

Synthesis of 6-Amino Acid Substituted 4,6,7,12-Tetrahydro-4-oxoindolo[2,3-*a*]quinolizines

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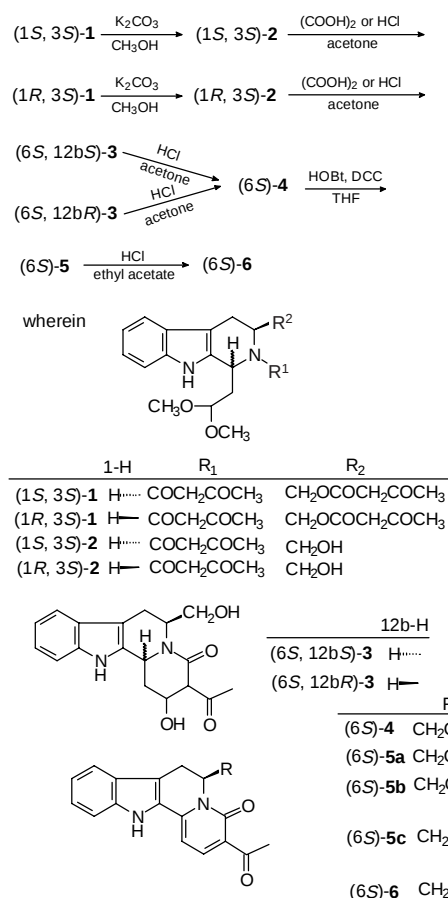
Abstract. In the presence of Na_2CO_3 (1*S*, 3*S*)- and (1*R*, 3*S*)-1-(2,2-dimethoxyethyl)-2-(1,3-dioxobutyl)-3-(1,3-dioxobutyl)oxymethyl-1,2,3,4-tetrahydrocarboline (**1**) were transformed into (1*S*, 3*S*)- and (1*R*, 3*S*)-1-(2,2-dimethoxyethyl)-2-(1,3-dioxobutyl)-3-hydroxymethyl-1,2,3,4-tetrahydrocarboline (**2**) which were cyclized to (6*S*)-3-acetyl-6-hydroxymethyl-4,6,7,12-tetrahydro-4-oxoindolo[2,3-*a*]quinolizine (**4**), via (6*S*, 12*bS*)- and (6*S*, 12*bR*)-3-acetyl-2-hydroxyl-6-hy-

droxymethyl-1,2,3,4,6,7,12,12*b*-octahydro-4-oxoindolo[2,3-*a*]quinoline (**3**). (6*S*)-**4** was coupled with Boc-Gly, Boc-*L*-Asp (β -benzyl ester), or Boc-*L*-Gln to give 6-amino acid substituted (6*S*)-3-acetyl-4,6,7,12-tetrahydro-4-oxoindolo[2,3-*a*]quinolizines **5a**, **5b**, or **5c**, respectively. After the removal of Boc from (6*S*)-**5a** (6*S*)-3-acetyl-6-glycyl-4,6,7,12-tetrahydro-4-oxoindolo[2,3-*a*]quinolizine (**6**) was obtained. The anticancer activities of (6*S*)-**5** and (6*S*)-**6** *in vitro* were tested.

In a previous paper we showed that the *in vitro* anti-HL₆₀ activity of 3-acetyl-4,6,7,12-tetrahydro-4-oxoindolo[2,3-*a*]quinolizines depended on the 6-substituent [1]. For the metabolism and action of amino acid analogs as anticancer agents there is a general rule that the most potent amino acid antimetabolites are those which interrupt nucleic acid biosynthesis. In the assembly of purine and pyrimidine rings only three amino acids are required, namely Gly, *L*-Asp and *L*-Gln [2]. Considering the effect of 6-substituents on the anticancer activity of 3-acetyl-4,6,7,12-tetrahydro-4-oxoindolo[2,3-*a*]quinolizine and the general rule of amino acid antimetabolites just mentioned, in the design of the antagonists of Gly, *L*-Asp and *L*-Gln we introduced them into the 6-position of 3-acetyl-4,6,7,12-tetrahydro-4-oxoindolo[2,3-*a*]quinolizine.

