Supporting Information for:
Characterization of Spiroiminodihydantoin as a Product of One-Electron Oxidation of 8-Oxoguanosine
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Experimental Section

Materials. Guanosine hydrate and 4-dimethyl-aminopyridine were purchased from Acros, bromine, cobalt chloride and guanidine hydrochloride from Fisher Scientific, benzyl alcohol and palladium (10% on activated carbon) from Aldrich, sodium from Mallinckrodt, potassium monoperoxysulfate (Oxone) from Sigma, sodium hexachloroiridate from Alfa Aesar and $H_2{}^{18}O$ from Icon. The nucleoside 2',3',5'-triacetoxy-8-oxoguanosine (**1**) was synthesized by a modified method of Holmes[1] and Matsuda.[2]

^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian VXR-500MHz or a VXR-300 MHz spectrometer. FAB mass spectra were recorded on a Finnigan MAT 95 mass spectrometer, ESI mass spectra were recorded on a Micromass Quatro II mass spectrometer. HPLC analyses were carried out using a Beckman System Gold 126NM solvent module attached to a Beckman 168NM diode arrray detector using an Alltech Alltima C-18 Nuc analytical reverse phase column (5 μm, 250 mm x 4.6 mm). All the solvents used for HPLC were HPLC grade and filtered and sonicated before use. All aqueous solutions utilized purified water (Nanopure, Sybron/Barnsted).

Oxidation of 1 with Na_2IrCl_6 or $CoCl_2/KHSO_5$. In a final volume of 30 μL of 75 mM potassium phosphate buffer (pH 7), **1** (7.5 mM) was incubated with Na_2IrCl_6 (7.5 mM) or $CoCl_2$ (0.125 mM)/$KHSO_5$(7.5 mM) at room temperature for 40 min. The reaction mixture was directly injected for HPLC or LC-ESI-MS analysis (5 μL).

HPLC and LC-ESI-MS analysis. The reaction mixture was analyzed by HPLC with a linear gradient of 10% solvent B to 20% solvent B in 20 min at 1 mL/min. Solvent A was water and solvent B was acetonitrile. In the case of LC-ESI-MS, Solvent A was 0.1% trifluoroacetic acid (TFA) in water, solvent B was 0.08% TFA in acetonitrile and the flow rate was 0.7 mL/min. The UV spectra were recorded at 220 nm.

Chromatographic Purification of 2',3',5',-triacetoxyribofuranosylspiroimino-dihydantoin, 4. Compound **1** (100 mg, 0.235 mmol) was dissolved in 31 mL 75 mM potassium phosphate buffer (pH 7) with stirring. $CoCl_2 \bullet 6H_2O$ (0.93 mg, 3.92 μmol) and Oxone (108 mg, including $KHSO_5$ 0.353 mmol) were added and the reaction solution was stirred for 1 h at room temperature (22°C). The reaction solution then was concentrated under vacuum at room temperature. The residue was suspended in $CHCl_3$:MeOH/1:1 (v:v) and purified by a short silica gel column using $CHCl_3$:MeOH/1:1 (v:v) as mobile phase. The residue obtained was purified again by a short silica gel column using $CHCl_3$:MeOH/8:1 (v:v) as mobile phase. The purity of 2',3',5-triacetoxyribofuranosylspiroiminodihydantoin was >90% by HPLC. Diastereomer A and B (ratio A:B/1.1:1): ^{1}H NMR (DMSO-d_6) δ 8.45, 8.38, 8.30, 8.28, 8.11 (b, 4Hx2, NH), 5.66 (dd, 1H, 2'A), 5.43 (dd, 1H, 2'B), 5.16-5.19 (m, 2H, 3'A, 3'B), 4.94-4.95 (d, 1H, 1'B), 4.91-4.93 (d, 1H, 1'A), 3.98-4.22(m, 6H, 4'A, 4'B, 5'A, 5'B, 5"A, 5"B), 1.14-1.18 (m, 9Hx2, CH_3) ppm. ^{13}C NMR (CD_3OD) δ 172.70, 171.67, 169.84, 156.87, 156.41 (C=O, C=N), 85.98 (C-1'), 85.05 (C-spiro), 80.58, 80.41 (C-4'), 72.52, 72.07, 71.72 (C-3', C-2'), 64.89, 64.31(C-5'), 20.56 (CH_3) ppm (see

Figures A-C). UV Absorbance: λ_{max} 230 nm, ϵ=4900 L•$M^{-1}cm^{-1}$ (see Figure D). FAB-HRMS: m/z Calcd for $C_{16}H_{20}N_5O_{10}$ 422.12102, found 422.12100.

$H_2{}^{18}O$ Labeling Experiments. After 30 μL of 75 mM potassium phosphate buffer (pH 7.0) with **1** (7.5 mM) was lyophilized to dryness, 30 μL $H_2{}^{18}O$ was added. This solution was added to dry Na_2IrCl_6 which made its final concentration 7.5 mM. After 40 min, the reaction mixture was analyzed by LC-ESI-MS (see Figure E).

Synthesis of Spiroiminodihydantoin, 4 (R=H). Alloxan (4.8g, 30 mmol) was dissolved in 13.5 mL water upon heating. A solution of guanidine hydrochloride (4g, 42 mmol) and sodium hydroxide (1.7g, 42 mmol) in 10 mL water was added slowly with stirring, and the reaction mixture was left overnight at room temperature. A precipitate was collected and recrystallized from water to provide **12** (61 mg, yield 1%) as a prismatic crystal (see x-ray crystallography). ESI-MS: m/z 202 ($M+H^+$). Compound **12** was dried under vacuum at room temperature overnight and then suspended in 5 mL trifluoroacetic anhydride and refluxed for 5 h. The reaction mixture was concentrated and the light yellow oil was analyzed by ESI-MS without purification**.** ESI-MS: m/z 184 ($M+H^+$) (see Figure F).

