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Staake, Michael D
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McMorris, Trevor C
et al.

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Hydroxyurea derivatives of irofulven with improved antitumor efficacy

Michael D. Staake [a], Alisala Kashinatham [a], Trevor C. McMorris [a], Leita A. Estes [b], Michael J. Kelner [b,*]

[a] Department of Chemistry & Biochemistry, University of California, San Diego, La Jolla, CA 92093-0358, USA
[b] Department of Pathology, University of California, San Diego, MC 7721, La Jolla, CA 92093-7721, USA

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ABSTRACT

Irofulven is a semi-synthetic derivative of Illudin S, a toxic sesquiterpene isolated from the mushroom *Omphalotus illudens*. Irofulven has displayed significant antitumor activity in various clinical trials but displayed a limited therapeutic index. A new derivative of irofulven was prepared by reacting hydroxyurea with irofulven under acidic conditions. Acetylation of this new compound with acetic anhydride produced a second derivative. Both of these new derivatives displayed significant antitumor activity in vitro and in vivo comparable to or exceeding that of irofulven.

The toxic mushroom *Omphalotus illudins* is the source of the highly toxic sesquiterpenes Illudin S **1** and Illudin M **2** (Fig. 1).[1] These compounds were tested by the National Cancer Institute and displayed significant antitumor activity but displayed a poor therapeutic index in xenograft studies.[2] Semisynthetic derivatives of these compounds were developed which displayed improved efficacy in xenograft studies including multidrug resistant tumor models.[3] One derivative, irofulven **3**[4] has been extensively investigated in numerous clinical trials and displayed significant activity against ovarian, prostate, and gastrointestinal cancers including hepatocellular tumors.[5,6]

Irofulven has proven to be a potent antitumor agent, but its efficacy is limited by the hematologic side effects that reduce the maximum dose that can be administered to patients. Modifying the drug to increase its selectivity towards tumor cells versus normal cells should in theory reduce adverse side effects and allow administration of higher doses. We describe here the preparation of two new hydroxyurea derivatives of irofulven with improved in vitro antitumor activity.

The drug hydroxyurea **4** (Fig. 2) is an inhibitor of DNA synthesis and has been used clinically to treat cancer.[7] Hydroxyurea, however, has limited efficacy as an anticancer drug as it displays a short half-life in humans and cancer cells readily develop resistance to the drug. The hydroxyurea derivative BWB70C **5** was developed and demonstrated superior inhibition of tumor growth in vivo.[8]

Another hydroxyurea derivative, Zileuton[9] **7** has been prepared from benzo[*b*]thiophen-2-yl-ethanol **6** via an S_N1 displacement of the secondary hydroxyl by hydroxyurea under acidic conditions (Scheme 1).[10] Alkylation occurred specifically at the nitrogen bearing the hydroxyl group.

We followed an analogous approach to obtain the hydroxyurea derivative of irofulven. The primary hydroxyl group of irofulven was previously demonstrated to undergo S_N1 displacement by a variety of nucleophiles under acidic conditions.[11,12] In a 1:1 mixture of 2 M H_2SO_4 and acetone the primary hydroxyl group of irofulven was rapidly replaced by hydroxyurea to yield **8** in 83% yield (Scheme 2). Alkylation was also observed to occur exclusively at the nitrogen bearing the hydroxy group. An acetylated derivative

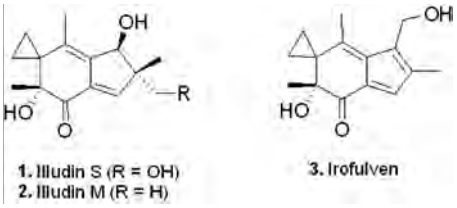

Figure 1. Structures of Illudin S, Illudin M, and irofulven.

* Corresponding author. Tel.: +1 858 657 5731; fax: +1 858 657 5807.
E-mail address: mkelner@ucsd.edu (M.J. Kelner).

