An Improved Method for the Preparation of ω-Cyclohexyl Esters of Aspartic and Glutamic Acid

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An improved synthesis of β-cyclohexyl L-aspartate and γ-cyclohexyl L-glutamate and its *N-tert*-butoxycarbonyl (Boc) derivatives is described.

It has been known for more than ten years that the use of benzyl esters of aspartic and glutamic acid can cause numerous side reactions during the syntheses of peptides containing these amino acid residues.[1-4] In attempts to suppress these side reactions, many other carboxy protecting groups have been introduced into peptide chemistry, e.g., the cyclohexyl,[5] cyclopentyl,[6] cycloheptyl,[7] menthyl,[8] cyclododecyl,[9] and allyl[10] esters in the case of aspartic and glutamic acids.

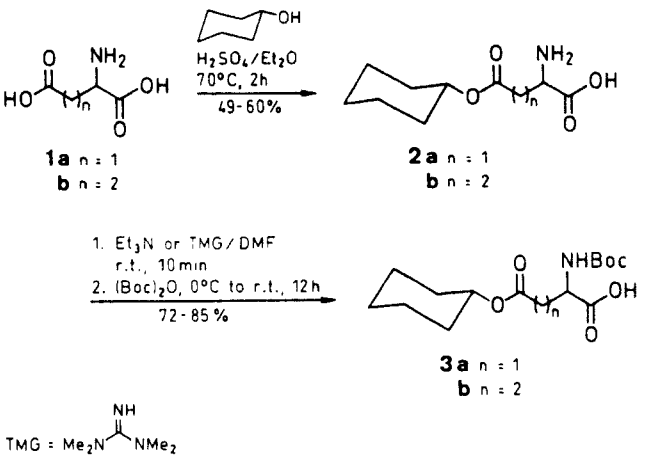
Of these protecting groups, the cyclohexyl esters seem to be the best choice for use in solid-phase peptide synthesis, and this protecting group has been used since 1979. However, the original syntheses[5] of the desired compounds [Boc-Asp(OcHex)-OH and Boc-Glu(OcHex)-OH] are rather complicated. This method involves a lengthy, multistep procedure and no optimization of these syntheses has been described in the literature.

In order to develop a rational synthesis procedure for the ω-cyclohexyl esters of aspartic and glutamic acid, we set out to prepare the required compounds by direct esterification, and we have found a simple and cheap method[11] for the production of these amino acid derivatives in high purity and large amount. The well-known procedure for the synthesis of ω-benzyl esters of the above amino acids[12] can be used with certain minor modifications to produce the ω-cyclohexyl esters of these amino dicarboxylic acids. The two most important conditions are as follows:

1. Only an appropriate temperature and time led to sufficiently site-specific reactions. Without a rigorous control, a mixture of the dicarboxylic acid, the ω-mono ester and the diester can easily be obtained. If the proportions of the above compounds are comparable, isolation of the target compound by a simple and cheap laboratory technique is impossible. Therefore, we need a reaction mixture containing at least 70–80% of the desired product. Only a narrow temperature and time ranges are applicable.

2. The presence of the residual unreacted cyclohexyl alcohol hinders the isolation of the desired compound, and therefore the complete removal of the alcohol component is absolutely necessary.

Although both compounds prepared by this method were obtained in yields of over 50% and were homogeneous (TLC, HPLC and NMR investigations), the elemental analyses showed the presence of some (7–20%) sodium sulfate. However, this salt content does not disturb the introduction of the Boc-group, it disappears during the work-up of the next step. (After RP HPLC desalting of an aliquot part we got excellent element analysis.) Of course, all traces of water must be removed from the crystalline ω-esters of the amino dicarboxylic acids in a desiccator, as otherwise the unwanted side reactions occur.



All reagents were of commercial quality from freshly opened containers. Cyclohexyl alcohol *i*-Pr_2EtN, Et_3N and H_2SO_4 (d = 1.84) were purchased from Aldrich Chemical Co.; di-*tert*-butyl dicarbonate from Fluka Chemical Co., and L-aspartic and L-glutamic acid from Reanal Fine Chemicals. Reagent quality solvents were used without further purification. Analytical TLC plates were purchased from E. Merck. For TLC the following solvent systems were used: a, BuOH/AcOH/H_2O (4:1:1); b, EtOAc/BuOH/AcOH/H_2O (44:1.5:0.2:2.4). Melting points were taken with a PHMK apparatus and are uncorrected. HPLC investigations were performed on a Knauer apparatus with a C-18 column (4 × 250 mm). Microanalyses were carried out with a Heraeus element analyser and rotations at the Na-D-line were obtained at 25°C with a Polamat A polarimeter. IR spectra were recorded with a Nicolet IR 80 spectrophotometer, and ^1H NMR spectra with a Bruker Aspect 3000 (400 MHz) spectrometer.

β-Cyclohexyl L-Aspartate (2a):

H_2SO_4 (25 mL, 500 mmol) was added to cooled Et_2O (250 mL) under stirring, and cyclohexyl alcohol (250 mL, 2500 mmol) and L-aspartic acid (33 g, 250 mmol) were then added. The resulting suspension was heated in a rotary evaporator at 70°C for 2 h under vacuum. The bulk of the solvent was removed during this procedure. The resulting oil was partitioned between EtOAc (250 mL) and aq $KHCO_3$ (5%, 300 mL). The pH was adjusted to 7.0 with 4 N aq NaOH. The aqueous layer was concentrated under vacuum until precipitation occured. The resulting suspension was chilled overnight and filtered to give L-aspartic acid β-cyclohexyl ester. Yield: 32 g (60%), containing 93% of the desired ester and 7% of salt (Na_2SO_4).

