p-AZOBENZENECARBOXAMIDOMETHYL ESTERS – NEW COLORED HYDROPHOBIC CARBOXYL PROTECTING GROUPS IN PEPTIDE SYNTHESIS

Valentin G. ZHURAVLEV, Anatolii A. MAZUROV*, Sergei A. ANDRONATI

A. V. Bogatsky Physico-Chemical Institute,
Academy of Sciences of the Ukrainian SSR, Chernomorskaya doroga 86, 270080 Odessa, Ukrainia

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p-Azobenzenecarboxamidomethyl esters (OAbc esters) of several α-amino acids have been synthesized and characterized. Their application in peptide chemistry as a colored alkali labile carboxyl protecting group was demonstrated. The esters are compatible with commonly used protecting groups. They are removed with aqueous potassium carbonate in 15 – 20 min.

Carboxyl protection in peptide liquid phase synthesis should, among other things, promote the solubility of reactant and to facilitate purification of product.

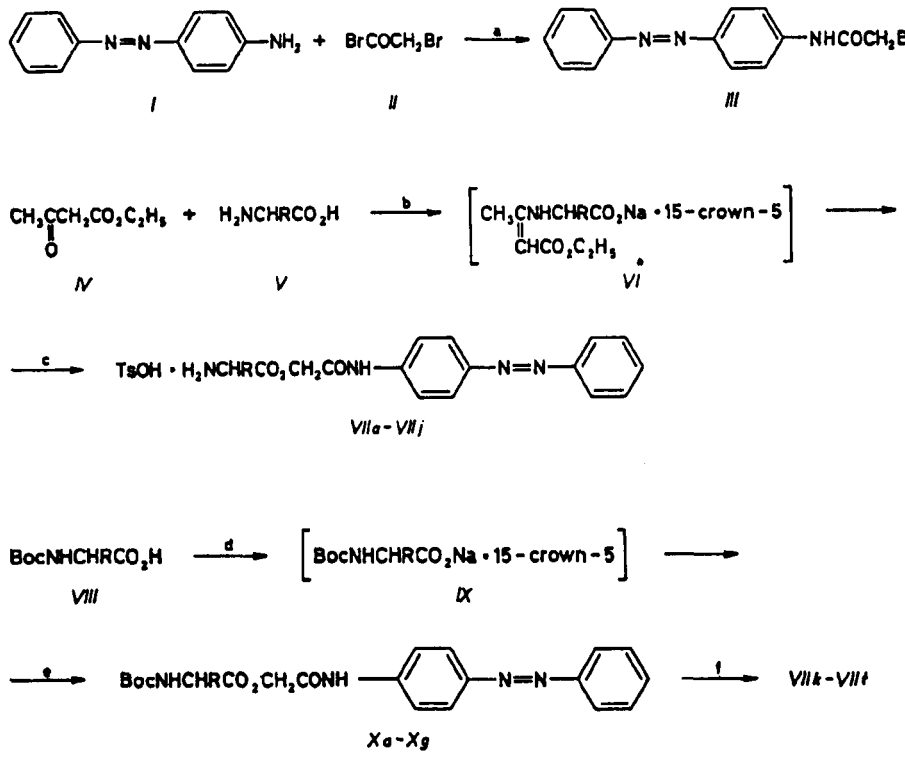
In this paper we describe the preparation and utilization of *p*-azobenzenecarboxamidomethyl esters which increase the solubility of the synthetic fragments in nonpolar solvents and allow to monitor colorometrically or visually their purification by chromatography or countercurrent distribution. Carboxamidomethyl esters[1 – 3] showed good stability during removal of usual N-protecting groups and were cleanly and selectively removed in mild alkaline media.

p-Azobenzenecarboxamidomethyl esters (OAbc** esters) *VII* were prepared by two methods. Esters *VIIa – VIIk* (Table I) were synthesized by modification of a known method[4] as one-pot procedure (Scheme 1). A boiling mixture of benzene and dimethylformamide dissolved amino acids *V* readily in the presence of sodium methylate, crown ether and ethyl acetoacetate. Complex of 15-crown-5 with the sodium salt of enamine was formed in several minutes and then the alkylating agent *III* was added to the mixture. *p*-(Bromoacetamido)azobenzene (*III*) was prepared by acylation of

* The author to whom correspondence should be sent.

