Synthesis of N^1- and N^8-(γ-L-Glutamyl)spermidines and (γ-L-Glutamyl) putrescine

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Abstract: (γ-L-Glutamyl)putrescine and N^1- and N^8-(γ-L-glutamyl)spermidines, possible regioisomeric products of the polyamine glutamyl transferase of bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*, were synthesized from *N*-Boc-protected putrescine and 1,3-propanediamine.

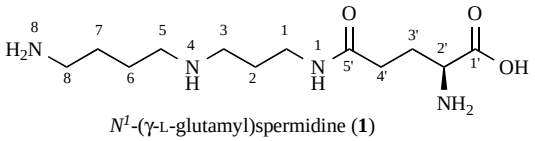
Keywords: glutamylspermidine, glutamylputrescine, *Pseudomonas aeruginosa*, polyamine biosynthesis, polyamine glutamyl transferase.

INTRODUCTION

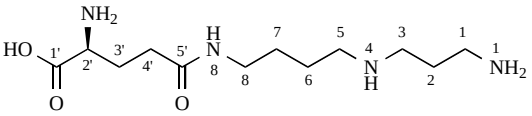
Polyamines (spermine, spermidine, putrescine etc.) are a group of ubiquitous polycations necessary for optimal cell growth of procalyotic and eukaryotic cells [1], and thus it is important to elucidate their metabolic pathways. Recently, γ-glutamylation was found to be a key reaction in the metabolism. N^1-(γ-L-Glutamyl)spermidine (**1**, Fig. **1**) and N^8-(γ-L-glutamyl)spermidine (**2**), plausible metabolic intermediates, were firstly reported by Folk as unnatural compounds prepared by enzymatic procedure [2]. Later, (γ-L-glutamyl)spermidine conjugates were identified *in vitro* as a major protein secreted from the rat seminal vesicles [3]. Folk *et al*. also showed their ion-exchange chromatogram using HPLC method [2, 4]. γ-L-Glutamylputrescine (**3**), one of the key compounds in polyamine metabolism, was found in mammalian brain [5], and recently in *Escherichia coli* K-12 [6, 7]. We report here the synthesis and spectroscopic data of three γ-L-glutamylated polyamines (**1**, **2** and **3**) as standards for the metabolic studies.

RESULTS AND DISCUSSION

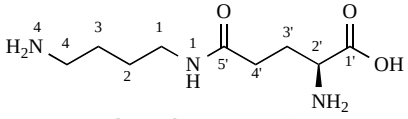
The synthesis of the compounds is shown in Scheme **1**. Recently, Jeitner *et al*. reported the synthesis of HCl salts of **1** and **3** in a similar manner as we describe here [8]. The free N^1 amino group of N^4,N^8-bis(Boc)-spermidine (**4**) [9, 10] was condensed with *N*-Boc-*O*-*t*-Bu-L-glutamic acid (**5**) [11] using water-soluble carbodiimide (EDCI) in 55% yield to give **6** [8]. Finally, acidic deprotection of the three Boc and one *t*-Bu group afforded **1**. Similarly, N^8-(γ-L-glutamyl)spermidine (**2**) and (γ-L-glutamyl)putrescine (**3**) were prepared from N^1,N^4-bis(Boc)-spermidine (**7**) [12] and *N*-Boc-putrescine (**9**) [13], respectively. Most of the ^{1}H and ^{13}C NMR resonances (Table **1**) of the target compounds were assigned by the HH-COSY, HMQC and HMBC spectra. Positions of each amide bond were confirmed by the observation of ^{1}H-^{13}C three bond correlations from H(1) to C(5') for **1** and from H(8) to C(5') for **2** in the HMBC spectra.



N^1-(γ-L-glutamyl)spermidine (**1**)



N^8-(γ-L-glutamyl)spermidine (**2**)



N-(γ-L-glutamyl)putrescine (**3**)

Fig. (1). N^1- and N^8-γ-L-glutamylspermidines, and (γ-L-glutamyl) putrescine.

