Tetrahydro-4(1*H*)-pyrimidinone Ring Formation of Bellenamine, a Biogenic Amine, with Carbonyl Compounds

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Bellenamine, (*R*)-3,6-diamino-*N*-(aminomethyl)hexanamide in a neutral aqueous solution at 37 °C formed a new tetrahydro-4(1*H*)-pyrimidinone compound, N^1,N^3-methylenebellenamine by the reaction with self-generated formaldehyde. The structure was elucidated by NMR spectral analyses. Two tetrahydro-4(1*H*)-pyrimidinone compounds, N^1,N^3-methylenebellenamine and (*R*)-6-(3-aminopropyl)tetrahydro-4(1*H*)-pyrimidinone (tentatively named cyclized bellenamine) were synthesized from bellenamine and D-β-lysinamide, respectively, with an equimolar amount of formalin in good yields. However, formation of a 5-membered ring 4-imidazolidinone compound by the reaction of L-lysinamide with formalin was a very low yield. Reaction of bellenamine with acetaldehyde gave a mixture of four diastereomeric aldol adducts. By the reaction with acetone or diacetone alcohol, two stereoisomeric diacetone adducts, (2*R*,9a*S*)- and (2*R*,9a*R*)-2-(3-aminopropyl)-8,8,9a-trimethyloctahydro-4*H*-pyrimido[1,6-*a*]pyrimidin-4-one, were obtained. Although these adducts were difficult to separate, the (2*R*,9a*R*) diastereomer was more stable than the other in acidic aqueous solution.

A biogenic amine, bellenamine produced by *Streptomyces nashvillensis* MD743-GF4 has immuno-enhancing activity and potent inhibitory effect of infection of T-cell with human immunodeficiency virus. The absolute structure was confirmed to be (*R*)-3,6-diamino-*N*-(aminomethyl)hexanamide by total synthesis.[1,2] In the biosynthetic studies, two interesting findings, the presence of new 2,3-aminomutase forming D-β-lysine from L-lysine and the incorporation of glycine into the *gem*-diamine structure, were reported.[3—5] Bellenamine in aqueous solutions at pH 3.7—10.6 was stable in the cold room for 3 weeks, but unstable by heating. In a neutral solution at 37 °C for 2—3 weeks, bellenamine was easily converted into (*R*)-6-(3-aminopropyl)tetrahydro-4(1*H*)-pyrimidinone (tentatively named cyclized bellenamine) by deamination of 1′-NH_2 as reported in our previous paper,[5] but keeping the solution for 1 week gave a new tetrahydro-4(1*H*)-pyrimidinone compound, N^1,N^3-methylenebellenamine in more than 20% yield. The new compound was prepared from bellenamine with formalin in a good yield. In this paper, the formation and structural elucidation of N^1,N^3-methylenebellenamine and new tetrahydro-4(1*H*)-pyrimidinone compounds prepared by reactions of bellenamine with formaldehyde, acetaldehyde and acetone, are reported.

Results and Discussion

Isolation and Structure of N^1,N^3-Methylenebellenamine. N^1,N^3-Methylenebellenamine was isolated from a neutral aqueous solution of bellenamine after heating at 37 °C for 6 d in 20% yield, along with bellenamine[1] (34%), cyclized bellenamine[5] (27%), and D-β-lysinamide[5] (19%) (Fig. 1). The structure of newly isolated N^1,N^3-methylenebellenamine was determined to be (*R*)-3-aminomethyl-6-(3-aminopropyl)tetrahydro-4(1*H*)-pyrimidinone by the ^{1}H and ^{13}C NMR spectra and HMBC experiment (Fig. 2), and by formation of its

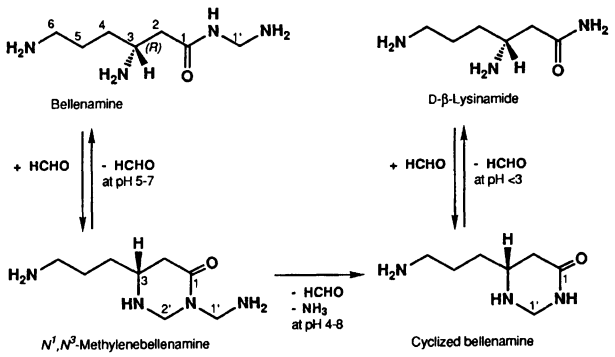

Fig. 1. Formation of N^1,N^3-methylenebellenamine and cyclized bellenamine. Numberings are assigned according to those of bellenamine.

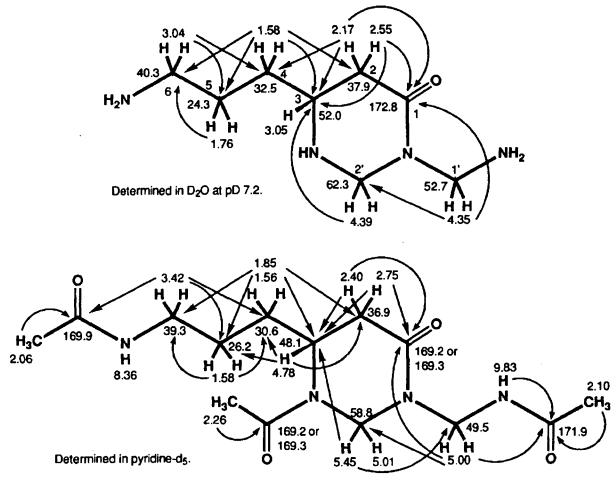

Fig. 2. Summary of HMBC experiments of N^1,N^3-methylenebellenamine and its tri-*N*-acetyl derivative.

tri-*N*-acetyl derivative and bis(Schiff bases). The deuterium isotope effect[6] of N^1,N^3-methylenebellenamine on ^{13}C NMR in D_2O and H_2O solutions was not observed at carbonyl carbon, showing that no amide pro-

ton exists, as shown in Fig. 3. Furthermore, NOE between 3-H at $\delta=3.24$ and 2′-H at $\delta=4.71$ was clearly shown in a pyridine-d_5/D_2O (1 : 1) solution.

N^1,N^3-Methylenebellenamine was unstable in an acidic solution at 50 °C; it was converted into cyclized bellenamine in more than 50% yield and regenerated a small amount of bellenamine at pH 4.8 for 4 h (Table 1, Fig. 1). N^1,N^3-Methylenebellenamine has antibacterial activity against *Bacillus subtilis* PCI219, but weaker than that of bellenamine.

Arylmethylene Schiff Bases of Bellenamine and N^1,N^3-Methylenebellenamine. Reactions of bellenamine with salicylaldehyde, 5-chlorosalicylaldehyde and 2-hydroxy-1-naphthalenecarbaldehyde[7] gave three crystalline 1′,3,6-tri-*N*-salicylidene-, 1′,3,6-tris[*N*-(5-chlorosalicylidene)]-, and 1′,3,6-tris[*N*-(2-hydroxy-1-naphthylmethylene)]bellenamine, respectively. While two bis(Schiff bases) (Fig. 4) were prepared from N^1,N^3-methylenebellenamine. Their ^{1}H NMR spectra in DMSO-d_6 showed that the salicylidene Schiff bases of bellenamine and N^1,N^3-methylenebellenamine have usual azomethine (phenol-imine) type linkages (−N=CH−), but the 2-hydroxy-1-naphthylmethylene Schiff bases are enamine (γ-keto-enamine) type tautomers[8,9] having two −NH−CH= linkages: in ^{1}H NMR of 1′,6-bis[*N*-(2-hydroxy-1-naphthylmethylene)]-N^1,N^3-methylenebellenamine, the methine signals at $\delta=9.09$ and 9.16 coupling with 6-NH and 1′-NH ($J=10.4$ and 9.1 Hz, respectively), and 1′-methylene

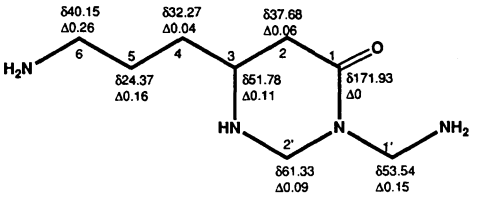

Fig. 3. Deuterium isotope effects of N^1,N^3-methylenebellenamine on ^{13}C NMR. δ (ppm) in D_2O at pD 9.0. Δ (ppm)=(δ in H_2O at pH 9.0) − (δ in D_2O at pD 9.0).