** Abbreviations used are in accordance with the rules of IUPAC–IUB Comission on Biochemical Nomenclature (Biochem. J. *219*, 345 – 373 (1984)). Other abbreviations: Abc, *p*-azobenzenecarboxamidomethyl; Abu, γ-aminobutyric acid; DCCI, dicyclohexylcarbodiimide; DCHA, dicyclohexylamine; DCU, dicyclohexylurea; Dho, dihydrocorotic acid; DMF, dimethylformamide; DMSO, dimethylsulfoxide; EtOAc, ethyl acetate; MeOH, methanol; NMM, N-methylmorpholine; Pfp, pentafluorophenyl; TEA, triethylamine.

commercially available and cheap *p*-aminoazobenzene* (*I*) with bromoacetyl bromide (*II*) of Schotten–Baumann conditions.



a) $NaHCO_3$, H_2O. b) MeONa, 15-crown-5, MeOH. c) 1. *III*, 2. TosOH. d) MeONa, 15-crown-5, MeOH. e) *III*, MeOH, 50°C. f) HCl, dioxane.

SCHEME 1

Removal of the acetoacetate N-protecting group was easily achived using solution of *p*-toluenesulfonic acid in ethyl acetate. Application of equimolar amounts of 15-crown-5 or 18-crown-6 ethers in respect to amino acid sodium salts promoted their dissolution and allowed to prepare not only esters of most lipophilic amino acids: phenylalanine, leucine, valine and phenylglycine, as by ref.[4], but also another (Table I).

Hydrophilic amino acids failed to dissolve in the benzene–dimethylformamide mixture, and cystine was decomposed at these conditions. Therefore, esters *VIIl* – *VIIt*

* *p*-Aminoazobenzene is classified as cancer suspect agent.

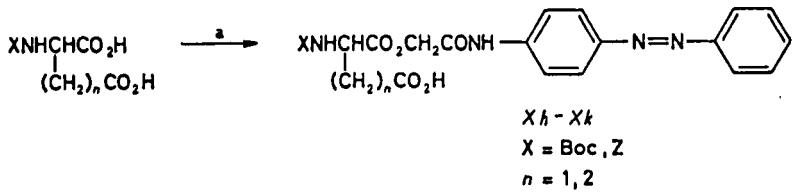
(Table I) were synthesized from N-tert-butoxycarbonyl amino acids *VIII* by a procedure demonstrated[5] a few years ago. 15-Crown-5 complexes of N-protected amino acid sodium salts (*IX*) were alkylated at the carboxyl group by bromoacetanilide *III* (Scheme 1), followed by a selective acidolytic cleavage of N-tert-butoxycarbonyl amino acid *p*-azobenzenecarboxamidomethyl esters (*X*).

N-Protected dicarboxylic amino acid α-esters *Xh – Xk* were obtained by this way via monosodium salts (Scheme 2). α-Carboxyl groups in aspartic and glutamic acid are more acidic than ω-carboxyl moieties[6]; therefore, these acids form with one equivalent of a base α-salts, mainly. Sodium salts are ionized to a greater extent than, for example, triethylammonium salts, and their application leads to the selective formation of α-(*p*-azobenzenecarboxamidomethyl) esters with good yields. The formation of the α-esters has been confirmed by synthesis of tert-butoxycarbonylglutamic acid α-(*p*-azobenze-

