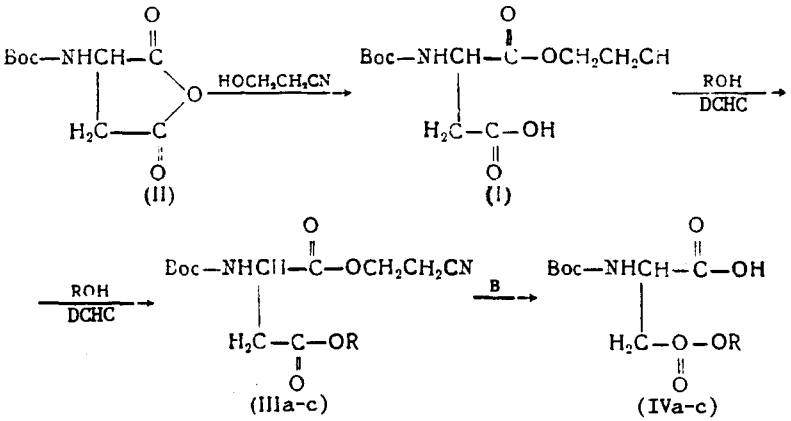
USE OF α-2-CYANOETHYL tert-BUTOXYCARBONYLASPARTATE AS AN INTERMEDIATE FOR SYNTHESIS OF β-ESTERS

V. V. Kalashnikov and V. V. Samukov

UDC 547.466:547.964.4

The use of α-2-cyanoethyl Boc-aspartate is proposed for the first time for the synthesis of β-esters of Boc-aspartic acid. The α-2-cyanoethyl protective group is selectively split out by strong organic bases under hydrolytic conditions without the β-ester bond being affected. Using the α-2-cyanoethyl derivative, β-cyclohexyl, β-benzyl, and β-tert-butyl Boc-aspartates have been synthesized in good yields.

The main method of protecting the β-carboxy group of aspartic acid in the synthesis of peptides is esterification. Supplementing the β-benzyl and β-tert-butyl esters traditionally used in the synthesis, in recent years new protective groups have been introduced into practice – the β-cyclohexyl [1] and β-cycloheptyl [2] groups, which possess increased resistance to the formation of aspartimide derivatives on the treatment of the protected peptides with acids and alkalis. These protective groups are promising alternatives to the β-benzyl group, since they permit a considerable lowering of the level of side reactions in the synthesis and deblocking of peptides [2]. One of the factors favoring the wide use of a particular protective group in practical synthesis is the existence of effective and reproducible methods of introducing it into an amino acid. The synthesis of β-tert-butyl and β-cycloalkyl aspartates is usually performed by the esterification of an N(α)-protected α-benzyl or α-methyl aspartate followed by the selective cleavage of the α-ester by hydrogenolysis or alkaline saponification. Since β-cycloalkyl esters are insufficienty resistant to alkaline saponification, they are synthesized only via the α-benzyl esters [1, 2].



IIIa, IVa: R=—C_6H_{11}-cyclo
IIIb, IVb: R=—$CH_2C_6H_5$
IIIc, IVc: R=—$C(CH_3)_3$
B = piperidine or 1,8-diazobicyclo [5, 4, 0] undec-7-ene

In the present paper we propose a universal method of synthesizing β-esters of aspartic acid (scheme) which includes the use of a new intermediate compound – α-2-cyanoethyl N(α)-tert-butoxycarbonyl(Boc)aspartate (I). The method is based on the capacity of 2-cyanoethyl (Cet) esters for being cleaved by a β-elimination mechanism under the action of strong organic bases in aprotic solvents [3].

All-Union Scientific-Research Institute of Molecular Biology. Vektor Scientific-Industrial Association. Ministry of the Medical and Biochemical Industries, Koltsovo, Novosibirsk Province. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 241-245, March-April, 1990. Original article submitted April 5, 1989; revision submitted August 23, 1989.