Table 1. Stability of N^1, N^3-Methylenebellenamine in Aqueous Solution at 50 °C

Time		Compound/mol%		
		Methylene-	Cyclized	
h	pH	bellenamine	bellenamine	Bellenamine
	8.7	89	11	0
2	7.5	78	20	3
	4.8	46	42	12
	8.7	82	18	0
4	7.5	74	25	2
	4.8	35	58	7

The aqueous solution of N^1,N^3-methylenebellenamine (500 μg ml^{-1}) adjusted to each pH with 0.01 M HCl were used. Compounds were determined by HPLC.

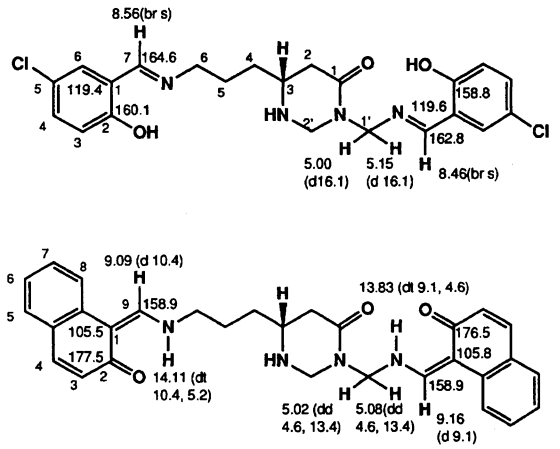

Fig. 4. Structures of two Schiff bases from N^1,N^3-methylenebellenamine. ^{1}H and ^{13}C NMR chemical shifts (δ) of Schiff base-related protons and carbons in DMSO-d_6 are shown. Multiplicities and coupling constants (J/Hz) are in parentheses.

protons at $\delta=5.02$ and 5.08 coupling with 1′-NH ($J=4.6$ Hz). The structure of the enamine tautomer was also confirmed by deuterium isotope effects[6] in the DMSO-d_6 and $CDCl_3$ solutions (data are not shown).

Formation of N^1,N^3-Methylenebellenamine from Bellenamine with Formaldehyde. A new 6-membered ring amine, N^1,N^3-methylenebellenamine could be formed by the reaction of bellenamine with an equimolar amount of formaldehyde in an alkaline solution at 50 °C (Table 2, Fig. 1). The synthetic N^1,N^3-methylenebellenamine was identical with the compound isolated from a neutral aqueous solution of bellenamine, in all respects including the optical activity. A similar cyclocondensation of formaldehyde to give hexahydropyrimidine compound was reported by Ganem,[10—12] when spermine or spermidine was treated with formalin. The newly formed 2′-methylene group was introduced by intermolecular reaction with formaldehyde generated from the 1′-methylene group in bellenamine. It was confirmed by incorporation of ^{13}C into the 2′-methylene from [1′-^{13}C]bellenamine which was prepared by fermentation of *Streptomyces nashvillensis* MD743-GF4 in a synthetic medium by feeding of [2-^{13}C]glycine.[4] [1′-^{13}C]Bellenamine (29% enrichment) in an aqueous solution (pH 7.7) at 37 °C for 6 d was converted into [1′, 2′-$^{13}C_2$]N^1,N^3-methylenebellenamine (each 30% enrichment) in 12% yield. While, treatment with an equimolar amount of formaldehyde (pH 8.5, 50 °C, 2 h) gave [1′-^{13}C]N^1,N^3-methylenebellenamine (26% enrichment) in 74% yield.

Reaction of D-β-Lysinamide and L-Lysinamide with Formaldehyde. Reaction of D-β-lysinamide with an equimolar amount of formaldehyde in an aqueous solution at 50 °C for 2 h gave cyclized bellenamine in a good yield (Fig. 1). However, a 5-membered imidazolidine formation by the reaction of L-lysinamide was

Table 2. Formation of N^1, N^3-Methylenebellenamine from Bellenamine with Formaline at 50 °C

Time	HCHO		Compound/mol%			
h	(1 equiv)	pH	Bellenamine	Methylene-bellenamine	Cyclized bellenamine	D-β-Lysinamide
	−	10.6	100	0	0	0
1	−	7.6	96	3	1	0
	+	10.6	43	56	1	0
	+	7.6	32	59	9	0
	−	10.6	99	1	0	0
2	−	7.6	91	4	1	4
	+	10.6	32	65	3	0
	+	7.6	25	58	16	0
	−	10.6	98	2	0	0
4	−	7.6	83	7	2	7
	+	10.6	27	68	6	0
	+	7.6	16	52	33	0

The aqueous solution (pH 10.6) of bellenamine (1740 μg ml^{-1}) and the neutralized solution (pH 7.6 with 1 M HCl) were used. Compounds were determined by HPLC.

poor (6% yield) (Fig. 5). In general, imidazolidine formation from 1,2-diamines with formaldehyde was easier than hexahydropyrimidine formation from 1,3-diamines, and more than 7-membered diazaheterocycles could not be formed.[10] L-Lysinamide was difficult to form a 5-membered imidazolidine ring for existence of its amide carbonyl group.

Reactions of Bellenamine with Acetaldehyde and Acetone. Reaction of bellenamine with acetaldehyde in an aqueous solution gave a mixture of four diastereomeric aldol adducts (condensation products with 2 moles of acetaldehyde) (Fig. 5). Reaction with acetone afforded a mixture of two diastereomeric diacetone adducts (condensation products with 2 moles of acetone) (Fig. 5) in a poor yield. However, the diacetone adducts were prepared by the reaction with diacetone alcohol (4-hydroxy-4-methyl-2-pentanone) in a good yield. These two diastereomers were difficult to separate, but they were different in their stabilities, as shown in Table 3. The structure of the stable diastereomer was determined by ^1H and ^{13}C NMR (Table 4), and HMBC and NOE experiments to be (2*R*,9a*R*)-2-(3-aminopropyl)-8,8,9a-trimethyloctahydro-4*H*-pyrimido[1,6-*a*]pyrimidin-4-one (Fig. 6); NOEs between 9a-CH_3 (δ=1.60) and 2-H (δ=3.33), 9a-CH_3 and 8-CH_3 (β) (δ=1.34), and 9a-CH_3 and 6-H(β) (δ=3.86) were observed, but no NOE between 2-H and 9-H_2. Similarly, the structure of the unstable diastereomer was determined to be 9a*S* configuration (Fig. 6); NOEs between 2-H (δ=3.11) and 9-H(β) (δ=1.82), 9a-CH_3 (δ=1.62), and 8-CH_3(α) (δ=1.33), and 9a-CH_3 and 6-

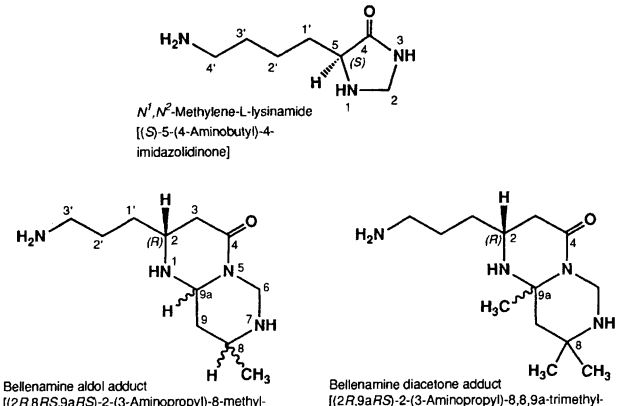

Fig. 5. Structures of N^1,N^2-methylene-L-lysinamide and two addition derivatives of bellenamine.

