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Chemo-enzymic synthesis of protected cyano derivatives of glutamate

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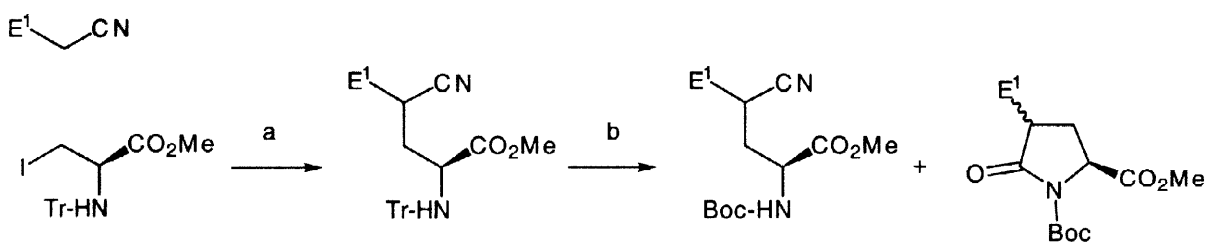
Abstract: (2S,4RS)-Boc-4-cyanoglutamate γ-methyl ester (ee = 95%) and (2S)-Boc-2-amino-4-bis(cyano)butyrate (ee = 96%) were synthesised in respectively 43% and 40% overall yields by addition of sodium malonate derivatives onto Boc-dehydroalanine methyl ester followed by regio- and stereoselective hydrolysis of α-methyl ester by α-chymotrypsin. These regio-and stereoselectivities were strongly dependent on the nature of the γ-substituents.

We recently described the stereoconservative synthesis of orthogonally protected glutamate derivatives[1] starting from N-trityl-3-iodoalanine benzyl ester.[2,3] Hydrogenolysis of trityl and benzyl groups, followed by subsequent reprotection, afforded the Boc- or Fmoc- protected compounds readily usable in classical peptide synthesis. However, mild acidolysis of compounds bearing a cyano group on the γ-position followed by treatment with di-*tert*-butyldicarbonate and triethylamine gave the Boc- protected derivatives in low yields, along with the corresponding lactams (scheme 1). This presumably resulted from the nucleophilic attack of the nitrile by the intermediary free amine and, despite several attempts, we could not optimise this step.

Scheme 1



$E^1 = CO_2Me$	74%	31%	22%
$E^1 = CN$	77%	10%	13%

a: see ref 1; b: 5% HCO_2H / dichloroethane then Boc_2O, NEt_3 / dichloromethane

We report herein on the chemoenzymatic preparation of Boc-protected 4-cyanoglutamates (Boc-CNGlu)[4] and 2-amino-4-bis(cyano)butyrate (Boc-CN_2Abu) from the easily available serine β-cation equivalent Boc-dehydroalanine methyl ester **1**.

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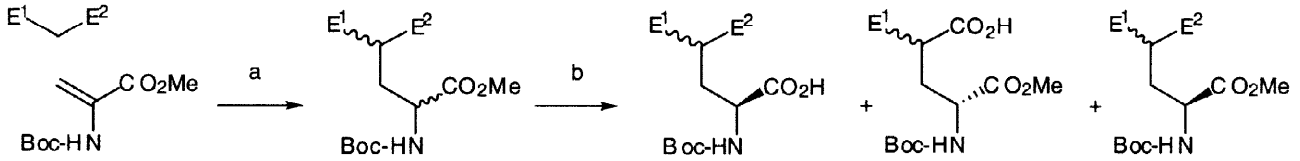
Compound **1**, obtained in high yield from D,L-serine,[5] was treated by the sodium salts of dimethyl malonate, methyl cyanoacetate or malononitrile in a THF-HMPA mixture[6] and gave the corresponding racemic glutamate derivatives in very good yields (scheme 2, table 1). Formation of the parasitic lactam was minimised either by rapid addition of compound **1** or by cooling the reaction medium to -18°C. Compound **3** was obtained as a 1:1 mixture of diastereomers (as determined by NMR) which could not be differenciated by silica gel flash chromatography.