 0009-3130/90/2602-0196$12.50

TABLE 1. Boc-aspartic Acid Derivatives Synthesized

Formula of the compound	Yield, %	R_f		$[\alpha]_D$, deg (c 2; DMFA)	mp, °C
		System 1	System 2		
Boc-Asp(OH)-OCet·DCHA	57	0,43	0,47	−11,5[a]	144–146
Boc-Asp(OCet)-OH·DCHA[b]	—	0,33	0,43	—	—
I. Boc-Asp(OH)-OCet	96	0,43	0,47	−41,9	119–121
IIIa. Boc-Asp(OChx)-OCet	95	0,63	0,74	−25,3	57–59
IVa. Boc-Asp(OChx)-OH[c]	92	0,58	0,55	−16,3	89–91
IIIb. Boc-Asp(OBzl)-OCet[b]	—	0,74	0,78	—	—
IVb. Boc-Asp(OBzl)-OH[d,e]	91	0,52	0,54	−17,6	98–101
IIIc. Boc-Asp(OtBu)-OCet[b]	—	0,75	0,69	—	—
IVc. Boc-Asp(OtBu)-OH·DCHA[f]	62	0,56	0,44	+8,3	122–124

[a] c 2; water.
[b] Substance not isolated specially.
[c] According to the literature, mp 93-95°C [1].
[d] Commercial preparation (Fluka) Boc-Asp(OBzl)-OH: R_f 0.52 (system 1); 0.54 (system 2); $[\alpha]_D^{20}$ −19.2° (c 2; DMFA), mp 100-101°.
[e] Boc-Asp(OH)-OBzl. DCHA was obtained as in [4], R_f 0.59 (system 1); 0.58 (system 2).
[f] Commercial preparation (Reanal) Boc-Asp(OtBu)-OH·DCHA: R_f 0.59 (system 1); 0.44 (system 2); $[\alpha]_D^{20}$ +9.2° (c 2; DMFA), mp 124-126°.

α-Cet Boc-aspartate (I) was obtained by the reaction of Boc-aspartic anhydride (II) with 3-hydroxypropionitrile and was readily separated from the accompanying β-Cet ester by crystallization in the form of the dicyclohexylammonium salt.

By esterifying compound (I) with various alcohols under the action of dicyclohexylcarbodiimide (DCHC) in the presence of catalytic amounts of 4-dimethylaminopyridine the mixed diesters of Boc-aspartic acid (IIa-c)were obtained (Table 1). The β-benzyl ester (IIIb) can also be obtained in high yield by the alkylation of the ester (I) with benzyl bromide in the presence of a base.

When the diesters (IIIa-c) were treated with a twofold excess of 1,8-diazobicyclo[5.4.0]-undec-7-ene in dioxane (40-60 min) or with 30% piperidine in acetonitrile (10-14 h) at room temperature, the α-Cet group was split off quantitatively with the formation of the corresponding β-esters (IVa-c). Under such conditions, the ester groupings in the β-position are completely stable. The second product of the cleavage of a Cet ester – acrylonitrile – is bound by the excess of base. To simplify the process of splitting out the α-Cet group it is possible to operate without isolating the intermediate diesters (IIIa-c) in the pure form. The desired β-esters of Boc-aspartic acid were isolated with high yields, and with respect to their chromatographic phyiscochemical properties they were identical with commercial preparations produced by Reanal (Hungary) and Fluka (Switzerland).

EXPERIMENTAL

L-Aspartic acid, di-tert-butyl pyrocarbonate, DCHC, and 4-dimethylaminopyridine from Fluka were used, the other reagents and solvents being of domestic production.

Thin-layer chromatography was conducted on Kieselgel 60 F_{254} plates (Merck, FRG) in the systems: 1) chloroform–ethanol–acetic acid (95:5:3); and 2) benzene–acetone–acetic acid (100:50:2). The spots were revealed in UV light and after treatment with the ninhydrin reagent. Optical rotations were measured on a DIP-360 automatic polarimeter (JASCO) in cells 10 cm long. The results of the elementary analyses of all the compounds corresponded to the calculated figures. Information on the products obtained is given in Table 1.

