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TOTAL SYNTHESIS OF GALANTIN I. REVISION OF THE ORIGINAL STRUCTURE

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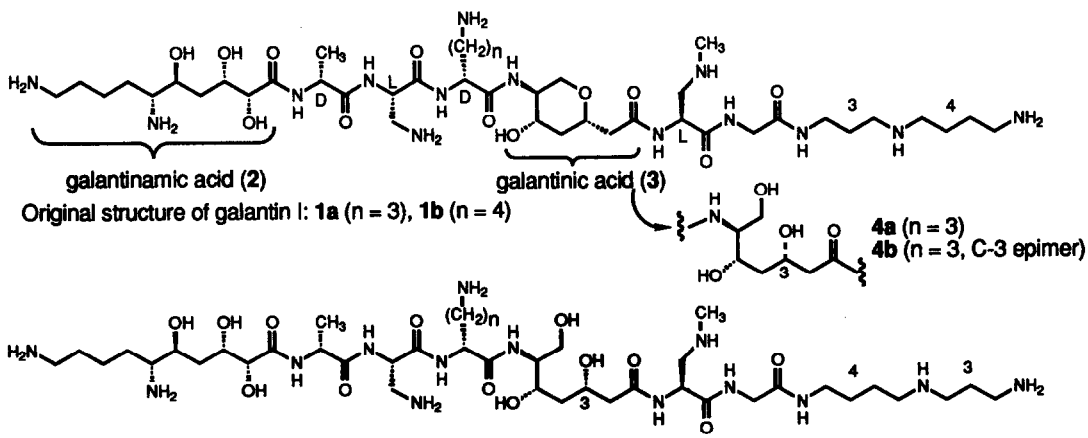
Summary: The proposed structure of galantin I, isolated as a mixture of homologs (**1a**/**1b** = 9/1), was shown to be incorrect by total synthesis and was revised twice to finally give **5a** and **5c**, respectively, by total synthesis.

Galantin I, a peptide antibiotic isolated from a culture broth of *Bacillus pulvifaciens* as an inseparable mixture of homologs with the D-ornithine residue replaced with D-lysine (Orn/Lys = 9/1), has attracted significant interest owing to its unique structure and its potent anti-bacterial activity.[1] The structure of galantin I, possessing several unusual amino acid moieties named galantinamic acid (glm, **2**) and galantinic acid (gla, **3**), had been assigned to be **1a** and **1b** by chemical degradation studies[1b,2] and by the syntheses of **2** and **3**.[3,4] However, the structures, **1a** and **1b**, had to be revised twice. Our total synthesis of the structure **1a** showed it to be different from natural galantin I and further suggested that the sub-structure **3** and the sequence of the spermidine moiety were incorrect. We wish to report here the correct structures of galantin I as being **5a** and **5c**, respectively, which were confirmed by total synthesis.

Our approach to the synthesis of **1a** was by the fragment coupling of protected **2**, **3a**, (2*S*)-α,β-diaminopropionic acid (L-A_2Pr) **6**,[5] (2*S*)-N$^{\beta}$-methyl-α,β-diaminopropionic acid (N$^{\beta}$-Me-L-A_2Pr) **7**[6] and glycyl spermidine [Gly-Spe(3,4)] **9**.[7] The N-terminus of all amino acids was protected by the benzyloxycarbonyl (Z) group and the amino group on the side chains by the *t*-butoxycarbonyl (Boc) group. Protected **7** was prepared, efficiently, via an N-monomethylation of **6** using Grieco's procedure.[6,8] Thus, the protected form of **1a** was constructed by the use of WSC, DEPC, or DPPA as the coupling reagents in a convergent manner and was deprotected with TFA at once to give **1a** (Scheme I)[9] whose spectroscopic data [MS and ^{1}H