$C_{10}H_{17}NO_4$	calc.	C 55.81	H 7.91
(215.3)	found*	56.03	8.02

* Salt content (Na_2SO_4) 7%, corrected.

0.1 g of the salt-containing β-ester was dissolved in H_2O (2 mL) and loaded to an RP-HPLC column (16 × 250 mm, Lichrosorb RP 18, 10 μ). Elution was performed using a linear gradient of MeCN from 0% to 30% in 60 min in 0.1% TFA (flow: 4 mL/min). Pure fractions were pooled, lyophilized and dried in a desiccator; mp 220–223°C; R_f = 0.55 (a); $[\alpha]_D^{24}$ + 20.1° (*c* = 2, H_2O).

$C_{10}H_{17}NO_4$	calc.	C 55.81	H 7.91
(215.3)	found	55.61	7.99

^{1}H NMR: δ = 4.85 (m, 1 H, CO_2CH), 4.45 (t, 1 H, CH, J = 6.1 Hz), 3.19 and 3.09 (dd, 2 H, CH_2, J = 12.5 Hz), 1.90–1.20 (m, 10 $H_{cyclohexyl}$).

β-Cyclohexyl *N*-(*tert*-Butoxycarbonyl)-L-aspartate (3a):
Et_3N (9.8 mL, 70 mmol) was added to a stirred suspension of L-aspartic acid β-cyclohexyl ester (16 g, 70 mmol) in DMF (150 mL) and stirring was continued at r.t. for 10 min. After cooling to 0 °C, di-*tert*-butyl dicarbonate (16.4 mL, 80 mmol) was added under stirring. After reaction overnight (12 h), the solvent was removed under vacuum and the oily residue dissolved in EtOAc (150 mL). This solution was washed with $KHSO_4$ (3 × 100 mL, 5 %) dried (Na_2SO_4) and concentrated at reduced pressure to a pale oil, which crystallized from hexane. Yield: 18.8 g (85 %); mp 91–92 °C (Lit.[5] mp 93–95°); R_f = 0.57 (b); $[\alpha]_D^{24}$ −21.7° (c = 2, DMF).

$C_{15}H_{25}NO_6$	calc.	C 57.14	H 7.94
(315.4)	found	57.54	7.65

γ-Cyclohexyl L-Glutamate (2b):
H_2SO_4 (25 mL, 500 mmol) was added to cooled Et_2O (250 mL) under stirring, and cyclohexyl alcohol (250 mL, 2500 mmol) and L-glutamic acid (36.5 g, 250 mmol) were then added. The resulting suspension was heated in a rotary evaporator at 70 °C for 2 h under vacuum. The bulk of the solvent was removed during this procedure. The resulting oil was partitioned between EtOAc (250 mL) and aq $KHCO_3$ (5 %, 300 mL). The pH was adjusted to 7.0 with 4 N aq NaOH solution. The aqueous layer was concentrated under vacuum until precipitation occured. The resulting suspension was chilled overnight and filtered to give L-glutamic acid γ-cyclohexyl ester. Yield: 28 g (49 %), containing 80 % of ester and 20 % of salt.

$C_{11}H_{19}NO_4$	calc.	C 57.64	H 8.29
(229.3)	found*	56.95	8.20

* Salt content (Na_2SO_4) 20 %, corrected.

The salt-containing γ-ester (0.1 g) was dissolved in H_2O (2 mL) and loaded to an RP-HPLC column (16 × 250 mm, Lichrosorb RP 18, 10 µ). Elution was performed using a linear gradient of MeCN from 0 % to 30 % in 60 min in 0.1 % TFA (flow: 4 mL/min). Pure fractions were pooled, lyophilized and dried in a desiccator; mp 195–198 °C; R_f = 0.53 (a); $[\alpha]_D^{24}$ +10.2° (c = 2, H_2O).

$C_{11}H_{19}NO_4$	calc.	C 57.64	H 8.29
(229.3)	found	57.39	8.37

^{1}H NMR: δ = 4.78 (m, 1 H, CO_2CH), 4.17 (t, 1 H, CH, J = 6.1 Hz), 2.64 and 2.25 (mm, 4 H, CH_2, J = 12.5 Hz) 1.90–1.20 (m, 10 $H_{cyclohexyl}$).

γ-Cyclohexyl *N*-(*tert*-Butoxycarbonyl)-L-glutamate (3b); Isolated as its Dicyclohexylammonium Salt:
1,1,3,3-Tetramethylguanidine (0.63 mL, 5 mmol) was added to a stirred suspension of L-glutamic acid γ-cyclohexyl ester (2.76 g, 10 mmol) in DMF (20 mL) and stirring was continued at r.t. for 10 min. After cooling to 0 °C, di-*tert*-butyl dicarbonate (2.54 mL, 11 mmol) was added under stirring. After reaction overnight (12 h), the solvent was removed under vacuum and the oily residue dissolved in EtOAc (30 mL). This solution was washed with $KHSO_4$ (3 × 20 mL, 5 %), dried (Na_2SO_4) and concentrated at reduced pressure to a pale oil. After adding dicyclohexylamine, the product crystallized as DCHA-salt from hexane. Yield: 3.6 g (72 %); mp 129–132 °C (Lit.[5] mp 135–136°); R_f = 0.70 (b); $[\alpha]_D^{24}$ −6.8° (c = 1, AcOH).

$C_{28}H_{50}N_2O_6$	calc.	C 66.01	H 9.63
(510.6)	found	66.39	9.24

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