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Partial assignment of the ^{15}N NMR spectrum of sulfomycin-I at natural abundance

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ABSTRACT: Early efforts to utilize ^{15}N NMR spectroscopy in structure elucidation studies were often frustrated by the low gyromagnetic ratio (γ_N) and the low natural abundance (0.37%) of the nuclide. The advent of ^{1}H and inverse-detected 2D NMR methods has eliminated many of the difficulties inherent to the use of ^{15}N as a structural probe. This paper reports the partial assignment of the ^{15}N NMR resonances of the thiopeptide antibiotic sulfomycin-I produced by *Streptomyces viridochromogenes*. With the exception of two tertiary nitrogen resonances that had no two- or three-bond coupling pathways, assignments were made either through direct correlation ^{1}H–^{15}N GHSQC or one-bond optimized ^{1}H–^{15}N GHNMQC or via two or three bonds using ^{1}H–^{15}N GHNMQC spectra. Assignments are also reported for the heterocyclic nitrogen resonances of two thiazole and one oxazole moiety contained in the structure of the antibiotic via ^{3}J(N,H) coupling from the heterocyclic ring protons. Despite the suggestion that these coupling pathways, suspected to be *ca.* 2 Hz, might be difficult to observe since they are comparable to the linewidths of the thiazole and oxazole protons in question, they were still exploitable for assignment purposes. © 1998 John Wiley & Sons Ltd.

KEYWORDS: NMR; ^{15}N NMR; GHSQC; GHNMQC; sulfomycin-I; thiopeptide antibiotic; ^{1}H–^{15}N correlation; long-range ^{1}H–^{15}N correlation; ^{15}N natural abundance

INTRODUCTION

Sulfomycin-I[1–4] belongs to a family of about 20 thiopeptide antibiotics elaborated by various *Streptomyces* species. Members of the group include thiostrepton,[5] thiopeptin,[6] nosiheptide,[7–9] micrococin,[10] siomycin,[11] berninamycin,[12,13] promothiocins,[14] geninthiocin,[15] thiotipin[16] and promoinducin.[17] Sulfomycin-I is most closely related to sulfomycin-II and -III,[18] from which it differs only in the constitution of the oxazole-C (Oxa-C) residue side-chain. The next most closely related congeners are promoinducin,[17] which differs only in that the dehydroalanine-A (Deala-A) residue appears as a free carboxylic acid rather than a primary amide, and geninthiocin,[15] whose dehydroalanine 'tail' is comprised of two rather than three dehydroalanine residues. In this regard, the proliferation of trivial names is unfortunate; without careful comparison, it might not be noticed in a casual literature search that two molecules with seemingly very different names, sulfomycin-I and promoinducin, are, in fact, closely related.

Early efforts at the characterization of sulfomycin-I preceded two-dimensional NMR methods and were largely based on chemical degradation followed by structural identification of hydrolysis fragments.[1] Two-dimensional NMR methods have subsequently been applied, leading to total assignments of the ^{1}H and ^{13}C NMR spectra of the molecule.[20,21] Total ^{1}H and ^{13}C NMR resonance assignments have also been reported for the closely related molecules sulfomycin-II and -III, [18] geninthiocin[15] and promoinducin.[17] Within the thiopeptide antibiotic family, total ^{1}H and ^{13}C NMR assignments have also been reported for nosiheptide,[22] which has some structural homology with sulfomycin-I. Studies of the biosynthesis of sulfomycin-I,[21] nosiheptide,[23–25] thiostrepton[26] and berninamycin[13] have also been reported.

Heteronuclear ^{1}H–^{13}C shift correlation methods have long been a mainstay for the elucidation of complex natural product structures, first via ^{13}C-detected methods and more recently via inverse-detected methods. The latter experiments, exploiting the much greater sensitivity afforded by ^{1}H detection,[27,28] have almost completely supplanted their less efficient heteronucleus-detected predecessors. In contrast, ^{15}N-detected heteronuclear shift correlation experiments have essentially never been employed because of the abysmal sensitivity associated with direct ^{15}N observation. There have, however, been some early natural abundance ^{15}N direct observation one-dimensional NMR studies. The pioneering efforts of Bax *et al.*[29,30] in developing multiple quantum-based, inverse-detected

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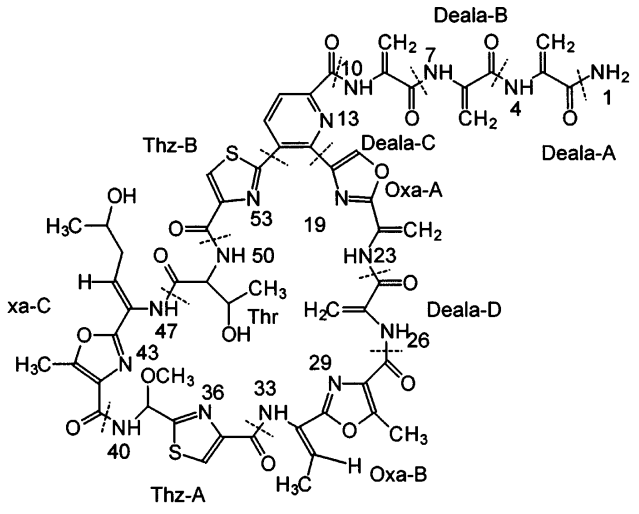

