Unusually Large ^{13}C NMR Chemical Shift Differences between Neutral and Protonated Glycocyamidines. New Insights on Previously Reported Chemical Shift Assignments and Chemical Properties

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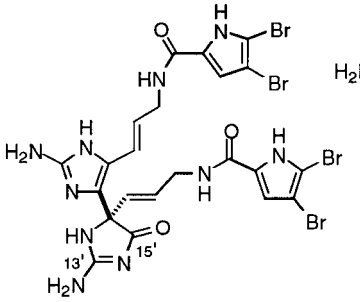
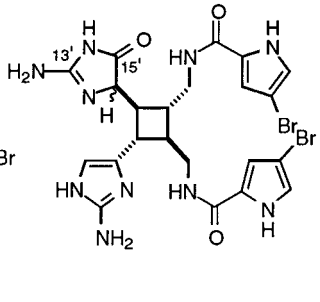
Unusually large ^{13}C chemical shift differences are observed between neutral and protonated glycocyamidine (2-aminoimidazolinone) derivatives. Upon protonation, the guanidine (N−C═N) and carbonyl (C═O) carbons undergo an upfield shift of approximately 12 and 17 ppm, respectively, relative to the neutral species. For neutral glycocyamidine derivatives, the ^{13}C chemical shifts for the carbonyl carbon reside above 190 ppm. In the protonated form, the carbonyl resonances are in the mid to lower 170s. These values are indicative of the protonation state of the glycocyamidine moiety. The present study serves as a useful reference in assisting the characterization of future glycocyamidine-based natural products and derivatives.

We recently completed a synthesis of the potent antifouling agent mauritiamine (**1**).[1] When spectral data of synthetic **1** as the dihydrochloride salt were compared against those reported for natural **1** as the ditrifluoroacetic acid salt,[2] significant differences were found in the ^{13}C chemical shift values of C13′ and C15′ of the glycocyamidine moiety. For synthetic **1**·2HCl, values of 158.9



mauritiamine (**1**)



oxysceptrin (**2**)

^{13}C NMR Chemical Shifts (ppm)[a]

	C13'	C15'
natural mauritiamine (**1**)·2TFA	149.0	194.7
synthetic mauritiamine (**1**)·2HCl	158.9	172.2
oxysceptrin (**2**)·2HOAc	172.4	191.1
oxysceptrin (**2**)·2HCl	159.7	176.1

[a] Data from refs. 1, 2, and 4. All shifts were recorded in CD_3OD.

and 172.2 ppm (CD_3OD) were obtained for C13′ and C15′, respectively, whereas the values for these same carbons in natural **1**·2TFA are reported as 149.0 and 194.7 ppm (CD_3OD). This represents a difference of approximately 10 ppm for C13′ and 22 ppm for C15′. We also found similar differences in the literature values reported for the dihydrochloride and diacetic acid salts of the glycocyamidine-containing metabolite, oxysceptrin (**2**).[3,4] Values of 159.7 (C13′) and 176.1 ppm (C15′) are listed for oxysceptrin (**2**)·2HCl,[4] whereas the values for oxysceptrin (**2**)·2HOAc are given as 172.4 (C13′) and 191.1 (C15′).[4] The difference in this case is approximately 12 ppm for C13′ and 15 ppm for C15′. While the large chemical shift differences in the above examples are the apparent result of different counterions (e.g., chloride vs acetate), the magnitude of this difference does not seem to be compatible with a simple counterion effect. In this paper, we describe unusually large ^{13}C NMR chemical shift differences between neutral and protonated forms of glycocyamidines. The ramifications of these findings on previously published reports of glycocyamidine metabolites are discussed.