TABLE I
Physical data of amino acid *p*-azobenzenecarboxamidomethyl esters *VII*

Compound	Yield, %	M. p., °C	$[\alpha]_{545}^{20}$ (*c*, MeOH)	R_F (Solvent)
VIIa, TsOH.H-Gly-OAbc	93	208	–	0.43 (C)
VIIb, TsOH.H-Ala-OAbc	75	195	−5.7 (1)	0.49 (C)
VIIc, TsOH.H-Met-OAbc	76	198 – 201	+15.0 (1)	0.67 (C)
VIId, TsOH.H-Phe-OAbc	82	242	+8.3 (1)	0.31 (B)
				0.51 (D)
VIIe, TsOH.H-Leu-OAbc	87	258	+13.5 (1)	0.24 (B)
				0.48 (D)
VIIf, TsOH.H-Ile-OAbc	85	232	+11.7 (1)	0.26 (B)
				0.45 (D)
VIIg, TsOH.H-Tyr-OAbc	70	246	+7.8 (1)	0.26 (B)
				0.32 (D)
VIIh, TsOH.H-Trp-OAbc	65	128 – 131	+3.5 (1)	0.81 (C)
VIIi, TsOH.H-Val-OAbc	88	137	+6.0 (1)	0.27 (B)
VIIj, TsOH.H-Pro-OAbc	79	165	+15.5 (1)	0.69 (C)
VIIk, TsOH.H-Abu-OAbc	92	152	–	0.21 (D)
VIIl, HCl.H-Ala-OAbc	94	205	+20.1 (1)	0.19 (C)
VIIm, HCl.H-Ser-OAbc	95	209	+17.3 (1)	0.50 (C)
VIIn, HCl.H-Asn-OAbc	87	180 – 189	+49.4 (0.95)	0.43 (C)
VIIo, HCl.H-Gln-OAbc	90	185 – 187	+40.2 (1)	0.35 (C)
VIIp, HCl.H-Arg(NO_2)-OAbc	92	hygr.	–	0.44 (C)
VIIq, HCl.H-Thr-OAbc	94	foam	–	0.38 (C)
VIIr, HCl.H-His(Dnp)-OAbc	90	185	+17.7 (1)	0.35 (C)
VIIs, HCl.H-Lys(Z)-OAbc	85	foam	–	0.87 (C)
VIIt, HCl.H-Cys-OAbc HCl.H-Cys-OAbc	89	125	+30.0 (1)	0.70 (B)

necarboxamidomethyl) ester γ-benzyl ester (*XI*) from *Xi*. Methylene protons of γ-benzyl group of N-protected glutamic acid γ-benzyl esters appear as singlet in ^{1}H NMR spectra as distinct from α-benzyl ester, which gives doublet for the same protons in hexadeuteriodimethylsulfoxide[7]. Also an equivalence of γ-benzyl methylene protons remains for diester *XI*.



a) 1. MeONa, 15-crown-5, MeOH, 2. *III*

SCHEME 2

The amino acid *p*-azobenzenecarboxamidomethyl esters prepared are very pure and can be used for peptide synthesis without further purification. Their structure was confirmed by ^{1}H NMR spectroscopy (some examples are listed in Table II).

TABLE II
^{1}H NMR data (δ, ppm) of some amino acid *p*-azobenzenecarboxamidomethyl esters

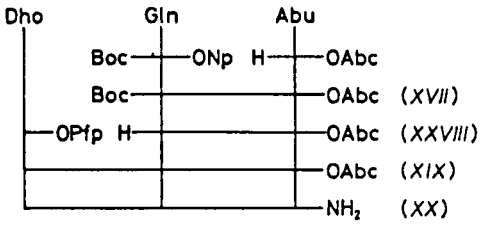
Compound	Amino acid					Abc		Ar	CH_3
	$C^\alpha H$	$C^\beta H$	$C^\gamma H$	$C^\delta H$	NH	CH_2	NH		
VIIc	4.33	2.19	2.76			4.97	10.71	8.00 – 7.15	2.33
VIIh[a]	4.48	3.45				4.93	10.62	7.99 – 7.05	2.33
VIIm[b]	4.10	3.78				4.97	10.86		
VIIr	4.09	3.42				4.65	11.02	9.03 – 8.83 8.18 – 7.60	
Xe	4.13	1.69	1.69	3.20	7.60	4.82	10.50	7.98 – 7.84 7.67 – 7.59	1.41
Xi	4.20	1.93	2.10		7.50	4.77	10.50	8.04 – 7.50	1.44
XI	4.24	1.95	2.16		7.60	4.83 5.10	10.50	7.97 – 7.40	1.43
XIIf[c]	4.26	3.76			9.51	4.70	10.85	7.35 – 7.07 6.56 – 6.51	1.43

Other protons: [a] 11.13(NH). [b] 4.35(OH). [c] 5.20(OH).

The enantiomeric purity of the amino acid esters was checked by synthesis of the peptides described below. According to ^{1}H NMR spectral data no diastereomeric dipeptides could be detected.

p-Azobenzenecarboxamidomethyl esters are stable under conditions for removal of usual available protecting groups (Boc, tert-butyl) and are cleaved by potassium carbonate (or ammonium hydroxide) in 15 – 20 min at room temperature, to give corresponding amino acid derivatives. The reaction by-product, identified as *p*-(hydroxyacetamido)azobenzene (*XII*), was removed by ethyl acetate extraction. Also the compound *XII* was prepared in one-pot procedure from *III* via *p*-(acetoxyacetamido)-azobenzene by cleavage with ammonium hydroxide. While carboxyl moiety is not deblocked by the reductive methods used for removal of the benzyloxycarbonyl group, *p*-azobenzenecarboxamidomethyl esters are converted into *p*-aminophenylenecarboxamidomethyl esters: ester *Xb* is reduced into compound *XIII* by hydrogenation on palladium.