Table 3. Stability of Diastereomers of Bellenamine Diacetone Adduct in Aqueous Solutions

Time	pD	Compound/mol%[a]		
d		Unstable diastereomer (9a*S*)	Stable diastereomer (9a*R*)	Regenerated bellenamine
	4.7	56	37	<5
0	3.6	57	34	<5
	1.9	57	31	<5
	4.7	27	40	38
4	3.6	23	32	42
	1.9	16	25	43
	4.7	17	35	47
6	3.6	15	30	49
	1.9	15	23	51
	4.7	<5	33	52
12	3.6	<5	27	55
	1.9	<5	18	56

a) Each compound was calculated from the signal strength in the ^1H NMR (see text).

Table 4. ^{13}C and ^{1}H NMR Spectral Data of Diastereomers of Bellenamine Diacetone Adduct

Position	Unstable diastereomer (9a*S*)[a]			Stable diastereomer (9a*R*)[b]		
	δ_C	δ_H (J/Hz)		δ_C	δ_H (J/Hz)	
2	46.2	3.11 m		45.0	3.33m	
3 (α)	38.9	2.19 dd	(11.7, 17.6)	37.9	2.19 dd	(11.2, 17.6)
(β)		2.49 dd	(2.9, 17.6)		2.55 dd	(4.4, 17.6)
4	171.7			170.3		
6 (α)	50.0	3.97 d	(13.7)	49.2	5.23 d	(14.2)
(β)		5.24 d	(13.7)		3.86 d	(14.2)
8	50.7			49.9		
9 (α)	47.7	1.69 d	(14.2)	50.7	1.47 d	(13.7)
(β)		1.82 d	(14.2)		1.95 d	(13.7)
9a	71.8			70.9		
8-CH_3 (α)	26.0	1.33 s		32.9	1.16 s	
8-CH_3 (β)	32.7	1.15 s		24.6	1.34 s	
9a-CH_3	27.5	1.62 s		23.6	1.60 s	
1′	31.9	1.54 m		32.7	1.54 m	
2′	24.5	1.76 m		24.7	1.76 m	
3′	40.2	3.02 t	(7.8)	40.3	3.02 t	(7.3)

a) A 84:16 mixture (9a*S*:9a*R*) was measured in D_2O at pD 8.3. b) A 14:86 mixture was measured at pD 8.6. Signals of each minor diastereomer are omitted.

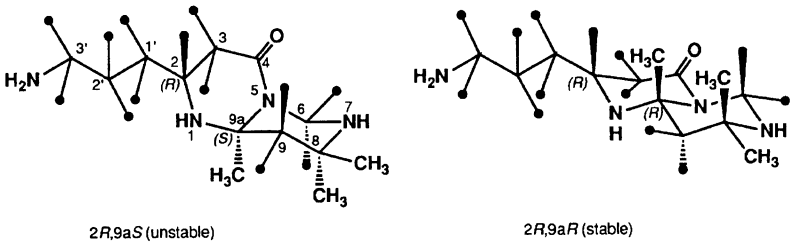

Fig. 6. Two diastereomers of bellenamine diacetone adduct.

H(α) (δ=3.97) were observed, but NOEs between 2-H and three CH_3 groups were not.

Experimental

Melting points were obtained using an Electrothermal IA9100 digital melting point apparatus and were not corrected. Optical rotations were recorded on a Perkin–Elmer 241 polarimeter. IR spectra were taken on a Hitachi 260-10 spectrophotometer. ^{1}H NMR spectra were recorded on a JEOL JNM-EX400 spectrometer at 400 MHz using tetramethylsilane (δ=0) or HOD (δ=4.80) as an internal standard and ^{13}C NMR spectra at 100 MHz were recorded with tetramethylsilane or dioxane (δ=67.4). MS were measured on a JEOL JMS-SX102 mass spectrometer in a FAB mode. HPLC on a Waters 600E system using Waters Optipak CE column (3.9×150 mm) with a guard column (3.9×35 mm) and 0.36% $HClO_4$ (pH 1.5) as a mobile phase, was performed at 15.0 °C and a flow rate of 0.4 ml min^{-1}, and the retention time (R_t) was recorded.[4] High-voltage paper electrophoresis (HVPE) was performed on a CAMAG HVE system at 3300 volts for 10 min, using HCOOH–CH_3COOH–H_2O (25:75:900, pH 1.8) as an electrolyte solution and the relative mobility (R_m) to alanine was calculated.[13] TLC was carried out on silica-gel plates (E. Merck, Art. 5715) developed with $CHCl_3$–CH_3OH–25% aqueous ammonia (2:2:1) and R_f values of ninhydrin-positive spots were calculated.

Isolation of N^1,N^3-Methylenebellenamine from Bellenamine Solution. An aqueous solution (2.0 ml) of bellenamine (285 mg, 1.64 mmol) was adjusted to pH 7.5 with 3.2 ml of 1 M (1 M=1 mol dm^{-3}) hydrochloric acid and kept at 37 °C for 6 d. The solution was passed through a column of Amberlite CG-50 (NH_4^+, 120 ml). The column was washed with water (200 ml) and eluted with 1.5% (1000 ml) and 2.5% (400 ml) aqueous ammonia, successively. Eluate fractions of 10 ml were collected. Fractions 18-23 showing R_t 9.7 min (HPLC), R_m 2.30 (HVPE), and R_f 0.38 (TLC) were concentrated to give a colorless solid of N^1,N^3-methylenebellenamine (60 mg, 0.32 mmol). Mp 128—140 °C (decomp); $[\alpha]_D^{26}$ −64° (c 0.6, H_2O); IR (KBr) 3380, 2950, 1620, 1510, 1480, 1400, 1340, 1160, 920, 880, and 830 cm^{-1}; ^{1}H NMR (D_2O, pD 5.9) δ=1.59 (2H, m, 4-H_2), 1.76 (2H, m, 5-H_2), 2.22 (1H, dd, J=10.9 and 17.8 Hz, 2-H), 2.58 (1H, dd, J=4.6 and 17.8 Hz, 2-H), 3.03 (2H, t, J=7.3 Hz, 6-H_2), 3.06 (1H, m, 3-H), 4.42 (2H, s, 2′-H_2), 4.54 (1H, d, J=12.9 Hz, 1′-H), and 4.56 (1H, d, J=12.9 Hz, 1′-H); (D_2O, pD 7.2) δ=1.58 (2H, m, 4-H_2), 1.76 (2H, m, 5-H_2), 2.17 (1H, dd, J=10.9 and 17.8 Hz, 2-H), 2.55 (1H, dd, J=4.6 and 17.8 Hz, 2-H), 3.04 (2H, t, J=7.3 Hz, 6-H_2), 3.05 (1H, m, 3-H), 4.35 (2H, m, 1′-H_2), and 4.39 (2H, s, 2′-H_2); (pyridine-d_5/D_2O, 1 :1) δ=1.85 (2H, m, 4-H_2), 2.13 (2H, m, 5-H_2), 2.50 (1H, dd, J=11 and 18 Hz, 2-H), 2.80 (1H, dd, J=4.4 and 18 Hz, 2-H), 3.24 (1H, m, 3-H), 3.39 (2H, m, 6-H_2), 4.71 (1H, d, J=11 Hz, 2′-H), 4.75 (1H, d, J=13 Hz, 1′-H), 4.82 (1H, d, J=11 Hz, 2′-H), and 4.88 (1H, d, J=13 Hz, 1′-H);