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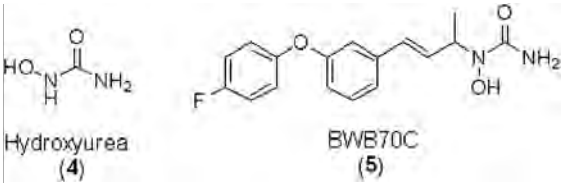
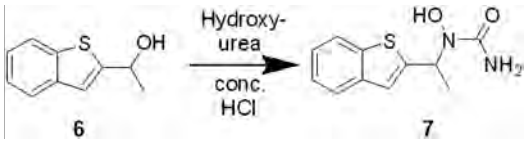
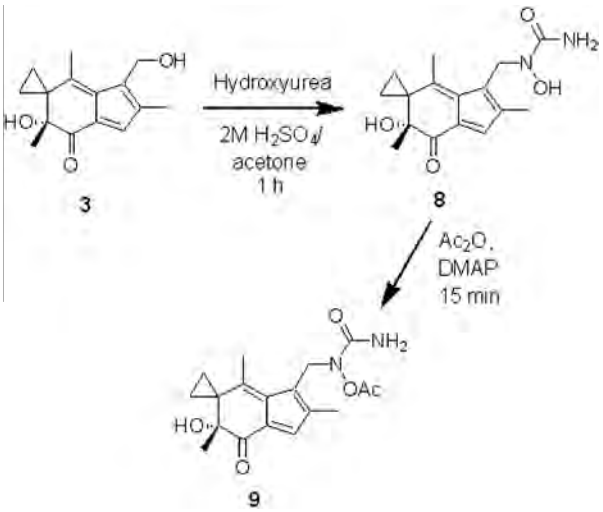


Figure 2. Structures of hydroxyurea and BWB70C.



Scheme 1. Synthesis of Zileuton.



Scheme 2. Synthesis of hydroxyurea irofulven derivatives.

9 was obtained in quantitative yield by treating **8** with acetic anhydride and catalytic DMAP.

The antitumor activity of compound **8** was compared to previous irofulven results in the NCI DTP 60 cell line screening assay. Results indicated that anticancer activity of compound **8** equaled or exceeded that of irofulven (Table 1) in 43 of 52 comparable cell lines (cell lines used in the screening assay have varied with time so that only 52 of the solid tumor lines were tested with both irofulven and compound **8**). Compound **8** retained activity against multidrug resistant cell lines, such as Ovarian NCI/ADR-RES, consistent with previous reports on the activity of acylfulvenes against multidrug resistant phenotypes.[3]

The antitumor activities of compounds **8** and **9** were then compared against that of irofulven using the MV522 adenocarcinoma cell line as the target cell line because these cells have previously been demonstrated to be resistant to a wide variety of conventional chemotherapeutic agents.[13] The megakaryocytic cell line CHRF 288-11 was chosen as the non-target cell line as the primary toxicity of irofulven is thrombocytopenia.[14] The in vitro cytotoxicity assay has been previously described.[15] While the hydroxyurea derivatives retained cytotoxicity towards the target MV522 adenocarcinoma cell line, the analogs were markedly less toxic to the megakaryocytic CHRF 288-11 cell line (Table 2).

Based on the screening results with MV522 and CHRF 288-11 cells, compound **8** was then tested in the MV522 xenograft model.

Table 1
Solid tumor IC_{50} values (nM) for irofulven and hydroxyurea derivative **8** from the NCI DTP 60 cell line analysis