In order to prove the usefulness if this carboxyl protecting group, we report synthesis of several di- and tripeptides. Carbodiimide mediated coupling of Boc-Ala-OH with H-Ala-OAbc yielded Boc-Ala-Ala-OAbc (*XIV*). Pyroglutamic acid pentafluorophenyl ester[8] reacted with asparagine *p*-azobenzenecarboxamidomethyl ester (*VIIn*) to give dipeptide Glp-Asn-OAbc (*XV*). Potassium carbonate mediated hydrolysis of *XV* led to Glp-Asn-OH (*XVI*). The tripeptide *XXII* was obtained by active ester method (Scheme 3). This synthesis reveals the possibility of generating peptide amides from *p*-azobenzenecarboxamidomethyl esters in DMF solution by treatment with aqueous ammonium hydroxide for short period of time. Rapid hydrazinolysis of *p*-azobenzenecarboxamidomethyl esters was shown by synthesis of Boc-Ala-$NHNH_2$ (*XXIII*) from ester *Xa*.



SCHEME 3

EXPERIMENTAL

Optical rotations were measured with a Perkin–Elmer 241 MC polarimeter. Proton magnetic resonance spectra (δ, ppm) were obtained with a Bruker 250 instrument in hexadeuteriosulfoxide with tetramethylsilane as internal standard. The melting points are uncorrected. Dried peptide samples were hydrolysed 24 h at 110 °C in 6M HCl and analyzed on an amino acid analyzer type AAA 881 (Mikrotechna, Prague). High performance liquid chromatography was performed on Du Pont Instruments Model 8800 and

Zorbax C8 analytical column (4.6 × 250 mm), methanol–water, 4 : 1, isocratic mode. For TLC precoated plates Silufol (Kavalier) and Kieselgel 60F-254 (Merck) were used with following solvent systems: (A) benzene–acetone–acetic acid, 100 : 50 : 1; (B) chloroform–ethyl acetate–methanol, 9 : 3 : 2; (C) butanol–acetic acid–water, 4 : 1 : 1; (D) chloroform–ethyl acetate–methanol–acetic acid, 9 : 3 : 2 : 1; (E) ethyl acetate–pyridine–acetic acid–water, 5 : 5 : 1 : 3.

p-(Bromoacetamido)azobenzene (*III*)

p-Aminoazobenzene (5.0 g, 25.3 mmol) was dissolved in chloroform (40 ml) and added water (40 ml). The reaction mixture was cooled to 10 °C and with vigorous stirring bromoacetyl bromide (2.3 ml, 27 mmol) was added dropwise at 10 °C. By slow addition of sodium carbonate pH of the solution was kept at 9. The mixture was stirred for additional 30 min. Precipitated amide *III* was filtered, washed with cold chloroform, dried and recrystallized from chloroform–methanol (4 : 1) to give 7.66 g (95%) of *III*, m.p. 166 – 168 °C, R_F 0.89 (A). ^{1}H NMR spectrum: 10.83 s, 1 H (NH); 7.99 – 7.85 and 7.66 – 7.57 m, 9 H (arom.); 4.15 s, 2 H (CH_2). For $C_{14}H_{12}N_3OBr$ (318.2) calculated: 52.8% C, 3.8% H, 13.2% N; found: 53.1% C, 3.5% H, 13.4% N.

Amino Acid *p*-Azobenzenecarboxamidomethyl Ester Salts (*VII*). General Procedure

Method A. A mixture of an amino acid (*V*; 10 mmol), DMF (10 ml), benzene (10 ml), 2M solution of sodium methoxide in methanol (5 ml), 15-crown-5 (2.2 g, 10 mol) and ethyl acetoacetate (*IV*; 1.5 ml, 11 mmol) was refluxed for 20 min using Dean–Stark adapter containing benzene. Then, *III* (3.18 g, 10 mmol) was added, the mixture was stirred at 50 °C for 3 h and diluted with ethyl acetate (50 ml). The solution was washed with brine, dried over magnesium sulfate and acidified with anhydrous *p*-toluenesulfonic acid (1.72 g, 10 mmol). Precipitated orange crystals of salts *VIIa – VIIk* were filtered and washed with ether. Yields and physico-chemical data are given in Table I.

