Synthesis of N-[(β -carboline-3-yl)-formyl]-L-Amino Acid Derivatives

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Abstract: Four N-[(β -carboline-3-yl)-formyl]-L-Amino Acids have been synthesized by condensation of 3-carboxyl- β -carboline with corresponding protected amino-acids in the presence of dicyclohexylcarbodiimide(DCC), and removal of the protected groups.

Introduction

There are a large number of indole alkaloids which play a remarkable important role in plant antitumor drugs[1]. Many of them are able to bind to DNA by intercalation. It was found that some β -carboline analogs had the property of exact DNA intercalation in our earlier research[2]. Considering some amino acid residues in DNA binding protein are the key sites for groove binding in DNA double helix, we hope to develop a kind of excellent DNA binding agents by introducing the amino acids into the flat arometic chromophore.

Experimental

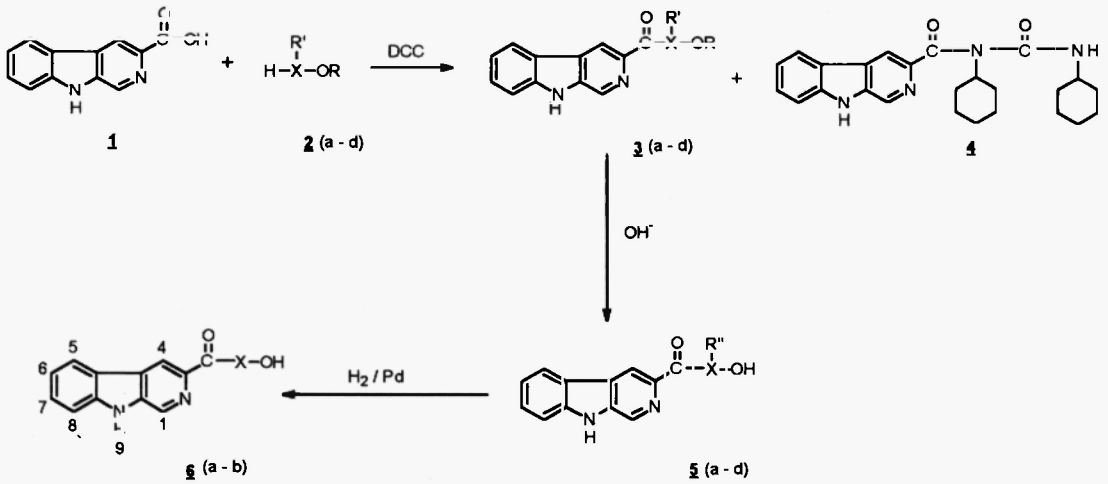
^{1}HNMR spectra were recorded on a Varian XL-300 spectrometer. IR spectra (KBr) were recorded on Perkin-Elmer 983. Mass spectra were obtained with VG-ZAB-MS spectrometer using FAB ionization. Elemental analyses were carried out on PE-2400. Molecular rotation was obtained with Polartronic-D. All melting points were uncorrected. Thin-layer chromatography (TLC) was carried out on Merk GF 254 silica gel. N,N-dimethylformamide (DMF) was distilled under reduced pressure and kept over molecular sieves prior to use. All other solvents were purchased from the manufacturer in analytical grade and used without further purification. Protected amino acids were purchased from Sigma Chemical Co..

3-Carboxyl- β -carboline (**1**) was prepared from tryptophan following the reported multistep synthesis[3]. O-benzyl-L-serine benzyl ester(**2a**), N -benzyloxycarbonyl-L-lysine benzyl ester(**2b**) and L-glutamic acid dibenzyl ester(**2c**) were prepared under mild conditions via cesium salts[4] from N-t-

butyloxycarbonyl-amino acid, and then N-t-butyloxycarbonyl- group was removed by treatment with hydrogen chloride in ethyl acetate. Glycine ethyl ester(**2d**) was synthesized by the introduction of hydrogen chloride into an alcoholic suspension of glycine[5].