^{13}C NMR (D_2O, pD 5.9) δ=24.1 (C-5), 32.3 (C-4), 37.8 (C-2), 40.1 (C-6), 51.2 (C-1′), 51.9 (C-3), 63.1 (C-2′), and 173.5 (C-1); (D_2O, pD 7.2) δ=24.2 (C-5), 32.5 (C-4), 37.9 (C-2), 40.2 (C-6), 52.0 (C-3), 52.7 (C-1′), 62.2 (C-2′), and 172.7 (C-1); MS (FAB, positive) m/z 187 (MH^+). Found: C, 46.63; H, 8.37; N, 26.03; O, 19.02%. Calcd for $C_8H_{18}N_4O \cdot 1/2H_2CO_3$: C, 46.99; H, 8.81; N, 25.79; O, 18.41%. Fractions 26—33 showing R_t 6.5 min (HPLC), R_m 2.10 (HVPE), and R_f 0.38 (TLC) were concentrated to obtain cyclized bellenamine[5] (70 mg, 0.45 mmol). Fractions 63—89 showing R_t 10.8 min (HPLC), R_m 2.50 (HVPE), and R_f 0.21 (TLC) led to the recovery of bellenamine (117 mg, 0.56 mmol). From fractions 108—119 showing R_t 6.8 min (HPLC), R_m 2.20 (HVPE), and R_f 0.22 (TLC), D-β-lysinamide[5] (45 mg, 0.31 mmol) was afforded.

Tri-*N*-acetyl-N^1,N^3-methylenebellenamine. A mixture of N^1,N^3-methylenebellenamine (19 mg), methanol (1 ml), and acetic anhydride (0.2 ml) was kept overnight at room temperature. After evaporation, the residue was washed with diethyl ether (3 ml) to give tri-*N*-acetyl-N^1,N^3-methylenebellenamine (25 mg) as a colorless syrup; $[\alpha]_D^{23}$ +22° (*c* 0.5, H_2O); IR (KBr) 3430, 3280, 3090, 2940, 1650, 1560, 1430, 1375, 1280, 1200, 1100, and 1040 cm^{-1}; ^{1}H NMR (pyridine-d_5) δ=1.56 (1H, m, 4-H), 1.58 (2H, m, 5-H_2), 1.85 (1H, m, 4-H), 2.06 (3H, s, 6-NCOCH$_3$), 2.10 (3H, s, 1′-NCOCH$_3$), 2.26 (3H, s, 3-NCOCH$_3$), 2.40 (1H, dd, *J*=5.0 and 16.5 Hz, 2-H), 2.75 (1H, dd, *J*=6.9 and 16.5 Hz, 2-H), 3.42 (2H, m, 6-H_2), 4.78 (1H, m, 3-H), 5.00 (2H, d, *J*=6.6 Hz, 1′-H_2), 5.01 (1H, d, 2′-H), 5.45 (1H, d, *J*=12.5 Hz, 2′-H), 8.35 (1H, br, 6-NH), and 9.83 (1H, br t, *J*=6.6 Hz, 1′-NH); ^{13}C NMR (pyridine-d_5) δ=21.9 (3-*N*-acetyl CH_3), 22.8 (1′-*N*-acetyl CH_3), 23.1 (6-*N*-acetyl CH_3) 26.2 (C-5), 30.6 (C-4), 36.9 (C-2), 39.3 (C-6), 48.1 (C-3), 49.5 (C-1′), 58.8 (C-2′), 169.2 (3-NCO or C-1), 169.3 (C-1 or 3-NCO), 169.9 (6-NCO), and 171.9 (1′-NCO) (all protons and carbons were assigned by HMBC experiment as shown in Fig. 2); MS (FAB, positive) m/z 313 (MH^+). Found: C, 54.04; H, 7.17; N, 17.80; O, 20.45%. Calcd for $C_{14}H_{24}N_4O_4$: C, 53.83; H, 7.74; N, 17.94; O, 20.49%.

***N*-(Arylmethylene)bellenamines.** A suspension of bellenamine (125.6 mg, 0.72 mmol) and salicylaldehyde (582.5 mg, 4.77 mmol) in a mixture of ethanol (30 ml) and methanol (2.2 ml) was stirred overnight at room temperature.[7] Pale yellow needles of 1′,3,6-tri-*N*-salicylidenebellenamine (338 mg, 97%) were collected by filtration and washed with diethyl ether (5 ml). Mp 120—121 °C; $[\alpha]_D^{25}$ −13° (*c* 0.2, $CHCl_3$); IR (KBr) 3325, 2950, 2880, 1660, 1645, 1610, 1590, 1550, 1510, 1475, 1430, 1360, 1290, 1220, 1160, 1130, 1050, 1030, 890, 865, 760, and 750 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ=1.64 (3H, m, 4-H and 5-H_2), 1.72 (1H, m, 4-H), 2.58 (1H, dd, *J*=8.8 and 14.7 Hz, 2-H), 2.66 (1H, dd, *J*=4.4 and 14.7 Hz, 2-H), 3.60 (2H, br t, 6-H_2), 3.77 (1H, m, 3-H), 4.85 (1H, ddd, *J*=2.0, 5.9, and 15.1 Hz, 1′-H), 4.91 (2H, ddd, *J*=2.0, 5.9, and 15.1 Hz, 1′-H), 6.77 (1H, t, *J*=7.8 Hz, $N^{1'}$-5-H), 6.84 (1H, d, *J*=7.8 Hz, $N^{1'}$-3-H), 6.86 (1H, t, *N*-5-H), 6.87 (1H, d, *N*-3-H), 6.88 (1H, t, *N*-5-H), 6.88 (1H, d, *N*-3-H), 7.05 (1H, dd, *J*=1.5 and 7.8 Hz, $N^{1'}$-6-H), 7.30, 7.31, 7.34 (3H, each t, *N*-4-H), 7.41 (2H, each dd, *J*=1.5 and 7.8 Hz, N^3- and N^6-6-H), 8.26 (1H, s, $N^{1'}$-7-H), 8.54 (2H, s, N^3- and N^6-7-H), 8.71 (1H, br t, *J*=6.2 Hz, amide NH), 13.03 (1H, br, OH), 13.42 (1H, br, OH), and 13.56 (1H, br, OH); ^{13}C NMR (DMSO-d_6) δ=27.1 (C-5), 33.0 (C-4), 42.0 (C-2), 57.9 (C-6), 59.0 (C-1′), 65.2 (C-3), 116.2, 116.4, 116.4 (*N*-C-3), 118.2, 118.3, 118.4 (*N*-C-1), 118.5, 118.6, 118.6 (*N*-C-5), 131.5, 131.6, 131.7 (*N*-C-6), 132.1, 132.3, 132.3 (*N*-C-4), 160.0, 160.4, 160.7 (*N*-C-2), 162.9 ($N^{1'}$-C-7), 165.3 (N^3-C-7), 165.8 (N^6-C-7), and 170.6 (C-1); MS (FAB, positive) m/z 487 (MH^+). Found: C, 68.81; H, 6.27; N, 11.63; O, 13.16%. Calcd for $C_{28}H_{30}N_4O_4$: C, 69.12; H, 6.21; N, 11.51; O, 13.15%.