Scheme 2



1 **2-4** **5-7** **8** **2-4**

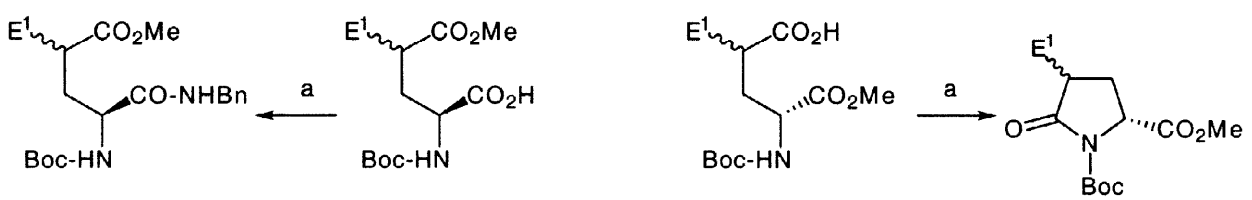
a: E^1-CH_2-E^2, NaH / THF-HMPA 80-20, 1.5 hrs. at r.T.; b: α-chymotrypsin / 0.1M KH_2PO_4-DMSO 95-5 pH 7.6, 37°C, 2 hrs.

Table 1

E^1	E^2	Michael adduct	α-Monoacid	γ-Monoacid	residual adduct
CN	CN	**2** (90%) (D,L)	**5** (45%); ee = 96% (L)	-	**2** (48%); ee = 95%
CN	CO_2Me	**3** (92%) (D,L)	**6** (47%); ee = 95% (L)	**8** (40%); ee = 80% (D)	**3** (3%)
CO_2Me	CO_2Me	**4** (93%) (D,L)	**7** (82%); ee = 0% (D,L)	- (0%)	**4** (<1%)

Resolution of the racemates was tempted by treatment of the Michael adducts with α-chymotrypsin which has been described to hydrolyse hydrophobic Boc-protected glutamate esters with satisfactory regio and stereoselectivities.[7] The reaction conducted in a pH 7.6 phosphate buffer containing 5% DMSO was monitored by thin layer chromatography.[8] Monoacids **5-8** were isolated in good yields[9] (table 1) and their structures were unambigously established as depicted in scheme 3. The enantiomeric purities of monoacids **5-8** were determined as follows: acidic hydrolysis (4 hrs. in refluxing 6N HCl) and subsequent decarboxylation of monoacids led to glutamic acid which was quantitatively derivatized with FLEC-Cl®. The enantiomeric excess were assessed by HPLC as previously reported.[10]

Scheme 3



9 65% E^1 = CN
10 72% E^1 = CO_2Me

6 E^1 = CN
7 E^1 = CO_2Me

8 E^1 = CN

11 76% E^1 = CN

a: DCC, HOBT, NEt_3, $BnNH_2$ / dichloromethane.

As anticipated, the relatively hydrophobic Boc-CN_2Abu **2** was hydrolysed readily with an almost complete stereoselectivity and gave a mixture of monoacids **5** (2S) along with the residual methyl ester (2R). In contrast, enzymatic hydrolysis of Boc-CNGlu **3** led to an easily separable mixture of monoacids **6** and **8**[11] which were identified as the α- and γ-monoacids respectively: treatment of compound **6** with benzylamine and DCC / HOBT led to benzylamide derivative **9** whereas compound **8** lactamised readily (scheme 3).

Interestingly, the regioselectivity of the chymotrypsin hydrolysis was strictly related to the configuration of the α-carbon. Highly selective hydrolysis of the α-methyl ester into the (2S) isomer (ee = 95%) and of the γ-methyl ester in the (2R) isomer (ee = 80%) was observed.

Surprisingly, the carboxyglutamate triester **4** showed a completely different behaviour. The hydrolysis was totally regioselective, leading exclusively to the α-monoacid **7** (table 1). No trace of γ-monoacid could be detected since coupling with benzylamine gave the corresponding benzylamide **10** without lactamisation. On the other hand, no stereoselectivity was observed since the α-monoacid **7** was racemic. No significative difference in optical purity was observed at approximately half-conversion of compound **4** (**7**: ee = 4%).

We checked that this lack of stereoselectivity could not be attributed to a concurrent saponification by the slightly alcaline buffer. As anticipated, no significant saponification of triester **4** was detected after 2 hours at 37°C in the phosphate buffer. Moreover, malonic esters are much more sensitive to saponification than α-amino esters: hence, a putative saponification should lead to the γ-monoacid rather than the α-monoacid. [12]

Steric and electronic effects of the leaving group on the reactivity of α-chymotrypsin have been extensively investigated.[13,14] Previous studies have also underscored that hydrolysis efficiency (k_{cat}/K_M) increases with the leaving group hydrophobicity. However, long-range influence of substituents on the enzyme specificity, as revealed by our results, is not well documented. Our results show that (i) the enantioselectivities are strongly dependent on the nature of the γ-substituent; (ii) the α-γ regioselectivity is directed by the C-α configuration.