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^{1}H–^{15}N heteronuclear correlation methods have made studies involving ^{15}N as a structural probe feasible. Despite the availability of inverse-detected methods, there have been only relatively sparse applications of these experiments in natural product structural studies. The earliest work from our laboratories involved ^{1}H–^{15}N direct correlation of the alkaloids cryptospirolepine[32] and quindolinone[33] at natural abundance using ^{1}H–^{15}N HMQC, the latter work utilizing micro inverse methods on a sample of only 800 μg. The first long-range ^{1}H–^{15}N HMBC study of which we are aware was the 1990 study of the ^{15}N-labeled marine alkaloid tantazole-A by Carmeli *et al.*[34] We believe the first reported attempts to long-range correlate ^{1}H–^{15}N at natural abundance were reports using non-gradient methods by the present authors in 1993[35] and a report that used gradient methods by Uzawa *et al.* in 1993.[36] These early efforts were followed in 1995 by the successful application of gradient methods to study the long-range ^{1}H–^{15}N correlation pathways of the alkaloid ajmaline at natural abundance[37] and a survey paper reporting several examples by Koshino and Uzawa.[38] The study of ajmaline has been followed by a number of other applications to alkaloids,[39–45] a study of a pyrrolobenzofuran antibiotic,[46] a cytotoxic thiazoline-based antibiotic,[47] a protonation study of an aminopyrazole system,[48] a study of the reverse transcriptase inhibitor delavirdine[49] and most recently a method for the unequivocal location of *N*-oxides based on natural abundance long-range ^{1}H–^{15}N methods.[50]

Nitrogen NMR studies of the thiopeptide antibiotics, while spanning a period of 20 years, are relatively few in number and are easily reviewed. The first ^{15}N direct observation studies are contained in reports dealing with the thiopeptide antibiotics nosiheptide,[8] thiostrepton and siomycin.[31] These efforts were primarily ^{15}N chemical shift tabulations with only partial assignments of the spectra. More recently, still utilizing ^{15}N direct observation of ^{15}N biosynthetically labeled material, Mocek *et al.*[25] were able to completely assign the ^{15}N spectrum of nosiheptide. The only contemporary application of inverse-detected ^{1}H–^{15}N heteronuclear correlation to a thiopeptide antibiotic at natural abundance of which we are aware of was the recent work of Gasmi *et al.*[51] which utilized nosiheptide as a model compound in their efforts to develop new inverse-detected NMR methods.

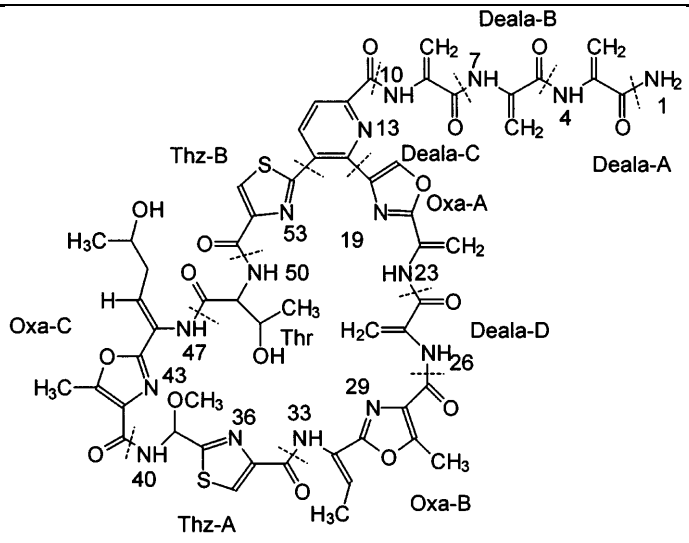
We now report the use of gradient enhanced direct- and long-range inverse-detected ^{1}H–^{15}N heteronuclear shift correlation techniques to assign all but two of the 16 nitrogen resonances contained in the structure of sulfomycin-I (**1**). The remaining two resonances, N-29 in the Oxa-B and N-43 in the Oxa-C residues, have no two- or three-bond long-range ^{1}H–^{15}N coupling pathways available that could be exploited for assignment purposes using the methods in this study. The plausible, but invariably much weaker four-bond couplings were not observed under any conditions employed in this study.

RESULTS AND DISCUSSION

Assignment of the ^{15}N NMR spectrum of sulfomycin-I (**1**) (Table 1) is based on the utilization of responses that are subdivisible into four strategic categories of successively increasing observational difficulty. We have arbitrarily labeled these as Groups I–IV, which are comprised as follows. Group I consists of the protonated amide nitrogens characterized by their direct or one-bond correlation responses. Observation of responses in this group in inverse-detected 2D ^{1}H–^{15}N NMR spectra is a facile undertaking; assignment of the amide nitrogens follows straightforwardly from the amide NH proton resonance assignments. Protonated nitrogen responses may be observed either as singlets at the intersection of their respective ^{15}N and amide ^{1}H chemical shifts in a GHSQC spectrum with ^{15}N decoupling, or as unsuppressed direct response *ca.* 90 Hz doublets in long-range optimized experiments. Group II is composed of the two- and three-bond correlation responses to the protonated amide nitrogens.

Again, these correlations are readily observed in GHNMQC spectra optimized for long-range couplings of 6–10 Hz (83–50 ms) and provide convenient, secondary confirmation of the ^{15}N resonance assignments. Group III responses consist exclusively of three-bond correlation pathway responses to the non-protonated heteroaromatic nitrogen resonances. It has been suggested that these coupling pathways would be difficult to observe in long-range correlation experiments by Gasmi *et al.*[51] based on the prior work of Chen *et al.*[52] establishing these couplings at *ca.* 2 Hz, which is comparable to the thiazole proton linewidths. Finally, the Group IV responses involve coupling via more than three bonds to the non-protonated heteroaromatic nitrogens. These coupling pathways are intuitively expected to be very difficult to observe in conventional ^{1}H–^{15}N inverse-detected GHNMQC spectra, if they are observable at all.