1′,3,6-Tris[*N*-(5-chlorosalicylidene)]bellenamine: Yellowish crystals (320 mg, 90%) were obtained from bellenamine (105 mg, 0.60 mmol) and 5-chlorosalicylaldehyde (427 mg, 2.73 mmol). Mp 172—173 °C; $[\alpha]_D^{28}$ +1.2° (*c* 0.5, $CHCl_3$); IR (KBr) 3320, 2925, 2860, 1660, 1645, 1580, 1540, 1485, 1380, 1280, 1215, 880, 830, and 695 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ=1.64 (2H, m, 4-H_2), 1.67 (1H, m, 5-H), 1.72 (1H, m, 5-H), 2.57 (1H, dd, *J*=8.9 and 14.4 Hz, 2-H), 2.65 (1H, dd, *J*=4.3 and 14.4 Hz, 2-H), 3.61 (2H, br t, *J*=6.4 Hz, 6-H_2), 3.76 (1H, m, 3-H), 4.84 (1H, dd, *J*=4.7 and 15.0 Hz, 1′-H), 4.92 (1H, dd, *J*=4.7 and 15.0 Hz, 1′-H), 6.87 (1H, d, *J*=8.8 Hz, $N^{1'}$-3-H), 6.88 (1H, d, *J*=8.5 Hz, N^6-3-H), 6.88 (1H, d, *J*=8.9 Hz, N^3-3-H), 7.32 (1H, dd, *J*=2.7 and 8.8 Hz, N^3-4-H), 7.34 (2H, dd, *J*=2.8 and 8.8 Hz, N^6- and $N^{1'}$-4-H), 7.37 (1H, d, *J*=2.4 Hz, $N^{1'}$-6-H), 7.49 (1H, d, *J*=2.8 Hz, N^3-6-H), 7.51 (1H, d, *J*=2.7 Hz, N^6-6-H), 8.33 (1H, br s, $N^{1'}$-7-H), 8.51 (1H, s, N^3-7-H), 8.53 (1H, s, N^6-7-H), 8.74 (1H, t, *J*=6.1 Hz, amide NH), 13.1 (1H, br, OH), 13.4 (1H, br, OH), and 13.7 (1H, br, OH); ^{13}C NMR (DMSO-d_6) δ=26.8 (C-5), 32.8 (C-4), 41.7 (C-2), 57.6 (C-6), 59.1 (C-1′), 65.1 (C-3), 118.3, 118.4, 118.6 (*N*-C-3), 119.3, 119.4, 119.4 (*N*-C-1), 121.5, 121.9, 122.0 (*N*-C-5), 130.3, 130.4, 130.5 (*N*-C-6), 131.8, 131.9, 132.0 (*N*-C-4), 158.9 ($N^{1'}$-C-2), 159.2 (N^3-C-2), 159.9 (N^6-C-2), 162.0 ($N^{1'}$-C-7), 164.2 (N^3-C-7), 164.7 (N^6-C-7), and 170.5 (C-1); MS (FAB, positive) m/z 589 (MH^+). Found: C, 56.85; H, 4.58; N, 9.31; O, 10.59%. Calcd for $C_{28}H_{27}N_4O_4Cl_3$: C, 57.01; H, 4.61; N, 9.50; O, 10.85%.

1′,3,6-Tris[*N*-(2-hydroxy-1-naphthylmethylene)]bellenamine (γ-Keto–Enamine Tautomer): Yellowish needles (467 mg, 99%) were obtained from bellenamine (129 mg, 0.74 mmol) and 2-hydroxy-1-naphthalenecarbaldehyde (575 mg, 3.34 mmol). Mp 192—196 °C; $[\alpha]_D^{28}$ −77° (*c* 0.2, $CHCl_3$); IR (KBr) 3200, 3050, 2950, 1680, 1630, 1540, 1490, 1445, 1400, 1360, 1280, 1215, 1190, 1160, 1100, 995, 860, 840, and 755 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ=1.75 (4H, m, 4-H_2 and 5-H_2), 2.72 (2H, m, 2-H_2), 3.67 (2H, br m, 6-H_2), 4.13 (1H, br, 3-H), 4.86 (1H, ddd, *J*=4.8, 6.8, and 12.8 Hz, 1′-H), 4.96 (1H, ddd, *J*=4.8, 6.8, and 12.8 Hz, 1′-H), 6.70 (1H, d, *J*=9.8 Hz, *N*-3-H), 6.71 (1H, d, *J*=9.3 Hz, *N*-3-H), 6.75 (1H, d, *J*=9.3 Hz, *N*-3-H), 7.15 (1H, dd, *N*-6-H), 7.20 (2H, dd, *N*-6-H), 7.38 (1H, dd, *N*-7-H), 7.40 (1H, dd, *N*-7-H), 7.41 (1H, dd, *N*-7-H), 7.61 (1H, d, *N*-5-H), 7.63 (2H, d, *N*-5-H), 7.71 (1H, d, *N*-4-H), 7.73 (2H, d, *J*=9.3 Hz, *N*-4-H), 7.99 (1H, d, *J*=8.8 Hz, *N*-8-H), 8.03 (1H, d, *J*=8.3 Hz, *N*-8-H), 8.10 (1H, d, *J*=7.8 Hz, *N*-8-H), 8.98 (1H, t, *J*=6.1 Hz, amide NH), 9.09 (1H, d, *J*=10.5 Hz, *N*-9-H), 9.10 (1H, d, *J*=9.3 Hz, *N*-9-H), 9.20 (1H, d, *J*=8.3 Hz, *N*-9-H), 13.90 (1H, dt, *J*=3.1 and 7.1 Hz, OH), 14.12 (1H, dt, *J*=3.9 and 7.8 Hz, OH), and 14.43 (1H, dd, *J*=6.1 and 7.1 Hz, OH); ^{13}C NMR (DMSO-d_6) δ=26.8 (C-5), 31.9 (C-4), 40.9 (C-2), 50.3 (C-6), 54.8 (C-1′), 59.5 (C-3), 105.6, 105.7, 106.0 (*N*-C-1), 118.3, 118.4, 118.7 (*N*-C-8), 122.0, 122.3, 122.4 (*N*-C-6), 124.2, 124.9, 125.1 (*N*-C-3), 125.4, 125.5, 125.5 (*N*-C-4a), 127.7, 127.7, 127.9 (*N*-C-7), 128.7, 128.7, 128.9 (*N*-C-

5), 133.8, 134.0, 134.2 (*N*-C-8a), 136.5, 136.9, 137.3 (*N*-C-4), 158.4, 158.9, 159.0 (*N*-C-9), 170.6 (C-1), 174.5, 176.3, and 177.2 (*N*-C-2); MS (FAB, positive) m/z 637 (MH^+). Found: C, 75.14; H, 5.62; N, 8.75; O, 10.38%. Calcd for $C_{40}H_{36}N_4O_4$: C, 75.45; H, 5.70; N, 8.80; O, 10.05%.