General procedure for synthesis of compounds **3**(**a-d**):

3-Carboxyl- β -carboline (**1**) (212mg, 1.0mmol) was suspended in DMF(4ml) with stirring, and to this mixture, DCC (227mg, 1.1mmol) and corresponding protected amino acid (**2a-2d**) (1.0mmol) was added at 0℃. After stirring at 0℃ for 2 hours, the reaction mixture was warmed to room temperature and stirred overnight. The reaction mixture was filtrated and the filtrates were evaporated to dryness under reduced pressure. The residue was washed with ethyl ether and then purified by column chromatography ($CHCl_3$-MeOH-AcOH, 20:1.0:0.4) to give compound **3a-3d**, and a secondary compound which was identified as **4**.



1 **2** (a - d) **3** (a - d) **4** **5** (a - d) **6** (a - b)

	X	R	R'	R"
a	Ser	Bzl	Bzl	Bzl
b	Lys	Bzl	Z	Z
c	Glu	Bzl	Bzl	
d	Gly	Et		

Bzl: $-CH_2-C_6H_5$ Z: $-C(=O)-O-CH_2-C_6H_5$ Et: $-CH_2CH_3$

General procedure for synthesis of compounds **5**(**a-d**):

A solution of one of **3a-3d**(1.0mmol) in methanol (5ml) was cooled in a ice-water bath, and 2N NaOH

A solution of one of **3a**-**3d**(1.0mmol) in methanol (5ml) was cooled in a ice-water bath, and 2N NaOH (0.5ml) was added with stirring. This continued for one hour at 0℃ and an additional hour at room temperature. When TLC ($CHCl_3$-MeOH, 10:1) showed that the reaction was completed, the mixture was evaporated to dryness under reduced pressure. The residue was acidified with dilute hydrochloric acid to PH 6. After storage in the cold for two hours, the precipitate was collected on a filter, thoroughly washed with water and dried in air. After recrystallization from methanol-ether, the pure compounds **5**(a-**d**) were obtained.

General procedure for synthesis of compounds **6**(**a**, **b**):

A solution of **5a** or **5b** (1.0mmol) was dissolved in methanol (5-10ml). Distilled water (5ml) was added followed by 5% palladium-on-charcoal (40mg). The mixture was stirred with a magnetic stirrer in an atmosphere of hydrogen until no more gas was absorbed. The catalyst was removed by filtration and the filtrate was concentrated in vacuo to dryness. After redissolved in 0.5N hydrochloric acid (0.5ml), the solution was dried in air. Recrystallization from water-ethanol-ether to afford the objective products.

N-[(β -Carboline-3-yl)-formyl]-O-benzyl-L-serine benzyl ester (**3a**):

Yield: 41%. mp 113-115℃. $[\alpha]_D$ =+24 (c=0.5, $CHCl_3$). MS: m/e 480 $[M+H]^+$. IR (cm^{-1}): 3394, 3219 (NH), 1709, 1645 (C=O). ^{1}HNMR ($CDCl_3$) δ 9.08 (s, 1H, H-9); 8.97(d, J=8.0, 1H, NH-Ser); 8.80 (s, 1H, H-1); 8.75(s, 1H, H-4); 8.10 (d, J=7.8, 1H, H-8); 7.60 (t, J=6.9, 1H, H-7); 7.50 (d, J=7.8, 1H, H-5); 7.30 (t, J=6.9, 1H, H-6); 7.40-7.20 (m, 10H, H-Ar); 5.27 (dd, J=15.6, 10.5, 2H, CH_2-Bzl); 5.10 (m, 1H, α CH-Ser); 4.55 (m, 2H, CH_2-Bzl); 4.10 (d, J=8.4, 1H, β CH_2-Ser); 3.88(d, J=9.3, 1H, β CH_2-Ser).