Table 1. ^{15}N resonance assignments and direct and long-range proton–nitrogen correlations for sulfomycin-I observed using the GHNMQC pulse sequence[a]



Position	Residue	^{15}N chemical shift	^{1}H proton shift (direct)	Long-range coupled protons
N-1	Deala-A	99.8	H-1 7.52 H-1 7.94	—
N-4	Deala-A	121.5	H-5 9.07	H-4 5.65 H-4 6.12
N-7	Deala-B	119.7	H-7 10.08	H-6 5.72
N-10	Deala-C	118.6	H-10 10.42	H-9 5.95
N-13	Pyridine	302.8	—	H-17 8.30 (weak)
N-19	Oxazole-A	255.8	—	H-21 8.64
N-23		117.0	H-23 9.97	H-22 5.79
N-26	Deala-D	121.0	H-26 9.17	—
N-29	Oxazole-B	No response	—	—
N-33		115.7	H-33 10.0	6.49
N-36	Thiazole-A	310.6	—	H-38 8.45 H-39 6.46
N-40		123.0	H-40 8.35	—
N-43	Oxazole-C	No response	—	—
N-47		117.3	H-47 9.44	6.27
N-50	Threonine	110.3	H-50 8.18	—
N-53	Thiazole-B	314.3	—	H-55 8.55

[a] Spectra were acquired in DMSO-d_6 using a sample containing *ca.* 2 mmol of sulfomycin-I dissolved in 160 µl of solvent in a 3 mm NMR tube or using a sample containing *ca.* 8 mmol of sulfomycin-I dissolved in 500 µl of solvent in a 5 mm NMR tube.

Protonated amide nitrogen resonance assignments: Group I responses

Spectroscopically, observing ^{1}H–^{15}N direct correlation responses is a facile, high-sensitivity undertaking. There are several ways of observing ^{1}H–^{15}N direct responses. Since the GHNMQC[49] pulse sequence does not employ a low-pass *J*-filter to eliminate the direct responses, they will generally be observed in ^{1}H–^{15}N long-range GHNMQC correlation spectra, characterized by their intrinsic 85–95 Hz doublets. It should be noted, however, that the intensity of the direct responses will be strongly modulated, resulting in varying response intensity, as a function of the match/mismatch of a harmonic multiple of the one-bond coupling to the optimization of the long-range delay used to perform the experiment.[49] Obviously, the GHNMQC experiment can also be optimized for one-bond couplings by setting the delays to $1/2[^{1}J(\mathrm{N,H})]$ or *ca.* 5.5 ms and essentially converting the experiment to a simplified, ^{15}N-coupled version of ^{1}H–^{15}N HMQC. Direct magnetization components can also be refocused following the final gradient with decoupling then applied as in the normal GHMQC experiment. It should also be noted here that this experimental variant is the so-called D-HMBC experiment of Furihata and Seto[53] when the delays are nominally optimized for long-range rather than one-bond coupling pathways. Our experience with this

experiment when used for $^{1}H-^{15}N$ heteronuclear correlation has been disappointing. We have generally found the sensitivity to be very poor even relative to the long-range experiment in which direct responses are observed as suppressed doublets. Finally, $^{1}H-^{15}N$ direct correlation experiments can also be performed using a gradient-enhanced HSQC (GHSQC) pulse sequence[54] with the gradient ratio optimized for $^{1}H-^{15}N$ rather than $^{1}H/^{13}C$ gyromagnetic ratios (γ).

The labeled ^{1}H reference spectrum of sulfomycin-I (**1**) is shown in Fig. 1; the $^{1}H-^{15}N$ GHNMC spectrum of sulfomycin-I is shown in Fig. 2. The response density when direct correlation responses are observed as doublets in the one-bond optimized GHNMQC spectrum is reasonably high. Some of the multiplets are overlapped in F_2 and not particularly well resolved in F_1 when normal levels of F_1 digitization are employed. The spectrum is manageable but borders on becoming unmanageable if either the molecule were more complicated or the ^{1}H spectrum more congested. In contrast, a $^{1}H-^{15}N$ GHSQC spectrum, by virtue of ^{15}N decoupling during acquisition, would be far more manageable and also readily acquired. An investigator will obviously be in a position to make an informed decision as to whether or not the acquisition of a GHSQC spectrum is necessary if the long-range spectrum in which the direct responses appear is recorded first. Generally, we have found it convenient to opt for the long-range experiment first.

Of the 10 protonated nitrogen resonances observed in the sulfomycin-I $^{1}H-^{15}N$ correlation spectra shown in Fig. 1, only one can be assigned directly, the primary amide resonance of Deala-A (dehydroalanine-A) resonating at 99.8 ppm. Assignment is made based on the inequivalence of the two amide proton resonances of this residue, a feature not repeated elsewhere in this molecule. The observed chemical shift is between the values reported by Gasmi *et al.*[51] (96.5 ppm, not shown in Fig. 2) and Mocek *et al.*[25] (106.6 ppm) for the terminal dehydroalanine residue contained in the structure of nosiheptide.

The remaining nine protonated nitrogen resonances were all readily assigned on the basis of the $^{1}H-^{15}N$ correlation to their assigned proton resonances. Working in from the terminal primary amide, the N-4 resonance of Deala-A was assigned at 121.5 ppm, its directly bound proton resonating at 9.07 ppm. The N-7 resonance of Deala-B was slightly upfield, resonating at 119.7 ppm, H-7 resonating at 10.08 ppm. The final protonated nitrogen contained in the 'tail' of the molecule, N-10 in Deala-C, resonated still further upfield at 118.6 ppm while its directly bound proton, H-10, resonated the furthest downfield of any of the amide protons at 10.42 ppm. It is interesting, but probably not significant, to note the upfield trend of the nitrogen shifts progressing from the terminus of the tail to the point of attachment at the pyridine and the corresponding downfield shift of the amide protons. Finally, in contrast, the N-26 resonance of the Deala-D residue, which is contained in the macrocyclic ring of the antibiotic, resonated at 121.0 ppm, its directly bound proton resonating at 9.17 ppm.