References:

(1) Holmes, R.; Robins, R. K. *J. Am. Chem. Soc.* **1965**, 87, 1772-1776.
(2) Matsuda, A. *Synthesis.* **1986**, 385-386.

Mass spectrometry of 2',3',5'–triacetoxyribofuranosylspiroiminodihydantoin. The labeled and unlabeled nucleosides were analyzed by positive electrospray ionization (ESI) on a Micromass Quattro II tandem mass spectrometer equipped with a Zspray API source. Samples were dissolved in acetonitrile and water (1:1, v/v) with 0.1% TFA. Approximately 100 μL were placed in a 300 μL glass insert of a Waters (Milford, Mass.) autosampler vial. Sample introduction was *via* a Waters Alliance 2690 Sample Module fitted with an Alltech (Deerfield, Illinios) Altima C18-Nuc (5 μm, 250X4.6mm) reversed phase column. A Waters 2487 Dual Absorbance Detector was placed in line between the Alliance 2690 Separations Module and the Zspray probe of the ion source. UV spectra were accumulated at 220 Å. Chromatographic and mass spectrometric conditions follow:
Solvent A – Water (0.10% TFA); Solvent B – Acetonitrile (0.08% TFA)
Flow 0.7 mL/min

HPLC Pump Gradient Table

Time	%A	%B	Flow Rate (ml/min)
0.0	90	10	0.7
20	80	20	0.7
25	80	20	0.7
27	90	10	0.7
37	90	10	0.7

The flow rate was split to allow a total flow of 200μL/min into the Zspray probe. Dry nitrogen gas was used as a pneumatic spray aid, also as a blanket gas to assist with desolvation. The source and desolvation temperatures were 80 degrees and 250 degrees respectively. The capillary

voltage was set to 3.1 kV, sampling cone voltage to 60 or 65V, and the extractor cone to 3V. The collision energy was set to 26 eV. Argon, used as a collision gas for the CID experiments, was adjusted to a pressure of 1.7×10^{-4} mBar.

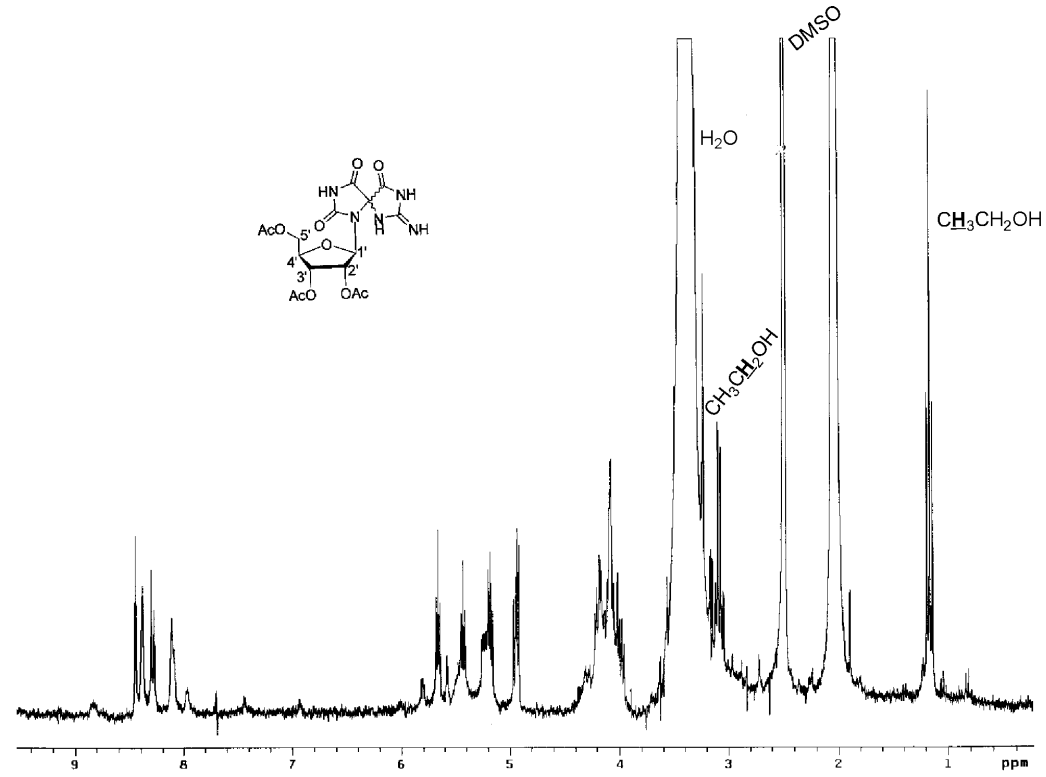
Labeled (^{18}O) and unlabeled spiroiminodihydantoin nucleosides were used to study the CID fragmentation of the base. Appropriate cone voltages (60V for the former and 65V for the latter) were chosen to optimize the separation of the base from the sugar by in-source fragmentation.

In a representative experiment, the first scanning analyzer (MS1) was scanned between 50 and 1000 daltons to record full-scan spectra of the fragmented nucleoside. Masses of the bases obtained in this manner were used in subsequent CID experiments. The scan duration and the interscan delay times were 3.0 and 0.1 seconds respectively.

In the following experiment, run alternately with the above, MS1 was set to the mass of the nucleoside base of interest (186.2 for labeled and 184.2 for unlabeled). The precursor ion was subjected to CID in the static quadrupole and the resulting spectrum of the products recorded by scanning the second scanning analyzer (MS2) between 50 and 250 daltons. The scan duration and the interscan delay times were 1.0 and 0.1 seconds respectively. The instrument was operated and data accumulated with Micromass Masslynx software (version 3.2). HPLC spectra were also recorded.