EXPERIMENTAL

General

Optical rotation: *Horiba Sepa-300* apparatus. FT-IR: *Jasco 4100* apparatus (ATR, Zn-Se); in cm^{-1}. ^{1}H and ^{13}C NMR: *Varian Inova 600* (600 MHz for ^{1}H and 150 MHz for ^{13}C) and *Inova 500* apparatuses (500 MHz for ^{1}H and 126 MHz for ^{13}C); rel. to Me_4Si (= 0 ppm). Mass spectra: *Jeol JMS–700*; in *m/z*.

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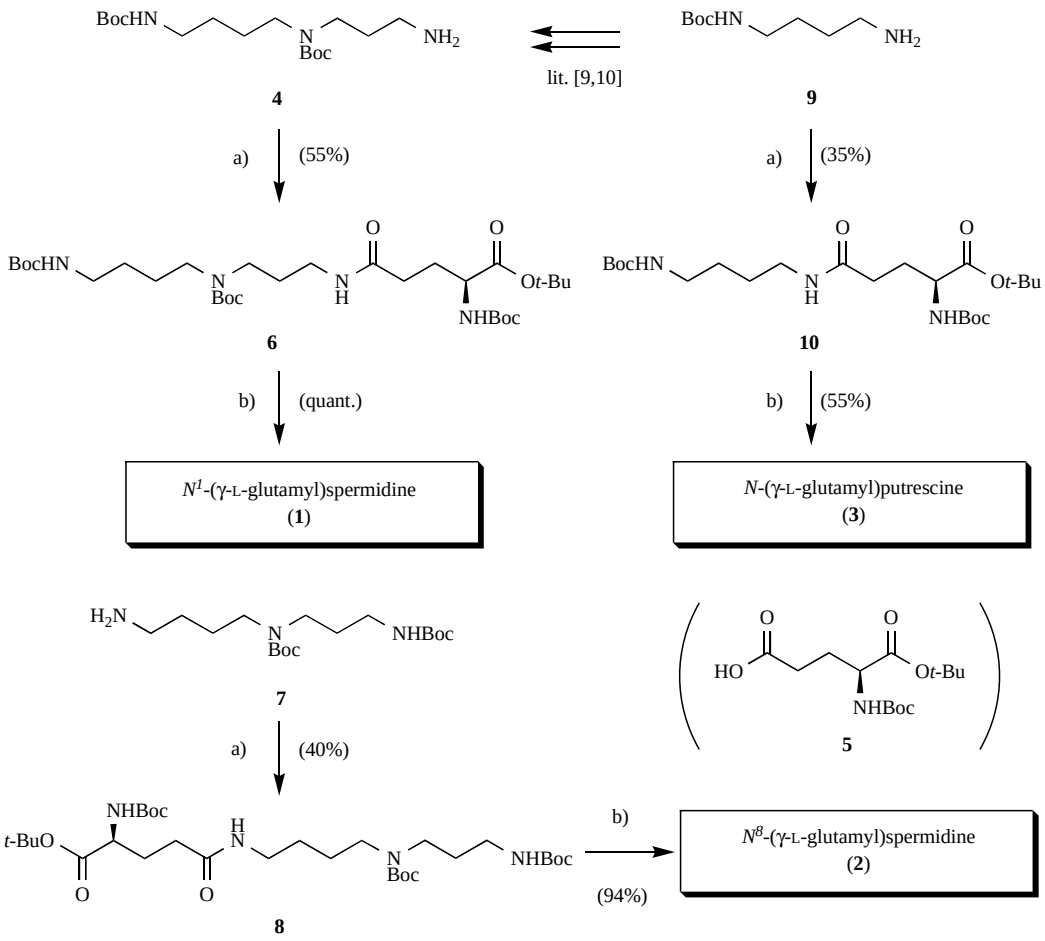

Scheme 1. Synthesis of N^1- and N^8-γ-L-glutamylspermidines, and (γ-L-glutamyl)putrescine.

a) *N*-Boc-*O*-*t*-Bu-L-Glu (**5**), EDCI, CH_2Cl_2. **b**) CF_3CO_2H, CH_2Cl_2, 0°C.