Continuing with the oxazole protonated nitrogen resonances, N-23, N-33 and N-47 contained in Oxa-A, -B and -C, respectively, we find them to be clustered,

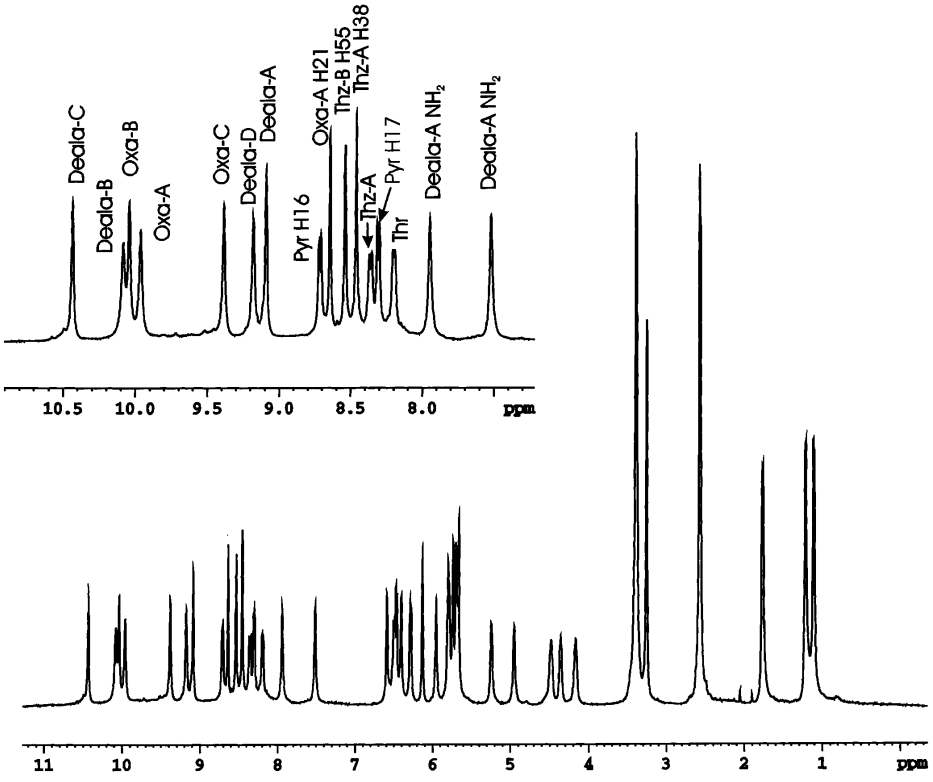


Figure 1. Proton reference spectrum of sulfomycin-I (**1**) recorded at 500 MHz in DMSO-d_6. Key resonances needed for the assignment of the various ^{15}N resonances of the molecule are labeled in the inset.