In the presence of sodium carbonate (1*S*, 3*S*)- and (1*R*, 3*S*)-1-(2,2-dimethoxyethyl)-2-(1,3-dioxobutyl)-3-(1,3-dioxobutyl)oxymethyl-1,2,3,4-tetrahydrocarboline (**1**) was saponified at the 3-position selectively to provide the corresponding (1*S*, 3*S*)- or (1*R*, 3*S*)-3-hydroxymethylcarboline, respectively. The solvent and the base had critical effect on the saponification. With a strong base, for instance NaOEt, the saponification exhibited no selectivity and both the ester and the amide were cleaved. In aprotic solvents such as acetone or THF with Na_2CO_3 as the catalytic base no product was obtained although the reaction mixture was stirred for 10 days at room temperature (see Table 1).

Using oxalic acid or hydrochloric acid (2 mol/l) as the catalyst (1*S*, 3*S*)-**2** or (1*R*, 3*S*)-**2** was cyclized to corresponding (6*S*, 12*bS*)- or (6*S*, 12*bR*)-3-acetyl-2-hydroxyl-6-hydroxymethyl-1,2,3,4,6,7,12,12*b*-octahydro-4-oxoindolo[2,3-*a*]quinolizine (**3**) which was easy to be converted into (6*S*)-3-acetyl-6-hydroxymethyl-4,6,7,12-tetrahydro-4-oxoindolo[2,3-*a*]quinolizine (**4**) via dehydrogenation and dehydration. In the presence of HOBt and DCC (6*S*)-**4** was coupled with Boc-Gly or Boc-*L*-Asp- β -OBzl or Boc-*L*-Gln giving the protected ami-



Scheme 1 Synthesis of 6-amino acid ester of (6*S*)-3-acetyl-4,6,7,12-tetrahydro-4-oxoindolo[2,3-*a*]quinolizine, **5** and **6** from (1*S*, 3*S*)-**1** and (1*R*, 3*S*)-**1** by saponification, cyclization, dehydrogenation, dehydration, esterification and deprotection.

Tab. 1 Effect of base and solvent on the saponification of **1**

	Base ^{a)}	Solvent ^{a)}	Time/h	saponified group
(1S, 3S)- 1	Na ₂ CO ₃	MeOH	16	3-ester group
	Na ₂ CO ₃	acetone	240	no reaction
	NaOEt	MeOH	4	2-amide & 3-ester groups
	NaOEt	acetone	120	2-amide & 3-ester groups
(1R, 3S)- 1	Na ₂ CO ₃	MeOH	8	3-ester group
	Na ₂ CO ₃	acetone	240	no reaction
	NaOEt	MeOH	2	2-amide & 3-ester groups
	NaOEt	acetone	120	2-amide & 3-ester groups

^{a)} With K₂CO₃ instead of Na₂CO₃, ethanol instead of methanol, or THF instead of acetone the same results were obtained

no acid esters of (6S)-3-acetyl-6-hydroxymethyl-4,6,7,12-tetrahydro-4-oxoindolo[2,3-*a*]quinolizine **5a–c**. Treating (6S)-**5a** with hydrochloride in ethyl acetate its Boc group was removed and (6S)-3-acetyl-6-glycyloxymethyl-4,6,7,12-tetrahydro-4-oxoindolo[2,3-*a*]quinolizine (**6**) was obtained in 92% yield (Scheme 1).

The anticancer activities of (6S)-**5a–c** and (6S)-**6** *in vitro* were determined with the modified method of Denizot and Lang [3]. The data are listed in Table 2.

The results indicate that the esterification with Boc-Gly or Boc-*L*-Asp-β-OBzl or Boc-*L*-Gln or Gly at the 6-position of (6S)-**4** may have an important influence upon their anticancer activities *in vitro*. The potencies obviously depend on both the amino acids and the cell strain. At 10^{−5} mol/l (6S)-**5b** with 6-Boc-*L*-Asp-β-OBzl the inhibiting action to HCT-8 and Bel-7402 may reach 93.78% and 91.83%, respectively. On the

other hand, however, the anticancer activities of (6S)-**6** were not improved significantly as compared with that of (6S)-**5a**. As one can see in table 2 in most cases the data of (6S)-**6** are approximately same as that of (6S)-**5a**. This observation suggested that the removal of Boc from (6S)-**5** perhaps had no obvious influence on the anticancer activities *in vitro* thus the introduction of hydrophilic group into (6S)-**4** may be not necessary for the modification.