Method B. To a N-tert-butoxycarbonyl amino acid (*VIII*; 10 mmol) in DMF (10 ml), 2M solution of sodium methoxide in methanol (5 ml), 15-crown-5 (2.2 g, 10 mmol) and *III* (3.18 g, 10 mmol) were added.

TABLE III
Physical data of N-protected amino acid *p*-azobenzenecarboxamidomethyl esters

Compound	Yield, %	M. p., °C	$[\alpha]_{546}^{20}$ (c, MeOH)	R_F[a]
Xa, Boc-Ala-OAbc	86	112	+18.5 (1)	0.80
Xb, Boc-Ser-OAbc	74	85	−20.5 (1)	0.74
Xc, Boc-Asn-OAbc	76	113 – 115	−17.2 (1)	0.44
Xd, Boc-Gln-OAbc	82	162 – 164	−7.5 (1)	0.72
Xe, Boc-Arg(NO_2)-OAbc	69	92	−5.3 (0.74)	0.35
Xf, Boc-Thr-OAbc	85	118	−31.8 (1)	0.70
Xg, Boc-Lys(Z)-OAbc	72	98 – 102	+2.4 (1)	0.71
Xh, Z-Glu-OAbc DCHA	68	180	+15.0 (1)	0.40
Xi, Boc-Glu-OAbc	65	130	+17.4 (1)	0.65
Xj, Boc-Asp-OAbc DCHA	62	187	−27.0 (1)	0.42
Xk, Boc-Asp-OAbc DCHA	50	125	+5.0 (1)	0.55

[a] In solvent system (A).

The solution was kept at 50 °C for 30 min and then diluted with ethyl acetate (50 ml). Organic phase was washed by 50 ml portions of saturated sodium hydrogen carbonate and brine and dried over magnesium sulfate. After removal of the solvent the residue was triturated with diethyl ether. The orange crystals were collected by filtration, washed with ether and dried in vacuum to give ester *X*. Yields and physical data of ester *X* are given in Table III and elemental analyses in Table IV. Derivatives *X* were N-deprotected by 4M HCl in dioxane (5 ml) for 30 min at room temperature. Then, the solution was evaporated to dryness in vacuum at 50 °C. The residue was washed with ether and dried in vacuum to give salts *VIIi – VIIt*. Yields and physico-chemical data are given in Table I.

TABLE IV
Elemental analyses of N-protected amino acid *p*-azobenzenecarboxamidomethyl esters *X*

Compound	Formula	M. w.	Calculated/Found		
			% C	% H	% N
Xa	$C_{22}H_{26}N_4O_5$	426.2	64.0	6.1	10.2
			63.9	6.3	10.3
Xb	$C_{22}H_{26}N_4O_6$	442.2	64.0	6.1	10.2
			64.3	6.5	10.6
Xc	$C_{23}H_{26}N_4O_6$	454.2	60.8	5.7	12.3
			61.2	5.9	12.3
Xd	$C_{24}H_{28}N_4O_6$	468.2	61.6	6.0	12.0
			61.3	6.3	11.9
Xe	$C_{25}H_{31}N_7O_7$	541.2	55.5	5.7	18.1
			55.2	6.0	17.7
Xf	$C_{23}H_{27}N_3O_6$	441.2	62.6	6.1	9.5
			62.6	6.0	9.9
Xg	$C_{33}H_{38}N_4O_7$	602.3	65.8	6.3	9.3
			66.2	6.7	9.4
Xh	$C_{39}H_{48}N_4O_7$	642.3	72.9	7.5	8.7
			73.1	7.5	9.0
Xi	$C_{24}H_{30}N_3O_7$	472.2	61.0	6.4	8.9
			61.1	6.5	8.5
Xj	$C_{38}H_{46}N_4O_7$	670.3	68.1	6.9	8.4
			68.0	7.2	8.5
Xk	$C_{35}H_{48}N_4O_7$	636.3	66.1	7.5	8.8
			66.4	7.8	8.8

