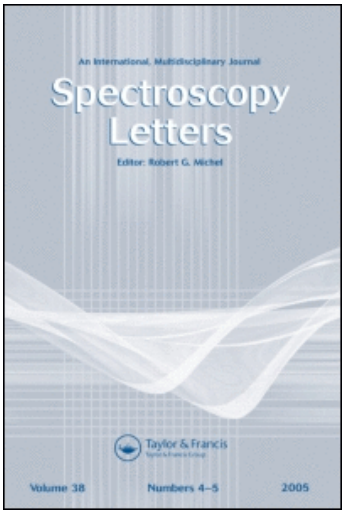

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Two Dimensional NMR Study of Aspidospermine

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TWO DIMENSIONAL NMR STUDY OF ASPIDOSPERMINE

Key Words: Aspidospermine, Aspidospermidine, NMR.

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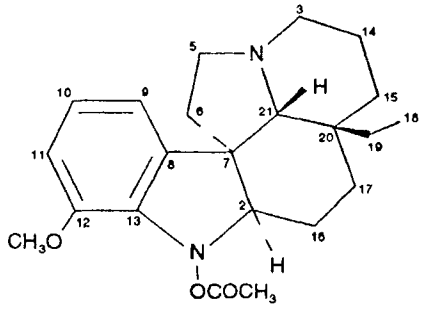
ABSTRACT

Two dimensional COSY, HETCOR and inverse HMBC experiments were performed on a natural alkaloid, Aspidospermine, which allowed complete ^{1}H and ^{13}C spectral assignments to be made. These results suggest that the reversal of certain ^{13}C literature assignments of related compounds is required.

INTRODUCTION

The alkaloid aspidospermine has been isolated from the bark of various *Aspidosperma* species[1]. The molecular structure (I), indicated by chemical studies, was confirmed by X-ray crystal structure analysis of the N-1 methiodide derivative of the title compound[2]. Only features of this structure were confirmed by ^{1}H nmr analysis conducted at 60 MHz[3], although full ^{1}H



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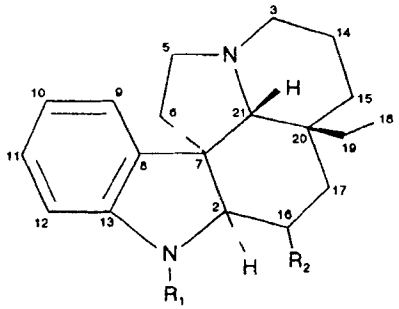

II ($R_1 = R_2 = H$)

III ($R_1 = CH_3$, $R_2 = CO_2CH_3$)

assignments for aspidospermane-type alkaloids have been published[4] and the ^{13}C resonances of aspidospermidine (II) identified[5].

DISCUSSION

The ^1H and ^{13}C assignments of aspidospermine were made using COSY[6], HETCOR[7] and inverse HMBC[8] experiments in order to corroborate the proposed structure (I) and thereby confirm literature values assigned for related compounds prior to the advent of 2D nmr methods. Our study suggests that the reversal of a pair of ^{13}C assignments for II is required.

The following assignments were made from the ^1H (Fig 1), COSY (Fig 2), HETCOR (Fig 3), ^{13}C (Fig 4), and inverse HMBC (Fig 5) spectra. The ^1H nmr spectrum (Fig 1) clearly shows the high field triplet at δ 0.56 ($CH_2\underline{CH_3}$), singlets at δ 2.15 ($COC\underline{H_3}$) and 3.82 ($OC\underline{H_3}$), and the aromatic signals at ~ δ 7 as previously reported[3]. The assignments presented here will proceed from the most downfield proton signals.