Table 1. ^{1}H and ^{13}C NMR Spectral Data of 1, 2 and 3 (600 MHz for ^{1}H and 150 MHz for ^{13}C in D_2O)

	1			**2**			**3**		
No	**^{1}H**	**mult. (Hz)**	**^{13}C**	**^{1}H**	**mult. (Hz)**	**^{13}C**	**^{1}H**	**mult. (Hz)**	**^{13}C**
1	3.21	t (6.5)	36.26	3.09*	t (7.8)	36.65	3.20	t (6.5)	38.78
2	1.82	quint (7.3)	25.99*	2.03	quint (7.3)	23.81	1.56	quint (7.0)	25.47
3	2.98	m	45.23	3.04*	t (8.7)	45.54	1.65	quint (7.3)	24.26
4							2.98	t (7.3)	39.22
5	2.99	m	47.03	3.02	t (8.1)	47.46			
6	1.690	m	22.86	1.64	quint (7.3)	25.50			
7	1.679	m	23.99	1.52	quint (7.3)	23.07			
8	2.97	m	38.88	3.16	t (6.9)	38.69			
1’	-		171.49	-		171.45	-		171.82*
2’	4.03	t (6.5)	52.30	4.04	t (6.6)	52.54	4.04	m	52.62
3’	2.13 2.17	dt (15.0, 7.2) dt (15.0, 6.6)	25.65*	2.14 2.19	dt (15.0, 7.3) dt (15.0, 7.3)	25.78	2.19	m	25.89
4’	2.40	dt (15.5, 7.6)	31.11	2.41	dt (15.3, 7.6)	31.24	2.46	m	31.32
	2.44	dt (15.5, 7.3)		2.44	dt (15.5, 7.6)		-		
5’	-		174.56	-		174.19	-		174.24*
NH	7.57 8.14 8.30 10.8	br							

Unable to redefine the ambiguous assignments (*) due to the overlapping and/or lack of the key HMBC/HMQC correlation.

N^4,N^8-Bis(tert-butoxycarbonyl)-N^1-(N-tert-butoxycarbonyl-O-tert-butyl-γ-L-glutamyl)spermidine (6) [8]

To a solution of amine **4** (FW: 345.48, 80 mg, 0.22 mmol), **5** (FW: 303.35, 64 mg, 0.21 mmol) and *i*-Pr_2NEt (FW: 129.24, 39 mg, 0.23 mmol) in dry CH_2Cl_2 (2 ml) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI•HCl, FW: 191.71, 50 mg, 0.26 mmol) at 0°C and the mixture was stirred at room temp. for 12 h. Then the mixture was diluted with EtOAc, washed three times with water, dried with $MgSO_4$ and concd. *in vacuo*. The residue was chromatographed on silica gel ($CHCl_3$/MeOH = 100:1) to give **6** (FW: 630.81, 78 mg, 0.12 mmol, 55%) as a colorless oil, $[\alpha]_D^{22}$ +1.6° (*c* 3.9, $CHCl_3$). R_f 0.40 ($CHCl_3$/MeOH = 20:1). IR: 3350*m* (N–H), 1699*s* (C=O), 1679*s* (C=O), 1366*s*, 1258*s*, 1152*s*, 749*vs*. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.44 (*s*, 2 x *t*-Bu), 1.454 (*s*, *t*-Bu), 1.462 (*s*, *t*-Bu), 1.4-1.6 (*m*, CH_2(6, 7)), 1.65 (*br s*, CH_2(2)), 1.91 (*br s*, 1 H-(3')), 2.15 (*m*, 1 H-(3')), 2.27 (*m*, CH_2(4')), 3.12 & 3.14 (*br s*, CH_2(5, 8)), 3.20 (*br s*, CH_2(1)), 3.26 (*br s*, CH_2(3)), 4.14 (*br s*, 1 H, H-(2')), 4.57 (*br*, 0.75 H, H-(N^8)), 4.68 (*br*, 0.25 H-(N^8)), 5.27 (*pseudo d*, *J* = 7.3, H-($NCHC_2$)), 6.41 (*br*, 0.75 H -(N^1)), 6.89 (*br*, 0.75 H-(N^1)). ^{13}C NMR ($CDCl_3$, 126 MHz) δ: 27.9, 28.3, 28.4, 32.7, 35.7, 40.0, 43.3, 46.6, 53.6, 53.8, 79.1, 79.7, 81.9, 82.2, 155.6, 155.9, 171.5, 172.0. FAB-MS: 631 (*[M+H]*$^+$), 531 (*[M+2H–t-BuOC=O]*$^+$), 475, 275. HR-FAB-MS: 631.4286 (*[M+H]*$^+$, $C_{31}H_{59}O_9N_4$; calc. 631.4282).