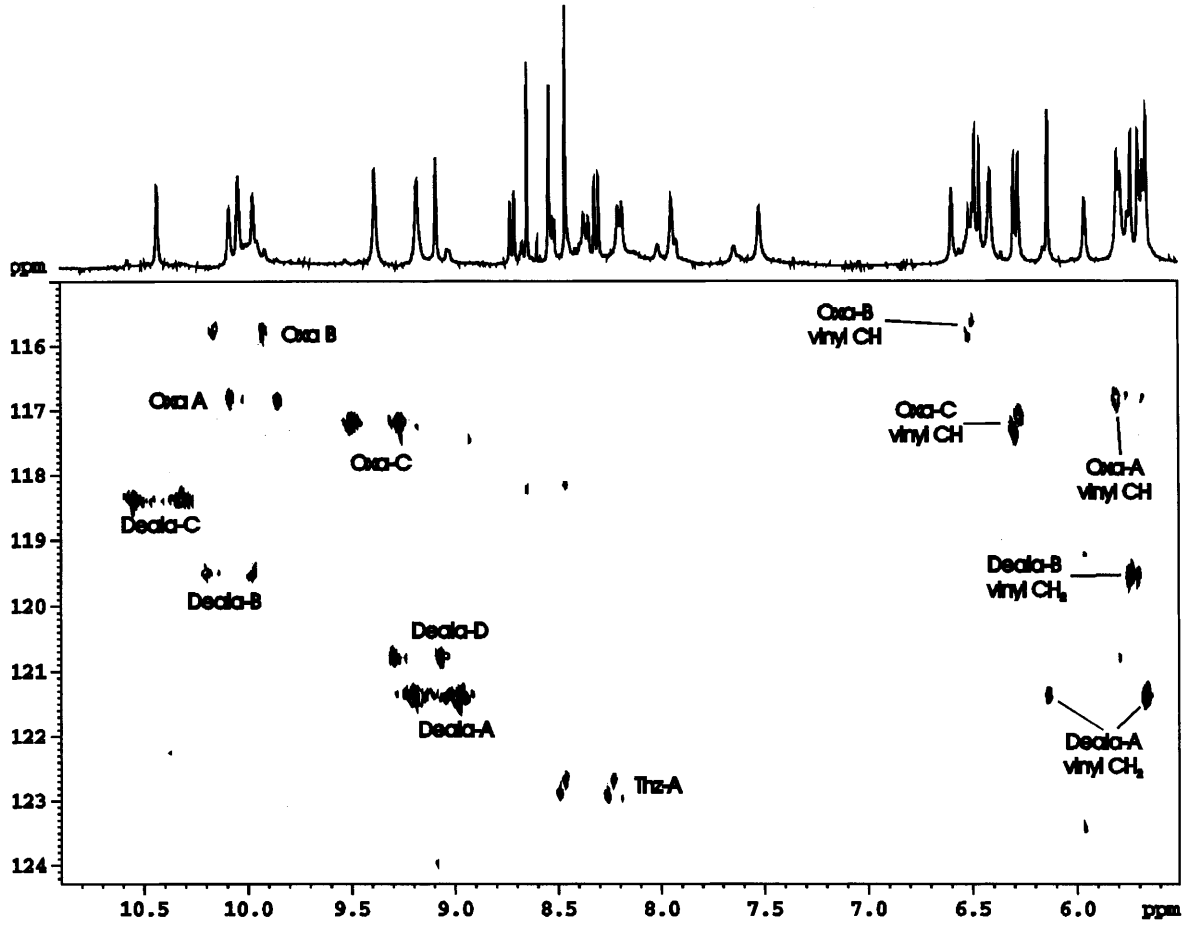


Figure 2. GHNMQC spectrum of sulfomycin-I (**1**) optimized for a 6 Hz (83 ms) long-range ^{1}H–^{15}N coupling recorded at 400 MHz in DMSO-d_6. Both direct and long-range coupling responses are shown, labeled according to Fig. 1. Data were acquired overnight using a sample prepared by dissolving *ca.* 2 mmol of sulfomycin-I in 160 μl of DMSO-d_6 (99.992% D) in a 3mm micro NMR tube.

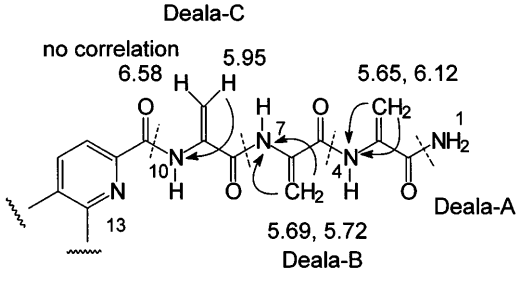
resonating at 117.0, 115.7 and 117.3 ppm, respectively. Their corresponding directly bound protons resonate at 9.97, 10.00 and 9.44 ppm, respectively.

Remaining to be assigned are the thiazole-A N-40 and threonine N-50 nitrogen resonances. Whereas all of the aforementioned amide protons were singlets, those attached to N-40 and N-50 are doublets resonating reasonably far upfield of their non-coupled secondary amide proton counterparts at 8.35 and 8.18 ppm, respectively. The directly bound nitrogen resonances defined the downfield and upfield limits of the nitrogen shifts of the protonated macrocyclic ring nitrogens, resonating at 123.0 and 110.3 ppm, respectively. Finally, it is also worth noting that in the 6 Hz optimized spectrum the direct response for the Thr N-50 resonance (this region of the spectrum is not shown in Fig. 2) was completely suppressed owing to modulation.[49]

Long-range response to the protonated amide nitrogen resonances: Group II responses

Long-range responses providing redundant secondary confirmation of the protonated nitrogen resonance assignments were also readily observed using a sample of *ca.* 2 mmol in a 3 mm tube at 400 MHz. The 6 Hz optimized GHNMQC spectrum of **1** is shown in Fig. 2. Long-range responses were observed for most of the protons having two- or three-bond coupling pathways to the protonated nitrogen resonances. Again, we will begin by considering responses correlating the protonated nitrogens in the 'tail' of the molecule.

Beginning with N-4 that resonates at 121.5 ppm and is directly correlated to the amide proton resonating at 9.07 ppm, as shown by **2**, N-4 has two long-range coupling responses possible to the exocyclic methylene protons resonating at 5.65 and 6.12 ppm in the Deala-A residue. Both exhibit correlations to N-4 as shown in Fig. 2. In a similar fashion, the exocyclic methylene protons of the Deala-B residue resonating at 5.69 and 5.72 ppm long-range couple to N-7 resonating at 119.7 ppm. The directly coupled N-7 amide proton resonates



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at 10.07 ppm. In contrast, for the Deala-C protons, which resonate at 6.58 and 5.95 ppm, only the upfield member of the pair long-range couples to N-10 resonating at 118.6 ppm. The H-10 amide proton directly coupled to N-10 resonates at 10.42 ppm. Although none of the dehydroalanine exomethylene protons has been assigned in terms of *cis–trans* regiochemistry, the proton resonating at 5.95 ppm that long-range couples to N-10 may be tentatively assigned as *trans* to the nitrogen based on Karplus considerations inherent to ^{1}H–^{15}N coupling pathways. Support for this assignment is provided by the long-range couplings observed for the Oxa-B and -C residues of sulfomycin-I considered below.

As noted above, the remaining long-range couplings for this group of responses are those to the oxazole residues A–C. Beginning with the Oxa-A residue, the vinyl methylene proton resonating at 5.79 ppm was long-range coupled to N-23, which resonated at 117.0 ppm, as shown by **3**. The other vinyl proton, resonating at 5.66 ppm, did not long-range couple to N-23. Again, the regiochemical assignment of the vinyl protons was based on Karplus considerations supported by behavior observed for the Oxa-B and -C residues.

In contrast to the dehydroalanine and oxazole-A residues, all of which contain two exomethylene protons, the Oxa-B and -C residues (see **4** and **5**, respectively) each contain only a single exomethylene proton specifically known to be oriented '*trans*' to the nitrogens of interest, N-33 and N-47, respectively. In both cases, the protons long-range couple to their respective nitrogens, N-33 and N-47 resonating at 115.7 and 117.3 ppm, respectively. Based on the absent couplings observed for the dehydroalanine-C and oxazole-A residues of selected exomethylene vinyl protons, we can reasonably assume that couplings would also have been absent for the Oxa-B and -C residues if the orientation of the protons in these residues were '*cis*' to their respective nitrogens. It is specifically on the basis of this observation that we have made the assignments for the other residues discussed above.