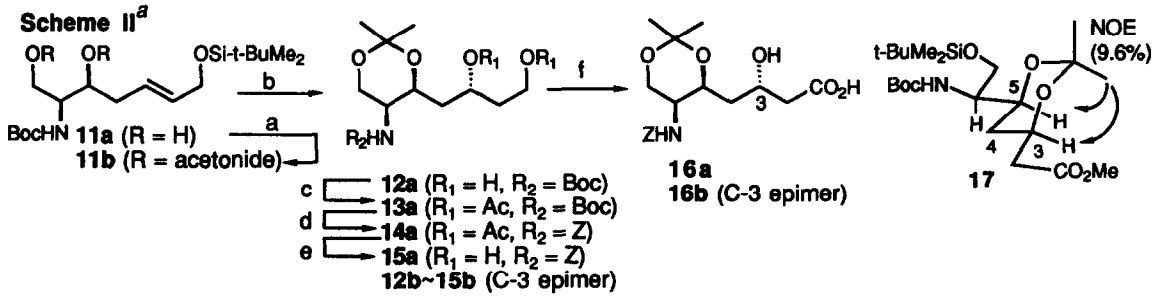


NMR (500 MHz)][10] were in agreement with those of the proposed structure **1a**. In order to carry out spectroscopic identification of the synthetic **1a** with natural ornithine congener, we examined the separation of each congener from natural galantin I which was donated by Shiba as a crude form. Fortunately, we found HPLC conditions capable of separating each congener which was preparatively fractionated: (galantin I with Orn; 2 mg and with Lys 100 μg).[11] Both congeners were spectroscopically characterized. However, it was found that MS (SIMS) data of each congener showed the parent ion peak at 981 $(M+H)^+$ for galantin I (Orn) and 995 $(M+H)^+$ for galantin I (Lys), respectively, which corresponds to the original molecular formula plus H_2O.[12] In combination with ^1H COSY studies, these data[10] led to the conclusion that previously isolated **3** is an artifact, probably dehydrated during the chemical degradation studies.[1b] The correct structure of galantin I should be either acyclic 3*S*-**4a** or 3*R*-**4b**.[1b]

The synthesis of gla with an acyclic structure was carried out starting from **11a**, a precursor of the previous synthesis of **3** (Scheme II).[4,13] The configuration of C-3 in **16a** and **16b** was determined by converting **12b** into the acetonide **17** in which the structure having (3*R*,5*S*) stereochemistry as depicted was proven by ^1H NMR studies (coupling constants, decoupling and NOE experiments).[14,15] Each diastereomer was introduced into the synthetic sequence of **4a** and **4b** in essentially the same manner as that of **1a**.[10,11] However, comparison of synthetic with natural galantin I still showed them to be different; based on ^1H NMR the spermidine sequence [Spe(3,4)] was believed to be incorrect.[16]

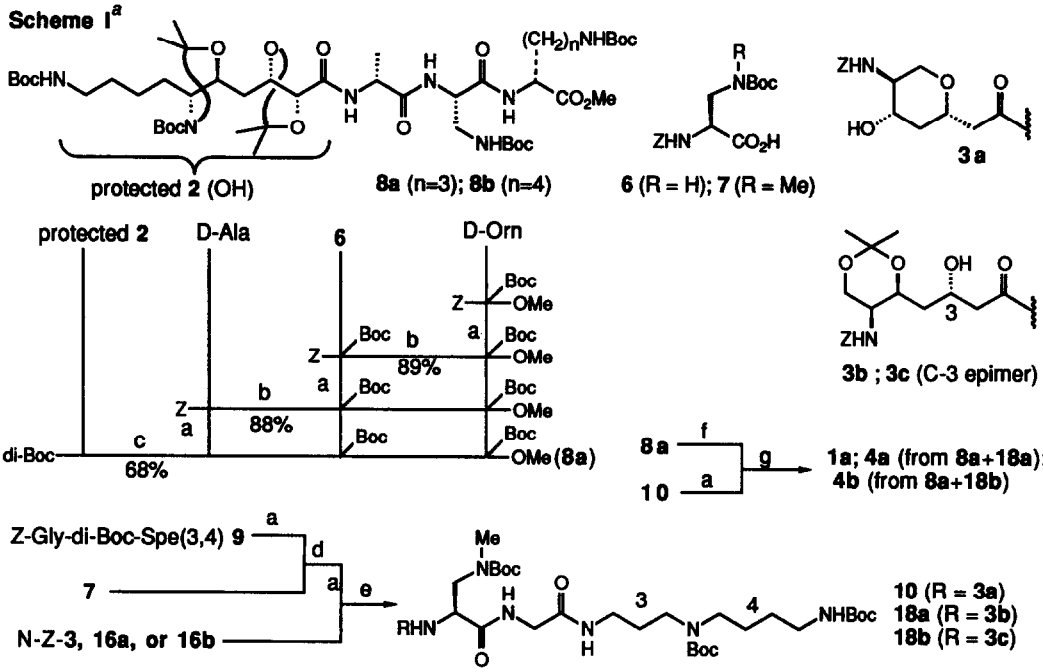
Therefore, the structure of galantin I had to be revised again to be **5a** or **5b** with a different sequence of the spermidine residue [Spe(4,3)].[17] Scheme III illustrates our third synthesis. It is noted that the use of DPPA/powdered $NaHCO_3$[18] for the final stage of the coupling sequences yielded the protected form of **5a** and **5b** in satisfactory yields while DPPA/Et_3N or other coupling reagents provided only ~10% of the desired product. Removal of all protecting groups with TFA proceeded smoothly to give **5a** and **5b**. The synthetic 3*S*-**5a** was found to be completely identical with natural galantin I (Orn) in all respects (^1H NMR, MS, and HPLC).[11,19] 3*R*-**5b** was slightly different from **5a**.[10,11] Galantin I (Lys) **5c** was prepared in the same manner as above (**8b** + **20a**). Thus, the structures of both galantin I (Orn) and its Lys component were revised to be **5a** and **5c**, respectively, by the present synthetic and spectroscopic studies.