1,3-Dicyclohexyl-1-[(β -carboline-3-yl)-formyl]-urea (**4**):

Yield: 40-70%. mp 188-190 ℃. MS: m/e 419 $[M+H]^+$. IR (cm^{-1}): 3335, 3229 (NH), 1672, 1618 (C=O). ^{1}HNMR ($CDCl_3$) δ 9.04 (s, 1H, H-9); 8.65 (s, 1H, H-1); 8.34(s, 1H, H-4); 7.98 (d, J=7.8, 1H, H-8); 7.57 (t, J=7.8, 1H, H-7); 7.49 (d, J=8.4, 1H, H-5); 7.31 (t, J=7.8, 1H, H-6); 5.82 (d, J=8.4, 1H, NHCO); 4.25 (m, 1H, HC-N); 3.53 (m, 1H, HC-N); 0.98-1.98 (m, 20H, $(CH_2)_{10}$).
Anal: Calcd. for $C_{25}H_{30}O_2N_4 \cdot 1/4CH_3COOH$: C, 70.65; H, 7.21; N, 12.92. Found: C, 70.92; H, 6.96; N, 12.64.

N-[(β -Carboline-3-yl)-formyl]-N ε -benzyloxycarbonyl-L-lysine benzyl ester (**3b**):

Yield: 23%. mp 56-58 ℃. $[\alpha]_D$ =+13 (c=0.5, $CHCl_3$). MS: m/e 587 $[M+Na]^+$. IR (cm^{-1}): 3321,

(NH), 1692 (C=O). ^{1}HNMR ($CDCl_3$) δ 9.08 (s, 1H, H-9); 8.80 (s, 1H, H-1); 8.78(s, 1H, H-4); 8.68(d, J=8.0, 1H, α NH-Lys); 8.11 (d, J=8.4, 1H, H-8); 7.59 (t, J=7.2, 1H, H-7); 7.55 (d, J=7.8, 1H, H-5); 7.34 (t, J=7.2, 1H, H-6); 7.35-7.25 (m, 10H, H-Ar); 5.23 (m, 2H, CH_2-B_Zl); 5.05 (s, 2H, CH_2-B_Zl); 4.92 (m, 1H, α CH-Lys); 3.15(m, 2H, ε CH_2-Lys); 1.95-1.30 (m, 6H, β , γ , δ CH_2-Lys).

N-[(β -Carboline-3-yl)-formyl]-L-glutamic acid dibenzyl ester (**3c**):

Yield: 51%. mp 81-83 ℃. $[\alpha]_D$ =+48 (c=0.5, $CHCl_3$). MS: m/e 298 $[M+H]^+$. IR (cm^{-1}): 3370, 3319(NH), 1729, 1656 (C=O). ^{1}HNMR ($CDCl_3$) δ 9.98 (s, 1H, H-9); 8.80 (s, 1H, H-1); 8.75(s, 1H, H-4); 8.73(d, J=7.0, 1H, NH-Glu); 8.13 (d, J=7.2, 1H, H-8); 7.60 (t, J=7.5, 1H, H-7); 7.50 (d, J=7.2, 1H, H-5); 7.32 (t, J=7.0, 1H, H-6); 7.37-7.24 (m, 10H, H-Ar); 5.24 (s, 2H, CH_3-B_Zl); 5.07 (s, 2H, CH_2-B_Zl); 4.98 (m, 1H, α CH-Glu); 2.63-2.23(m, 4H, β , γ CH_2-Glu).

N-[(β -Carboline-3-yl)-formyl]-glycine ethyl ester (**3d**):

Yield: 18%. mp 123-125 ℃. MS: m/e 298 $[M+H]^+$. IR (cm^{-1}): 3370, 3319 (NH), 1729,1656 (C=O). ^{1}HNMR (DMSO-d_6) δ 11.98 (s, 1H, H-9); 9.01(t, 1H, NH-Gly); 8.91 (s, 1H, H-1); 8.84(s, 1H, H-4); 8.39 (d, J=7.8, 1H, H-8); 7.64 (t, J=8.4, 1H, H-7); 7.50 (d, J=7.8, 1H, H-5); 7.30 (t, J=7.2, 1H, H-6); 4.14 (d, J=6.6, 2H, α CH_2- Gly); 4.10 (q, J=6.3, 2H, CH_2-Et); 1.21 (t, J=7.5, 3H, CH_3-Et).