Natural products in which glycocyamidine (**3**) comprises the parent structural unit are relatively rare. Of the glycocyamidine derivatives examined thus far, creatinine (**4**) has commanded the most attention due to its abundance as a prominent end-product of nitrogen metabolism present in human urine. Recently, scattered reports have appeared describing the occurrence of glycocyamidine metabolites from marine origin.[5] They are found primarily within the "oroidin" group of secondary metabolites produced by marine sponges. Representative members of this metabolic group include the *Agelas* alkaloids, mauritiamine (**1**),[2] oxysceptrin (**2**),[3,4] and the dispacamides (**5**).[6] A previous ^{13}C NMR study on glycocyamidine derivatives has been done by Kenyon and coworkers,[7] who showed that ^{13}C NMR spectroscopy is a

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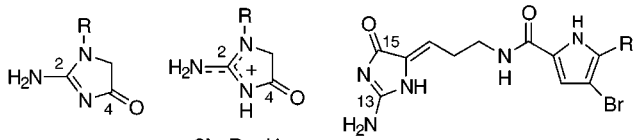


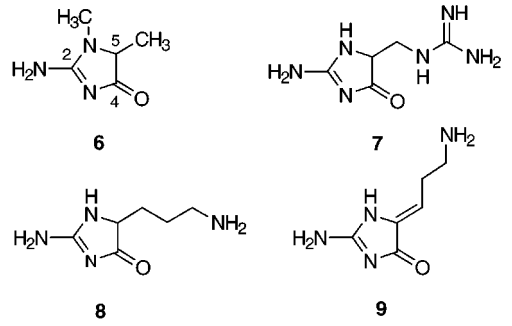
3a R = H
4a R = CH_3

3b R = H
4b R = CH_3

5 R = H or Br

useful method to distinguish between the ring-open (creatine) analogues and cyclized products, creatinines. In their study, all derivatives examined were analyzed as the hydrochloride salts. A search of the literature did not reveal any reports on ^{13}C chemical shifts of neutral glycocyamidines.

In our recent synthesis of mauritiamine (**1**),[1] the data obtained for C13′ (158.9) and C15′ (172.2) of mauritiamine (**1**)·2HCl are in good agreement with the corresponding carbon values of 157.1 and 177.3 ppm reported by Kenyon[7] for D,L-alacreatinine (**6**)·2HCl and to the hydrochloride salt of imidazolinone derivative **7**[8] reported by Rapoport. They also correspond well with values reported by Kobayashi[3] and Rinehart[4] for the protonated glycocyamidine unit found in oxysceptrin (**2**)·2HCl. Our analysis of glycocyamidine derivatives **3**, **4**, and **8** as their hydrochloride salts produced similar results (Table 1). For all compounds examined, the ^{13}C resonances are within the range of 172−175 ppm for the carbonyl carbon (C═O) and 158−160 ppm for the guanidino carbon (N−C═N). These same carbons, however, resonate at significantly lower field in the corresponding free base ranging from 190 to 193 ppm (C═O) and 170−173 ppm (N−C═N). A similar trend was observed for the neutral and protonated forms of unsaturated glycocyamidine derivative **9**.



6 **7** **8** **9**

Attempts were made to examine the effect of changing the counterion from Cl^- to $CH_3CO_2^-$. While the diHCl salts of **8** and **9** were readily isolated by the addition of HCl to the free base, the corresponding diHOAc salts could not be obtained in a similar fashion. Attempts to prepare and isolate the diacetic acid salt of glycocyamidines **8** and **9** consistently afforded less than two molecules of acetic acid per molecule of base as evidenced by ^1H NMR. Integration typically revealed the presence of 1.3−1.5 equiv of HOAc per molecule of base. These results are consistent with the relatively weak basicity of glycocyamidines ($pK_a < 4.9$).[9−12] The ^{13}C chemical shift data obtained for C2 and C4 of the acetate salts of **8** and **9** resemble closely the data obtained for the free base,