***N*-(Arylmethylene)-N^1,N^3-methylenebellenamines.** A suspension of N^1,N^3-methylenebellenamine (54 mg, 0.29 mmol) and 5-chlorosalicylaldehyde (136 mg, 0.87 mmol) in a mixture of ethanol (15 ml) and methanol (1 ml) was stirred overnight at room temperature.[7] Yellowish crystals of 1′, 6-bis[*N*-(5-chlorosalicylidene)]-N^1,N^3-methylenebellenamine (63 mg, 48%) were collected by filtration and washed with diethyl ether (7 ml). Mp 147—148 °C; $[\alpha]_D^{28}$ −30° (c 0.25, $CHCl_3$); IR (KBr) 3400w, 3260w, 2930, 2860, 1660, 1645, 1580, 1485, 1390, 1380, 1350, 1330, 1280, 1195, 1180, 1100, 1045, 1000, 920, 900, and 845 cm^{-1}; ^1H NMR (DMSO-d_6) δ=1.44 (2H, m, 4-H_2), 1.71 (1H, m, 5-H), 1.78 (1H, m, 5-H), 2.03 (1H, dd, J=10.7 and 17.1 Hz, 2-H), 2.35 (1H, dd, J=4.4 and 17.1 Hz, 2-H), 2.51 (1H, m, 3-NH), 2.94 (1H, m, 3-H), 3.62 (2H, br t, J=6.6 Hz, 6-H_2), 4.23 (2H, m, 2′-H_2), 5.00 (1H, d, J=16.1 Hz, 1′-H), 5.15 (1H, d, J=16.1 Hz, 1′-H), 6.90 (1H, d, J=8.8 Hz, N^6-3-H), 6.93 (1H, d, J=8.8 Hz, $N^{1'}$-3-H), 7.34 (1H, dd, J=2.9 and 8.8 Hz, N^6-4-H), 7.38 (1H, dd, J=2.9 and 8.8 Hz, $N^{1'}$-4-H), 7.54 (1H, d, J=2.9 Hz, N^6-6-H), 7.65 (1H, d, J=2.9 Hz, $N^{1'}$-6-H), 8.46 (1H, s, $N^{1'}$-7-H), 8.56 (1H, s, N^6-7-H), 12.97 (1H, br s, OH), and 13.78 (1H, br s, OH); ^{13}C NMR (DMSO-d_6) δ=26.6 (C-5), 32.6 (C-4), 38.5 (C-2), 51.7 (C-3), 57.7 (C-6), 62.2 (C-2′), 63.5 (C-1′), 118.4 ($N^{1'}$-C-3), 118.6 (N^6-C-3), 119.4 (N^6-C-1), 119.6 ($N^{1'}$-C-1), 121.4 (N^6-C-5), 122.0 ($N^{1'}$-C-5), 130.4 (N^6-C-6), 130.7 ($N^{1'}$-C-6), 131.8 (N^6-C-4), 132.1 ($N^{1'}$-C-4), 158.8 ($N^{1'}$-C-2), 160.1 (N^6-C-2), 162.8 ($N^{1'}$-C-7), 164.6 (N^6-C-7), and 168.2 (C-1) (all protons and carbons were assigned by HMBC experiment); MS (FAB, positive) m/z 463 (MH^+). Found: C, 56.83; H, 5.50; N, 12.24; O, 10.10%. Calcd for $C_{22}H_{24}N_4O_3Cl_2$: C, 57.03; H, 5.22; N, 12.09; O, 10.36%.

1′, 6- Bis[*N*- (2- hydroxy- 1- naphthylmethylene)]-N^1,N^3-methylenebellenamine (γ-Keto–Enamine Tautomer): Yellowish crystals (145 mg, 69%) were obtained from N^1,N^3-methylenebellenamine (80 mg, 0.43 mmol) and 2-hydroxy-1-naphthalenecarbaldehyde (223 mg, 1.30 mmol). Mp 180—181 °C; $[\alpha]_D^{23}$ −37° (c 0.2, $CHCl_3$); IR (KBr) 3460, 3330, 3100, 2980, 2920, 1660, 1570, 1520, 1425, 1380, 1340, 1300, 1235, 1190, 1160, 1060, 1030, 890, 865, and 770 cm^{-1}; ^1H NMR (DMSO-d_6) δ=1.44 (2H, m, 4-H_2), 1.72 (1H, m, 5-H), 1.78 (1H, m, 5-H), 2.02 (1H, dd, J=10.7 and 17.1 Hz, 2-H), 2.36 (1H, dd, J=3.9 and 17.1 Hz, 2-H), 2.84 (1H, br, 2′-H), 2.90 (1H, br, 3-H), 3.65 (2H, m, 6-H_2), 4.28 (1H, br t, J=10.0 Hz, 2′-H), 4.36 (1H, br d, J=10.7 Hz, 3-NH), 5.02 (1H, dd, J=4.6 and 13.4 Hz, 1′-H), 5.08 (1H, dd, J=4.6 and 13.4 Hz, 1′-H), 6.70 (1H, d, J=9.4 Hz, N^6-3-H), 6.74 (1H, d, J=9.1 Hz, $N^{1'}$-3-H), 7.18 (1H, br dd, N^6-6-H), 7.23 (1H, br dd, $N^{1'}$-6-H), 7.41 (1H, ddd, N^6-7-H), 7.47 (1H, ddd, $N^{1'}$-7-H), 7.62 (1H, dd, J=0.9 and 7.9 Hz, N^6-5-H), 7.66 (1H, dd, J=0.9 and 7.9 Hz, $N^{1'}$-5-H), 7.70 (1H, d, J=9.5 Hz, N^6-4-H), 7.76 (1H, d, J=9.4 Hz, $N^{1'}$-4-H), 8.05 (1H, d, J=8.2 Hz, $N^{1'}$-8-H), 8.06 (1H, d, J=8.2 Hz, N^6-8-H), 9.09 (1H, d, J=10.4 Hz, N^6-9-H), 9.16 (1H, d, J=9.1 Hz, $N^{1'}$-9-H), 13.83 (1H, dt, J=4.6 and 9.2 Hz, 1′-NH), and 14.11 (1H, dt, J=5.2 and 10.4 Hz, 6-NH); ^{13}C NMR (DMSO-d_6) δ=26.7 (C-5), 32.0 (C-4), 38.5 (C-2), 50.4 (C-6), 51.5 (C-3), 59.0 (C-1′), 62.2 (C-2′), 105.5 (N^6-C-1), 105.8 ($N^{1'}$-C-1), 118.3 (N^6-C-8), 118.6 ($N^{1'}$-C-8), 121.9 (N^6-C-6), 122.5 ($N^{1'}$-C-6), 124.8 ($N^{1'}$-C-3), 125.0 (N^6-C-4a), 125.5 ($N^{1'}$-C-4a), 125.6 (N^6-C-3), 127.7 (N^6-C-7), 128.0 ($N^{1'}$-C-7), 128.7 (N^6-C-5), 128.9 ($N^{1'}$-C-5), 134.0 ($N^{1'}$-C-8a), 134.2 (N^6-C-8a), 136.9 (N^6-C-4), 137.4 ($N^{1'}$-C-4), 158.9 ($N^{1'}$- and N^6-C-9), 168.6 (C-1), 176.5 ($N^{1'}$-C-2), and 177.5 (N^6-C-2) (all protons and carbons were assigned by HMBC experiment); MS (FAB, positive) m/z 495 (MH^+). Found: C, 72.87; H, 6.29; N, 11.51; O, 9.69%. Calcd for $C_{30}H_{30}N_4O_3$: C, 72.85; H, 6.11; N, 11.33; O, 9.70%.