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Experimental

All reactions were carried out under nitrogen (1bar). ¹H NMR spectra were recorded at 300 MHz with a VXR-300 instrument in deuteriochloroform with tetramethylsilane as internal standard. IR spectra were recorded with a Perkin-Elmer 983 instrument and mass spectra with a ZAB-MS (70 eV) spectrometer. Optical rotations were determined on Schmidt+Haensch Polartronic D at 20 °C. Chromatography was performed with Qingdao silica gel H.

(1S, 3S)- and (1R, 3S)-1-(2,2-Dimethoxyethyl)-2-(1,3-dioxobutyl)-3-hydroxymethyl-1,2,3,4-tetrahydrocarboline (2)

a) The suspension of 0.49 g (0.88 mmol) of (1S, 3S)-**1**[1], 25 ml of methanol and 200 mg of anhydrous K₂CO₃ was stirred at room temperature for 18 h, then TLC (CHCl₃ : CH₃OH, 30 : 1) indicated complete disappearance of (1S, 3S)-**1**. After filtration and evaporation the residue was purified by chromatography (CHCl₃ : CH₃OH, 50 : 1) to give 290 mg (92.1%) of (1S, 3S)-**2**, as white needles; *m.p.* 164–165 °C. [α]_D = 12.8° (c = 2, CHCl₃). – IR (KBr): ν /cm^{−1} = 3440 (NH), 3390 (OH), 2931 and 2818 (CH, CH₂ and CH₃), 1712 (C=O), 1614 and 1446 (aromatic C=C), 1356 and 1320 (C–O–C), 750 (1,2-disubstituted phenyl). – ¹H NMR: δ /ppm = 2.16–2.30 (m, 2H, (CH₃O)₂CHCH₂), 2.22–2.31 (m, 3H, COCH₃), 2.72–2.83 (m, 1H, CH₂CH CH₂OH), 2.76 (s, 1H, OH), 3.05–3.27 (m, 1H, CH₂CH CH₂OH), 3.37–3.38 (m, 2H, COCH₂COCH₃), 3.38–3.40 (m, 2H, CH₂OH), 3.39 (s, 3H, OCH₃), 3.46 (s, 3H, OCH₃), 4.06 (m, 1H, CH₂CHCH₂OH), 4.74 (m, 1H, NCHCH₃CH₂CH (OCH₃)₂), 5.07–5.72 [(m, 1H, CH(OCH₃)₂), 7.09 (t, *J* = 7.0 Hz, 1H, aromatic H), 7.16 (d, *J* = 7.3 Hz, 1H, aromatic H), 7.37 (d, *J* = 7.9 Hz, 1H, aromatic H), 7.45 (d, *J* = 7.0 Hz, 1H, aromatic H), 8.57 (s, 1H, pyrrole NH)]. – MS (ESI): *m/z* = 397 [M + Na]⁺.

Tab. 2 The anticancer activities of 6-amino acid esters of 3-acetyl-4,6,7,12-tetrahydro-4-oxoindolo[2,3-*a*]quinolizine *in vitro*

Compound	Cell strain	Inhibition ratio (%) at		
		10 ^{−7} mol/l	10 ^{−6} mol/l	10 ^{−5} mol/l
(6S)- 5a	BIU	26.06	28.30	31.75
	ET	−21.58	−4.29	27.25
	HCT-8	8.45	11.83	14.19
	Bel-7402	−1.48	−0.36	−5.58
	HL-60	−38.80	−22.20	4.46
	K-562	−18.66	−10.71	−0.06
(6S)- 5b	BIU	10.35	16.16	34.43
	ET	7.88	6.92	23.10
	HCT-8	5.31	13.37	93.78
	Bel-7402	1.86	4.11	91.83
	HL-60	−28.60	−7.00	25.50
	K-562	−44.44	−8.09	65.58
(6S)- 5c	BIU	9.32	3.88	3.71
	ET	−12.31	2.07	9.41
	HCT-8	7.31	6.17	13.17
	Bel-7402	2.29	−8.13	−0.66
	HL-60	−45.80	−31.8	−14.00
	K-562	−34.55	−7.12	−2.83
(6S)- 6	BIU	0.94	13.73	20.31
	ET	−29.05	−19.36	−30.15
	HCT-8	5.00	3.63	−0.43
	Bel-7402	1.09	−10.81	4.14
	HL-60	−28.60	−19.10	−10.20
	K-562	−8.15	−20.94	−40.43