Panel/cell line	Log_{10} IC_{50}	
	Irofulven	Compound **8**
Non-small cell lung		
A549	−6.9	<−8.0[a]
EKVX	−6.7	−6.5
HOP-62	−6.8	−7.6[a]
HOP-92	−6.5	−7.4[a]
NCI-H226	−6.7	−6.7[a]
NCI-H23	−7.1	−7.8[a]
NCI-H322M	−6.6	−7.0[a]
NCI-H460	−7.3	<−8.0[a]
NCI-H522	−6.8	−6.0
Colon		
COLO 205	−6.8	−7.6[a]
HCC-2998	−6.8	−7.9[a]
HCT-116	−6.7	−6.8[a]
HCT-15	−6.5	−6.9[a]
HT29	−6.6	−6.9[a]
KM12	−6.2	−6.4[a]
SW-620	−6.2	−4.8
CNS		
SF-268	−6.6	−7.7[a]
SF-295	−6.5	−7.3[a]
SF-539	−6.8	−7.7[a]
SNB-19	−6.5	−6.9[a]
SNB-75	−6.7	−7.5[a]
U251	−6.5	−6.9[a]
Melanoma		
LOX IMVI	−6.2	−6.5[a]
MALME-3M	−6.7	−6.9[a]
M14	−6.6	−6.7[a]
MDA-MB-435	−5.8	−6.4[a]
SK-MEL-2	−6.7	−6.4
SK-MEL-28	−6.2	−6.9[a]
SK-MEL-5	−6.8	−7.2[a]
UACC-257	−6.6	−6.6[a]
Ovarian		
IGROV1	−6.8	−7.6[a]
OVCAR-3	−6.6	−7.3[a]
OVCAR-4	−6.6	−7.0[a]
OVCAR-5	−6.8	−7.6[a]
OVCAR-8	−6.7	−6.5
NCI/ADR-RES	−6.6	−6.8[a]
SK-OV-3	−6.6	−6.5
Renal		
786-O	−6.7	−6.9[a]
A498	−6.7	−7.5[a]
ACHN	−6.6	−7.5[a]
CAKI-1	−6.6	−7.3[a]
RXF 393	−7.3	−7.8[a]
SN12C	−6.4	−6.5[a]
TK-10	−6.6	−7.5[a]
UO-31	−6.7	−7.6[a]
Prostate		
PC-3	−6.6	−6.4
DU-145	−7.0	<−8.0[a]
Breast		
MCF-7	−6.6	−5.9
MDA-MB-231	−6.5	−6.6[a]
HS 578T	−6.5	−6.3
BT-549	−6.5	−6.8[a]
T-47D	−6.7	−7.2[a]
Mean	−6.38	−7.02[a]

[a] Designates equal or increased cytotoxicity by derivative **8**.

As a comparator irofulven was tested at 10 mg/kg, the maximum tolerated dose when administered intraperitoneally at 3 doses per week for 3 weeks. Compound **8** was less toxic than irofulven, in agreement with the screening results (Table 2) and could be

Table 2
IC_{50} values (nM) for irofulven and hydroxyurea derivatives when tested against MV522 adenocarcinoma cells and CHRF 288-11 megakaryocytic cells[a]

Compound	MV522	CHRF 288-11	Ratio[b]
Irofulven **3**	70 ± 10	290 ± 20	4.1
8	210 ± 20	8800 ± 1700	41.9
9	130 ± 10	1900 ± 100	14.6

[a] IC_{50} is the concentration of the irofulven derivative at which 50% inhibition of growth occurs as measured by the trypan blue exclusion assay for a 48 h exposure.[15]
[b] The ratio is defined as the CHRF IC_{50} value/MV522 IC_{50} value.

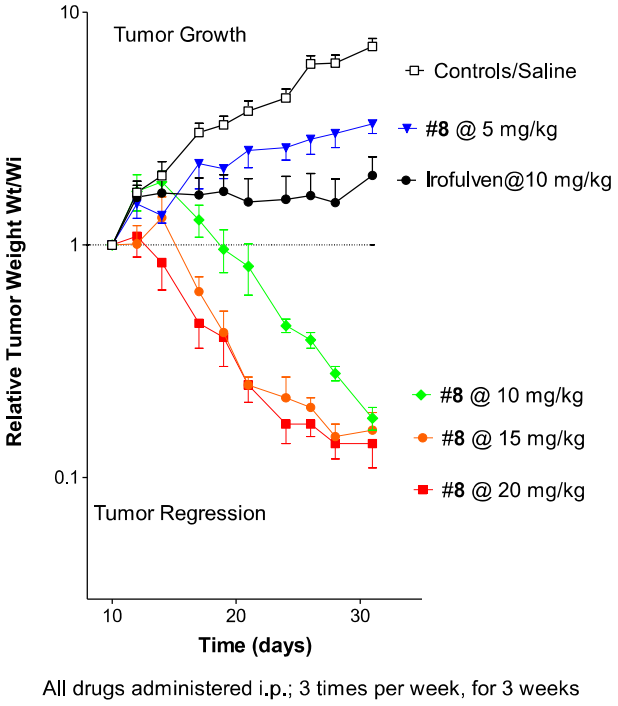


Figure 3. Activity of compound **8** in the drug-resistant MV522 Balb/c nu/nu mouse xenograft model[13] as compared to irofulven. All drugs were administered intraperitoneally, 3 times a week, for 3 consecutive weeks as previously described ($N = 8$).[13]

administered at higher doses (Fig. 3). The in vivo antitumor activity of compound **8** was superior to irofulven when administered at an equivalent dose of 10 mg/kg (the maximum tolerated dose for irofulven), and compound **8** could be administered at a twofold higher dose than irofulven.

