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SYNTHESIS OF A TRIPROTECTED DL-β-CARBOXYASPARTIC ACID (Asa) DERIVATIVE SUITABLE FOR THE SYNTHESIS OF Asa-CONTAINING PEPTIDES

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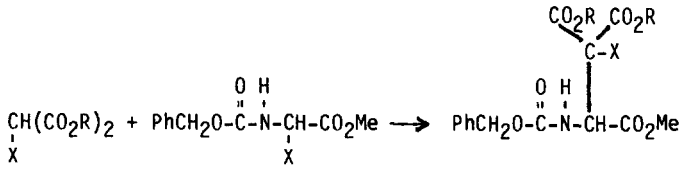
Summary: A new method for synthesizing DL-β-carboxyaspartic acid (Asa) derivatives suitable for use in peptide synthesis is reported.

β-Carboxyaspartic acid (Asa), the lower homologue of γ-carboxyglutamic acid (Gla), was first detected in the acid and base hydrolysates of E. coli ribosomal proteins.[1] Gla is biosynthesized *via* a post-translational vitamin K-dependent carboxylation of glutamyl residues in polypeptide precursors of prothrombin, bone and kidney proteins,[2-4] and Asa may be formed by related reactions.[5] Two syntheses of racemic Asa have been reported. Koch[1] utilized a tris (carbobenzyloxy)ethylene derivative which upon reaction with hydrazoic acid followed by reduction gave Asa in 46% yield overall, while Hauschka et al. synthesized Asa (< 2% overall yield) by alkylation of malonate anion with chlorohydantoin followed by hydrolysis;[6] neither route afforded selectively protected Asa derivatives.

The present communication describes an efficient synthesis of a racemic Asa derivative (7) suitably protected for use in the synthesis of Asa-containing peptides (Scheme I). Alkylation of the sodium salt of malonate diesters 1 and 2 (2 eq., NaH, THF, room temperature) with methyl α-chloro-N-benzyloxycarbonyl glycinate 4[7] (1 eq., THF, 1.5 h) gave the corresponding Asa ester derivatives 5 and 6 (48-50%, purified by column chromatography on silica gel).[8] For the synthesis of 7 the anion 3a, generated from di-t-butylmalonate[9] (1 eq., NaH, THF, 6 h), was added to the N-acyl imine formed *in situ* by dehydrochlorination of 4 (1 eq., triethylamine, THF, 1.5 h) to give triprotected Asa derivative 7 [39% yield; 50% conversion based on recovery of 3; m.p. 62-63°; TLC (hexane:ethyl acetate (7:3), Rf 0.56; ^{1}H NMR ($CDCl_3$), δ 1.40 (s, 9 H, t-Bu), 1.46 (s, 9 H, t-Bu), 3.73 (s, 3 H, OCH_3), 3.83 (d, 1 H, J=4.4 Hz, β-H), 4.98 (dd, 1 H, α-H, (J=4.4, 9.8 Hz)), 5.06 (s, 2 H, benzyl CH_2), 5.95 (d, 1 H, NH, J=10 Hz), 7.33 (s, 5 H, Ar-H).[10]

Other products obtained from the reaction were the carbamate 8 and the pentaester 10 in 15% and 10% yields respectively,[8,11] which also were formed when anion 3a (2 eq., NaH, THF, room temperature) was reacted with 4 (1 eq., THF, 1.5 h) for 16 h. Presumably compound 8 and 9 are formed by β-deamination of anion 7a (Scheme I), since Koch et al. have reported that substituted β-carboxyaspartic acid derivatives undergo deamination *via* β- elimination in the presence of excess base.[12] The formation of 10 follows *via* Michael addition of the anion 3a to the olefin 9. However selective saponification of triester 7 (1 N NaOH, 1.2 eq., dioxane, 1.5 h) gave the triprotected acid 12 in good yield (85%)[13] without significant deamination occurring (Scheme II).

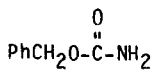
The successful application of Asa derivative 7 to the synthesis of Asa-containing peptides was established by the following experiments. The free acid 12 (1 eq.) was converted to the



1: R = C_2H_5, X = H
2: R = CH_2Ph, X = H
3: R = ^tBu, X = H
3a: R = ^tBu, X = anion

4: X = Cl
11: X = OH

5: R = C_2H_5, X = H
6: R = CH_2Ph, X = H
7: R = ^tBu, X = H
7a: R = ^tBu, X = anion



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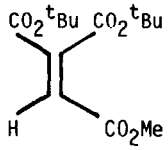