$C_{20}H_{26}N_2O_5$ Calcd.: C 64.16 H 7.00 N 7.48
(374.40) Found: C 63.97 H 6.98 N 7.29.

b) The suspension of 0.49 g (0.88 mmol) of (1R, 3S)-1, 25 ml of methanol and 200 mg of anhydrous K_2CO_3 was stirred at room temperature for 16 h. Using procedure a) the reaction mixture was worked up to give 300 mg (95%) of (1R, 3S)-2, as colorless needles; *m.p.* 145–146 °C. $[\alpha]_D^{20} = -2.7^\circ$ (c = 2, $CHCl_3$). – IR (KBr): $\nu/cm^{-1} = 3450$ (NH), 3392 (OH), 2931 and 2820 (CH, CH_2 and CH_3), 1713 (C=O), 1616 and 1439 (aromatic C=C), 1358 and 1321 (C–O–C), 748 (1, 2-disubstituted phenyl). – 1H NMR: $\delta/ppm = 2.13$ – 2.25 [(m, 2H, $CH_2CH(OCH_3)_2$), 2.26–2.30 (m, 3H, $COCH_3$), 2.74 (s, 1H, OH), 3.40 (s, 3H, OCH_3), 3.53 (s, 3H, OCH_3), 3.54–3.65 (m, 2H, CH_2CHCH_2OH), 3.72–3.84 (m, 2H, $COCH_2COCH_3$), 3.87 (m, 2H, CH_2OH), 4.32 (m, 1H, $CHCH_2OH$), 4.94 (m, 1H, $CHCH_2CH(OCH_3)_2$), 5.97 (m, 1H, $CH(OCH_3)_2$), 7.07 (t, $J = 7.1$ Hz, 1H, aromatic H), 7.14 (d, $J = 7.5$ Hz, 1H, aromatic H), 7.32 (d, $J = 8.0$ Hz, 1H, aromatic H), 7.42 (d, $J = 7.0$ Hz, 1H, aromatic H), 8.75 (s, 1H, pyrrole NH). – MS (ESI): $m/z = 397$ [M + Na] $^+$.

$C_{20}H_{26}N_2O_5$ Calcd.: C 64.16 H 7.00 N 7.48
(374.40) Found: C 64.22 H 6.89 N 7.39.

(6S, 12bS)- and (6S, 12bR)-3-Acetyl-2-hydroxy-6-hydroxymethyl-1,2,3,4,6,7,12,12b-octahydro-4-oxoindolo [2,3-a]quinolizine (3)