It is not entirely clear why the hydroxyurea derivatives **8** and **9** of irofulven display an improved therapeutic index towards solid tumor cells and not cells of a megakaryocytic lineage. One could argue that these derivatives are simply functioning as an equimolar mixture of irofulven and hydroxyurea, however, hydroxyurea is nontoxic to MV522 cells (IC50 > 650 μM). Further biological studies will be required to clarify the mechanism of increased therapeutic ratio for the hydroxyurea derivatives of irofulven.

Acknowledgment

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Supplementary data

Supplementary data (experimental details for the synthesis and ^{1}H NMR and ^{13}C NMR spectra of compounds **8** and **9**. Compound **8** has been selected for phase I clinical trials and now carries the drug designation LP-184 for these trials.) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.02.028.

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Supporting Information

Hydroxyurea derivatives of irofulven with improved antitumor efficacy

Michael D. Staake, Kashinatham Alisala, Trevor C. McMorris,

Leita A. Estes, and Michael J. Kelner*

Contents:

General Experimental Methods. Reactions were conducted under N_2 atmosphere in oven-dried glassware employing standard air-free manipulation techniques.

Reaction solvents were dried and distilled prior to use. Methylene chloride (CH_2Cl_2) was distilled from CaH_2 under N_2. All other reagents and solvents were used as received from commercial sources. Solvents were removed under reduced pressure using a rotary evaporator.

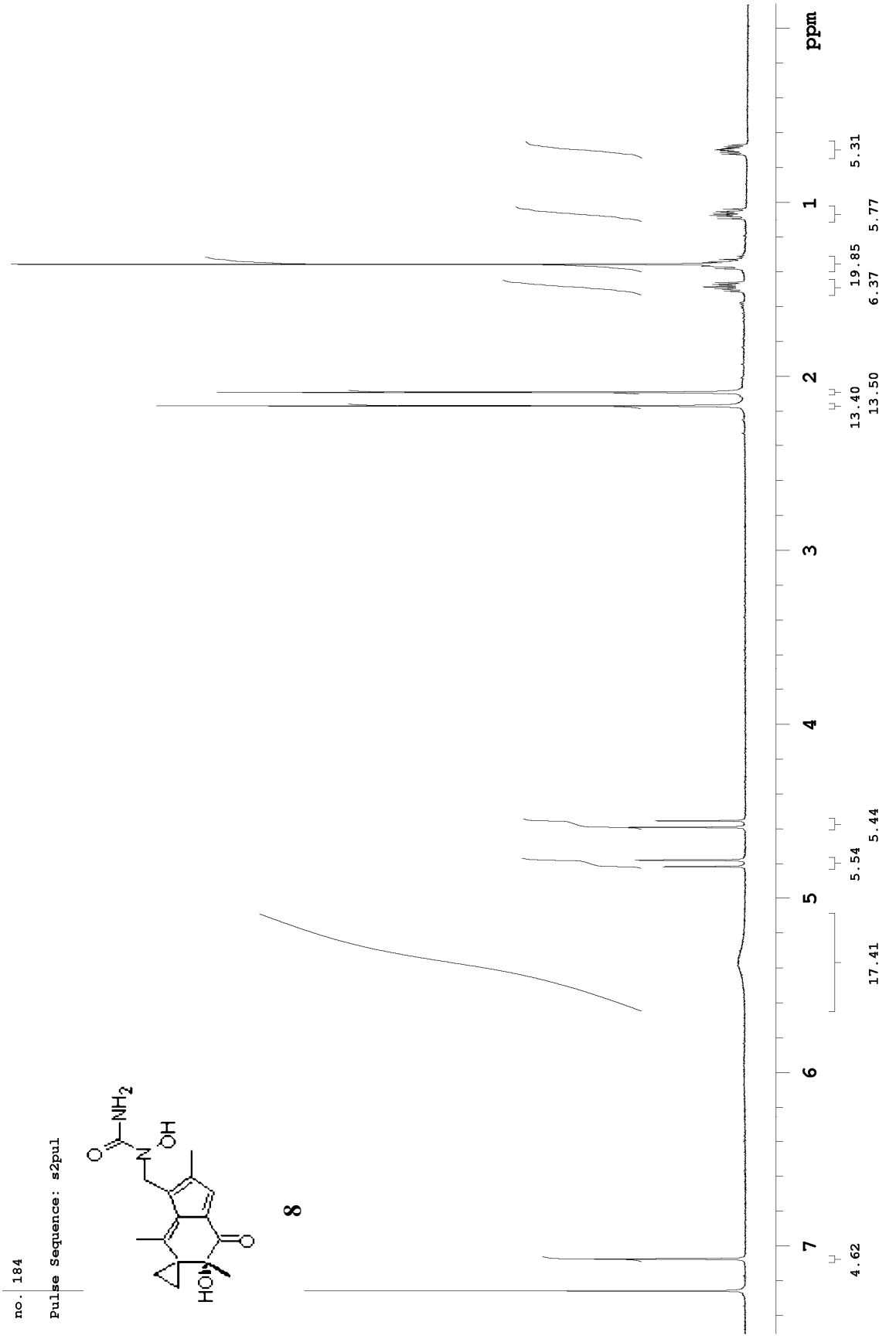
All chromatography was carried out with silica gel. Analytical TLC was carried out on silica gel plates. Reactions were routinely monitored by TLC.