α-2-Cyanoethyl N(α)-tert-Butoxycarbonyl-L-aspartate (I). A solution of 6.03 g (28 mmole) of the anhydride of Boc-aspartic acid (obtained as in [4]) in 50 ml of ethyl acetate was

treated with 2.11 ml (30.8 mmole) of freshly distilled 3-hydroxypropionitrile. The mixture was stirred at room temperature for 1 h, and then 6.12 ml (30.8 mmole) of dicyclohexylamine was added to the resulting homogeneous solution. The mixture was stirred for another 0.5 h and the viscous precipitate that had deposited was suspended in 200 ml of dry ether and left at 0°C for 4 h. The solid matter was filtered off and washed on the filter with ether. Yield 5.7 g.

The filtrate and the ether washings after supplementary working up yielded 3.9 g of a product containing approximately equal amounts of the dicyclohexylammonium salts of α- and β-2-cyanoethyl Boc-aspartates.

On the repeated performance of this reaction, the yield of the desired product (I), in the form of its dicyclohexylammonium salt, was 55-62%.

A suspension of 5.7 g of the dicyclohexylammonium salt of compound (I) in ethyl acetate was treated in a separatory funnel with a 10% solution of citric acid. The organic layer was washed with water, dried with anhydrous sodium sulfate, and evaporated in vacuum. The residue after evaporation crystallized spontaneously, and it was dried in vacuum over P_2O_5. The yield of compound (I) was 4.4 g. Where necessary, it was recrystallized from aqueous alcohol.

β-Cyclohexyl N(α)-tert-Butoxycarbonyl-L-aspartate (IVa). A. A solution of 5.7 g (20 mmole) of compound (I) and 2.3 g of cyclohexanol (22 mmole) in 50 ml of dry acetonitrile was cooled in an ice bath and, with stirring, 4.9 g (24 mmole) of DCHC and 0.24 g (2 mmole) of 4-diethylaminopyridine were added. The mixture was stirred at 0°C for 1 h and was left at +5°C for 12 h. The precipitate of dicyclohexylurea was separated off by filtration and was washed on the filter with ethyl acetate, and the filtrate was evaporated in vacuum. The residue was dissolved in ethyl acetate and the solution was washed in a separatory funnel with a 5% solution of sodium bicarbonate, water, 10% citric acid, and water again and was dried with anhydrous sodium sulfate and evaporated in vacuum. The residue was re-evaporated several times with hexane and was triturated under a layer of hexane with cooling. The resulting precipitate of compound (IIIa) was filtered off. Yield 7.0 g.

In the preparative production of compound (IVa), substance (IIIa), was used without isolation in the reaction for splitting out the cyanoethyl group.

B. A solution of 7.0 g (19.1 mmole) of compound (IIIa) in 40 ml of piperidine–acetonitrile (3:7) was left at room temperature for 12 h. The reaction mixture was evaporated to the state of an oil which was re-evaporated several times with ethyl acetate, and the residue was dissolved in ethyl acetate; this solution was washed with 10% citric acid solution and with water, and the organic layer was dried with anhydrous sodium sulfate. The residue after the evaporation of the ethyl acetate crystallized spontaneously. Yield 5.5 g.

β-Benzyl N(α)-tert-Butoxycarbonyl-L-aspartate (IVb). This was obtained in a similar manner to (IVa) starting from 5 mmole of compound (I). The overall yield of compound (IVb) calculated from the initial (I) was 91%.

β-tert-Butyl N(α)-tert-Butoxycarbonyl-L-aspartate (IVc). This was synthesized in a similar manner to (IVa), starting from 10 mmole of compound (I). To isolate compound (IVc) in the crystalline form its solution in ether was treated with 10 mmole of dicyclohexylamine. The yield of the dicyclohexylammonium salt of compound (IVc) was 62%, calculated on the initial compound (I).

LITERATURE CITED

1. J. P. Tam, T.-W. Wong, M. W. Reimen, F. S. Tjoeng, and R. B. Merrifield, Tetrahedron Lett., No. 42, 4033 (1979).
2. N. Fujii, M. Nomizu, S. Futaki, A. Otaka, S. Funakoshi, K. Akaji, K. Watanabe, and H. Yajima, Chem. Pharm. Bull, 34, No. 2, 864 (1986).
3. V. V. Kalashnikov and V. V. Samukov, Khim. Prir. Soedin., No. 3, 412 (1988).
4. E. Schröder and E. Kleiger, Ann. Chem., 673, 208 (1964).