a) To the solution of 200 mg (0.54 mmol) of (1S, 3S)-2 and 10 ml of acetone 50 mg of oxalic acid was added. The suspension obtained was stirred at room temperature for 120 h; then TLC analysis (ethyl acetate) indicated complete disappearance of (1S, 3S)-2. The reaction mixture was adjusted to pH 8 with aqueous solution of Na_2CO_3 (10%) and extracted with chloroform (5 ml $\times$ 3). The organic phases were combined and evaporated. The residue was purified by chromatography (ethyl acetate) to give 140 mg (86%) of (6S, 12bS)-3, as colorless needles; *m.p.* 188–189 °C. $[\alpha]_D^{20} = 48.9^\circ$ (c = 1.2, MeOH). – IR (KBr): $\nu/cm^{-1} = 3400$ (NH), 3298 (OH), 2894 and 2840 (CH, CH_2 and CH_3), 1714 (ketone C=O), 1630 (amide C=O), 1464 and 1439 (aromatic C=C), 750 (1,2-disubstituted phenyl). – 1H NMR: $\delta/ppm = 2.05$ (q, $J = 12.5$ Hz, 1H, $CH_2CH(N)CH_2OH$), 2.15 (q, $J = 26.4$ Hz, 1H, $CH_2CH(N)CH_2OH$), 2.30 (s, 3H, CH_3COCH_2), 2.80 (m, 1H, CH_2CHOH), 3.01 (s, 1H, CH_2CHOH), 3.17 (s, 1H, CH_2OH), 3.22 (d, $J = 14.1$ Hz, 1H, CH_2OH), 3.27 (d, $J = 2.6$ Hz, 1H, CH_2OH), 3.44 (d, $J = 7.0$ Hz, 1H, CH_2CHOH), 3.48 (d, $J = 8.2$ Hz, 1H, CH_2CHOH), 3.82 (d, $J = 3.0$ Hz, 1H, $CH_3COCHCO$), 4.78 (d, $J = 11.9$ Hz, 1H, CH_2CHCH_2OH), 5.22 (d, $J = 6.6$ Hz, 1H, $CHCH_2CHOH$), 7.00 (m, 1H, aromatic H), 7.08 (m, 1H, aromatic H), 7.33 (d, $J = 7.9$ Hz, 1H, aromatic H), 7.45 (d, $J = 8.1$ Hz, 1H, aromatic H), 10.30 (s, pyrrole NH). – MS (ESI): $m/z = 351$ [M + Na] $^+$.

$C_{18}H_{20}N_2O_4$ Calcd.: C 65.84 H 6.14 N 8.53
(328.15) Found: C 65.89 H 6.07 N 8.41.

b) To the solution of 200 mg (0.54 mmol) of (1S, 3S)-2 and 10 ml of acetone 0.01 ml of hydrochloric acid (2 mol/l) were added. The reaction mixture was stirred at room temperature for 48 h and worked up according to procedure a) to give 140 mg (86%) of (1S, 3S)-3, as colorless needles.

c) Using procedure a) 200 mg (0.54 mmol) of (1R, 3S)-2 gave 163 mg (92%) of (6S, 12bR)-3, as colorless needles; *m.p.* 175–176 °C. $[\alpha]_D^{20} = 21.5^\circ$ (c = 2, $CHCl_3$). – IR (KBr): $\nu/cm^{-1} = 3420$ (NH), 3310 (OH), 2901 and 2846 (CH, CH_2 and CH_3), 1720 (ketone C=O), 1641 (amide C=O), 1470 and 1450 (aromatic C=C), 740 (1,2-disubstituted phenyl). – 1H NMR: $\delta/ppm = 2.11$ (q, $J = 12.0$ Hz, 1H, $CH_2CH(N)CH_2OH$), 2.20 (q, $J = 24.1$ Hz, 1H, $CH_2CH(N)CH_2OH$), 2.31 (s, 3H, CH_3COCH_2), 2.81 (m, 1H, CH_2CHOH), 3.03 (s, 1H, CH_2CHOH), 3.20 (s, 1H, CH_2OH), 3.25 (d, $J = 13.8$ Hz, 1H, CH_2OH), 3.30 (d, $J = 3.0$ Hz, 1H, CH_2OH), 3.41 (d, $J = 7.2$ Hz, 1H, CH_2CHOH), 3.46 (d, $J = 8.0$ Hz, 1H, CH_2CHOH), 3.80 (d, $J = 3.4$ Hz, 1H, $CH_3COCHCO$), 4.80 (d, $J = 11.5$ Hz, 1H, CH_2CHCH_2OH), 5.50 (d, $J = 7.0$ Hz, 1H, $CHCH_2CHOH$), 7.01 (m, 1H, aromatic H), 7.10 (m, 1H, aromatic H), 7.35 (d, $J = 8.0$ Hz, 1H, aromatic H), 7.44 (d, $J = 8.0$ Hz, 1H, aromatic H), 10.50 (s, 1H, pyrrole NH). – MS (ESI): $m/z = 351$ [M + Na] $^+$.

$C_{18}H_{20}N_2O_4$ Calcd.: C 65.84 H 6.14 N 8.53
(328.14) Found: C 65.80 H 6.10 N 8.31.