Elemental analysis of 2',3',5'–triacetoxyribofuranosylspirodihydantoin was obtained with a Finnigan (San Jose, California) MAT95 high-resolution mass spectrometer. FAB mass spectra were acquired via an electric scan (ESCAN). The instrument was equipped with a Cs ion gun that was operated at 20 keV with an emission current of 2uA. Samples were prepared from 1μL of glycerol and 1μL of a solution in acetonitrile: water (1:1, 0.1% TFA) solutions of the analyte. A solution of polyethylene glycol – 400 (Aldrich, Milwaukee, Wis.) in glycerol (1:50, v/v, 1% TFA) was used as an internal standard. The 415 and 459 ions from the standard were used to calculate the mass of the analyte. Data were accumulated at high resolution (10K) by scanning between 413 and 461 Daltons. The results were within +/- 5 mmu tolerance. Calcd for $C_{16}H_{20}N_5O_{10}$: 422.12102; found: 422.1210.

Fig. A. [1]H NMR spectrum (300 MHz) of **4** in d_6-DMSO.



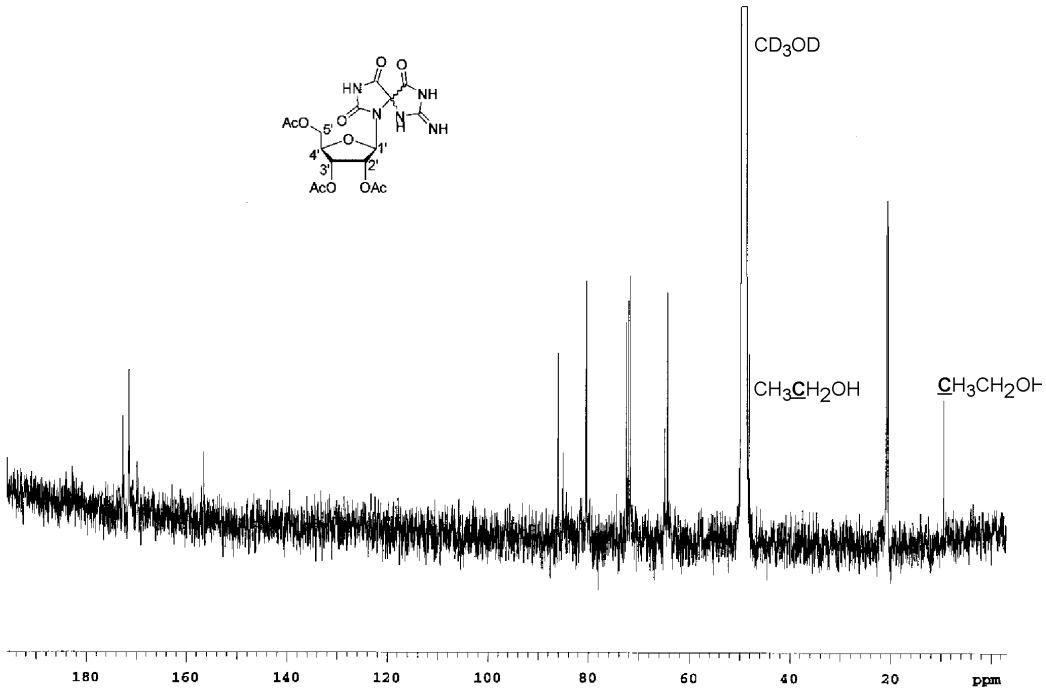


Fig. B. [13]C NMR spectrum (125 MHz) of **4** in CD_3OD.

Fig. C. 2D-COSY NMR spectrum of **4** in d_6-DMSO.

