The Complete ^1H and ^{13}C Chemical Shift Assignments of a Cyclopropylpyrroloindole Analog: Adozelesin

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The ^1H and ^{13}C nmr spectral assignments of [7bR]-*N*-[2-[(4,5,8,8a-tetrahydro-7-methyl-4-oxocyclopropa[*c*]pyrrolo[3,2-*e*]indol-2(1*H*)-ylcarbonyl]-1*H*-indol-5-yl]-2-benzofurancarboxamide (Adozelesin) (**1**) are described. Complete and unambiguous assignments of the hydrogen and carbon spectra were made using a combination of conventional homonuclear and gradient-selected inverse-detected heteronuclear nmr experiments: double quantum filtered ^1H-^1H correlation spectroscopy (COSY), gradient-selected heteronuclear single quantum coherence spectroscopy (gs-HSQC), and gradient-selected heteronuclear multiple bond coherence spectroscopy (gs-HMBC). The enhanced sensitivity of these experiments allowed a smaller sample concentration and shorter spectral collection times for a full nmr analysis of this compound. The nmr data corroborates the published structure of this compound.

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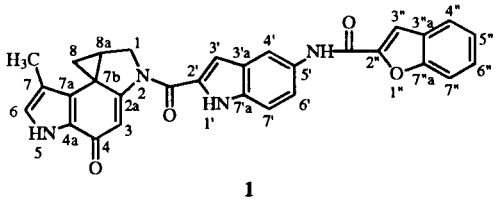
Introduction.

Adozelesin (U-73,975) is a synthetic analog of the cyclopropylpyrroloindole antitumor antibiotic, CC1065. This compound is under development as a novel chemotherapeutic agent that has been shown to bind in the minor groove of DNA and alkylate the N3 position of adenine [1,2]. Initial nmr characterization of this compound was limited due to its poor solubility at the concentrations required for direct-detected two-dimensional nmr experiments. Subsequently, two inverse-detected nmr experiments (gs-HSQC, gradient-selected heteronuclear single quantum coherence spectroscopy [3,4] and gs-HMBC, gradient-selected heteronuclear multiple bond coherence spectroscopy [5]) have been implemented which allow full analysis and assignment of both ^1H and ^{13}C chemical shifts with smaller amounts of sample. Accordingly, a more detailed analysis of the chemical shifts (^1H and ^{13}C) and spin connectivities of Adozelesin was undertaken. This report is the result of these studies.



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Results and Discussion.

For nmr spectral assignments Adozelesin can be divided into three distinct subunits: a cyclopropylpyrroloindole, an indole, and a benzofuran. The labeling shown in structure **1** and used in the following discussion reflects this classification. The ^1H and ^{13}C chemical shift assignments for each subunit were made separately; the ^1H spin systems within each subunit did not display homonuclear coupling between adjacent subunits.

The assignment of the cyclopropylpyrroloindole subunit was initiated with hydrogens H-8A, H-8B, H-8a, and H-1A and H-1B. The resonances at 1.47/2.02, 3.22, and 4.56/4.63 ppm were assigned to these hydrogens, respectively, on the basis of chemical shift, COSY connectivities, and the unique coupled spin system (Table 1). The gs-HSQC spectrum correlates these hydrogens with ^{13}C resonances at 21.63, 21.92,