N-[(β -Carboline-3-yl)-formyl]-O-benzyl-L-serine (**5a**):

Yield: 88%. mp 139-141 ℃. $[\alpha]_D$ =+20 (c=0.5, DMF). MS: m/e 390 $[M+H]^+$. IR (cm^{-1}): 3371, (OH), 1721, 1622 (C=O). ^{1}HNMR ($CDCl_3$) δ 12.02 (s, 1H, H-9); 8.94 (s, 1H, H-1); 8.86(s, 1H, H-4); 8.76(d, J=6.9, 1H, H-8); 7.64 (t, J=8.4, 1H, H-7); 7.58 (d, J=7.8, 1H, H-5); 7.30 (t, J=6.9, 1H, H-6); 7.28 (m, 5H, H-Ar); 4.76 (m, 1H, α CH-Ser); 4.55 (m, 2H, CH_2-B_Zl); 3.98 (dd, J=9.9, 3.9, 1H, β CH_2-Ser); 3.82 (dd, J=9.6, 3.6, 1H, β CH_2-Ser).

N-[(β -Carboline-3-yl)-formyl]-N ε -benzyloxycarbonyl-L-lysine (**5b**):

Yield: 62%. mp 89-91 ℃. $[\alpha]_D$ =+8 (c=0.5, $CHCl_3$). MS: m/e 475 $[M+H]^+$. IR (cm^{-1}): 3314, (OH), 1692, 1644 (C=O). ^{1}HNMR (DMSO-d_6) δ 11.99 (s, 1H, H-9); 8.92 (s, 1H, H-1); 8.84(s, 1H, H-4); 8.66(d, J=7.8, 1H, NH-Lys); 8.39 (d, J=7.8, 1H, H-8); 7.64 (t, J=7.2, 1H, H-7); 7.57 (d, J=8.4, 1H, H-5); 7.28 (t, J=7.8, 1H, H-6); 7.30-7.23 (m, 5H, H-Ar); 4.96 (s, 2H, CH_2-B_Zl); 4.51 (m, 1H, α CH-Lys); 2.97 (m, 2H, ε CH_2-Lys); 1.87(m, 2H, β CH_2-Lys); 1.48-1.30 (m, 4H, γ , δ CH_2-Lys).

CH_2-Lys).

N-[(β -Carboline-3-yl)-formyl]-L-glutamic acid (**5c**):

Yield: 89%. mp 196-198 ℃. $[\alpha]_D$=+37 (c=0.5, DMF). MS: m/e 342 $[M+H]^+$. IR (cm^{-1}): 3070, (NH), 1719, (C=O). ^{1}HNMR (DMSO-d_6) δ 11.99 (s, 1H, H-9); 8.92 (s, 1H, H-1); 8.84(s, 1H, H-4); 8.77(d, J=7.5, 1H, NH-Glu); 8.39 (d, J=8.1, 1H, H-8); 7.65 (t, J=8.4, 1H, H-5); 7.59 (t, J=7.2, 1H, H-7); 7.30 (t, J=6.9, 1H, H-6); 4.55 (m, 1H, α CH-Glu); 2.32(t, J=6.9, 2H, γ CH_2-Glu), 2.20-2.00(m, 2H, β CH_2-Glu). Anal: Calcd. for $C_{17}H_{15}O_5N_3 \cdot 0.8HCl$: C, 55.11; H, 4.30; N, 11.34. Found: C, 55.29; H, 4.27; N, 11.01.