Table 1. ^{13}C NMR Chemical Shifts of 3 and 4 in D_2O,[a] 8, and 9 in CD_3OD

	3	**3** + AcOH[b]	**3** + AcOH[c]	**3**·HCl[d]	**4**	**4** + AcOH[e]	**4** + AcOH[f]	**4**·HCl[d]	**8**	**8**·1.4AcOH	**8**·2HCl	**9**	**9**·1.4AcOH	**9**·2HCl	**9**[h]	**9**·2HCl[h]
C-2	172.4 s	166.1 s	160.3 s	159.6 s	170.3 s	167.9 s	159.9 s	158.0 s	172.6 s	172.6 s	159.8 s	168.9 s	168.3 s	157.2 s	166.9 s	155.4 s
C-4	192.4 s	180.4 s	176.1 s	175.4 s	189.9 s	181.7 s	175.6 s	173.7 s	192.8 s	192.2 s	175.2 s	179.8 s	178.6 s	163.4 s	176.2 s	162.4 s
C-5	51.4 t	50.4 t	49.5 t	49.5 t	57.4 t	57.0 t	55.4 t	55.1 t	62.4 d	61.7 d	59.8 d	137.5 s	138.2 s	131.2 s	137.1 s	129.7 s
C-6									29.0 t[g]	24.0 t	23.7 t	112.0 d	107.9 d	115.7 d	107.0 d	114.9 d
C-7									30.0 t[g]	29.4 t	28.9 t	31.6 t	26.5 t	26.2 t	29.4 t	24.9 t
C-8									42.3 t	40.3 t	40.2 t	41.8 t	39.7 t	39.3 t	48.6 t	37.5 t
CH_3					31.1 q	31.4 q	31.7 q	31.9 q								
AcOH		184.1 s	177.6 s			186.7 s	178.1 s			180.1 s			179.9 s			
AcOH		23.1 q	21.4 q			23.9 q	21.8 q			24.0 q			23.8 q			

[a] Sample concentration 20 mg/mL. [b] Addition of 0.7 equiv of AcOH. [c] Addition of 10 equiv of AcOH. [d] Reference 14. [e] Addition of 0.25 equiv of AcOH. [f] Addition of 3.5 equiv of AcOH. [g] Tentative assignment. [h] In DMSO-d_6.

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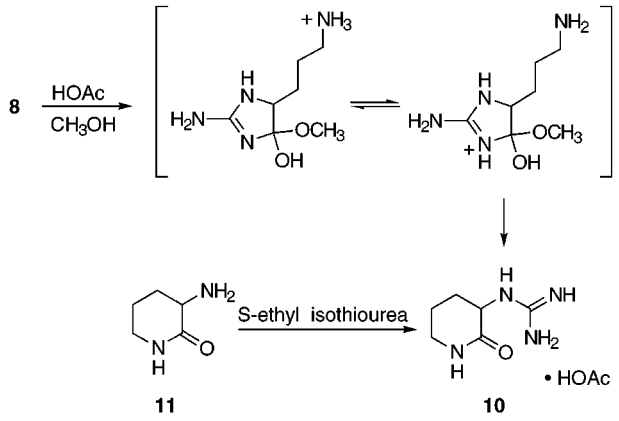
Scheme 1



thus indicating a neutral glycocyamidine moiety in CD_3OD. Similar results were obtained for glycocyamidines **3** and **4**. Neither derivative could be isolated as the monoacetate salt, but an NMR sample of glycocyamidine (**3a**) containing a drop of HOAc, however, showed a chemical shift of 182 ppm for C15′ that is intermediate between the fully protonated and neutral forms. By extension, these data suggest that the reported ^{13}C shifts for C13′ and C15′ in natural mauritiamine (**1**)·2TFA and oxysceptrin (**2**)·2HOAc correlate with a neutral glycocyamidine moiety.[13] The assignment of values >190 ppm for a carbonyl carbon (C15′) in a protonated glycocyamidine is inconsistent with our data. Chemical shift values >190 for the carbonyl carbon (C15′) of this type should be associated with a neutral glycocyamidine species. For protonated glycocyamidine derivatives, chemical shift values for the carbonyl carbon fall in the mid-170 range while those for the guanidino carbon fall in the upper-150 range, which is similar to values seen in isolated guanidinium salts.