N^1-(γ-L-Glutamyl)spermidine (1)

A solution of **6** (FW: 630.81, 70 mg, 0.11 mmol) in CF_3CO_2H (2.8 ml) and CH_2Cl_2 (1.7 ml)) was stirred at 40°C for 12 h. Then the mixture was concd. *in vacuo* and the residue was chromatographed on silica gel ($CHCl_3$/MeOH/aq. NH_3 = 2:2:1) to give **1** (FW: 274.36, 38 mg, 0.12 mmol, quant.) as a colorless oil, $[\alpha]_D^{22}$ +6.1° (*c* 1.3, MeOH). R_f 0.05 ($CHCl_3$/MeOH/aq. NH_3 = 2:2:1). IR: 3330*m*, (N–H), 3190*m* (N–H), 2958*s*, 1696*m*, (C=O), 1625*s* (C=O), 1235*m*, 1153*w*. FAB-MS: 297 (*[M+Na]*$^+$), 275 (*[M+H]*$^+$), 180, 153, 135, 115, 72, 61. HR-FAB-MS: 275.2080 (*[M+H]*$^+$, $C_{12}H_{27}O_3N_4$; calc. 275.2083).

N^1,N^4-Bis(tert-butoxycarbonyl)-N^8-(N-tert-butoxycarbonyl-O-tert-butyl-γ-L-glutamyl)spermidine (8)

In a similar manner as described for **6**, **7** (FW: 345.48, 205 mg, 0.593 mmol) was converted to **8** (FW: 630.81, 151 mg, 0.239 mmol, 40.3%) as a colorless oil, $[\alpha]_D^{27}$ +1.7° (*c* 1.1, $CHCl_3$). R_f 0.40 ($CHCl_3$/MeOH = 20:1). IR: 3330*m* (N–H), 1692*vs* (C=O), 1678*vs* (C=O), 1365*s*, 1249*s*, 1151*vs*, 752*vs*. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.438 (*s*, *t*-Bu), 1.443 (*s*, *t*-Bu), 1.461 (*s*, 2 x *t*-Bu), 1.50 (*m*, 2 H), 1.56 (*m*, 2 H), 1.65 (*br s*, 2 H), 1.95 (*br s*, 1 H), 2.15 (*m*, 1 H), 2.26 (*t*, *J* = 6.8, 2 H), 3.10 (*br s*, 2 H), 3.6 (*m*, 2 H), 3.18-3.31 (*m*, 4 H), 4.13 (*br s*, 1 H-(2')), 4.82 (*br s*, 0.5 H), 5.27 (*br d*, *J* = 6.8, 1 H), 5.32 (*br s*, 0.5 H), 6.43 (*br s*, 0.5 H), 6.54 (*br s*, 0.5 H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ: 28.0, 28.3, 28.5, 32.8, 37.4, 37.7, 39.2, 43.7, 44.3, 46.1, 46.6, 53.4, 78.9, 79.2, 79.6, 79.9, 80.0, 82.2, 82.4, 155.6, 156.0, 156.2, 171.4, 172.2. FAB-MS: 631 (*[M+H]*$^+$), 531 (*[M+2H–t-BuOC=O]*$^+$), 475, 275. HR-FAB-MS: 631.4282 (*[M+H]*$^+$, $C_{31}H_{59}O_9N_4$; calc. 631.4282).