N-Protected Dicarboxylic Amino Acid α-(*p*-Azobenzenecarboxamidomethyl) Esters *Xh – Xk*. General Procedure

To a solution of N-protected dicarboxylic amino acid (*IX*; 10 mmol) in DMF (20 ml), 15-crown-5 (2.2 g, 10 mmol), 2M solution of sodium methoxide in methanol (5 ml) and *III* (3.18 g, 10 mmol) was added. The reaction mixture was kept at 50 °C for 30 min and diluted with ethyl acetate (50 ml). The organic solution was washed with water (50 ml), 0.01M potassium carbonate (3 × 25 ml), dried over magnesium sulfate and evaporated under reduced pressure. Esters *Xh*, *Xj*, *Xk* were isolated as DCHA salts from ethyl acetate. Ester *Xi* was recrystallized from ethyl acetate–hexane.

p-(Hydroxyacetamido)azobenzene (*XII*)

The mixture of *III* (477 mg, 1.5 mmol) and fused sodium acetate (130 mg, 1.6 mmol) was stirred at 50 °C for 3 h. Then, 5 ml of 25% NH_4OH was added. After stirring at room temperature for 30 min, the solution was diluted with ethyl acetate (20 ml), washed with water, dried over magnesium sulfate and evaporated. The residue was recrystallized from ethyl acetate–hexane (3 : 1) to yield 286 mg (75%) of *XII*, m.p. 169 – 170 °C, R_F 0.55 (A). ^{1}H NMR spectrum: 10.12 s, 1 H (NH); 8.0 – 7.89 and 7.71 – 7.55 m, 9 H (arom.); 5.79 s, 1 H (OH); 4.10 d, 2 H (^2J = 6.0 Hz, CH_2). For $C_{14}H_{13}N_3O_2$ (255.3) calculated: 65.9% C, 5.1% H, 16.5% N; found: 66.3% C, 5.0% H, 16.1% N.

Boc-Ser-$OCH_2CONHC_6H_4NH_2$-*p* (*XIII*)

Compound *Xb* (0.35 g, 0.79 mmol) was hydrogenated for 24 h in 6 ml methanol in the presence of 10% Pd/C (50 mg) catalyst at room temperature and atmospheric pressure. The solvent was evaporated to dryness. Anilide *XIII* was isolated by flash-chromatography (ethyl acetate–hexane 3 : 1). Yield 189 mg (68%), R_F 0.25 (D), m.p. 75 °C.

Boc-Ala-Ala-OAbc (*XIV*)

Boc-Ala-OH (0.25 g, 1.32 mmol) was dissolved in THF. To this solution, cooled to −10 °C, N-hydroxysuccinimide (166 mg, 1.4 mmol), DCCI (298 mg, 1.4 mmol), TsOH.H-Ala-OAbc (*VIIb*; 656 mg, 1.3 mmol) and NMM (0.15 ml, 1.3 mmol) were added. After 6 h stirring at room temperature, precipitated DCU was filtered off, and filtrate was evaporated. The residue was dissolved in ethyl acetate (50 ml), washed with 0.1M HCl (2 × 50 ml), brine, 5% $NaHCO_3$, brine and dried over $MgSO_4$. The solution was evaporated to give 533 mg (81%) of derivative *XIV*, R_F 0.58 (B), m.p. 93 – 95 °C, $[\alpha]_{578}^{20}$ −35.5° (*c* 1, MeOH).

Glp-Asn-OAbc (*XV*)

The solution of HCl.H-Asn-OAbc (*VIIa*; 3.13 g, 7.7 mmol), NMM (0.86 ml, 7.7 mmol) and Glp-OPfp (2.5 g, 8.5 mmol) in DMF (20 ml) was kept overnight at room temperature and then diluted with ethyl acetate (30 ml) and water (30 ml). The precipitated solid was treated by hot methanol to yield 2.3 g (62%) of dipeptide *XV*, m.p. 260 °C, R_F 0.50 (C).

Glp-Asn-OH (*XVI*)

A solution of K_2CO_3 (1.3 g, 9.4 mmol) in water (5 ml) was added to a suspension of ester *XV* (2.3 g, 4.7 mmol) in DMF (20 ml). The reaction mixture was stirred for 30 min and after dilution with water (20 ml) extracted with ethyl acetate (3 × 20 ml). The aqueous layer was adjusted to pH 4 – 5 and evaporated to dryness. The residue was extracted by hot anhydrous ethanol (2 × 30 ml). The solution was filtered and evaporated to afford 1.0 g (86%) of dipeptide *XVI*, m.p. 262 – 264 °C, $[\alpha]_{578}^{24}$ −40.3° (*c* 1, H_2O), R_F 0.26 (C).