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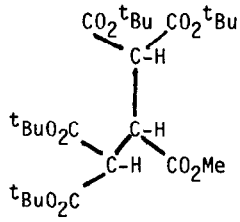

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Scheme 1

mixed anhydride (i-butylchloroformate, N-methylmorpholine, THF, -15°) and reacted with a pre-cooled solution of the dipeptide, H-Glu(O^tBu)-Leu-OMe (1 eq., obtained by hydrogenating Z-Glu(O^tBu)-Leu-OMe; N-methylmorpholine, THF) to give the tripeptide 13 as an oil[14] (75%, purified by chromatography over silica gel in 98:2 chloroform-methanol). The ^1H and ^{13}C NMR spectra of 13 showed the presence of two diastereomers in a 2:1 ratio. Treatment of 13 with TFA-CH_2Cl_2 (7:3) gave the triacid tripeptide 14 (82%).[15]

The nitrogen of 7 can also be selectively deprotected and coupled. Hydrogenation of 7 (10% Pd-C, t-butanol, acetic acid, 6 h) gave the acetate salt which was condensed with the mixed anhydride of Z-Ala-OH (i-butylchloroformate, N-methylmorpholine, THF, -15°) to give the dipeptide 15 [70% yield purified by chromatography].[8,16]

Thus, compound 7 is a useful derivative of β-carboxyaspartic acid that is suitable for the synthesis of peptides containing racemic Asa. The application of these methods to the synthesis of products formed by reaction of Asp-peptides with vitamin K-dependent carboxylase is in progress as are attempts to resolve racemic Asa.

ACKNOWLEDGEMENTS

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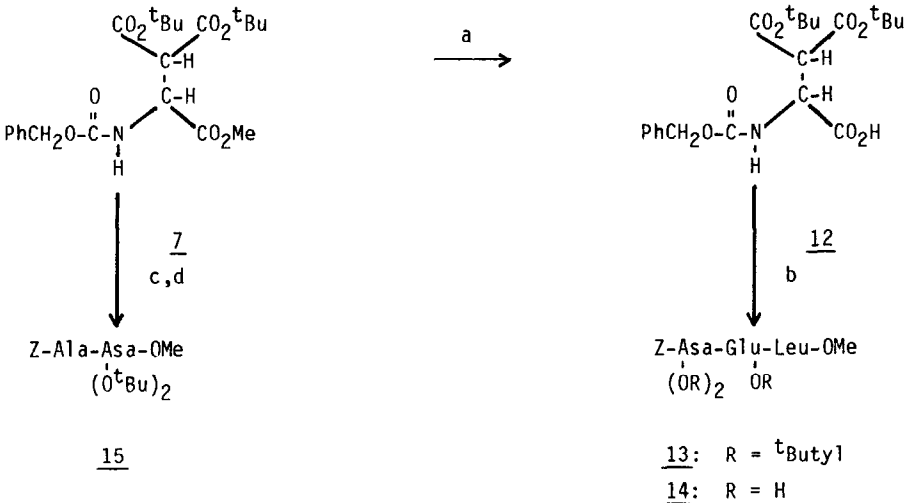

Scheme 2

a. 1.2 equiv NaOH, dioxane, water; b. i-butylchloroformate, N-methylmorpholine, THF, -15° C; 10% Pd/C, t-butanol, acetic acid; d. i-butylchloroformate; N-methylmorpholine, THF, -15° C, Z-Ala-OH.

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8. All new compounds reported gave satisfactory elemental analysis and produced ^{1}H, ^{13}C NMR spectra and mass spectra in accord with the assigned structure.
9. Di-t-butylmalonate was prepared by a new procedure in one step from malonic acid (1 eq.), t-butyl alcohol (3 eq.), 4-dimethylaminopyridine (0.3 eq.), N,N'-dicyclohexylcarbodiimide (2.3 eq.) in ethyl acetate; yield 86%, b.p. 72-73°/1.5 mm (reported 67-68°/1 mm).
10. Compound 7 had the following ^{13}C NMR and mass spectra: ^{13}C NMR ($CDCl_3$), δ (ppm), 170.48, 167.12, 165.88, 156.13, 136.35, 128.50, 128. 12, 82.88, 67.12 (t), 55.14 (d), 53.41 (q),

52.65 (d), 27.95 (q); MS m/e (relative intensity) 438 (M^+ + 1, 0.3), 416 (1), 382 (3), 326 (19), 325 (23), 282 (12), 218 (33), 204 (10), 175 (33), 146 (33), 131 (33) 128 (33), 91 (100). Anal. Calcd.: C, 60.39; H, 7.14; N, 3.20. Found: C, 60.61; H, 7.27; N, 3.16.