(6S)-3-Acetyl-6-hydroxymethyl-4,6,7,12-tetrahydro-4-oxoindolo[2,3-a]quinolizine (4)

a) The solution of 460 mg (1.4 mmol) of (6S, 12bS)-3 in 30 ml of acetone and 0.1 ml of hydrochloric acid (2 mol/l) was stirred at room temperature for 120 h, then TLC analysis ($CHCl_3$: MeOH, 20:1) indicated complete disappearance of (6S, 12bS)-3. The reaction mixture was adjusted with Na_2CO_3 to pH 8. After filtration and evaporation the residue obtained was purified by chromatography ($CHCl_3$: MeOH, 30:1) to give 410 mg (95%) of (6S)-4, as yellow needles.

b) Using procedure a (reaction time 120 h) from (6S, 12bR)-3 420 mg (97%) of (6S)-4 were obtained, as yellow needles; *m.p.* 201–202 °C. – IR (KBr): $\nu/cm^{-1} = 3440$ (NH), 3300 (OH), 2944 and 2840 (CH, CH_2 and CH_3), 1711 (ketone C=O), 1649 (amide C=O), 1604, 1582, 1567 and 1492 (aromatic C=C), 750 (1, 2-disubstituted phenyl). – 1H NMR: $\delta/ppm = 2.60$ (s, 3H, $COCH_3$), 3.14 (d, $J = 6.8$ Hz, 2H, CH_2CHCH_2OH), 3.45 (t, $J = 9.4$ Hz, 1H, CH_2OH), 3.47 (t, $J = 10.2$ Hz, 1H, CH_2OH), 3.54 (s, 1H, CH_2OH), 3.56 (m, 1H, CH_2CHCH_2OH), 6.79 (d, $J = 7.7$ Hz, 1H, C=CH=CH=C–CO– CH_3), 7.11 (m, 1H, aromatic H), 7.27 (m, 1H, aromatic H), 7.45 (d, $J = 8.4$ Hz, 1H, aromatic H), 7.66 (d, $J = 8.0$ Hz, 1H, aromatic H), 8.08 (d, $J = 7.8$ Hz, 1H, C=CH=CH=C–CO– CH_3), 8.56 (s, 1H, pyrrole NH). – MS (ESI): $m/z = 331$ [M + Na] $^+$.

$C_{18}H_{16}N_2O_3$ Calcd.: C 70.12 H 5.23 N 9.09
(308.12) Found: C 70.01 H 5.35 N 9.01.

(6S)-3-Acetyl-6-(N-tert-butoxycarbonylglycyl)oxymethyl-4,6,7,12-tetrahydro-4-oxoindolo[2,3-a]quinolizine (5a)

The solution of 18 mg (0.12 mmol) of Boc-Gly, 20 mg (0.1 mmol) of HOBt and 1 ml of anhydrous THF was stirred at room temperature for 0.5 h, to which a solution of 30 mg (0.1 mmol) of (6S)-4 in 1 ml of anhydrous THF was added. The reaction mixture was stirred at 0 °C and 24 mg (0.11 mmol) of DCC were added. The reaction mixture was stirred at 0 °C for 4 h and at room temperature for 120 h, then TLC analysis ($CHCl_3$: MeOH, 20:1) indicated complete dis-

appearance of (6S)-4. After evaporation the residue was diluted with 50 ml of ethyl acetate and filtered to remove the precipitate. The filtrate was extracted with water (2 m×5) and the organic phases were combined. After evaporation the residue was purified by chromatography (CHCl₃: MeOH, 100:1) to give 39 mg (86%) of (6S)-5a, as colorless crystals; *m.p.* 177–178 °C. [α]_D = 52.4° (c = 2, CHCl₃). – IR (KBr): ν /cm⁻¹ = 3307 (NH), 2970 and 2924 (CH, CH₂ and CH₃), 1750 (ester C=O), 1678 (ketone C=O), 1661 (amide C=O), 1586, 1568, 1494 and 1424 (aromatic C=C), 750 (1, 2-disubstituted phenyl). – ¹H NMR: δ /ppm = 1.57 (s, 9H, -C(CH₃)₃), 2.71 (s, 3H, COCH₃), 3.31 (m, 2H, CH₂NH COOC(CH₃)₃), 3.57 (m, 1H, CH₂CHNCO), 3.66 (m, 1H, CH₂CHNCO), 4.22 (t, *J* = 11.2 Hz, 1H, CH₂O₂CCH₂NH), 4.40 (m, 1H, CH₂O₂CCH₂NH), 4.81 (s, 1H, CH₂NH COOC(CH₃)₃), 5.83 (d, *J* = 6.2 Hz, 1H, CH₂CHNCO), 6.44 (d, *J* = 7.6 Hz, 1H, C=CH–CH=C–CO–CH₃), 7.19 (t, *J* = 14.9 Hz, 1H, aromatic H), 7.34 (m, 1H, aromatic H), 7.35 (t, *J* = 15.6 Hz, 1H, aromatic H), 7.59 (d, *J* = 8.4 Hz, 1H, aromatic H), 8.19 (d, *J* = 7.0 Hz, 1H, C=CH–CH=C–CO–CH₃), 8.64 (s, 1H, pyrrole NH). – MS (ESI): *m/z* = 488 [M + Na]⁺.