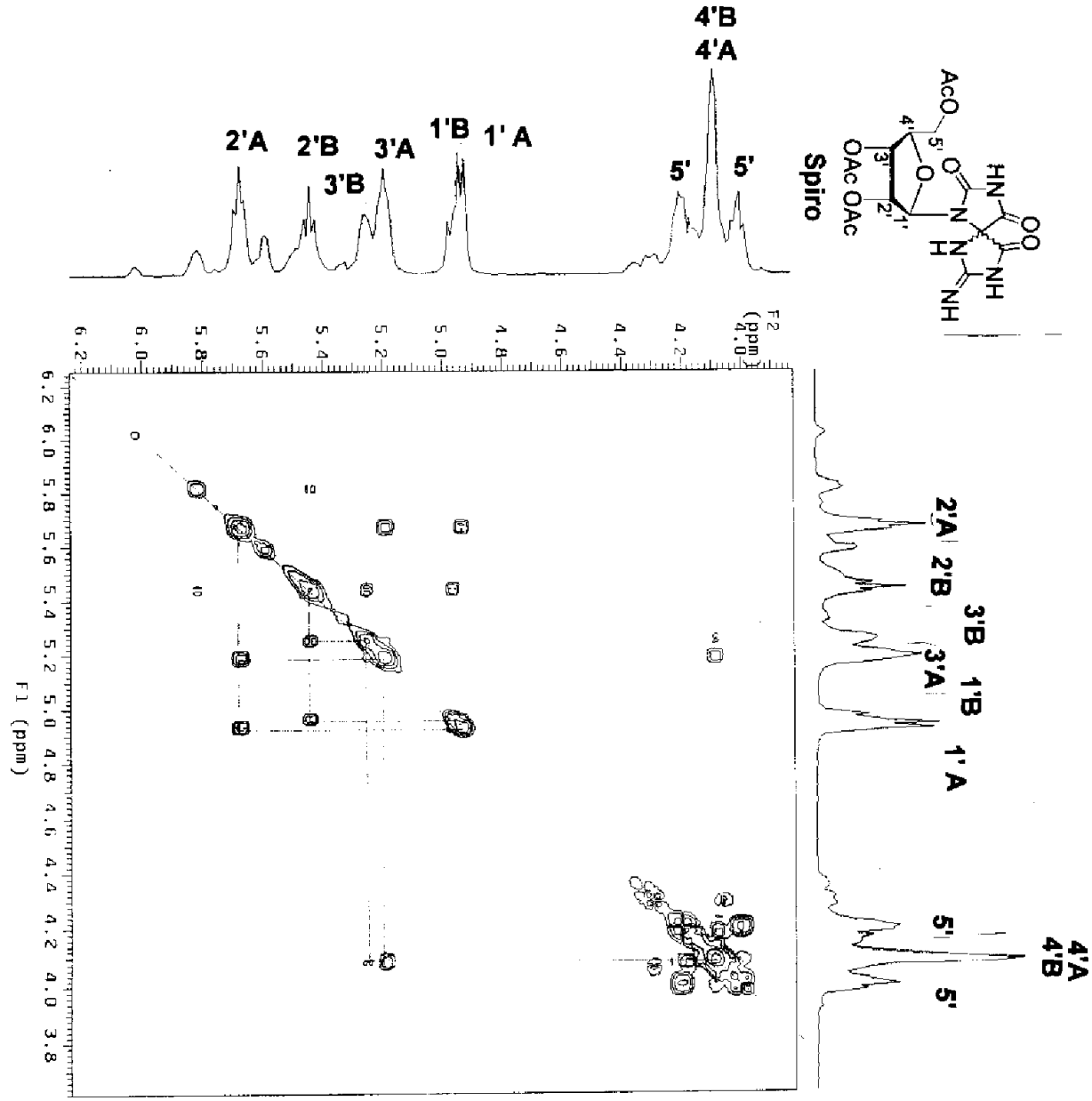


Fig. D. UV-vis spectrum of **4** in H_2O.

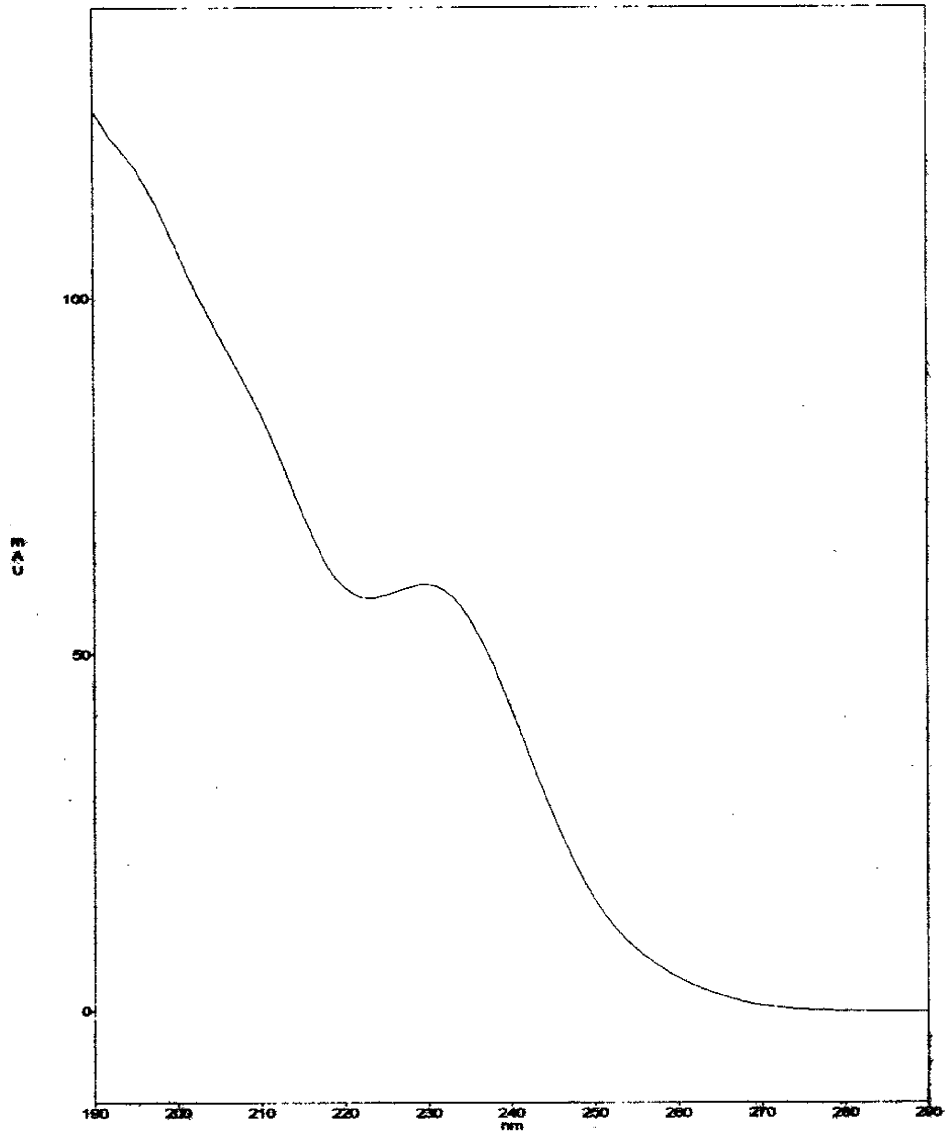


Fig. E. ESI-MS data for **4** in $H_2{}^{16}O$ vs. $H_2{}^{18}O$ experiments. $M+H^+$ ions were observed at 442 and 444 amu, respectively.