N-[(β -Carboline-3-yl)-formyl]-glycine (**5d**):

Yield: 81%. mp 295 ℃(dec.). MS: m/e 270 $[M+H]^+$. IR (cm^{-1}): 3409 (OH), 3322 (NH), 1622 (C=O). ^{1}HNMR (DMSO-d_6) δ 12.02 (s, 1H, H-9); 8.91 (s, 1H, H-1); 8.80(s, 1H, H-4); 8.65(t, J=6.5, 1H, NH-Gly); 8.38 (d, J=7.8, 1H, H-8); 7.60 (d, J=8.0, 1H, H-5); 7.57 (t, J=7.5, 1H, H-7); 7.28 (t, J=7.5, 1H, H-6); 3.60 (d, J=5.4, 2H, α CH_2- Gly). Anal: Calcd. for $C_{14}H_{11}O_3N_3 \cdot 1.1HCl$: C, 54.35; H, 3.94; N, 13.58. Found: C, 54.63; H, 4.14; N, 13.41.

N-[(β -Carboline-3-yl)-formyl]-L-serine (**6a**):

Yield: 62%. mp 310 ℃(dec). $[\alpha]_D$ =+19 (c=0.5, DMF). MS: m/e 300 $[M+H]^+$. IR (cm^{-1}): 3368 (OH), 1653 (C=O). ^{1}HNMR (DMSO-d_6) δ 12.00 (s, 1H, H-9); 8.93 (s, 1H, H-1); 8.85(s, 1H, H-4); 8.78(d, J=7.5, 1H, NH-Ser); 8.40(d, J=7.8, 1H, H-8); 7.65 (d, J=7.8, 1H, H-5); 7.59 (t, J=6.6, 1H, H-7); 7.30 (t, J=6.9, 1H, H-6); 4.48 (t, J=4.2, 1H, α CH-Ser); 3.91 (dd, J=10.5, 4.2, 1H, β CH_2-Ser); 3.74 (dd, J=10.5, 4.2, 1H, β CH_2-Ser). Anal: Calcd. for $C_{15}H_{13}O_4N_3 \cdot 2HCl$: C, 48.40; H, 4.06; N, 11.29. Found: C, 48.58; H, 4.31; N, 11.01.

N-[(β -Carboline-3-yl)-formyl]-L-lysine (**6b**):

Yield: 79%. mp 230-232 ℃. $[\alpha]_D$ =+11 (c=0.5, H_2O). MS: m/e 341 $[M+H]^+$. IR (cm^{-1}): 3429, (OH), 1619 (C=O). ^{1}HNMR (D_2O) δ 8.10 (s, 1H, H-1); 7.83(s, 1H, H-4); 7.68(d, J=7.8, 1H, H-8); 7.29 (t, J=7.2, 1H, H-7); 7.18 (d, J=7.2, 1H, H-5); 6.99 (t, J=6.9, 1H, H-6); 4.20 (t, J=3.0, 1H, α CH-Lys); 2.85 (t, J=6.6, 2H, ε CH_2-Lys); 1.77-1.28 (m, 6H, β , γ , δ CH_2-Lys). Anal: Calcd. for $C_{25}H_{30}O_2N_4 \cdot 1/4CH_3COOH$: C, 70.65; H, 7.21; N, 12.92. Found: C, 70.92; H, 6.96; N, 12.64.

Discussion

Compounds **3**(a-d) were formed along with additional byproducts which were identified as the

products of reaction of the acid **1** with DCC and subsequent transposition to give acylureas **4** . Formation of acylureas is known to be suppressed by carrying out the reaction in less polar solvents, e.g., dichloromethane. In our case, we have to use DMF because of the insolubility of the compound **1** in other suitable solvents. We tried to prepare **3a** and **3d** by suspending the acid **1** in DMF-THF (1:1) on this reaction, but the yields of desired compounds could not be improved obviously.

Several methods were examined to obtain the objective products. Formation of the linkage by condensation of the protected amino acid **2(a-d)** with the acyl chloride of **1** was unsuccessful because the acid **1** did not react with thionyl chloride. Another classical method usually used in peptide synthesis, i.e. active ester, was unsuccessful either. For example, reaction of **1** with *p*-nitrophenol in the presence of DCC did not yielded *p*-nitrophenol ester of **1** , but turn to compound **4** completely.

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