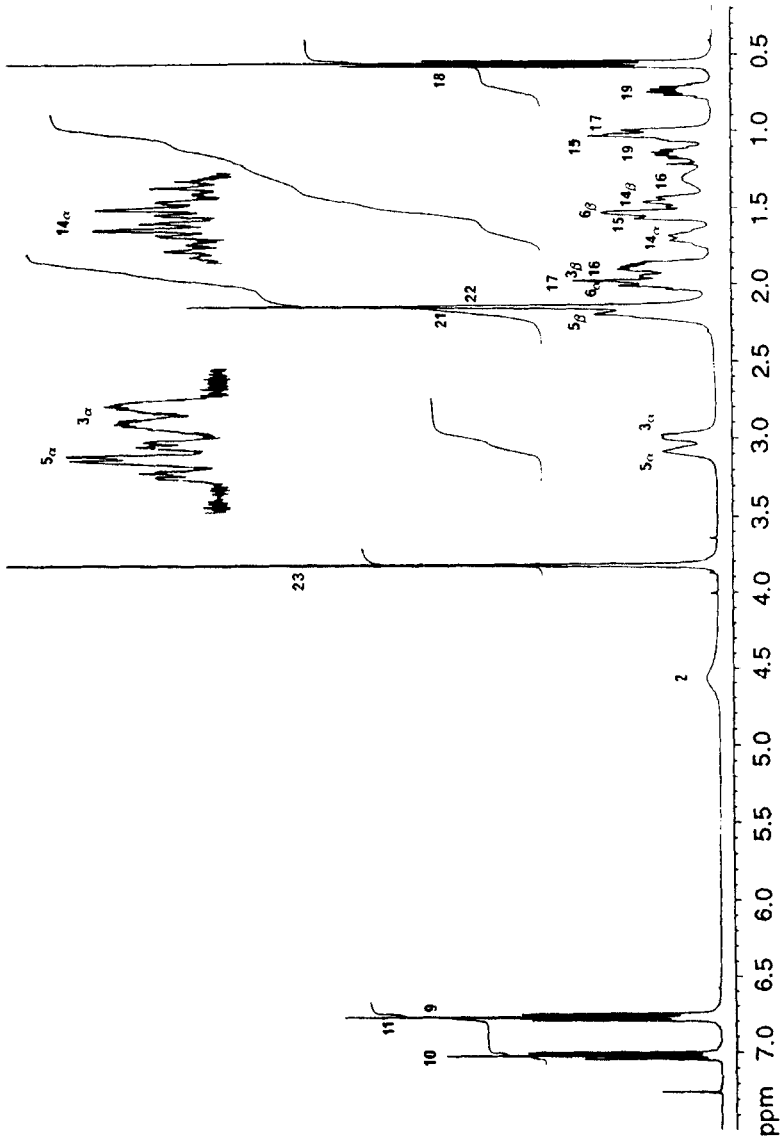

Fig 1: 400 MHz ^{1}H nmr spectrum of aspidospermine shown high resolution expansions of crucial regions recorded at 300 MHz.

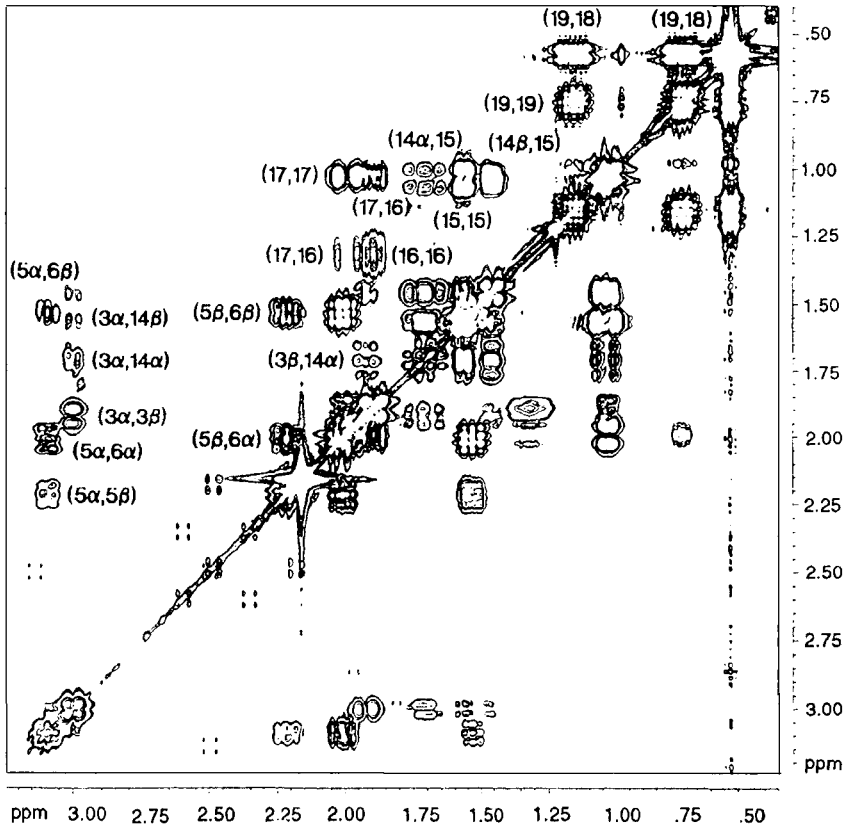

Fig 2: Expansion of the high field region of the COSY spectrum of aspidospermine recorded at 400 MHz. For clarity only some of the correlations have been labelled.