Finally, other plausible long-range three-bond couplings which were not observed in the case of sulfomycin-I (**1**) are those of the exomethylene group of the Deala-D residue to N-26. Why neither of these correlations was observed is not known. Although 500 MHz data were acquired (see the following discussion), it is worth noting that no response was observed from either proton to N-26 in experiments optimized for 1.75, 3 or 4 Hz.

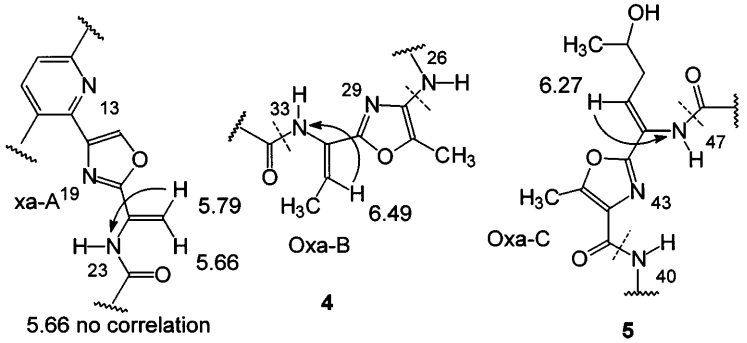
Long-range response to the non-protonated heteroaromatic nitrogen resonances: Group III responses

There were several three-bond long-range couplings to heteroaromatic nitrogen resonances that were unobservable in the 400 MHz spectra using *ca.* 2 mmol of **1** dissolved in 160 μl of DMSO-d_6. These responses include a three-bond coupling from H-17 to the N-13 pyridine nitrogen expected to resonate in the range 280–320 ppm. Likewise, three-bond couplings were also possible from the oxazole-A H-21 resonance to N-19 and from the thiazole-B H-55 proton to N-53. These couplings are all contained in the structural fragment represented by **6**. This component of sulfomycin has recently been synthesized and was given the trivial name sulfomycinamate.[19]

An additional three-bond coupling is also possible from H-38 to N-36 in the thiazole-A residue. In contrast, N-29 in the oxazole-B residue and N-43 contained in the oxazole-C residue do not have three-bond coupling possibilities since the position normally occupied by a proton on the oxazole heterocyclic ring is instead substituted by a methyl group, consigning correlations associated with these resonances to Group IV. These potential coupling pathways are shown by **7**.

Having identified the possible long-range coupling pathways in Group III to the heteroaromatic nitrogens, it is useful to consider the nitrogen resonance assignments that have been reported for the related thiopeptide antibiotic nosiheptide (**8**). Although the recent work of Gasmi *et al.*[51] utilized contemporary gradient-based inverse-detected NMR methods to establish the ^{15}N shifts of the protonated nitrogen resonances of nosiheptide (**8**), no assignments of the non-protonated heteroaromatic nitrogens were reported in their work. In contrast, the earlier work of Mocek *et al.*[22] that detailed the biosynthesis of nosiheptide using labeled precursors provided unequivocal ^{15}N chemical shift assignments for every resonance in the molecule. Hence the chemical shift range for the nitrogens at the 3-position of the thiazole residues is well established. Chemical shifts ranged from 303.9 ppm for the N-7 resonance contained in Thz-5 to 325.3 ppm for the N-18 resonance of Thz-4 (see **8** for the numbering and residue labeling scheme). The remaining thiazole 3-position nitrogen chemical shifts were 322.8 ppm for N-55 of Thz-1, 310.5 ppm for the Thz-2 N-44 resonance and 313.3 ppm for the N-62 resonance of Thz-3.

The 500 MHz GHNMQC spectrum of sulfomycin-I recorded with 4 Hz optimization contained three sets of long-range correlations to non-protonated heteroaromatic nitrogen resonances. The 4 Hz optimized spectrum is not shown. Instead, a 3 Hz optimized spectrum is presented in Fig. 3, which contained all the responses in the 4 Hz optimized spectrum, plus it importantly contains a response for the correlation from H-21 to N-19 in the Oxa-A residue not observed in the 4 Hz optimized spectrum. Responses are observed in the spectrum shown in Fig. 3 from the singlet resonating at 8.45 ppm (H-38) and the doublet resonating at 6.45 ppm (H-39) to a nitrogen resonating at 310.6 ppm which may be consequently assigned as N-36 in the Thz-A residue. A further singlet resonating at 8.55 ppm correlates with a nitrogen resonating at 314.3 ppm. Based on its chemical shift, the proton resonating at 8.55 ppm could be either H-21 in the Oxa-A residue or H-55 in the Thz-B residue. From the work of Fate *et al.*,[21] both the H-21



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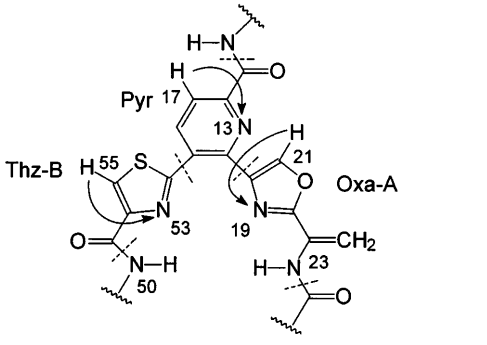

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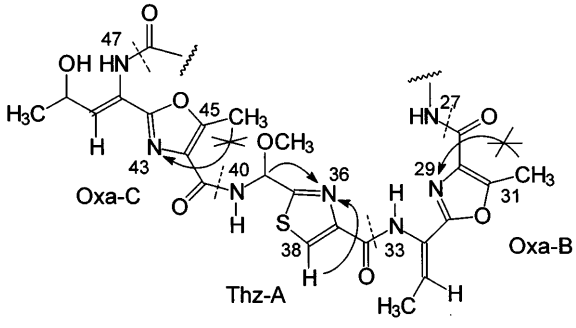