C₂₅H₂₇N₃O₆ Calcd.: C 64.51 H 5.85 N 9.03
(465.20) Found: C 64.41 H 5.75 N 8.99.

(6S)-3-Acetyl-6-(*N*-tert-butoxycarbonyl-*L*-monobenzyl-aspartyl)oxymethyl-4,6,7,12-tetrahydro-4-oxoindolo[2,3-*a*]quinolizine (5b)

Using the procedure for preparing (6S)-5a, from 41 mg (0.12 mmol) of Boc-*L*-Asp- β -OBzl 46 mg (75%) of (6S)-5b was obtained, as yellow syrup. [α]_D = 137.6° (c = 2, CHCl₃). – IR (KBr): ν /cm⁻¹ = 3350 (NH), 2978 and 2935 (CH, CH₂, and CH₃), 1730 (ester C=O), 1687 (amide and ketone C=O), 1583, 1544, 1495 and 1442 (aromatic C=C), 751 (1, 2-disubstituted phenyl). – ¹H NMR: δ /ppm = 1.35 (s, 9H, -C(CH₃)₃), 2.17 (d, *J* = 2.8 Hz, 2H, C₆H₅CH₂O₂CCH₂CH), 2.71 (s, 3H, COCH₃), 3.30 (m, 1H, C₆H₅CH₂O₂CCH₂CH), 3.60 (t, *J* = 9.6 Hz, 1H, CH₂CHNCO), 3.71 (d, *J* = 7.0 Hz, 1H, CH₂CHNCO), 3.76 (m, 2H, C₆H₅CH₂O₂CH₂CH), 4.36 (m, 2H, CH₂O₂CCHNH), 4.99 (m, 1H, CH₂O₂CCHNH), 5.79 (m, 1H, CH₂CHNCO), 6.47 (m, 1H, C=CH–CH=CCOCH₃), 7.13 (m, 1H, aromatic H), 7.31 (m, 1H, aromatic H), 7.32 (m, 5H, CH₂C₆H₅), 7.39 (d, *J* = 8.3 Hz, 1H, aromatic H), 7.54 (d, *J* = 7.7 Hz, 1H, aromatic H), 8.18 (d, *J* = 7.7 Hz, 1H, C=CH–CH=C–COCH₃), 9.80 (s, 1H, pyrrole NH). – MS (ESI): *m/z* = 636 [M + Na]⁺.

C₃₄H₃₅N₃O₈ Calcd.: C 66.55 H 5.75 N 6.85
(613.25) Found: C 66.47 H 5.63 N 6.74.

(6S)-3-Acetyl-6-(*N*-tert-butoxycarbonyl-*L*-glutamyl)oxymethyl-4,6,7,12-tetrahydro-4-oxoindolo[2,3-*a*]quinolizine (5c)

Using the procedure for preparing (6S)-5a, from 30 mg (0.16 mmol) of Boc-*L*-Gln 56 mg (85%) of (6S)-5c was obtained, as colorless sheets; *m.p.* 144–145 °C. [α]_D = 115.0° (c = 2, CHCl₃). – IR (KBr): ν /cm⁻¹ = 3420 and 3328, 3200 (NH and NH₂), 2968 and 2923 (CH, CH₂ and CH₃), 1735 (ester C=O and ketone C=O), 1653 (amide C=O), 1584, 1568,