Scheme II[a]



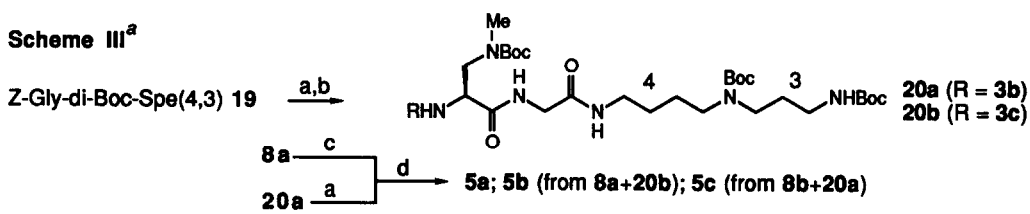
[a](a) 2,2-dimethoxypropane, acetone, CSA, room temperature, 3 h (88%); (b) (1) n-Bu_4NF, THF, room temperature, 3 h (51%); (2) MCPBA, CH_2Cl_2, 0 °C, 1.5 h (100%); (3) $LiAlH_4$, ether, 0 °C, 1 h (**12a**, 20%; **12b**, 28%); (c) Ac_2O, pyridine, room temperature, 14 h (80%); (d) (1)TBDMSOTf, 2,6-lutidine, CH_2Cl_2; (2) benzyl bromide, n-Bu_4NF, THF, 0 °C, 1 h (**14a**, 75%; **14b**, 56%); (e) 0.1 equiv K_2CO_3, MeOH, room temperature, 2 h (90%); (f) PtO_2, O_2, dioxane-H_2O (2/1), 45 °C, 4 h (60%).

Scheme I[a]



[a](a) H_2/10% Pd-C, room temperature, 16 h; (b) WSC, CH_2Cl_2, room temperature, 2 h ; (c) DPPA, Et_3N, DMF, 0 °C, 24 h; (d) DEPC, Et_3N, DMF, 0 °C, 16 h (61%); (e) DPPA, Et_3N, DMF, 0 °C, 22 h (**10**, 98%; **18a**, 42%; **18b**, 65%); (f) 0.5 N NaOH, 0 °C, 8 h; (g) (1) DPPA, Et_3N, DMF, 0 °C; (2) TFA, CH_2Cl_2, room temperature, 3 h (**1a**, 57%; **4a**, 15%; **4b**, 23%).