N^1,N^3-Methylenebellenamine from Bellenamine with Formaldehyde. Bellenamine (43 mg, 0.25 mmol) was dissolved in a dilute solution of formalin (0.37%, 2 ml, 0.25 mmol) and the solution was kept at 50 °C for 2 h. The products were purified by column chromatography on Amberlite CG-50 (NH_4^+, 20 ml) to yield 21 mg of N^1,N^3-methylenebellenamine (45%); $[\alpha]_D^{20}$ −63° (c 1.0, H_2O), along with 14 mg of recovered bellenamine (33%). The product was identical with the authentic sample in all respects.

[1′-^{13}C]Bellenamine. As already reported,[3—5] *S. nashvillensis* MD743-GF4 was cultured in a 500-ml baffled Erlenmeyer flask containing 110 ml of a synthetic medium containing D-galactose 2.0%, dextrin 2.0%. $(NH_4)_2SO_4$ 0.2% and $CaCO_3$ 0.2% (pH 7.4) at 28 °C on a rotatory shaker. Three d later, 25 mg of D-β-lysine and 12.5 mg of [2-^{13}C]-glycine (99% enrichment, Sigma) were added and the culture was continued for further 20 d. Purified [1′-^{13}C]bellenamine (67 mg) was isolated from the broth filtrate (8 flasks, 760 ml) by column chromatography on Amberline CG-50 (NH_4^+, 100 ml). ^1H NMR (D_2O, pD 6.0) δ=1.81 (4H, m, 4-H_2 and 5-H_2), 2.73 (1H, dd, J=8.3 and 17.2 Hz, 2-H), 2.87 (1H, dd, J=4.6 and 17.2 Hz, 2-H), 3.07 (2H, br, 6-H_2), 3.74 (1H, m, 3-H), and 4.53 (1.42H, s and 0.58H, d, J_{CH}=179 Hz, 1′-H_2, 29% enrichment for 1′-^{13}C); ^{13}C NMR (D_2O, pD 6.0) (rel intensity %) δ=23.7 (5.6%, C-5), 29.9 (4.2%, C-4), 37.0 (4.0%, C-2), 39.7 (4.5%, C-6), 46.1 (100%, C-1′), 48.8 (3.6%, C-3), and 173.8 (2.2%, C-1).

[1′,2′-$^{13}C_2$]N^1,N^3-Methylenebellenamine Prepared from [1′-^{13}C]Bellenamine. A solution (pH 7.7) of [1′-^{13}C]bellenamine (33 gm, 0.19 mmol) in 0.005 M (1 M=1 mol dm^{-3}) hydrochloric acid (2 ml) was kept at 37 °C for 7 d and [1′,2′-$^{13}C_2$]N^1,N^3-methylenebellenamine (4.1 mg, 12%) was purified by column chromatography on Amberlite CG-50 (NH_4^+, 20 ml). ^1H NMR (D_2O, pD 6.0) δ=1.59 (2H, m, 4-H_2), 1.76 (2H, m, 5-H_2), 2.23 (1H, dd, J=10.9 and 17.8 Hz, 2-H), 2.60 (1H, dd, J=4.6 and 17.8 Hz, 2-H), 3.03 (2H, t, J=7.6 Hz, 6-H_2), 3.10 (1H, m, 3-H), 4.44 (1.4H, s and 0.6H, d, J_{CH}=154 Hz, 2′-H_2, 30% enrichment for 2′-^{13}C), 4.27 (0.3H, ABq), 4.88 (0.3H, ABq), and 4.59 (1.4H, ABq, J_{HH}=13, J_{CH}=158 Hz, 1′-H_2, 30% enrichment for 1′-^{13}C); ^{13}C NMR (D_2O, pD 6.0) (rel intensity %) δ=24.1 (4.4%, C-5), 32.3 (3.3%, C-4), 37.8 (4.1%, C-2), 40.1 (3.7%, C-6), 51.0 (83.1% C-1′), 51.9 (4.4%, C-3), 63.2 (100%, C-2′), and 173.5 (2.5%, C-1).

[1′-^{13}C]N^1,N^3-Methylenebellenamine Prepared from [1′-^{13}C]Bellenamine with Formaldehyde. A solution of [1′-^{13}C]bellenamine (33 mg, 0.19 mmol) in a dilute solution of formalin (0.37%, 1.6 ml, 0.20 mmol) was kept at 50 °C for 2 h and [1′-^{13}C]N^1,N^3-methylenebellenamine (26 mg, 74%) was purified by column chromatography on Amberlite CG-50 (NH_4^+, 18 ml). ^1H NMR (D_2O, pD 6.0) δ=1.59 (2H, m, 4-H_2), 1.76 (2H, m, 5-H_2), 2.21 (1H, dd,

J=10.9 and 17.8 Hz, 2-H), 2.61 (1H, dd, J=4.6 and 17.8 Hz, 2-H), 3.02 (2H, t, J=7.6 Hz, 6-H_2), 3.06 (1H, m, 3-H) 4.42 (2H, s, 2′-H_2), 4.20 (0.26H, ABq), and 4.51 (1.48H, ABq, J_{HH}=13 Hz, J_{CH}=158 Hz, 1′-H_2, 26% enrichment for 1′-^{13}C); ^{13}C NMR (D_2O, pD 6.0) (rel intensity %) δ=24.1 (5.0%, C-5), 32.3 (4.4%, C-4), 37.8 (4.1%, C-2), 40.1 (4.1%, C-6), 51.5 (100%, C-1′), 51.9 (4.0%, C-3), 62.9 (5.6%, C-2′), and 173.3 (2.1%, C-1).

Formation of Cyclized Bellenamine from D-β-Lysinamide with Formaldehyde. D-β-Lysinamide (60 mg, 0.42 mmol) was dissolved in a dilute solution of formalin (0.37%, 3.4 ml, 0.42 mmol) and the solution was heated at 50 °C for 2 h. The product was purified by column chromatography on Amberlite CG-50 (NH_4^+, 10 ml) eluted with 2.5% aqueous ammonia to obtain cyclized bellenamine (55 mg, 82%); $[\alpha]_D^{20}$ −58° (c 1.0, H_2O), which was identical with the authentic sample in all respects.[5]

Reaction of L-Lysinamide with Formaldehyde. L-Lysinamide (66.3 mg, 0.46 mmol) was dissolved in a dilute solution of formalin (0.37%, 3.7 ml, 0.46 mmol) and the solution was heated at 50 °C for 2 h. The product was purified by column chromatography on Amberlite CG-50 (NH_4^+, 10 ml). The column was washed with water (25 ml) and eluted with 1.0% (50 ml) and 2.5% (50 ml) aqueous ammonia, successively. Fractions 14—19 were concentrated to give (*S*)-5-(4-aminobutyl)-4-imidazolidinone (4.4 mg, 6.1%) as a colorless hygroscopic solid; $[\alpha]_D^{24}$ +6° (c 0.35, H_2O); IR (KBr) 3400, 3200, 2940, 2870, 1705, 1560, 1520, 1480, 1430, 1390, 1300, 1180, 820, and 720 cm^{-1}; ^{1}H NMR (pyridine-d_5/D_2O 2: 1, pD 6.8) δ=1.78 (3H, m, 1′-H and 2′-H_2), 2.06 (3H, m, 1′-H and 3′-H_2), 3.35 (2H, t, J=7.3 Hz, 4′-H_2), 3.61 (1H, m, 5-H), 4.57 (1H, d, J=7.8 Hz, 2-H), and 4.64 (1H, d, J=7.8 Hz, 2-H); ^{13}C NMR (pyridine-d_5/D_2O 2:1, pD 6.8) δ=23.0 (C-2′), 27.3 (C-3′), 30.6 (C-1′), 39.8 (C-4′), 58.4 (C-5), 58.8 (C-2), and 179.9 (C-4). HRMS (FAB, positive). Found: m/z 158.1295 (MH^+). Calcd for $C_7H_{16}N_3O$: MH, 158.1293. Fractions 23—28 were concentrated to recover L-lysinamide (30 mg).