Closer inspection of the resolution enhanced aromatic proton signals permit the assignment of H-10 (dd at δ 7.02; J = 7.5, 7.6 Hz), H-9 (dd at δ 6.78; J = 7.5, 1.1 Hz) and H-11 (dd at δ 6.76; J = 7.6, 1.1 Hz) on the basis of expected splittings and the electronic effect of the methoxy substituent on C-12[9]. The COSY spectrum (data not shown) confirms the connectivity between H-10 and adjacent protons, while the HETCOR diagram straightforwardly identifies the attached carbon resonances (Table 1). The inverse HMBC experiment (Fig 5, Table 2) confirms these assignments and

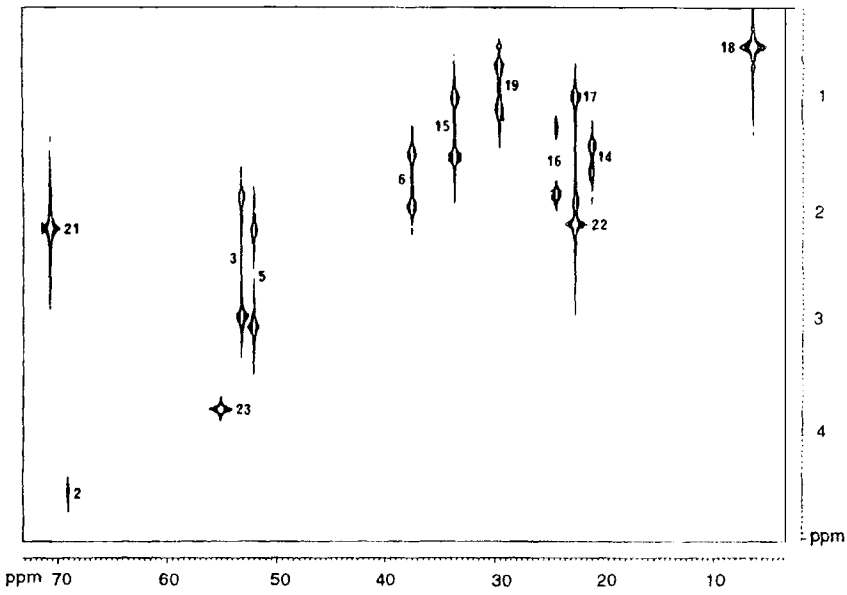

Fig 3: Expanded HETCOR diagram of aspidospermine (JC,H = 140 Hz). The crosspeaks have been labelled according to the attached carbon resonance.

permits identification of the ^{13}C resonances at 129.3, 143.4 and 149.5 ppm as being due to C-13, C-8 and C-12 respectively. Additionally C-12 is also correlated to the protons of the attached methoxy substituent.

The broad singlet at δ 4.57, connected to the carbon signal at 69.3 (Fig 3), was assigned to H-2 on the basis of chemical shift analysis (-CH-NH-COR at δ 4.1[9]) while the remaining carbon atom of the indoline skeleton (C-7) was assigned to the quartenary carbon signal at δ 53.4 by comparison with the ^{13}C data published for vindoline, another alkaloid from *Aspidosperma* (C-7 at 52.6 p.p.m., reference 10). This assignment was corroborated by the 3-bond coupling detected between this signal and H-9 (Fig 5).