N^8-(γ-L-Glutamyl)spermidine (2)

In a similar manner as described for **1**, **8** (112 mg, 0.178 mmol) was converted to **2** (FW: 274.36, 46.0 mg, 0.168 mmol, 94%) as a colorless oil, $[\alpha]_D^{27}$ +3.0° (*c* 1.1, MeOH). R_f 0.05 ($CHCl_3$/MeOH/aq. NH_3 = 2:2:1). IR: 3384*s* (N–H), 2948*s*, 1736*m* (C=O), 1634*m* (C=O), 1210*m*, 1048*m*. FAB-MS: 273 (*[M–H]*$^-$), 195, 155, 127, 97. HR-FAB-MS: 273.1925 (*[M–H]*$^-$, $C_{12}H_{25}O_3N_4$; calc. 273.1927).

N^4-(tert-Butoxycarbonyl)-N^1-(N-tert-butoxycarbonyl-O-tert-butyl-γ-L-glutamyl)putrescine (10)

In a similar manner as described for **6**, **9** (FW: 188.27, 80 mg, 0.43 mmol) was converted to **10** (FW: 473.60, 71 mg, 0.15 mmol, 35%) as a colorless oil, $[\alpha]_D^{27}$ +1.2° (*c* 0.70, $CHCl_3$). R_f 0.54 ($CHCl_3$/MeOH = 20:1). IR: 3341*m* (N–H), 2977*m*, 2934*m*, 1698*s* (C=O), 1652*m* (C=O), 1524*m*, 1366*m*, 1251*s*, 1172*s*. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.44 (*s*, *t*-Bu), 1.45 (*s*, *t*-Bu), 1.46 (*s*, *t*-Bu), 1.54 (*m*, CH_2(2, 3)), 1.85 (*m*, 1 H-(3')), 2.15 (*m*, 1 H-(3')), 2.25 (*t*, *J* = 7.1, CH_2(4')), 3.14 (*br s*, CH_2(NH)), 3.28 (*m*, CH_2(NH)), 4.14 (*m*, 1 H-(N)), 4.65 (*br s*, 1 H-(N)), 5.25 (*d*, *J* = 8.0, 1 H-(2')), 6.43 (*br s*, 1 H-(N)). FAB-MS: 474 (*[M+H]*$^+$), 374 (*[M+2H–(t-BuOC=O)]*$^+$), 318 (*[M+3H–(t-BuOC=O)–t-Bu]*$^+$), 262, 244, 218 (*[M+4H–2(t-BuOC=O)–t-Bu]*$^+$). HR-FAB-MS: 474.3186 (*[M+H]*$^+$, $C_{23}H_{44}O_7N_3$; calc. 474.3179).

N-(γ-L-Glutamyl)putrescine (3)

In a similar manner as described for **1**, **10** (70 mg, 0.15 mmol) was converted to **3** (FW: 217.27, 38 mg, 0.12 mmol, 55%) as an amorphous solid, $[\alpha]_D^{28}$ +4.62° (*c* 2.25, MeOH). R_f 0.3 ($CHCl_3$/MeOH/aq. NH_3 = 2:2:1). IR: 3330*m* (N–H), 3190*br vs* (O–H), 1736*m* (C=O), 1634*s* (C=O), 1561*m*, 1509*m*, 1399*s* (C–O), 1228*w*, 1160*w*, 1048*w*. FAB-MS (in D_2O): 225 (*[M–6H+7D]*$^+$) 176, 156, 154, 138, 136, 108, 89, 78; (in H_2O): 216 (*[M–H]*$^-$), 188, 151. HR-FAB-MS: 225.1952 (in D_2O, *[M–6H+7D]*$^+$, $C_9H_{13}D_7O_3N_3$; calc. 225.1944), 216.1347 (in H_2O, *[M–H]*$^-$, $C_9H_{18}O_3N_3$; calc. 216.1348).

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