Recently, we completed a synthesis of dispacamides (**5**).[14] The reported values for C13 and C15 for natural **5**[6] are 168.3 and 179.1 ppm, respectively. These values matched the values of 168.0 and 179.0 ppm that we obtained for synthetic **5** as the free base. Upon formation of the HCl salt, an upfield shift of 15 ppm for C15 and 11 ppm for C13 was observed. These data are consistent with the above results seen for saturated glycocyamidines **3**, **4**, **8**, and the unsaturated derivative **9**.

Deuterium exchange of ring protons in glycocyamidines is known.[15,16] Exchange at C5 took place in the free base of glycocyamidine **8** ($t_{1/2}$ = 1 d). However, the acetic acid salt of **8** underwent rearrangement ($t_{1/2}$ < 1 d) in CD_3OD to lactam **10**, which was accompanied by partial ^2H exchange at C5. The structure of **10** was confirmed independently through synthesis by converting 3-amino-2-piperidinone (**11**)[17] to **10** with *S*-ethylisothiourea (Scheme 1). In methanol, the rearrangement of **8** to **10** is believed to be initiated by the addition of CH_3OH to the carbonyl group, which in turn, generates a more basic guanidine moiety. This guanidine group can now accept a proton from the less basic aliphatic amine, which allows collapse of the presumed tetrahedral intermediate to the corresponding acyclic ester. Ring closure by the free amine affords lactam **10**. Finally, no exchange was observed at C5 for the hydrochloride salts of derivatives **3** and **4** or the dihydrochloride salt of **8**. These results suggest that epimer formation of natural oxysceptrin (**5**)[3,4] most likely occurs via a neutral or weakly protonated glycocyamidine unit and is accelerated in the presence of acetic acid.

Large chemical shift differences (>15 ppm) between neutral and protonated nitrogen heterocycles are relatively uncommon. One example can be seen for the ipso carbon of aniline·HCl relative to aniline. The ipso carbon of the protonated species resonates 17 ppm higher field than the neutral species.[18] The effect is believed to manifest itself in the π-polarization of the phenyl ring.

The physical basis for the chemical shift phenomenon of glycocyamidines is unknown at this time. Preliminary calculations of the chemical shift tensor of glycocyamidines from the Grant group have reproduced this effect.[19] It should be noted that this phenomenon is also observed in non-hydroxylic solvents such as DMSO-d_6 thus ruling out any solvent effects.[20] Grant has reported a similar trend with various protonated and neutral aromatic nitrogen heterocycles.[21] The difference in magnitude, however, is much smaller than that for glycocyamidines seen in the present study. Nevertheless, a good correlation was found between a decrease in C−N bond order and the upfield shift for carbons α to the site of protonation. For glycocyamidines, perhaps a combination of bond-order and polarization effects are responsible for the large chemical shift differences.

Glycocyamidines can exist, in principle, in different tautomeric forms. The most stable of these tautomers have been shown to be 2-amino-2-imidazolin-4-one (**3a**) and 1-methyl-2-amino-2-imidazolin-4-one (**4a**).[11] Upon protonation, tautomers **3b** and **4b** are the favored structures for the cation.[16,22] Although a previous report[22] indicated that conjugation to the carbonyl is present in the protonated form of creatinine (**4**), we find that, upon glycocyamidine protonation, conjugation to the carbonyl appears significantly diminished if not lost. This conclusion is supported by a blue shift in the UV data upon protonation of creatinine (**4**)[11] as well as a shift to higher wavenumber for the C=O stretch.[24]

In summary, large differences in ^{13}C chemical shifts were observed for the carbonyl (C=O) and guanidino (N−C=N) carbons in neutral and protonated glycocyamidine species. The data suggest that there is a significant change in the electronic state of the neutral glycocyamidine species upon protonation. The ^{13}C values are indicative of the protonation state of the glycocyamidine moiety. The present study should serve as a useful

(13) Reanalysis of the ^{13}C data for natural mauritiamine (**1**) by Fusetani has led to the conclusion that the data presented in ref 2 correspond to a neutral glycocyamidine unit that was found to be unstable. See footnote 19 of ref 1.