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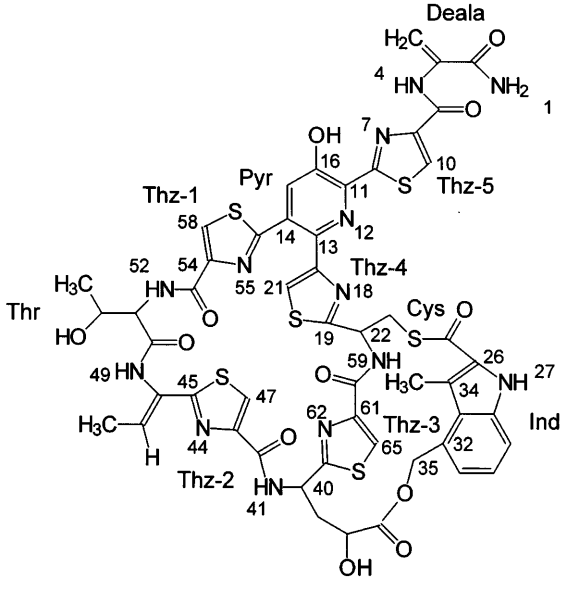

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and H-55 resonances of sulfomycin-I, at the concentration used in their NMR study, resonated at 8.64 ppm; no proton was observed to resonate at 8.55 ppm in that report, whereas resonances were observed at both 8.64 and 8.55 ppm in the present study. While it is certainly possible to assign unequivocally both H-21 and H-55 on the basis of the chemical shift of their directly attached carbons (C-21 and C-55 resonate at 140.2 and 127.5 ppm, respectively), the chemical shift of the nitrogen resonance now in question, 314.3 ppm, can readily be used for discriminatory purposes. Specifically, the observed nitrogen chemical shift is well within the range of expected ^{15}N chemical shifts for the thiazole residues of sulfomycin-I based on the previous nosiheptide ^{15}N assignments of Mocek *et al.*[22] In contrast, oxazole has an assigned ^{15}N chemical shift of 255.8 ppm in DMSO.[52] Hence, based on the ^{15}N chemical shift alone, the proton resonating at 8.55 ppm is assignable as H-55 and the nitrogen, correspondingly, as N-53 in the Thz-B residue.

Another important response observed in the 3 Hz optimized GHNMQC spectrum shown in Fig. 3 is that correlating the proton resonating at 8.64 ppm assigned as the Oxa-A H21 resonance to a nitrogen resonance observed at 255.8 ppm. As will be noted from the preceding discussion, this chemical shift is in excellent agreement with that of oxazole,[52] allowing the assignment of the nitrogen resonance as the Oxa-A N-19 resonance.

The remaining coupling in the 4 Hz optimized GHNMQC spectrum, which is observed weakly in the 3 Hz optimized spectrum, is the response correlating the H-17 doublet at the 3-position of the pyridine ring to a ^{15}N resonance at 302.8 ppm (see **6**). Referring again to the work of Mocek *et al.*,[22] the chemical shift of the pyridine nitrogen of nosiheptide (N-12 in **8**), which corresponds to N-13 in the case of sulfomycin-I (**1**), was assigned at 317.8 ppm. The assigned chemical shift of N-13 in sulfomycin-I is in reasonable accord with the corresponding nitrogen of nosiheptide, given the differences in substitution of the pyridine nucleus. In the present case, the pyridine is substituted with a thiazole and oxazole residue; whereas in the case of nosiheptide, both heteroaromatic substitutions are by thiazoles.

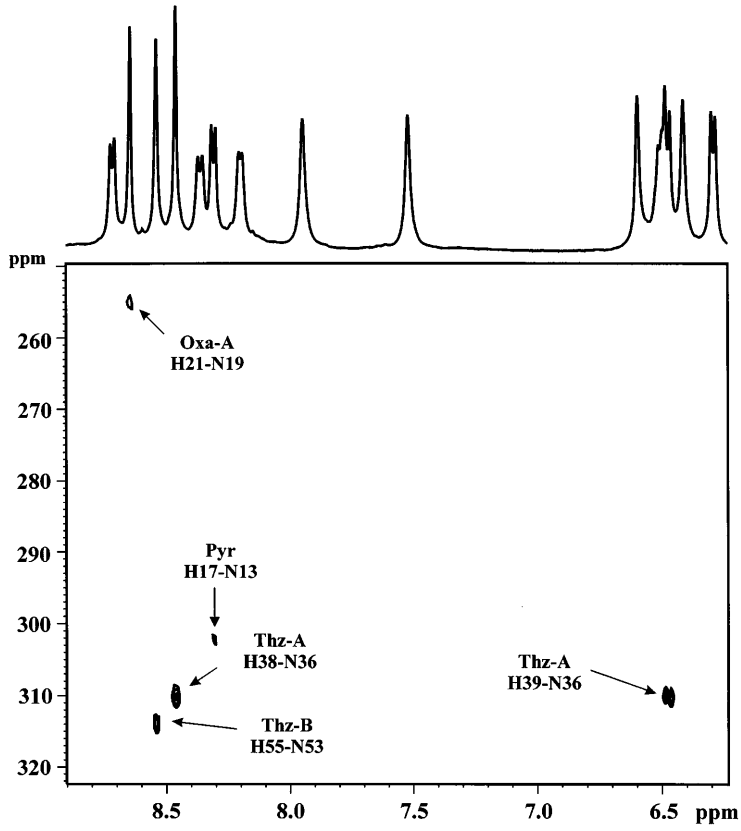

Figure 3. GHNMQC spectrum of sulfomycin-I (**1**) optimized for a 3 Hz (167 ms) long-range ^{1}H–^{15}N coupling recorded at 500 MHz in DMSO-d_6. Both direct and long-range coupling responses are shown, labeled according to Fig. 1. Data were acquired in 72 h using a sample prepared by dissolving *ca.* 4 mmol of sulfomycin-I in 500 μl of DMSO-d_6 (99.992% D) in a 5 mm NMR tube.