^{1}H NMR spectrum: 11.31 s, 1 H (COOH); 8.74 d, 1 H (NH, Asn); 7.94 s, 1 H (NH, Glp); 4.53 m, 1 H ($C^{\alpha}H$, Glp); 4.03 m, 1 H ($C^{\alpha}H$, Asn); 2.50 m 2 H ($C^{\beta}H_2$, Asn), 2.32 m, 2 H ($C^{\gamma}H_2$, Glp); 2.25 m, 2 H ($C^{\beta}H_2$, Glp).

Boc-Gln-Abu-OAbc (*XVII*)

Compound *VIIk* (5.647 g, 15 mmol) was dissolved in 15 ml of THF Et_3N (2.09 ml), HOBt (2.02 g, 15 mmol) and Boc-Gln-ONp (5.52 g, 15 mmol) were added to the solution. The mixture was stirred for 10 h at room temperature. Ethyl acetate (40 ml) was added and the organic layer was worked up as described for dipeptide *XIV*. Crude dipeptide *XVII* was isolated by flash-chromatography (hexane–acetone 1 : 2). Yield 2.0 g (37%), R_F 0.15 (A), 0.37 (B).

HCl.H-Gln-Abu-OAbc (*XVIII*)

Crude dipeptide XVII (2.0 g, 3.52 mmol) was treated by 4M HCl in dioxane (5 ml) for 40 min. Dioxane was evaporated under vacuum and the residue was triturated with diethyl ether to afford 1.56 g (97%) of hydrochloride *XVIII*, R_F 0.38 (C).

Dho-Gln-Abu-OAbc (*XIX*)

Dho-OPfp (0.9 g, 2.77 mmol) was added to a solution of hydrochloride *XVIII* (1.54 g, 3.07 mmol) and Et_3N (0.43 ml, 3.07 mmol) in 30 ml of THF. After stirring for 5 h, precipitate was filtered off and resuspended in 1M HCl. The product was separated and dried to yield 1.78 g (96%) of peptide *XIX*, R_F 0.45 (C).

Dho-Gln-Abu-NH_2 (*XX*)

Ester *XIX* (1.78 g, 2.93 mmol) was dissolved in 15 ml DMF and mixed with 12 ml of 25% NH_4OH. The mixture was stirred for 1 h at room temperature, then diluted with 20 ml of diethyl ether. Aqueous layer was separated and evaporated under vacuum. The residue was crystallized from aqueous ethanol. Yield 0.55 g (51%), R_F 0.62 (E), 0.18 (C), $[\alpha]_D^{20}$ +17° (*c* 1, H_2O). ^{1}H NMR spectrum: 8.22 d, 1 H (NH, Gln); 8.01 t, 1 H (NH, Abu); 7.67 and 7.59 s, 2 H (NH, Dho); 7.22 and 6.76 s, 2 H (NH_2); 4.19 m, 1 H ($C^{\alpha}H$, Gln); 4.12 m, 1 H ($C^{\alpha}H$, Dho); 3.11 m, 2 H ($C^{\alpha}H$, Abu); 2.78 m, 2 H ($C^{\beta}H_2$, Dho); 2.18 m, 2 H ($C^{\gamma}H_2$, Abu); 2.10 m, 2 H ($C^{\gamma}H_2$, Gln); 1.78 m, 2 H ($C^{\beta}H_2$, Gln); 1.64 m, 2 H ($C^{\beta}H_2$, Abu).

Boc-Ala-NHNH$_2$ (*XXI*)

To a solution of Boc-Ala-OAbc (*IXa*; 250 mg, 5.8 mmol) in DMF (5 ml) was added hydrazine hydrate (0.11 ml). After stirring at room temperature for 40 min, the mixture was evaporated to dryness. The residue was triturated with ether to afford 106 mg (90%) of hydrazide *XXI*, m.p. 110 – 112 °C, $[\alpha]_D^{20}$ –44.8° (*c* 1.0, 1M HCl), (ref.[9] $[\alpha]_D^{20}$ –45° (*c* 1, 1M HCl)).

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