Scheme III[a]



[a](a) H_2/10% Pd-C, MeOH, room temperature, 16 h; (b) (1) **7**, DEPC, Et_3N, DMF, 0 °C, 4 h (61%); (2) (a); (3) **16a** or **16b**, DPPA, Et_3N, DMF, 0 °C, 22 h (**20a**, 92%; **20b**, 53%); (c) 0.5 N NaOH, THF, 0 °C, 8 h; (d) (1) DPPA, powdered $NaHCO_3$, DMF, 0 °C (30 h for **5a**; 72 h for **5b**; 45 h for **5c**); (2) TFA, CH_2Cl_2, room temperature, 4 h (**5a**, 59%; **5b**, 70%; **5c**, 45%).

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6. Prepared from **6** by the following sequence of reactions: (1) CH_2N_2; (2) TFA, CH_2Cl_2; (3) CH_2O, cyclopentadiene, H_2O, room temperature, 1 h; (4) Et_3SiH, TFA, CH_2Cl_2;[8] (5) Boc_2O, Et_3N, CH_2Cl_2 (59% from **6**).
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8. Grieco, P. A.; Bahsas, A. *J. Org. Chem.* **1987**, *52*, 5746.
9. Abbreviations used in the text: WSC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; DEPC, diethylphosphorocyanidate; DPPA, diphenylphosphoryl azide; DMF, N,N'-dimethylformamide; Et_3N, triethylamine; TFA, trifluoroacetic acid; Boc_2O, di-*tert*-butyl dicarbonate; CSA, *dl*-camphorsulfonic acid; MCPBA, 3-chloroperoxybenzoic acid; TBDMSOTf, *tert*-butyldimethylsilyl trifluoromethanesulfonate.
10. Full spectroscopic data of this compound will be reported in a full paper.
11. HPLC conditions and retention time of natural galantin I and **1a**, **4a**, **4b**, **5a** and **5b** were as follows. Column: UNISIL PACK $5C_{18}$ (10.7Ø x 250 mm), available from Gasukuro Kogyo, Tokyo, Japan: flow rate; 1 ml/min (elution with 1% CH_3CN/0.1% TFA aqueous solution). Retention time of each compound: **1a**, 71min; **4a**, 54 min; **4b**, 57 min; natural galantin I(Orn) and **5a**, 61 min; **5b**, 65 min; natural galantin I (Lys) and **5c**, 75 min.
12. MS instruments which could measure such high molecular weight was not available at that time. MS (FD method) was used to determine the molecular formula of the fragment amino acids.[1b]
13. The N-Boc compounds **13a,b** were converted into the N-Z compounds **14a,b** via the N-*tert*-butyldimethylsilyloxycarbonyl intermediates: Sakaitani, M.; Ohfune, Y. *J. Org. Chem.* **1990**,*55*, 870.
14. Preparation of the acetonide **17** from **12b**: (1) PtO_2, O_2, dioxane-H_2O (2/1), 40 °C, 9 h; (2) CH_2N_2 (50% for 2 steps); (3) CSA, MeOH (69%); (4) TBDMSOTf, Et_3N, CH_2Cl_2, 0 °C, 5 min (58%); (5) 2,2-dimethoxypropane, CSA, benzene, room temperature, 10 min (65%).
15. ^1H NMR (500 MHz, C_6D_6) data of **17**: δ 0.07 (s, 3 H), 0.08 (s, 3 H), 0.98 (s, 9 H), 1.05 (ddd, *J* = 2.5, 2.5, 13 Hz), 1.33 (s, 3 H), 1.35 (s, 3 H), 1.48 (s, 9 H), 1.51 (ddd, 1 H, *J* = 13, 13, 13 Hz), 2.06 (dd, 1 H, *J* = 4.5, 11 Hz), 2.42 (dd, 1 H, *J* = 8.5, 11Hz), 3.31 (s,3 H), 3.63 (dd, 1 H, *J* = 9, 9 Hz), 3.76 (dd, 1 H, *J* = 5.5, 9 Hz), 3.92 (m, 1 H), 4.23 (ddd, 1 H, *J* = 2.5, 2.5, 13.0 Hz), 4.27 (dddd, 1 H, *J* = 2.5, 4.5, 8.5, 13 Hz), 4.95 (d, 1 H, *J* = 9 Hz).