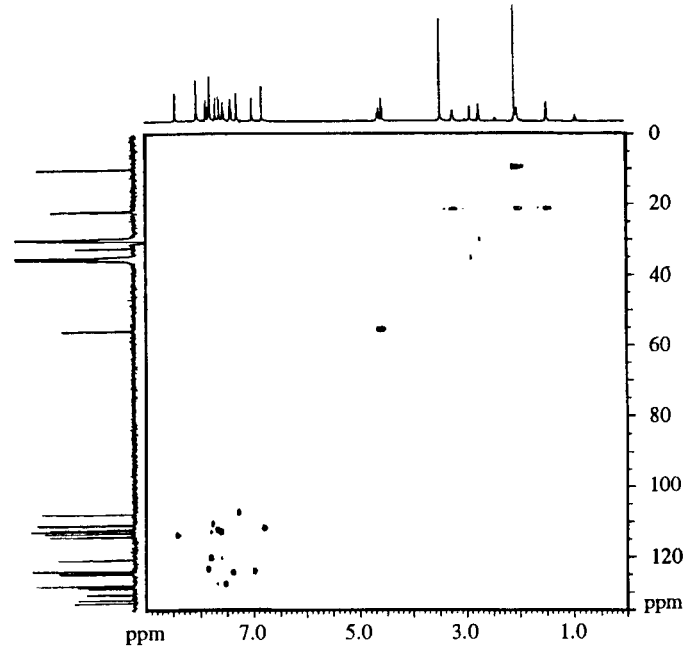

Figure 1. gs-HSQC spectrum of Adozelesin in DMF-d_7.

Table 1
^{1}H (400.13 MHz) and ^{13}C (100.61 MHz) NMR Assignments for Adozelesin

Position	^{1}H	^{13}C	COSY	gs-HMBC	DEPT135
8A	1.47	21.6	8a, 8B	7b [a], 1 [a], 2a [a]	CH_2
8B	2.02	21.6	8a, 8A		
7 methyl	2.07	9.8	—	7, 6, 7a	CH_3/CH
8a	3.22	21.9	1A, 8A, 8B	7b [a], 1 [a], 7a [a]	CH_3/CH
1A	4.56	55.8	1B, 8a	8a, 7b, 2a	CH_2
1B	4.63	55.8	1A, 8a		
3	6.80	112.0	—	7b, 4a, 2a	CH
6	6.98	124.1	5 [a]	7 methyl, 7, 7a, 4a, 4	CH
3'	7.27	107.5	1'	4', 3'a, 2', 7'a	CH
5"	7.39	124.5	4", 6"	3"a, 7"	CH
6"	7.51	127.7	5", 7"	4", 7"a	CH
7'	7.60	113.0	6'	3', 6', 3'a, 5'	CH
7"	7.67	112.4	6"	3"a, 7"a	CH
3"	7.77	110.7	—	3"a, 2", 7"a	CH
6'	7.81	120.4	7'	7'a	CH
4"	7.85	123.5	5"	7", 6", 7"a	CH
4'	8.42	114.0	—	3', 6', 5', 7'a	CH
2" NH	10.54	—	—	4', 6', 5', 2" carbonyl	—
5NH	11.51	—	6[a]	7, 6, 7a, 4a	—
1' NH	11.77	—	3'	3', 3'a, 2', 7'a	—
7b	—	32.3	—	—	C
7	—	114.1	—	—	C
7a	—	128.1	—	—	C
3'a	—	128.1	—	—	C
3"a	—	128.4	—	—	C
4a	—	130.2	—	—	C
2'	—	131.8	—	—	C
5'	—	132.7	—	—	C
7'a	—	135.0	—	—	C
2"	—	150.5	—	—	C
7"a	—	155.5	—	—	C
2" carbonyl	—	157.5	—	—	C
2a	—	161.1	—	—	C
2' carbonyl	—	162.1	—	—	C
4		177.5		—	C

[a] Weak cross peak.