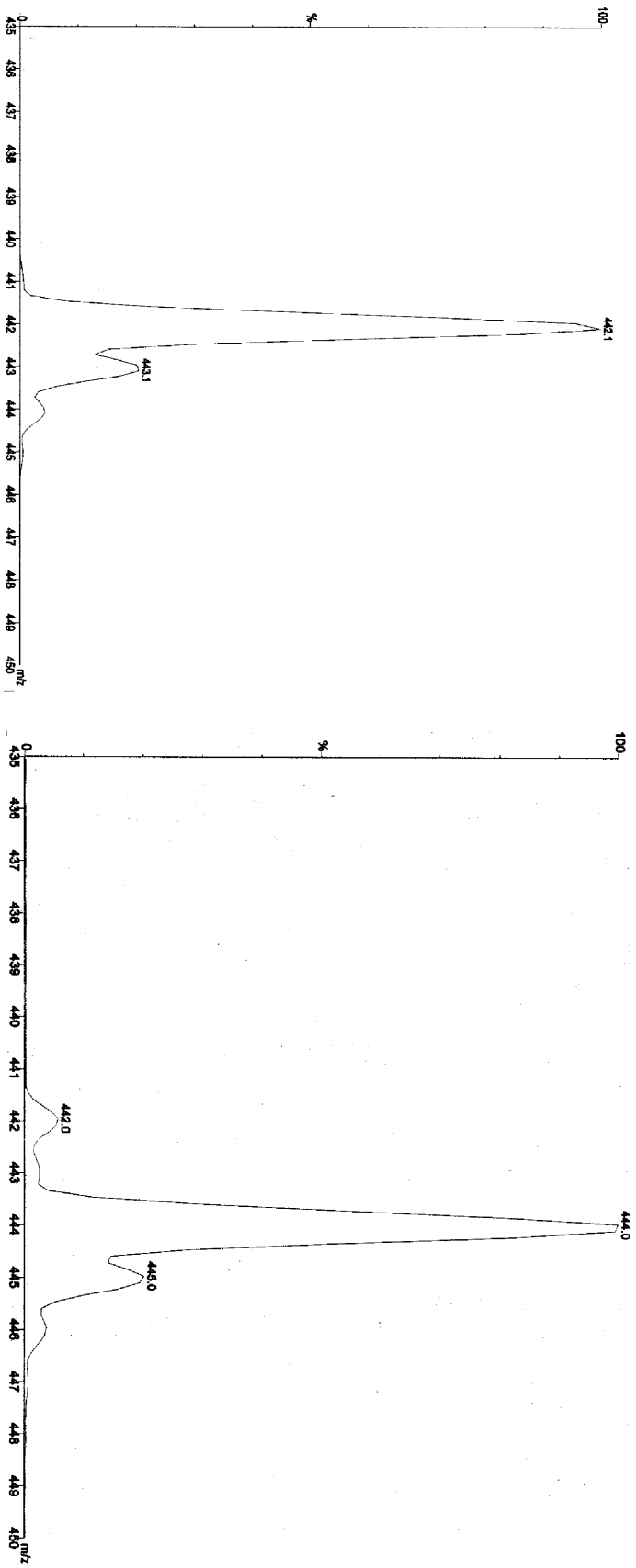
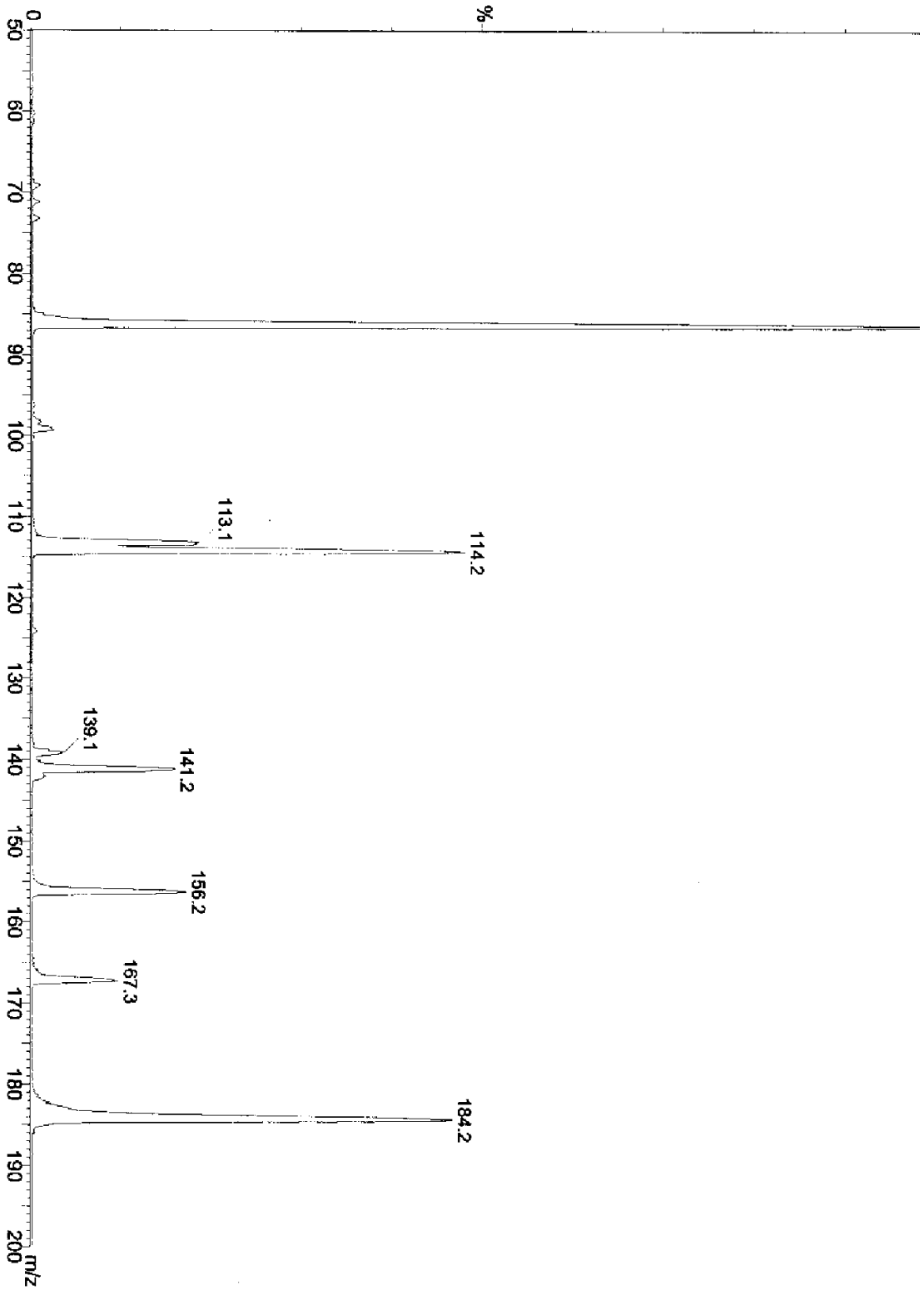


Fig. F. ESI-MS/MS spectrum of synthetic **4** (R = H). (Compare with Fig. 3 in manuscript.)