16. The sequence of Spe(3,4) residue in the original structure was elucidated by the microbial method.[1b] The ^1H NMR signal pattern and chemical shift of the methylene group centered at the 1,3-diamino propane residue were significantly different between **4a,b** (δ 1.91, m) and natural **5a** (δ 2.03, m).[10]
17. Z-Gly-di-Boc-Spe(4,3) **19** was prepared from Z-Gly-OSu: (1) 2 equiv putrescine, DMF, 80 °C, 10 min; (2) Boc_2O, Et_3N, DMF (50% for 2 steps); (3) TFA, CH_2Cl_2; (4) *N-t*-Boc-3-aminopropanal, $NaBH_3CN$, MeOH; (5) Boc_2O, Et_3N, dioxane (69% for 3 steps).
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19. CD, MS, HRMS, and ^1H NMR data of **5a**: Galantin I(Orn) (**5a**): MS(SIMS) 981 $(M+H)^+$, 491; CD (*c* 0.036, H_2O); λ_{max}199.6 (Δε -4.3), λ200 (Δε -4.2), λ220 (Δε -2.2); ^1H NMR (500 MHz, D_2O)* δ 1.46 (d, 3 H, *J* = 7.5 Hz; D-Ala, CH_3), 1.43-1.98 (m, 18 H), 2.07 (m, 2 H; Spe, N-CH_2-CH_2-CH_2-N), 2.49 (dd, 1 H, *J* = 10, 15 Hz; gla, 2-H), 2.59 (dd, 1 H, *J* = 3.5, 15 Hz; gla, 2-H), 2.78 (s, 3 H; N-CH_3), 3.01(t, 2 H, *J* = 8 Hz; N-CH_2), 3.02 (t, 2 H, *J* = 8 Hz; N-CH_2), 3.08 (t, 2 H, *J* = 8 Hz; N-CH_2), 3.09 (t, 2 H,*J* = 8 Hz; N-CH_2), 3.14 (t, 2 H, *J* = 8 Hz; N-CH_2), 3.23 (t, 2 H, *J* = 7 Hz; N-CH_2), 3.31 (dd, 1 H, *J* = 8,13 Hz; L-A_2Pr, 3-H), 3.35 (dd, 1 H, *J* = 8, 13 Hz; N$^\beta$-Me-L-A_2Pr, 3-H), 3.36 (ddd, 1 H, *J* = 4, 5,11Hz; glm, 6-H), 3.54 (dd, 1 H, *J* = 5.5, 13 Hz; L-A_2Pr, 3-H), 3.57 (dd, 1 H, *J* = 5.5, 13 Hz; N$^\beta$-Me-L-A_2Pr, 3-H), 3.62 (dd, 1 H, *J* = 7.5, 11.5 Hz; gla, 7-H), 3.73 (dd, 1 H, *J* = 5.5, 11.5 Hz; gla, 7-H), 3.89 (m, 1 H; gla, 6-H), 3.90 (d, 1 H, *J* = 18 Hz; Gly), 3.93 (d, 1 H, *J* = 18 Hz, Gly), 4.02 (m, 1 H; gla, 5-H), 4.11 (ddd, 1 H, *J* = 2, 2, 11 Hz; glm, 5-H), 4.14 (d, 1 H, *J* = 2 Hz; glm, 2-H), 4.20 (ddd, H, *J* = 2, 2, 10.5 Hz; glm, 3-H), 4.26 (m, 1 H; gla, 3-H), 4.39 (dd, 1 H, *J* = 6, 9 Hz; D-Orn, 2-H), 4.43 (q, 1 H, *J* = 7.5 Hz; D-Ala, 2-H), 4.88 (dd, 1 H, *J* = 5.5, 8 Hz; N$^\beta$-Me-L-A_2Pr, 2-H); HRMS(FAB) *m/z* calcd for $C_{41}H_{85}O_{13}N_{14}$ $(M+H)^+$ 981.6421, found 981.6440. *TSP was used as the external standard.
20. We are grateful to Professors T. Shiba and T. Wakamiya, Osaka University, for samples of galantin I and for their valuable suggestions, and to Dr. K. Tanaka, JEOL, for measurement of HRMS. We thank Prof. K. Nakanishi, Director, for encouragement. This work was supported in part by a grant-in-aid from the Ministry of Education, Science and Culture, Japan.

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