11. Pentaester 10 had following properties: m.p. 77-78°, TLC (8:2 hexane-ethyl acetate) Rf 0.33, ^{1}H NMR ($CDCl_3$), δ 1.40 (s, 36 H, t-Bu); 3.33-3.90 (m, 6 H, aliphatic H and OCH_3); ^{13}C NMR ($CDCl_3$), δ (ppm), 170.97, 166.80, 166.36, 82.33, 81.91, 53.63, 51.68, 43.61, 27.84. MS m/e (relative intensity) 335 (M^+, loss of three t-Bu groups, 0.2), 317 (0.5), 279 (3), 261 (7), 234 (8), 202 (4), 172 (11), 131 (15), 99 (33), 57 (100).
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13. Compound 12 was characterized as its cyclohexylamine salt (90%; m.p. 151-152°; ^{1}H NMR ($CDCl_3$), δ 1.40 (s, 9 H, t-Bu), 1.47 (s, 9 H, t-Bu), 3.97 (d, 1 H, β-H (J=3.6 Hz)), 4.98 (dd, 1 H, α-H (J=3.6, 9.3 Hz)), 5.13 (s, 2 H, benzyl CH_2), 5.70 (bs, 1 H, CO_2H), 6.0 (bd, 1 H, NH (J=9.4 Hz), 7.33 (s, 5 H, Ar-H); MS m/e (relative intensity) 311 (M^+, loss of 2 isobutylene groups, 0.1), 204 (0.2), 176 (0.5), 151 (2), 108 (33), 79 (56), 56 (100). Anal. Calcd.: C, 62.05; H, 8.10; N, 5.36. Found: C, 62.10; H, 8.39; N, 5.40.
14. Compound 13 had the following properties: TLC (9.6:0.4 chloroform-methanol) Rf 0.56, ^{1}H NMR ($CDCl_3$), 0.92 (m, 6 H, Leu δ-CH_3), 1.40 (s, 9 H, t-Bu), 1.45 (s, 18 H, t-Bu), 1.57-1.75 (m, 3 H, Leu β-CH_2 and γ-CH), 1.70-2.42 (m, 2 H, Glu β-CH_2), 2.32-2.48 (m, 2 H, Glu γ-CH_2), 3.70 (s, 3 H, OCH_3 major isomer), 3.72 (s, 3 H, OCH_3 minor isomer), 4.06 (d, 1 H, Asa β-H major isomer), 4.10 (d, 1 H, Asa β-H minor isomer), 4.38-4.59 (m, 2 H, α-H), 4.85 (dd, 1 H, α-H), 5.03-5.22 (m, 2 H, benzyl CH_2), 6.08 (d, 1 H, Z-NH, major isomer), 6.18 (d, 1 H, Z-NH minor isomer), 6.82 (m, 1 H, NH), 7.33 (s, 5 H, Ar-H), δ 7.38 (d, 1 H, NH, major isomer), δ 7.45 (d, 1 H, NH, minor isomer); ^{13}C NMR ($CDCl_3$), δ (ppm), 173.14, 172.87, 170.70, 169.78 (carbonyl, minor isomer), 169.56 (carbonyl, major isomer), 168.10 (carbonyl, minor isomer), 167.99 (carbonyl, major isomer), 166.53 (carbonyl, minor isomer), 166.42 (carbonyl, major isomer), 156.29, 136.08, 128.55, 128.23, 80.93, 82.93, 67.50, 54.49, 54.28, 52.76, 52.54, 52.11, 51.14, 41.01, 31.63, 28.11, 27.89, 24.97, 22.80, 21.94. Anal. Calcd.: C, 60.39; H, 7.80; N, 5.71. Found: C, 60.44; H, 8.00; N, 5.62.
15. Compound 14 had the following properties: M.P. 168-169°; Anal. Calcd. for $C_{25}H_{33}N_3O_{12}$: C, 52.90; H, 5.86; N, 7.40. Found: C, 52.77; H, 6.02; N, 7.45.
16. Compound 15 had the following ^{1}H NMR spectrum: ^{1}H NMR ($CDCl_3$), δ 1.32-1.67 (m, 21 H, 2 t-Bu and Ala-Me), 3.71 (s, 3 H, OCH_3), 3.85-4.18 (m, 2 H, α-H and β-H), 4.16-4.40 (m, 1 H, α-H), 5.12 (s, 2 H, benzyl CH_2), 5.16-5.47 (m, 1 H, NH), 7.0-7.23 (m, 1 H, NH), 7.34 (s, 5 H, Ar-H).

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