Reaction of Bellenamine with Acetaldehyde. A solution (pH 7.5) of bellenamine (206 mg, 1.18 mmol) and acetaldehyde (90% aqueous solution, 0.1 ml, 1.48 mmol) in a mixture of water (0.3 ml) and 1 M hydrochloric acid (2.5 ml) was kept at 50 °C for 2 h. The products were purified by column chromatography on Amberlite CG-50 (NH_4^+, 100 ml). The column was washed with water (100 ml) and eluted with each 500 ml of 1.5 and 2.0% aqueous ammonia, successively. Fractions of 10 ml were collected. Fractions 14—16 showing R_t 10.6 min (HPLC) were concentrated to give a diastereomeric mixture (3:2:2:1) of four bellenamine aldol adducts (79.7 mg, 29.7%) as a colorless hygroscopic solid. IR (KBr) 3400, 3250, 2950, 1620, 1495, 1390, 1330, 1290, 1150, 1100, 1080, and 830 cm^{-1}; ^{1}H NMR (D_2O, pD 7.7) δ=1.14, 1.28, 1.31 (each d, CH_3), 1.19, 1.49, 1.71, 1.88, 1.91, 2.01, 2.07 (each m, 9-H_2), 1.58 (m, 1′-H_2), 1.75 (m, 2′-H_2), 2.23, 2.54 (each dd, 3-H_2), 3.02, 3.46 (each m, 8-H), 3.04 (t, 3′-H_2), 3.04, 3.24 (each m, 2-H), 3.60, 3.92, 3.94, 5.05, 5.13, 5.32, 5.35 (each d, 6-H_2), and 4.40, 4.52, 4.61, 4.71 (3:2:2:1, each dd, 9a-H); ^{13}C NMR (D_2O, pD 7.7) δ=16.3, 16.6, 21.0 (CH_3), 23.9, 24.3 (C-2′), 30.8, 32.3 (C-1′), 37.5, 40.2, 40.4 (C-9), 37.7, 38.3 (C-3), 40.1 (C-3′), 46.0, 46.8, 50.0, 50.1 (C-8), 47.4, 47.5, 51.1 (C-2), 50.2, 51.1, 55.3, 55.8 (C-6), 63.6, 65.6, 67.8, 69.5 (C-9a), and 170.1, 170.2, 170.6 (C-4). HRMS (FAB, positive). Found: m/z 227.1874 (MH^+). Calcd for $C_{11}H_{23}N_4O$: MH, 227.1872. From fractions 66—82 showing R_t 11.1 min, bellenamine (98.6 mg, 47.9%) was recovered.

Reaction of Bellenamine with Acetone. To a solution of bellenamine (336 mg, 1.93 mmol) in water (1 ml) was added acetone (1.5 ml, 20.4 mmol) and the solution was kept at 37 °C for 1 week. The products were purified by column chromatography on Amberlite CG-50 (NH_4^+, 200 ml). The column was washed with water (400 ml) and eluted with each 1000 ml of 0.5 and 2.5% aqueous ammonia, successively. Fractions of 15 ml were collected. Fractions 23—29 showing R_f 0.68 (TLC), R_m 1.68 (HVPE), and R_t 13.7 min (HPLC) were concentrated to give a diastereomeric mixture (9a*S*:9a*R*, 84:16) of two bellenamine diacetone adducts (29.7 mg, 6.1%) as a colorless hygroscopic solid; $[\alpha]_D^{22}$ −23° (c 0.7, H_2O). IR (KBr) 3400, 3280, 2950, 1625, 1480, 1440, 1420, 1390, 1310, 1190, 1100, 830, and 780 cm^{-1}. ^{1}H and ^{13}C NMR of diastereomers are shown in Table 4. HRMS (FAB, positive). Found: m/z 255.2190 (MH^+). Calcd for $C_{13}H_{27}N_4O$: MH, 255.2185. From fractions 90—102 showing R_f 0.22, bellenamine (270 mg, 80.4%) was recovered.

Reaction of Bellenamine with Diacetone Alcohol. A large scale preparation of the diacetone adduct was carried out by the reaction of bellenamine with diacetone alcohol. A solution of bellenamine (503 mg, 2.89 mmol) and diacetone alcohol (1.8 ml, 14.6 mmol) in water (2 ml) was kept at 37 °C for 1 week and the products were purified by column chromatography on Amberlite CG-50 (NH_4^+, 200 ml) to yield a diastereomeric mixture (9a*S*:9a*R*, 65:35) of bellenamine diacetone adducts (266 mg, 36.2%); $[\alpha]_D^{23}$ −34° (c 0.5, H_2O). Bellenamine (106 mg, 21%) was recovered.

Stability of Diastereomers of Bellenamine Diacetone Adduct. A solution of a diastereomeric mixture of bellenamine diacetone adducts (6 mg) in D_2O (0.6 ml) was adjusted to pD 4.7, 3.6 or 1.9 with DCl and the ^{1}H NMR was recorded in a 5 mm sample tube. The amounts of 9a*S* and 9a*R* diastereomers were calculated from the signal strength of 6-H(α) at around δ=3.9 and 5.2, respectively, compared with all (100%) of 3′-H_2 in adducts and 6-H_2 in regenerated bellenamine. The amount of bellenamine was calculated from the signal strength of 1′-H_2. The solutions in sample tubes were kept at room temperature and the results of stability tests are shown in Table 3.

Preparation of a Stable Diastereomer of Bellenamine Diacetone Adduct. A solution of a diastereomeric mixture of bellenamine diacetone adducts (39.7 mg) in water (2.9 ml) was adjusted to pH 4.8 with 0.2 M hydrochloric acid (1.2 ml) and kept at room temperature for 12 d. A diastereomeric mixture was purified by column chromatography on Amberlite CG-50 (NH_4^+) to afford a 22:78 mixture (9a*S*:9a*R*) (12.8 mg); $[\alpha]_D^{25}$ −69° (c 0.4, H_2O). A solution of the mixture in water (pH 4.8) was kept at room temperature for further 3 d and the 9a*R*-abundant mixture (14:86) was obtained. $[\alpha]_D^{24}$ −77° (c 0.25, H_2O); HRMS (FAB, positive). Found: m/z 255.2178 (MH^+). Calcd for $C_{13}H_{27}N_4O$: MH, 255.2185. ^{1}H and ^{13}C NMR are shown in Table 4.

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