ml), solid Na_2CO_3 added to attain pH 7.5 and then Boc_2O (100 mg, 0.48 mmol) was added with ice-cooling. The resulting mixture was allowed to attain RT where it was stirred for 30 min. Then 2N NaOH was added to pH 8, followed by additional Boc_2O (100 mg). When the reaction was completed (30 min at RT) usual work-up gave crude product that was subjected to typical Mitsunobu esterification with BzlOH to give the corresponding benzyl ester with R_f(G) 0.1. Crude product was rid off triphenylphosphine oxide and diethyl 1,2-hydrazine-dicarboxylate by FCC using the solvent system G as eluant. HPLC examination of the thus obtained sample showed that it was a mixture of the dipeptides **19** and **20** in the ratio 88:12.

Acknowledgements : Financial support to C. A. in the form of a Human Capital and Mobility grant, Network Contract : CHRX-CT93-0286, is gratefully acknowledged.

REFERENCES

1. Barlos, K.; Mamos, P.; Papaioannou, D.; Patrianakou, S. *J. Chem. Soc. Chem. Commun.* **1987**, 1583.
2. Papaioannou, D.; Stavropoulos, G.; Sivas, M.; Barlos, K.; Francis, G.W.; Aksnes, D.W.; Maartmann-Moe, K. *Acta Chem. Scand.* **1991**, *45*, 99.
3. Barlos, K.; Papaioannou, D.; Patrianakou, S.; Tsegenidis, T. *Liebigs Ann. Chem.* **1986**, 1950.
4. Baldwin, J.E.; North, M.; Flinn, A.; Moloney, M.G. *Tetrahedron* **1989**, *45*, 1453 and references cited therein.
5. Papaioannou, D.; Athanassopoulos, C., Magafa, V.; Karigiannis, G.; Karamanos, N.; Stavropoulos, G.; Aksnes, D.W.; Francis, G.W. *Acta Chem. Scand.* **1994**, in press.
6. Barlos, K.; Theodoropoulos, D. *Z. Naturforsch.* **1982**, *B37*, 886.
7. Radwansky, A.; Elz, S.; Lehmann, J. *Arch. Pharm.* **1993**, *326*, 243.
8. Nestor, J.J.,Jr.; Ho,T.L.; Simpson, R.A.; Horner, B.L.; Jones, G.H.; McRae, G.I.; Vickery, B.H. *J. Med. Chem.* **1982**, *25*, 795.
9. Kayser, M.M.; Hatt, K.L.,Yu, H.; Hooper, D.L. *Can. J. Chem.* **1993**, *71*, 1010.
10. Barlos, K.; Papaioannou, D.; Theodoropoulos, D. *Int. J. Pept. Protein Res.* **1984**, *23*, 300.
11. (a) McGarvey, G.J.; Hiner, R.N.; Matsubara, Y.; Oh, T. *Tetrahedron Lett.* **1983**, *24*, 2733. (b) Bergmeier, S.C.; Lee. W.K.; Rapoport, H. *J. Org. Chem.* **1993**, *58*, 5019.
12. Sieber, P.; Riniker, B. *Tetrahedron Lett.* **1991**, *36*, 739.
13. Coste, J.; Frerot, E.; Jouin, P.; Castro, B. *Tetrahedron Lett.* **1991**, *32*, 1967 and references cited therein.
14. Fujii, N.; Otaka, A.; Ikemura, O.; Akaji, K.; Funakoshi, S.; Hayashi, Y.; Kuroda, Y.; Yajima, H. *J. Chem. Soc. Chem. Commun.* **1987**, 274.
15. Moroder, L.; Hallett, A.; Wuensch, E.; Keller, O.; Wersin, G. *Hoppe Seyler's Z. Physiol. Chem.* **1976**, *357*, 1651.
16. Mitsunobu, O. *Synthesis* **1983**, 1.

(*Received in UK* 21 *November* 1994; *revised* 20 *December* 1994; *accepted* 21 *December* 1994)