Crystal Structure Report for 12

Experimental:

A colorless, prism -shaped crystal 0.3 x 0.2 x 0.15 mm in size was mounted on a glass fiber with tiny traces of viscous oil and then transferred to a Nonius KappaCCD diffractometer equipped with Mo Kα radiation (λ = 0.71073 Å). Ten frames of data were collected at 200(0.1) K with an oscillation range of *1* deg/frame and an exposure time of *20* sec/frame.[1] Indexing and unit cell refinement based on all observed reflections from those ten frames, indicated a monoclinic P lattice. A total of 4734 reflections (θ_{max} = 34.33°) were indexed, integrated and corrected for Lorentz, polarization and absorption effects using DENZO-SMN and SCALEPAC.[2] Post refinement of the unit cell gave *a* = 6.3513(3) Å , b = 6.8636(2) Å, c = 17.6249(9) Å, β =98.5737(16), and V = 759.73(6) Å^3. Axial photographs and systematic absences were consistent with the compound having crystallized in the monoclinic space group *P* $2_1/n$.

The structure was solved by a direct method using SIR 97.[3] All of the non-hydrogen were refined with an anisotropic displacement coefficients. Hydrogen atoms were located and refined isotropically using SHELXL97.[4] The weighting scheme employed was $w = 1/[\sigma^2(F_o^2) + (0.0629P)^2 + 0.1962P]$ where $P = (F_o^2 + 2F_c^2)/3$. The refinement converged to R1 = 0.0422, wR2 = 0.1105, and S = 1.029 for 2175 reflections with $1 > 2\sigma(I)$, and R1 = 0.0539, wR2 = 0.1196, and S = 1.029 for 2655 unique reflections and 156 parameters.[5] The maximum Δ/σ in the final cycle of the least-squares was 0.001, and the residual peaks on the final difference-Fourier map ranged from -0.304 to 0.448 e/Å^3. Scattering factors were taken from the International Tables for Crystallography, Volume C.[6, 7]

See Fig. G for the ORTEP diagram.

References:

(1) COLLECT Data Collection Software. Nonius B.V. **1998**.
(2) Otwinowski, Z.; Minor, W., "Processing of X-ray Diffraction Data Collected in Oscillation Mode," *Methods Enzymol*. **1997**, *276*, 307-326.
(3) Altomare, A.; Burla, M. C.; Camalli, M.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A.; Moliteni, A. G. G.; Polidori, G.; and Spagna, R. SIR97 (Release 1.02) - A program for automatic solution and refinement of crystal structure.
(4) Sheldrick, G. M. SHELX97 [Includes SHELXS97, SHELXL97, CIFTAB]. Programs for Crystal Structure Analysis (Release 97-2). University of Göttingen, Germany. **1997**.
(5) $R1 = \Sigma(\| F_o | - |F_c \|) / \Sigma |F_o|$, $wR2 = [\Sigma(w(F_o^2 - F_c^2)2) / \Sigma(F_o^2)^2]^{1/2}$, and S = Goodness-of-fit on $F^2 = [\Sigma(w(F_o^2 - F_c^2)^2 / (n-p)]^{1/2}$, where n is the number of reflections and p is the number of parameters refined.
(6) Maslen, E. N.; Fox, A. G.; O'Keefe, M. A. International Tables for Crystallography: Mathemetical, Physical and Chemical Tables, Vol. C, Chapter 6, Wilson, A. J. C., Ed.; Kluwer, Dordrecht, The Netherlands, **1992**, 476-516.
(7) Creagh, D. C.; McdAuley, W. J., International Tables for Crystallography: mathematical, Physical and Chemical tables, Vol. C, Chapter 4 Wilson, A. J. C., Ed.; Kluwer, Dordrecht, The Netherlands, 1992; pp. 206-222.

Fig. G. ORTEP of **12**.