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reference in assisting the future characterization glycocyamidine-containing natural products and derivatives.

Experimental Section

General Methods. Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification except for solvents that were dried and distilled. Silica gel (particle size 32−63 μm) was used for flash chromatography. ^{1}H NMR and ^{13}C NMR spectra were measured at 300 and 75 MHz, respectively. When D_2O was used for ^{13}C measurements, CD_3OD was added as an internal standard.

Glycocyamidine (3). Preparation of **3**·HCl was accomplished as previously reported.[23] **3**·HCl: UV λ_{max} (MeOH) 217 nm. The free base of **3** was prepared from **3**·HCl by flash chromatography using methanol saturated with NH_3 to afford **3** as a white solid. **3**: mp > 205 °C; UV λ_{max} (MeOH) 227 nm.

Creatinine (4). Creatinine (**4**) was obtained from a commercial supplier. Creatinine **4**·HCl was prepared by addition of concentrated HCl to a methanol solution of the free base followed by concentration in vacuo to afford **4**·HCl as a white powder. **4**·HCl: mp > 205 °C; UV λ_{max} (MeOH) 214 nm. **4**: UV λ_{max} (MeOH) 235 nm.

2-Amino-5-(3-aminopropyl)-1*H*-imidazolin-4-one (8). Imidazolinone **8** and **8**·2HCl were prepared as previously reported.[14] **8**: UV λ_{max} (MeOH) 228 nm. **8**·2HCl: UV λ_{max} (MeOH) 215 nm. Addition of glacial acetic acid to a methanol solution of the free base of **8** and concentration in vacuo (0.01 Torr, 2 h) afforded **8**·1.4AcOH (by integration) as a colorless oil.

2-Amino-5-(3-aminopropylidene)-1*H*-imidazolin-4-one (9). **9** and **9**·2HCl were prepared as previously reported.[14] **9**: UV λ_{max} (MeOH) 245 and 287 nm. **9**·2HCl: UV λ_{max} (MeOH) 226 and 271 nm. Addition of glacial acetic acid to a methanol solution of the free base of **9** and concentration in vacuo (0.01 Torr, 2 h) afforded **9**·1.4AcOH (by integration) as a pale yellow oil.

3-Guanidino-2-piperidinone (10). To a solution of **8** (0.136 g, 0.87 mmol) in 10 mL of methanol at room temperature was added 2 equiv AcOH (1.7 mmol, 100 μL), and the reaction mixture was stirred for 7 d. After evaporation of the solvent, the residue was washed with EtOH to give **10**·AcOH (0.167 g, 80%) as a colorless solid. **10**·AcOH: ^{1}H NMR (CD_3OD) δ 4.14 (dd, 1H, J = 10.0, 6.2), 3.32−3.28 (m, 2H), 2.27−2.19 (m, 1H), 2.05−1.78 (m, 3H), 1.91 (s, 3H, AcOH); ^{13}C NMR (CD_3OD) δ 180.4 s, 171.9 s, 159.7 s, 52.8 d, 42.5 t, 28.5 t, 24.2 q, 21.8 t; IR (KBr) 3039, 1695, 1641, 1612, 1401 cm^{-1}; UV λ_{max} (MeOH) 210 nm; MS m/z (relative intensity) 157 ($M^+ + 1$, 100), 140 (15), 100 (15); HRMS calcd for $C_6H_{13}N_4O$ (MH^+) 157.1090, found 157.1087. From piperidinone **11**: To a stirred solution of **11**[17] (1.14 g, 10.4 mmol) in ethanol was added *S*-ethylisothiourea (1.29 g, 12.0 mmol). The reaction mixture was allowed to stir at room temperature under argon for 1 d. Concentration of the reaction mixture followed by chromatography and the addition of HOAc produced piperidinone **10** as the acetic acid salt.

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