and 55.75 ppm, respectively, Figure 1. These hydrogens can be further correlated with several ^{13}C resonances observed in the gs-HMBC spectrum: 32.26, 128.05, and 161.12 ppm, Figure 2. Using data from the ^{13}C DEPT135 experiment, all three of these resonances were determined to be from quaternary carbons. Because the gs-HMBC experiment correlates spins based on long-range ^{13}C-^{1}H coupling constants, the following carbon atoms may be correlated with these shifts: 8, 8a, 1, 7a, 7b, 2a, and the 2' carbonyl. Since C-8, C-8a, and C-1 have already been identified as methines or methylenes only C-7a, C-7b, C-2a, and the C-2' carbonyl remain unassigned. To unambiguously differentiate among these carbon resonances, long-range gs-HMBC connectivites from the H-7 methyl, H-6 methine, and cyclopropylpyrroloindole 5-NH hydrogens were used. The assignment of the 7 methyl hydrogens to the resonance at 2.07 ppm was based on chemical shift, integration, and multiplicity. The gs-HMBC data from these hydrogens shows a correlation to three carbon resonances at 114.1, 124.1, and 128.1 ppm. The resonance at 124.1 ppm was assigned to cyclopropylpyrroloindole C-6 using ^{13}C DEPT135 data, which indicated this carbon resonance is an aromatic methine. The 128.1 ppm ^{13}C was correlated in the gs-HMBC data to both H-8a and the H-7 methyl hydrogens; thus it was assigned as C-7a. The remaining correlated resonance from the gs-HMBC data of the 7-methyl hydrogens, 114.1 ppm, can then be assigned as C-7. Two ^{13}C shifts (32.3 ppm and 161.1 ppm) observed in the gs-HMBC data from H-8A/B, H-8a, and H-1A/B position hydrogens remain unassigned. Based on chemical shift, the 32.3 ppm resonance can be assigned as C-7b. The H-8a and H-1A/B hydrogens exhibit a correlation with the 161.1 ppm carbon resonance in the gs-HMBC data. Hence, the resonance at 161.1 ppm was assigned as C-2a. The cyclopropylpyrroloindole C-6 (assigned above) exhibits a connectivity in the gs-HSQC data with a hydrogen resonance at 6.98 ppm. The COSY data shows a weak coupling from the cyclopropyl-

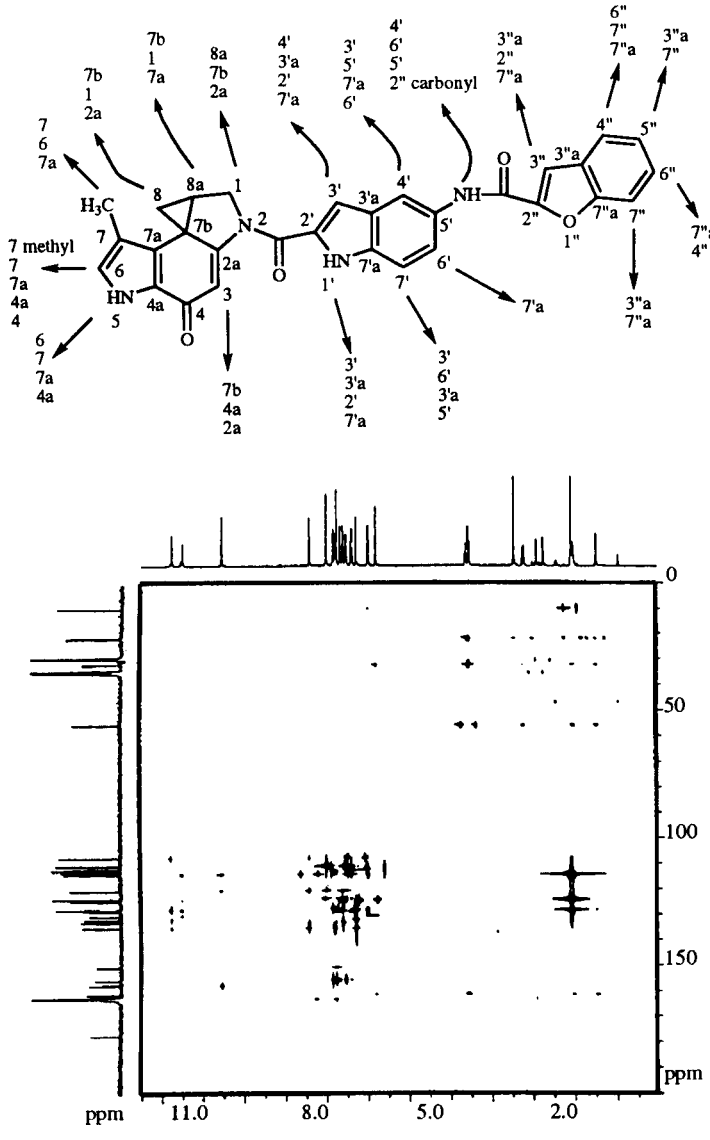

Figure 2. gs-HMBC of Adozelesin in DMF-d_7.