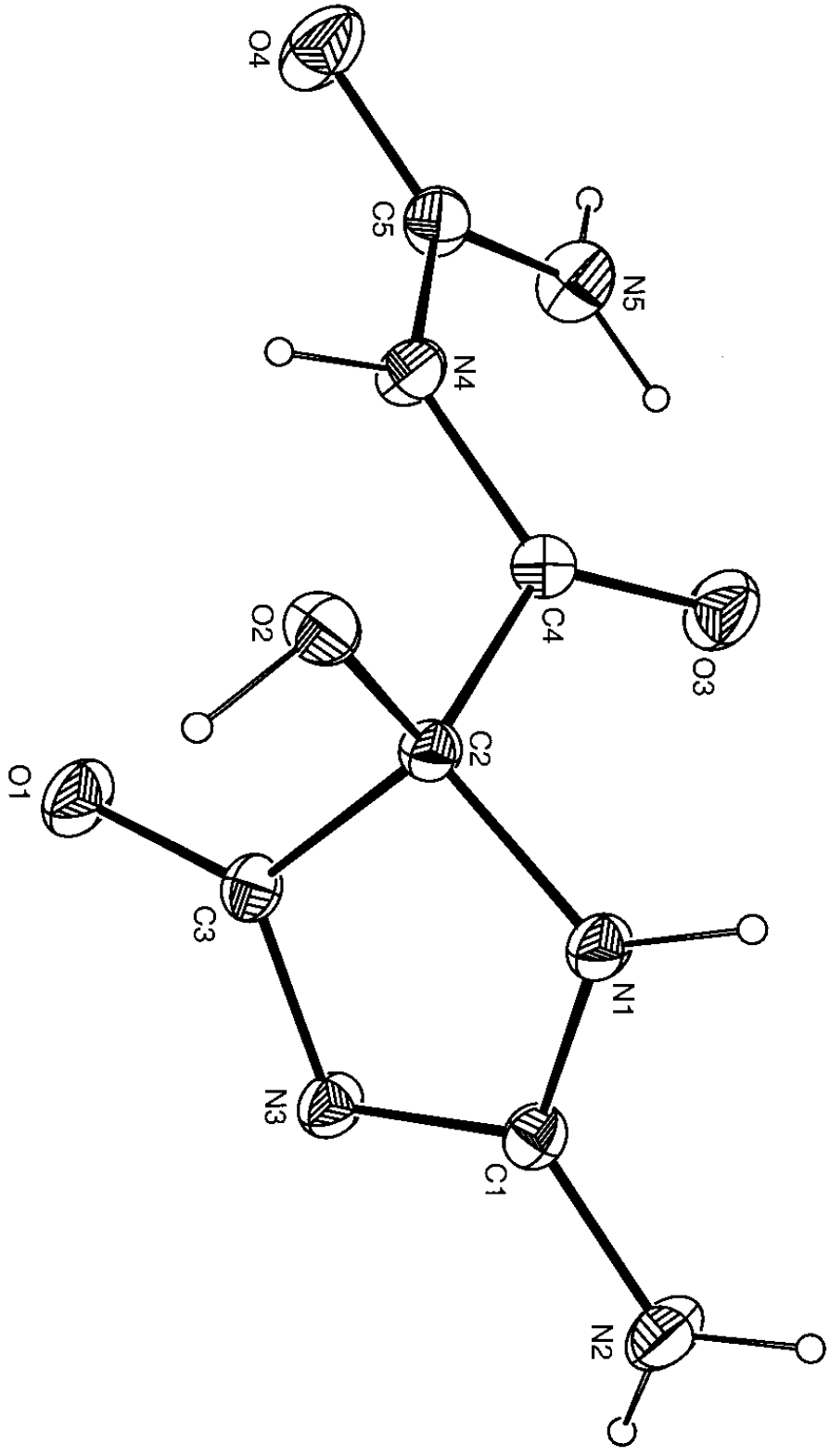


Table 1. Crystal data and structure refinement for shelxl.

Identification code	shelxl	
Empirical formula	C5 H7 N5 O4	
Formula weight	201.16	
Temperature	200(0.1) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	$P\ 2_1/n$	
Unit cell dimensions	a = 6.3513(3) Å	α= 90°
	b = 6.8636(2) Å	β= 98.5737(16)°
	c = 17.6249(9) Å	γ = 90°
Volume	759.73(6) $Å^3$	
Z	4	
Density (calculated)	1.759 Mg/m^3	
Absorption coefficient	0.153 mm^{-1}	
F(000)	416	
Crystal size	0.30 x 0.20 x 0.15 mm^3	
Theta range for data collection	4.40 to 34.33°	
Index ranges	-10<=h<=10, -7<=k<=7, -27<=l<=27	
Reflections collected	4734	
Independent reflections	2655 [R(int) = 0.0246]	
Completeness to theta = 34.33°	84.0 %	
Absorption correction	SCALEPACK	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2655 / 0 / 156	
Goodness-of-fit on F^2	1.029	
Final R indices [I>2sigma(I)]	R1 = 0.0422, wR2 = 0.1105	
R indices (all data)	R1 = 0.0539, wR2 = 0.1196	
Extinction coefficient	0.021(7)	
Largest diff. peak and hole	0.448 and -0.304 $e.Å^{-3}$	

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$ x 10^3) for shelxl. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

x		y	z	U(eq)
O(1)	347(1)	-88(1)	7900(1)	22(1)
O(2)	3794(1)	-2244(1)	8899(1)	16(1)
O(3)	5253(1)	2591(1)	9012(1)	24(1)
O(4)	704(2)	2131(1)	10450(1)	29(1)
N(1)	5862(1)	-502(1)	8092(1)	15(1)
N(2)	6522(2)	434(2)	6870(1)	21(1)
N(3)	3073(1)	305(1)	7181(1)	15(1)
N(4)	2614(2)	1040(2)	9544(1)	17(1)
N(5)	2559(2)	4328(2)	9852(1)	22(1)
C(1)	5211(2)	102(2)	7366(1)	14(1)
C(2)	4062(2)	-537(2)	8502(1)	13(1)
C(3)	2232(2)	-91(2)	7823(1)	14(1)
C(4)	4093(2)	1199(2)	9058(1)	15(1)
C(5)	1912(2)	2545(2)	9983(1)	17(1)

Table 3. Bond lengths [Å] and angles [°] for shelxl.

O(1)-C(3)	1.2249(12)
O(2)-C(2)	1.3881(13)
O(2)-H(2O)	0.89(2)
O(3)-C(4)	1.2164(14)
O(4)-C(5)	1.2388(14)
N(1)-C(1)	1.3499(13)
N(1)-C(2)	1.4413(13)
N(1)-H(1N)	0.869(18)
N(2)-C(1)	1.3132(13)
N(2)-H(2A)	0.90(2)
N(2)-H(2B)	0.89(2)
N(3)-C(3)	1.3488(13)
N(3)-C(1)	1.3555(13)
N(4)-C(4)	1.3663(13)
N(4)-C(5)	1.4028(14)
N(4)-H(4N)	0.896(16)
N(5)-C(5)	1.3226(16)
N(5)-H(5A)	0.880(17)
N(5)-H(5B)	0.85(2)
C(2)-C(4)	1.5400(15)
C(2)-C(3)	1.5692(14)