The methoxy protons, previously ascribed to the singlet at δ 3.82, are connected to the broad carbon signal at δ 55.4 (HETCOR). The broadness

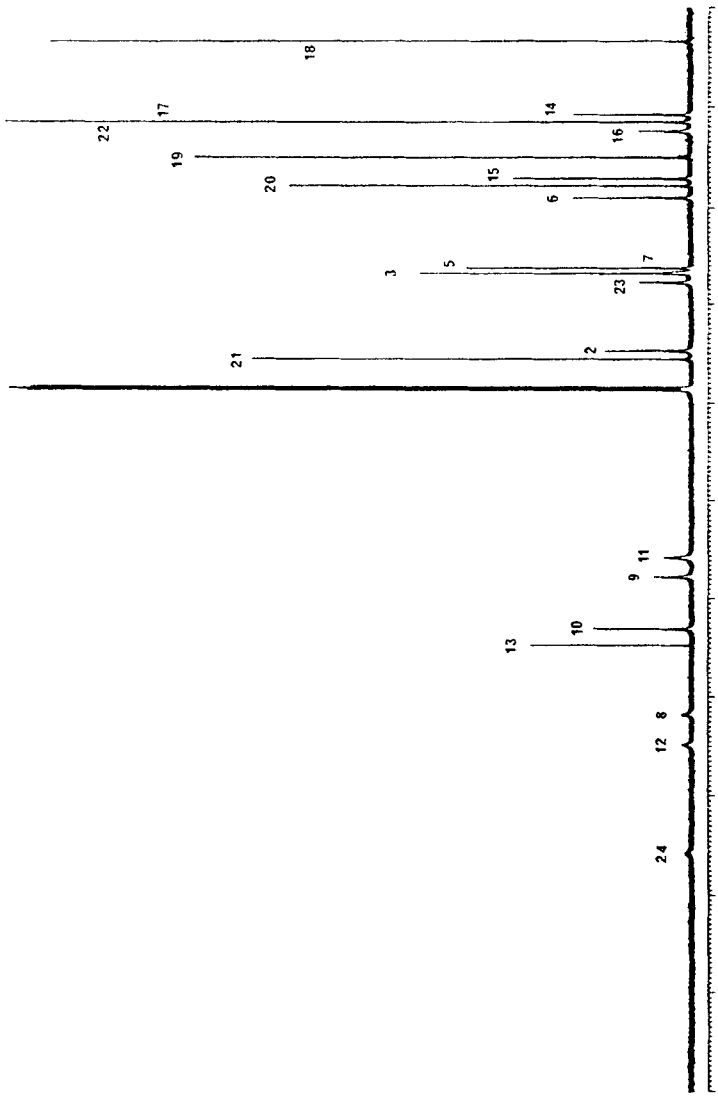

Fig 4: 100 MHz ^{13}C- nmr spectrum of aspidospermine.

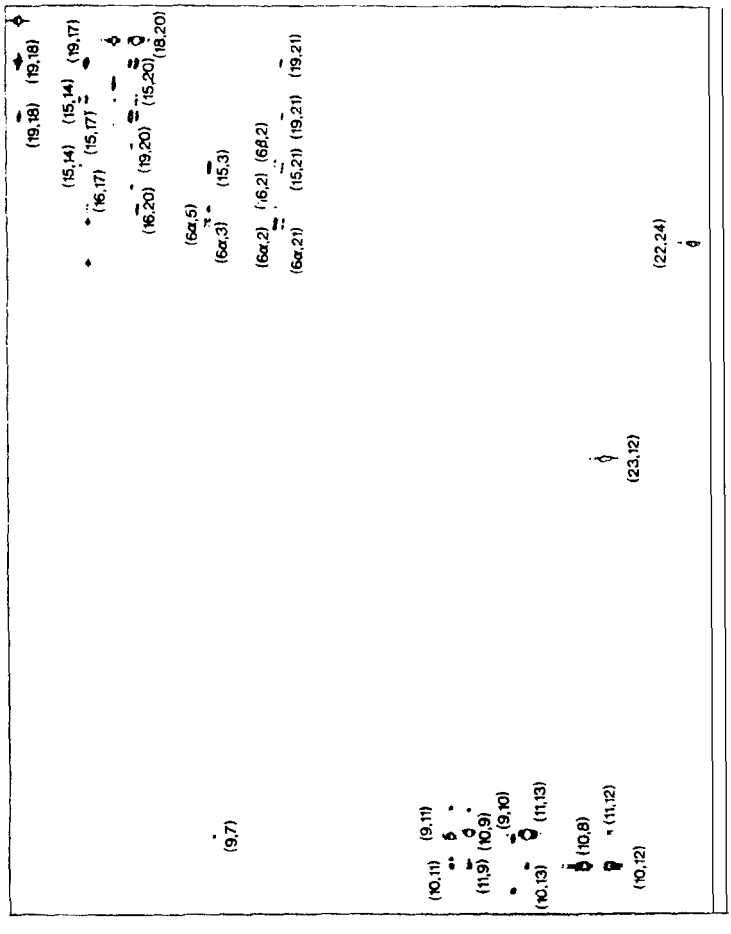

Fig 5: Inverse heteronuclear multiple bond coherence (HMBC) spectrum of aspidospermine. For clarity only some correlations have been indicated (H,C).