1497 and 1425 (aromatic C=C), 755 (1, 2-disubstituted phenyl). – ¹H NMR: δ /ppm = 1.27 (m, 2H, CHCH₂CH₂CONH₂), 1.59 (s, 9H, -C(CH₃)₃), 2.08 (m, 2H, CHCH₂CH₂CONH₂), 2.60 (s, 3H, COCH₃), 3.39 (m, 2H, CH₂CHNCO), 3.95 (m, 1H, CHCH₂CH₂CONH₂), 4.42 (m, 2H, CH₂O₂CCHNH), 4.94 (d, *J* = 8.2 Hz, 1H, CH₂O₂CCH₂NH), 5.80 (m, 1H, CH₂CHNCO), 6.54 (d, *J* = 8.2 Hz, 2H, CHCH₂CH₂CONH₂), 6.59 (t, *J* = 15.0 Hz, 1H, C=CH–CH=C–CO–CH₃), 7.15 (t, *J* = 14.9 Hz, 1H, aromatic H), 7.30 (t, *J* = 15.3 Hz, 1H, aromatic H), 7.44 (d, *J* = 8.2 Hz, 1H, aromatic H), 7.56 (d, *J* = 8.0 Hz, 1H, aromatic H), 8.21 (d, 1H, *J* = 7.6 Hz, 1H, C=CH–CH=C–CO–CH₃), 9.68 (s, 1H, pyrrole NH). – MS (ESI): *m/z* = 559 [M + Na]⁺.

C₂₈H₃₂N₄O₇ Calcd.: C 62.68 H 6.01 N 10.44
(536.24) Found: C 62.50 H 5.97 N 10.39.

(6S)-3-Acetyl-6-glycyloxymethyl-4,6,7,12-tetrahydro-4-oxoindolo[2,3-*a*]quinolizine hydrochloride (6)

At 0 °C to the stirred solution of 30 mg (0.07 mmol) of (6S)-5a in 1 ml of ethyl acetate 0.5 ml of hydrochloride–ethyl acetate (3 mol/l) was added dropwise. The reaction mixture was stirred at 0 °C for 3 h then TLC analysis (CHCl₃: MeOH, 9:1) indicated complete disappearance of (6S)-5a. After removal of the solvent the residue was purified by chromatography (CHCl₃: MeOH:HAc, 40:10:1) to give 23 mg (92%) of (6S)-6, as colorless crystals; *m.p.* 167–169 °C. [α]_D = 35.7° (c = 2, H₂O). – IR (KBr): ν /cm⁻¹ = 3400, and 2952 cm⁻¹ (NH and NH₂), 2952 and 2920 (CH, CH₂, and CH₃), 1753 (ester and ketone C=O), 1656 (amide C=O), 1584, 1543, 1494 and 1424 (aromatic C=C), 758 (1,2-disubstituted phenyl). – ¹H NMR (D₂O): δ /ppm = 2.36 (s, 3H, COCH₃), 3.43 (m, 2H, CH₂O₂CCH₂NH₂), 3.49 (m, 2H, CH₂O₂CCH₂NH₂), 4.06 (t, *J* = 11.6 Hz, 1H, CH₂CHNCO), 4.13 (t, *J* = 11.6 Hz, 1H, CH₂CHNCO), 5.48 (d, *J* = 6.6 Hz, 1H, CH₂CHNCO), 6.39 (d, *J* = 7.8 Hz, 1H, C=CH–CH=C–COCH₃), 6.96 (t, *J* = 14.5 Hz, 1H, aromatic H), 7.10 (t, *J* = 14.3 Hz, 1H, aromatic H), 7.18 (d, *J* = 8.0 Hz, 1H, aromatic H), 7.40 (d, *J* = 7.8 Hz, 1H, aromatic H), 7.87 (d, *J* = 7.9 Hz, 1H, C=CH–CH=C–COCH₃). – MS (ESI): *m/z* = 388 [M + Na]⁺.

C₂₀H₂₀N₃O₄Cl Calcd.: C 59.78 H 5.02 N 10.46
(401.12) Found: C 59.69 H 4.98 N 10.36.

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