In this paper, we reported on the synthesis of glutamate related compounds bearing one or two cyano groups on the γ-position. This two step chemoenzymic route was performed in good overall yields (typically 40-43% from the racemate) and satisfactory stereoselectivities (ee = 95-96%). It usefully complements the previous approaches to the synthesis of multifunctionalised glutamates.

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References and notes

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6- The Michael addition did not take place without HMPA (10 to 20%) as a cosolvent.

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8- General procedure for enzymic hydrolysis: the compound (5 mmol), dissolved in DMSO (50 mL) was added to 0.1M phosphate buffer (950 mL) and the pH was adjusted to 7.6. The mixture was treated with type II α-Chymotrypsin (250 mg, 54 u.mg^{-1}) at 37°C. The reaction was monitored by TLC. After acidification (pH 4) with citric acid, the product was extracted 4 times with dichloromethane. The organic layer was isolated by filtrating the emulsion through a sintered glass filter, washed with brine and reduced under vacuum.

All intermediary compounds described in this paper gave satisfactory spectral data and analysis;

2: IR: cm^{-1} 2260; ^{1}H NMR ($CDCl_3$): δ ppm 5.35 (b, 1H, NH), 4.47 (bm, 1H, N-CH), 4.11 (t, J = 7, 1H, NC-CH-CN), 3.84 (s, 3H, OCH_3), 2.75-2.65 (m, 1H, HCH), 2.49-2.37 (m, 1H, HCH), 1.47 (s, 9H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$): δ ppm 170.7 (CO_2), 156.8 (OCON), 112.7 (CN), 82.0 ($\underline{C}(CH_3)_3$), 53.9 (OCH_3) 51.8 (N-CH), 34.6 (NC-CH-CN), 28.8 ($C(\underline{C}H_3)_3$), 20.2 (CH_2); MS (DCI, NH_3): m/z = 285 (MNH_4^+, 100%), 268 (MH^+, 35%), 229 (MNH_4^+ - CH_2=$C(CH_3)_2$, 33%), 212 (MNH^+ - CH_2=$C(CH_3)_2$, 6%); Anal. calcd. for $C_{12}H_{17}N_3O_4$: C, 53.92; H, 6.41; N, 15.72; found: C, 53.65; H, 6.49; N, 15.76.

9- Characterisations of compounds **3**, **4**, **6**, **7** and **8** have been reported in reference 1.

5: $[\alpha]_D^{20}$ = +5 (c = 1, chloroform); IR: cm^{-1}2260, 2185; ^{1}H NMR ($CDCl_3$): δ ppm 5.40 (b, 1H, NH), 4.57 (bm, 1H, N-CH), 4.09 (t, J = 7, 1H, NC-CH-CN), 2.4-2.35 (2m, 2H, CH_2), 1.49 (s, 9H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$): δ ppm 173.2 (CO_2H), 156.2 (OCON), 112.7 (CN), 82.7 ($\underline{C}(CH_3)_3$), 52.1 (N-CH), 33.9 (NC-CH-CN), 28.8 ($C(\underline{C}H_3)_3$), 22.0 (CH_2); MS (DCI, NH_3): m/z = 271 (MNH_4^+, 100%), 215 (MNH_4^+ - CH_2=$C(CH_3)_2$, 15%); Anal. calcd. for $C_{11}H_{15}N_3O_4.H_2O$ C, 48.70; H, 6.32; N, 15.49; found: C, 48.94; H, 6.34; N, 14.99.

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11 The pH dependent exchange of the cyanomalonic proton of ester **7** in deuterated 1M phosphate buffer, monitored by ^{1}H NMR at 32°C ($t_{1/2}$ = 90 min at pH 7.4, 30 min at pH 5.4 and 10 min at pH 4.7), confirmed the expected lack of configurational stability of the γ-carbon and precluded a separation of the diastereomers.

12 In the same way, cyanoglutamic diester **3** was not saponified in the buffer and required more drastic conditions (1 equivalent LiOH in acetonitrile:water 3:1) for selective deprotection of the cyanoacetic moiety.

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