C(2)-O(2)-H(2O)	108.1(13)
C(1)-N(1)-C(2)	109.11(8)
C(1)-N(1)-H(1N)	122.1(12)
C(2)-N(1)-H(1N)	124.9(12)
C(1)-N(2)-H(2A)	118.8(13)
C(1)-N(2)-H(2B)	118.8(13)
H(2A)-N(2)-H(2B)	121.6(18)
C(3)-N(3)-C(1)	106.60(8)
C(4)-N(4)-C(5)	126.32(10)
C(4)-N(4)-H(4N)	117.9(10)
C(5)-N(4)-H(4N)	115.1(10)
C(5)-N(5)-H(5A)	122.4(11)
C(5)-N(5)-H(5B)	118.5(14)
H(5A)-N(5)-H(5B)	119.0(18)
N(2)-C(1)-N(1)	123.28(10)
N(2)-C(1)-N(3)	122.30(10)
N(1)-C(1)-N(3)	114.41(9)
O(2)-C(2)-N(1)	115.65(8)
O(2)-C(2)-C(4)	108.87(8)
N(1)-C(2)-C(4)	111.74(8)
O(2)-C(2)-C(3)	114.62(8)
N(1)-C(2)-C(3)	99.58(7)
C(4)-C(2)-C(3)	105.79(8)
O(1)-C(3)-N(3)	127.49(10)
O(1)-C(3)-C(2)	122.72(9)
N(3)-C(3)-C(2)	109.78(8)
O(3)-C(4)-N(4)	125.70(10)
O(3)-C(4)-C(2)	121.06(9)
N(4)-C(4)-C(2)	113.08(9)
O(4)-C(5)-N(5)	124.55(11)
O(4)-C(5)-N(4)	118.52(10)
N(5)-C(5)-N(4)	116.91(10)

Table 4. Anisotropic displacement parameters ($Å^2$ x 10^3) for shelxl. The anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U^{11} + ... + 2\ h\ k\ a^*\ b^*\ U^{12}]$

U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}	
O(1)	11(1)	32(1)	22(1)	3(1)	4(1)	1(1)
O(2)	20(1)	13(1)	14(1)	1(1)	1(1)	-2(1)
O(3)	25(1)	21(1)	28(1)	-5(1)	11(1)	-9(1)
O(4)	32(1)	25(1)	34(1)	-11(1)	21(1)	-9(1)
N(1)	10(1)	21(1)	14(1)	1(1)	2(1)	2(1)

N(2)	15(1)	29(1)	19(1)	6(1)	6(1)	1(1)
N(3)	12(1)	19(1)	14(1)	3(1)	2(1)	1(1)
N(4)	19(1)	14(1)	18(1)	-3(1)	8(1)	-3(1)
N(5)	25(1)	14(1)	29(1)	-2(1)	8(1)	0(1)
C(1)	13(1)	12(1)	15(1)	0(1)	3(1)	0(1)
C(2)	12(1)	15(1)	12(1)	0(1)	2(1)	1(1)
C(3)	12(1)	13(1)	15(1)	1(1)	2(1)	0(1)
C(4)	15(1)	17(1)	14(1)	-1(1)	2(1)	-1(1)
C(5)	16(1)	16(1)	19(1)	-3(1)	4(1)	0(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (Å^2 x 10^3) for shelxl.

x		y	z	U(eq)
H(2O)	3240(30)	-3130(30)	8563(11)	42(5)
H(1N)	7180(30)	-430(20)	8305(10)	29(4)
H(2A)	7930(30)	290(30)	7024(12)	41(5)
H(2B)	6020(30)	990(30)	6422(11)	40(5)
H(4N)	1870(30)	-70(20)	9535(9)	17(4)
H(5A)	3460(30)	4570(20)	9528(10)	25(4)
H(5B)	2160(30)	5260(30)	10113(12)	42(5)

Table 6. Torsion angles [°] for shelxl.

C(2)-N(1)-C(1)-N(2)	174.29(11)
C(2)-N(1)-C(1)-N(3)	-6.96(13)
C(3)-N(3)-C(1)-N(2)	-178.24(11)
C(3)-N(3)-C(1)-N(1)	2.99(13)
C(1)-N(1)-C(2)-O(2)	130.39(9)
C(1)-N(1)-C(2)-C(4)	-104.33(10)
C(1)-N(1)-C(2)-C(3)	7.04(11)
C(1)-N(3)-C(3)-O(1)	-179.33(11)
C(1)-N(3)-C(3)-C(2)	1.90(12)
O(2)-C(2)-C(3)-O(1)	51.58(14)
N(1)-C(2)-C(3)-O(1)	175.65(11)
C(4)-C(2)-C(3)-O(1)	-68.37(13)
O(2)-C(2)-C(3)-N(3)	-129.59(10)
N(1)-C(2)-C(3)-N(3)	-5.51(11)
C(4)-C(2)-C(3)-N(3)	110.47(10)

C(5)-N(4)-C(4)-O(3)	11.26(18)
C(5)-N(4)-C(4)-C(2)	-164.14(10)
O(2)-C(2)-C(4)-O(3)	143.36(10)
N(1)-C(2)-C(4)-O(3)	14.40(14)
C(3)-C(2)-C(4)-O(3)	-92.99(12)
O(2)-C(2)-C(4)-N(4)	-41.00(11)
N(1)-C(2)-C(4)-N(4)	-169.96(9)
C(3)-C(2)-C(4)-N(4)	82.65(10)
C(4)-N(4)-C(5)-O(4)	-173.47(11)
C(4)-N(4)-C(5)-N(5)	8.12(16)

Table 7. Hydrogen bonds for shelxl [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(2)-H(2O)...N(3)#1	0.89(2)	1.80(2)	2.6808(12)	173(2)
N(1)-H(1N)...O(1)#2	0.869(18)	2.242(17)	2.9321(12)	136.3(15)
N(2)-H(2A)...O(1)#2	0.90(2)	2.03(2)	2.8306(13)	148.1(18)
N(2)-H(2B)...O(4)#3	0.89(2)	2.13(2)	2.9903(14)	161.4(18)
N(4)-H(4N)...O(4)#4	0.896(16)	2.165(16)	3.0311(13)	162.5(14)

Symmetry transformations used to generate equivalent atoms:
#1 -x+1/2,y-1/2,-z+3/2 #2 x+1,y,z #3 x+1/2,-y+1/2,z-1/2
#4 -x,-y,-z+2