TABLE 1

^1H and ^{13}C Chemical Shifts for Aspidospermine(I)

	^{13}C			^1H
	I	**Lit(II)**	**Lit**	
C_2	69.5	65.4		4.57
C_3	53.5	53.7		2.99(α)
				1.95(β)
C_5	52.4	52.9		3.08(α)
				2.22(β)
C_6	37.9	38.7		2.01(α)
				1.53(β)
C_7	53.4	52.9		
C_8	143.4	135.6	134.2	
C_9	115.4	122.7	113.5	6.78
C_{10}	126.0	118.8	118.5	7.02
C_{11}	111.2	127.0	110.3	6.76
C_{12}	149.5	110.1	146.0	
C_{13}	129.3	149.3	136.8	
C_{14}	21.5	21.6		1.70(α)
				1.46(β)
C_{15}	34.1	34.3		1.57
				1.03
C_{16}	24.8	23.0		1.88
				1.31
C_{17}	22.9	28.1		1.98
				1.00
C_{18}	6.7	6.6		0.56
C_{19}	30.0	29.8		1.15
				0.74
C_{20}	35.5	35.5		
C_{21}	71.0	71.1		2.19
C_{22}	23.0			2.15
C_{23}	55.4			3.82
C_{24}	171.2			

TABLE 2

Correlations established using the HMBC experiment (Fig 5)

^{13}C		^{1}H	
δ	Assignment	δ	Assignment
6.7	C_{18}	0.42	•
		0.74	H-19
		1.15	H-19
21.5	C_{14}	1.03	H-15
		1.57	H-15
22.9	C_{17}	0.76	H-19
		1.03	H-15
		1.88	H-16
23.0	C_{22}	2.15	H-22
		2.01, 2.32	*
30.0	C_{19}	0.56	H-18
		0.92	*
		1.03	H-15
34.1	C_{15}	0.74	H-19
		1.15	H-19
		1.38	*
		1.70	H-14α
35.5	C_{20}	0.56	H-18
		0.74	H-19
		1.03	H-15
		1.15	H-19
		1.57	H-15
		1.88	H-16
52.4	C_5	1.53	H-6β
		2.01	H-6α
53.4	C_7	6.78	H-9
53.5	C_3	1.57	H-15
		1.88	H-16(?)
		2.01	H-6α

of the carbon resonance may be attributed to the rapid relaxation arising from the proximity of the oxygen lone pairs of electrons.

All the methylene carbon resonances were revealed by the DEPT experiment and the attached protons followed from the HETCOR spectrum. The most downfield proton signals (~ δ 3) were assigned to H-5α and H-3α due to their closest proximity to the N-4 lone pair of electrons. The individual assignments followed from the high resolution ^{1}H-nmr spectrum recorded at 300 MHz (inset Fig 2) using a dilute sample in order to improve resolution. This revealed the coupling pattern of a triplet of doublets at δ 3.08 (H-5α) and a doublet of triplets at δ 2.99, which was ascribed to H-3α. The latter assignment was confirmed by the COSY (Fig 2) crosspeak to the clearly resolved signal at δ 1.70 which has a unique coupling pattern of a quartet of triplets. This signal must be due to H-14α as C-14 is the only carbon atom adjacent to two methylene groups.

The remaining protons in the two spin systems were then identified from the COSY spectra using H-3α and H-5α as the starting points. The HMBC diagram confirmed these assignments and permitted the identification of C-21 at 71.0 ppm (correlated to H-6α and H-15β) and C-20 at 35.5 ppm (crosspeaks to H-15 and the ethyl side-chain protons).