pyrroloindole H-6 resonance to a hydrogen resonance at 11.51 ppm. The 11.51 ppm hydrogen does not exhibit a correlation in the gs-HSQC experiment, supporting the assignment of this hydrogen as cyclopropylpyrroloindole 5-NH. The gs-HMBC data from cyclopropylpyrroloindole 5-NH and H-6 establish and/or verify the 7, 7a, 4a, and 4 carbon chemical shifts as 114.1, 128.1, 130.2, and 177.5 ppm, respectively. The cyclopropylpyrroloindole C-3 and H-3 remain to be assigned. A hydrogen resonance at 6.80 ppm exhibits a gs-HMBC correlation to the C-7b, C-4a, and C-2a carbon resonances, thus assigning cyclopropylpyrroloindole H-3. The gs-HSQC data correlates this hydrogen resonance to a carbon resonance at 112.0 ppm. None of the hydrogens in the cyclopropylpyrroloindole subunit demonstrate any gs-HMBC correlations to the 2' carbonyl.

The H-6', H-7', and H-4' form a three spin system which is unique in Adozelesin: two strongly coupled aromatic hydrogens with one of these having a weak coupling to a third hydrogen. The three resonances which satisfied these conditions are at 7.81, 7.60, and 8.42 ppm. The 7.81 and 7.60 ppm resonances are strongly coupled to each other, while the 8.42 resonance shows a weak coupling to the 7.81 ppm resonance. The 7.60 ppm resonance shows no additional ^{1}H-^{1}H coupling. The 7.81, 7.60, and 8.42 ppm resonances are assigned as H-6', H-7', and H-4', respectively. Using the gs-HSQC data C-6', C-7', and C-4' are then assigned to 120.4, 113.0, and 114.0 ppm, respectively. The two hydrogen resonances at 10.54 and 11.77 ppm were identified as NH resonances based on their chemical shifts and their lack of a correlation in the gs-HSQC spectrum. The NH resonance at 10.54 ppm shows a gs-HMBC correlation to a ^{13}C chemical shift at 120.4 ppm (C-6'), assigning this resonance to the 2" amide. Assignment of the 2" amide hydrogen allowed the assignment of the other NH (11.77 ppm) as the indole 1'-NH. The gs-HMBC data from the 2" amide hydrogen shows a correlation with four carbon resonances: 114.0, 120.4, 132.7, and 157.5 ppm. Two of these resonances were assigned previously (120.4 and 114.0 ppm). The remaining two resonances, 132.7 and 157.5 ppm, may be assigned as C-5' and the 2" carbonyl respectively, based on chemical shift and the gs-HMBC data.

The gs-HMBC data from H-6' (7.81 ppm) shows a single correlation to a ^{13}C shift at 135.0 ppm; The ^{13}C DEPT135 data indicates this is a quaternary carbon. Because C-5' has already been assigned, the 135.0 ppm resonance is assigned as C-7'a. Hydrogen H-4' exhibits gs-HMBC correlations with carbon resonances at 107.5, 120.4, 132.7, and 135.0 ppm. The 120.4, 132.7, and 135.0 ppm resonances have already been defined as C-6', C-5', and C-7'a. The ^{13}C DEPT135 data indicates the 107.5 ppm resonance is a methine; thus it is assigned as C-3'. The gs-HSQC data correlates C-3' with a hydrogen at 7.27 ppm, which was assigned as H-3'. H-7' shows gs-HMBC correlations with four carbon resonances: 107.5, 120.4, 128.1, and 132.7 ppm; of these resonances only the 128.1 ppm resonance is unassigned. The ^{13}C DEPT135 data indicates this is a quaternary carbon, and it is therefore assigned as C-3'a. The only remaining unassigned spin of the indole subunit is C-2'. The gs-HMBC data from H-3' and the indole 1' NH hydrogen both reveal a correlation with a ^{13}C resonance at 131.8 ppm. Since this carbon is a quaternary carbon (^{13}C DEPT135 data), and because C-3'a and C-7'a have already been assigned, this resonance was assigned as C-2'.