Finally, although a 1.75 Hz optimized GHNMQC spectrum was also recorded, no new correlations were observed, nor were the correlations to the pyridine, oxazole or thiazole residues observed in the 3 and 4 Hz optimized spectra acquired for the same time. Weak correlations were observed in the 1.75 Hz optimized spectrum to the protonated nitrogens; some of the Group II responses discussed above were also observed. It is probable, given the long 286 ms delay necessary for the 1.75 Hz optimization, that extensive T_2 dephasing would have resulted, causing sufficient signal losses to preclude the observation of the already weak long-range correlations in the Group III responses or the Group IV responses discussed below.

Assignment of the non-protonated heteroaromatic nitrogen resonances without three-bond coupling pathways: Group IV responses

There remain to be assigned at this point only the N-29 and N-43 resonances of the Oxa-B and -C residues, respectively. The 3-position of the heteroaromatic ring in the case of both residues is methyl substituted, precluding the observation of a three-bond coupling in a GHNMQC spectrum. As noted above, optimization of the experiment at 1.75 Hz also failed to give any response for these potential correlation pathways despite the fact that they would be detected with the intrinsically greater sensitivity afforded by the three protons of the methyl singlets. Hence, the assignment of these resonances will require the development of an alternative approach, currently under investigation in our laboratories.

CONCLUSIONS

The studies described in this paper have demonstrated the facile assignment of the protonated ^{15}N resonances of a sample of *ca.* 2 mmol of sulfomycin-I (**1**) through the use of 3 mm gradient micro inverse probe technology. Although more effort and sample were required, it has also been shown that it is possible to assign the non-protonated heteroaromatic nitrogen resonances of sulfomycin-I when reasonable three-bond coupling pathways are present in the heteroaromatic amino acid subunits. With the higher intrinsic sensitivity of a 500 or 600 MHz instrument, coupled with micro inverse probe technology and restricted volume Shigemi micro sample tubes or, alternatively, a 1.7 mm gradient inverse submicro probe, it is reasonable to conclude that even the

most demanding experiments described in this paper could be accomplished on less material and in comparable or perhaps even shorter acquisition times.

EXPERIMENTAL

All of the 400 MHz studies were performed on a sample of 25 mg (*ca.* 2 mmol) of sulfomycin-I dissolved in 160 µl of 99.996% DMSO-d_6 (Cambridge Isotope Laboratories). The sulfomycin-I used to prepare NMR samples was obtained by extraction of fermentation broths of *Steptomyces argenensis* cultured in-house followed by preparative chromatographic isolation. By analytical HPLC methods, the sulfomycin-I used in this study was >98% pure. Experiments were performed using a Bruker AMX-400 spectrometer operating at a frequency of 400.13 MHz for routine ^{1}H observation. The instrument was equipped with gradients (single *z*-axis) and a Nalorac MIDG-400-3 micro gradient inverse-detection probe. The GHNMQC pulse sequence employed has been published previously.[49] Direct response experiments were performed by optimizing the 'long-range' delay for an assumed 90 Hz ^{1}H–^{15}N direct coupling (5.5 ms), affording only the direct responses with the respective one-bond couplings intact. Long-range experiments were performed using various optima ranging from 6 to 10 Hz (from 83 to 50 ms). Data sets were typically acquired using either 2048 or 4096 points in the F_2 frequency domain and from 128 to 384 files in the F_1(^{15}N) frequency domain, the choice based on the F_1 spectral width. Nitrogen chemical shifts were referenced using a sample of indole, the N-1 shift of which was taken as 132.2 ppm downfield of liquid ammonia, run at the same transmitter offset and spectral width as the sulfomycin-I data. (Nitrogen-15 chemical shifts referenced to liquid ammonia can be converted to the nitromethane chemical shift reference scale by subtracting the reported shift from that of liquid ammonia at +379.5 ppm. By convention, chemical shifts upfield of nitromethane, i.e. those with a chemical shift relative to liquid ammonia of <379.5 ppm, are reported with a positive chemical shift; the plus sign is usually specified. As an example, indole, which resonates at 132.2 ppm downfield of liquid ammonia, would have a ^{15}N chemical shift of + 247.3 ppm on the nitromethane scale.)

The 500 MHz data discussed here were acquired using samples containing either 25 or 100 mg (*ca.* 8 mmol) of sulfomycin-I dissolved in either 160 or 600 µl, respectively, of 99.992% DMSO-d_6 (Cambridge Isotope Laboratories). Experiments were performed on a Bruker AMX-500 spectrometer operating at a frequency of 500.13 MHz for routine ^{1}H observation. The instrument was equipped with gradients (single *z*-axis) and either a Nalorac 3 mm gradient triple resonance micro inverse probe or a Bruker 5 mm gradient inverse triple resonance probe. Direct response experiments were performed by optimizing the 'long-range' delay for an assumed 90 Hz ^{1}H–^{15}N direct coupling (5.5 ms). Long-range experiments were performed with delays ranging from 1.75 to 4 Hz (from 286 to 125 ms). Data sets were acquired using 2048 points in the F_2 frequency domain and 128 files in the F_1(^{15}N) frequency domain. Data were subsequently processed by zero-filling to 4096 points in F_2 prior to transformation and by linear prediction to 256 files in F_1 followed by zero-filling to 512 points prior to transformation.

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