C-20 also shows connectivity to the proton signal at δ 1.88 which in turn gives a COSY crosspeak to the proton resonance at 1.00 ppm, thereby accounting for the last pair of methylene groups. The attached carbons resonate at 24.8 and 22.9 ppm respectively and can be ascribed to C-17 and C-16 as the C-17 signal shows 3-bond coupling to H-15 and H-19. The broadness of the C-16 signal and that of H-16 at 1.31 ppm, may be attributed to the close proximity of the -NOCOCH$_3$ group.

The C-2 resonance at δ 69.3, previously assigned by inspection, is confirmed by long-range couplings to H-6α, H-6β and H-16 at δ 1.88 (Fig 5, Table 2). Finally, the HMBC experiment confirms the attachment of the

ethyl group to C-20 and also shows the correlation between the CH_3 protons at δ 2.15 and the carbonyl resonance at 171.2 ppm (C-24).

The ^1H and ^{13}C assignments are presented in Table 1, together with literature values for related compounds[5,11]. The ^1H assignments could not be usefully compared with those of the aspidospermane-type alkaloids[3], as these compounds all contain double bonds between C_2 and C_{16} and either an oxygen or double bond at C_{15}. Consequently the most appropriate model compound was aspidospermidine (II) for which ^{13}C nmr data is available[5].

The ^{13}C chemical shift values for I and II are presented in Table 1. They agree within 1 ppm of each other except for those shifts associated with the aromatic carbons and the carbon atoms in close proximity to N-1 (C_{12}, C_{16} and C_{17}). Unsurprisingly, better correlation is found between the aromatic signals and those reported for indoline derivatives containing a methoxy substituent at C-12 (See Table 1, reference 11).

The replacement of N_1-H (II) by N_1-$OCOCH_3$ (I) appears to have a deshielding effect on C_2(+3.9 ppm) and C_{16}(+1.9 ppm) and an unusually large shielding effect on C_{17} (-5.2 ppm). This suggests that the original assignments of C_{16} and C_{17}[5], should be reversed, leading to a deshielding of +3.3 ppm for C_{16} and a much smaller shielding for C_{17} (-0.1 ppm).

The literature assignment of II followed from the ^{13}C nmr data reported for 16-β carbomethoxy aspidospermine (III) *viz.* C_{16} at 37.9 ppm and C_{17} at 27.5 ppm[12]. Implicit in the assignment of the signals of II at δ 23.0 (C_{16}) and δ 28.1 (C_{17}), is the assumption that the CO_2CH_3 group has a large α-effect (+14.9 ppm, C_{16}) and a very small shielding β-effect (-0.6 ppm, C_{17}). This appears to be at variance with literature values[13] in which both the α- and β-effects are positive, with $\alpha > \beta$. For example, the effect of an axial CO_2CH_3 on C_2 of trans-decalin[14] results in an α-effect of +10.2 ppm (C_2) and a β-effect of +2.5 ppm (C_3). Reversal of the literature assignments for C_{16} and C_{17} of II gives $\Delta\delta$ values of +9.9 (C_{16}) and +4.5 ppm (C_{17}) which are more in accordance with expected shifts.

EXPERIMENTAL

Aspidospermine was a gift from E. Merck (SA).

Experiments were performed as a $CDCl_3$ solution in a 5 mm sample on a Bruker AMX 400 spectrometer as well as a Varian VXR 300 machine. Chemical shifts are relative to deuterochloroform (7.25 ppm for ^1H and 77.0 ppm for ^{13}C). Spectra were recorded at either 25°C (Varian VXR 300) or 30°C (Bruker AMX 400).

Cosy spectra were recorded at 400 MHz using a 90° observe pulse. An acquisition time of 0.3 sec was used at a digital resolution of 1.6 Hz in the F2 domain and 6.3 Hz in the F1 domain. Data were processed as a 2048 × 1024 matrix without folding about the diagonal.

The two dimensional HETCOR spectrum was recorded using the standard conventional Bruker program hxco. Bruker pulse program hxco. 256 Increments, 200 transients per increment, were recorded at an F1 resolution of 12.5 Hz and an acquisition time of 0.14 sec. A relaxation delay of 1.5 sec was used resulting in a total acquisition time of 22 h. Data were processed as a 2048 × 512 matrix using QSINE apodization in both dimensions. The inverse variant HMBC was recorded with an acquisition time of 0.64 sec and an F1 resolution of 40.7 Hz. 48 Transients were recorded for each of 512 F1 increments in a time of 11.5 h. Data were processed as a 1024 × 2048 data matrix using QSINE apodization.

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