The five remaining hydrogens were assigned to the benzofuran ring system: 7.39, 7.51, 7.67, 7.77, and 7.85 ppm. The gs-HSQC data correlates these hydrogens with 124.5, 127.7, 112.4, 110.7, and 123.5 ppm ^{13}C chemical shifts, respectively. The COSY data shows sequential

coupling between the following resonances: 7.85, 7.39, 7.51, and 7.67 ppm, while the resonance at 7.77 ppm does not exhibit any homonuclear coupling. Since the benzofuran ring contains the only isolated single spin remaining, the 7.77 ppm resonance was assigned as H-3". The gs-HMBC data from the 7.77 ppm hydrogen exhibits correlations with ^{13}C resonances at 128.4, 150.5, and 155.5 ppm. The ^{13}C DEPT135 experiment indicates each of these carbons is quaternary. Based on chemical shift, the 128.4 ppm resonance is assigned as C-3"a. The remaining two resonances cannot be unambiguously assigned as C-2" or C-7"a until the remaining hydrogens are assigned (H-4", H-5", H-6", and H-7"). The gs-HMBC data from the 7.39 ppm hydrogen exhibits a correlation to a quaternary carbon with a chemical shift of 128.4 ppm, C-3"a, as well as two large (ortho) homonuclear couplings. To satisfy this data and the gs-HMBC data, the resonance at 7.39 ppm was assigned as H-5". Identifying this hydrogen, in combination with the COSY data, correlates the remaining resonances (7.85, 7.51, and 7.67) with the H-4", H-6", and H-7" positions, respectively. The gs-HMBC data from H-6" exhibits a correlation to a quaternary carbon resonance at 155.5 ppm. Assuming this correlation reflects a three bond coupling, this resonance can be assigned as C-7"a.

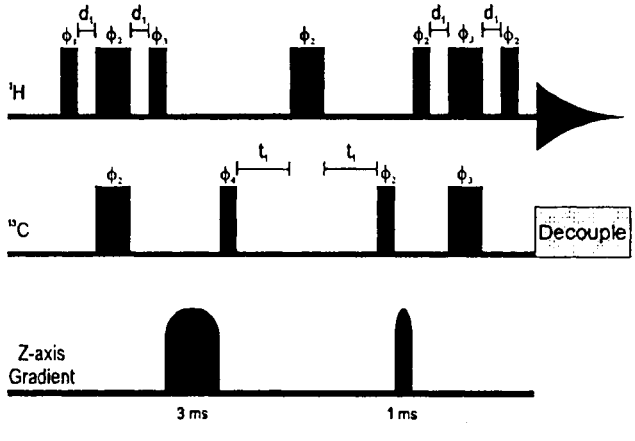

Figure 3. Pulse scheme for inverse detected gradient-selected heteronuclear single quantum coherence (gs-HSQC) spectroscopy. Narrow and wide pulses denote 90° and 180° flip angles, respectively. The experiment was performed using 32 step sine-bell shaped pulsed field gradients with a ratio of 1:1 and a strength of 5.7 G/cm at the center. Quadrature detection was obtained using the States-TPPI method of phase incrementation [7]. This pulse sequence produces phase-sensitive data in both dimensions. The ^{13}C decoupling during ^{1}H acquisition was accomplished using a GARP decoupling scheme. The phase cycling is as follows: $\phi_1 = y; \phi_2 = y; \phi_3 = -x; \phi_4 = y, -y$.

The carbon resonance at 150.5 ppm can be assigned as C-2" by the gs-HMBC correlation from H-3" to this carbon resonance. The 2' carbonyl was assigned as the only remaining quaternary carbon resonance at 162.1 ppm.

EXPERIMENTAL

General.

1-D Hydrogen, COSY, carbon, and ^{13}C DEPT135 nmr spectra were recorded on a Bruker AMX-400 spectrometer equipped with a 9.4 T magnet, operating at 100.61 MHz for ^{13}C and 400.13 MHz for ^{1}H. A Bruker 5 mm dual $^{13}C/^{1}H$ probe was used for these experiments. The gs-HSQC and gs-HMBC experiments were recorded on a Bruker AMX-400 spectrometer equipped with a 9.4 T magnet, operating at 399.95 MHz for ^{1}H and 100.53 MHz for ^{13}C. A Bruker 5 mm inverse $^{1}H/^{13}C/^{15}N$ probe equipped with a z-axis gradient was used for these experiments. The nmr sample was prepared using 9 mg of Adozelesin in 0.6 ml of dimethyl formamide-d_7 (DMF), resulting in a 29.9 m*M* solution. Hydrogen spectra were referenced using residual DMF-d_6 ($\delta = 2.91$ relative to tetramethylsilane (TMS), $\delta = 0.0$, the most shielded resonance). Carbon spectra were referenced using DMF carbon ($\delta = 35.2$ relative to TMS, $\delta = 0.0$, the most shielded resonance). Two-dimensional experiments were referenced using the methyl hydrogens and the 7 methyl carbon. All data was acquired at 300°K.

^{1}H NMR and ^{13}C NMR Spectra.

The 1D-^{1}H spectrum was recorded using 32 K real data points, a spectral width of 6024 Hz, and a recycle time of 10.4 s. The 1D-^{1}H decoupled ^{13}C spectrum was recorded using WALTZ-16 decoupling, 64 K real data points, a spectral width of 23809 Hz, and a recycle time of 3.4 s. A Lorentzian line broadening factor of 2 Hz was applied prior to Fourier transformation. The ^{13}C DEPT spectrum was recorded using a 135° read pulse which makes CH and CH_3 carbon resonances of opposite phase with respect to CH_2 carbon resonances. The spectrum was acquired using WALTZ-16 decoupling during acquisition, 64 K real data points, a spectral width of 23809 Hz, and a recycle time of 2.2 s.

Double Quantum Filtered ^{1}H-^{1}H Correlation (COSY).

The 2-D COSY spectrum was recorded using States-TPPI quadrature detection and the modified phase cycling of Derome and Williamson [6]. A 1K x 512 data matrix was acquired using 8 scans, 4 dummy scans, and a spectral width of 6024 Hz. The data were zero-filled to a size of 1K x 1K and were processed using a 90° shifted sine bell window in F_1 and a 90° shifted sine bell in F_2. A delay between transients of 1.2 s was used.

Gradient-Selected Heteronuclear Single Quantum Coherence (gs-HSQC).

The gs-HSQC spectrum was acquired using the pulse sequence shown in Figure 3. The sequence combines the absorption-mode HSQC sequence reported by Bodenhausen and Ruben [3] with the modifications developed by Bax and Pochapsky [4]. The experiment includes two pulsed field gradients of 3 msec and 1 msec duration added during the INEPT transfer from ^{1}H to ^{13}C and the reverse INEPT transfer from ^{13}C to ^{1}H, respectively. This gradient implementation uses coherence rejection instead of coherence selection [4,8] and yields the same intrinsic signal-to-noise as the original non-gradient HSQC sequence, but greatly suppresses t_1 noise from hydrogens bonded to ^{12}C. In addition, this pulse sequence produces phase sensitive data in both dimensions. The data were acquired using 16 scans and 128 dummy scans for 512 data points in each of the 256 t_1 incre-

ments. The data matrix was zero-filled to 1K x 1K and was processed using a 180° shifted sine bell squared window in F_1 and a 180° shifted sine bell squared window in F_2. The delay, d1 ($1/2^{1}J_{CH}$), was optimized for 142 Hz and the delay between transients was 1.2 s.

Gradient-Selected Heteronuclear Multiple Bond Coherence (gs-HMBC).

The gs-HMBC experiment was acquired using a published gradient pulse sequence [5]. The acquisition parameters were 1K x 512 data points using 128 scans and 4 dummy scans. Hydrogen and carbon spectral widths were 6024 and 22123 Hz, respectively. The data were zero-filled to 1K x 1K, processed using a 180° shifted sine bell squared window in F_1 and F_2, and presented in magnitude mode. The one-bond J-filter was optimized for 142 Hz $^{1}J_{CH}$ couplings and the delay for evolution of long-range carbon-hydrogen couplings was set to 65 ms. A delay between transients of 1.3 s was used.

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