^{1}H-NMR and ^{13}C-NMR spectra were measured at 400 and 100 MHz, respectively.

N-hydroxy-N-(methylacylfulvene)urea (8). To a solution of irofulven (200 mg, 0.812 mmol) in a 1:1 mixture (8.0 mL) of acetone and 2M H_2SO_4 was added hydroxyurea (125 mg, 1.64 mmol). The mixture was stirred for 1 h at rt then partitioned between ethyl acetate and water. The organic extract was washed with saturated $NaHCO_3$ and brine then dried with $MgSO_4$ and concentrated. The crude product was chromatographed (1:3 hexanes-ethyl acetate) to give 206 mg of **8** (83%) as an orange gum: ^{1}H-NMR ($CDCl_3$) δ 0.70 (m, 1H), 1.07 (m, 1H), 1.36 (m, 1H), 1.36 (s, 3H), 1.48 (m, 1H), 2.08 (s, 3H), 2.17 (s, 3H), 4.57 (d, J = 14.8 Hz, 1H), 4.80 (d, J = 14.8 Hz, 1H), 5.37 (br, 2H), 7.07 (s, 1H) ^{13}C-NMR ($CDCl_3$) δ 10.0, 13.7, 14.7, 16.8, 27.9, 37.9, 46.0, 76.3, 126.4, 128.9, 135.1, 138.5, 144.6, 160.8, 162.1, 198.2; HRMS for $C_{16}H_{21}N_2O_4$ (MH^+) calcd 305.1501, found 305.1505; UV λ_{max} (EtOH) 332 nm (ε 6621).

N-Acetoxy-N-(methylacylfulvene)urea (9). To a solution of **8** (20.4 mg, 67.0 μmol) and Ac_2O (7.0 μL, 74 μmol) in CH_2Cl_2 (2.0 mL) was added DMAP (2.0 mg, 16 μmol). The mixture was stirred for 15 min then added directly to a silica gel column and chromatographed (10:1 hexanes-ethyl acetate) to give 23.2 mg of **9** (quant.) as an orange

gum: ^{1}H-NMR ($CDCl_3$) δ ^{1}H-NMR ($CDCl_3$) δ 0.71 (m, 1H), 1.06 (m, 1H), 1.35 (m, 1H), 1.36 (s, 3H), 1.46 (m, 1H), 1.91 (s, 3H), 2.10 (s, 3H), 2.13 (s, 3H), 3.90 (br s, 1H), 4.77 (br, 2H), 5.25 (s, 2H), 7.04 (s, 1H); ^{13}C-NMR ($CDCl_3$) δ 9.7, 13.2, 14.5, 17.0, 18.3, 27.6, 38.0, 46.1, 76.4, 126.4, 127.1, 133.5, 138.5, 144.0, 159.5, 161.7, 167.6, 198.0; HRMS for $C_{18}H_{22}N_2O_5$ calcd 346.1529, found 346.1519; UV λ_{max} (EtOH) 332 nm (ε 6673).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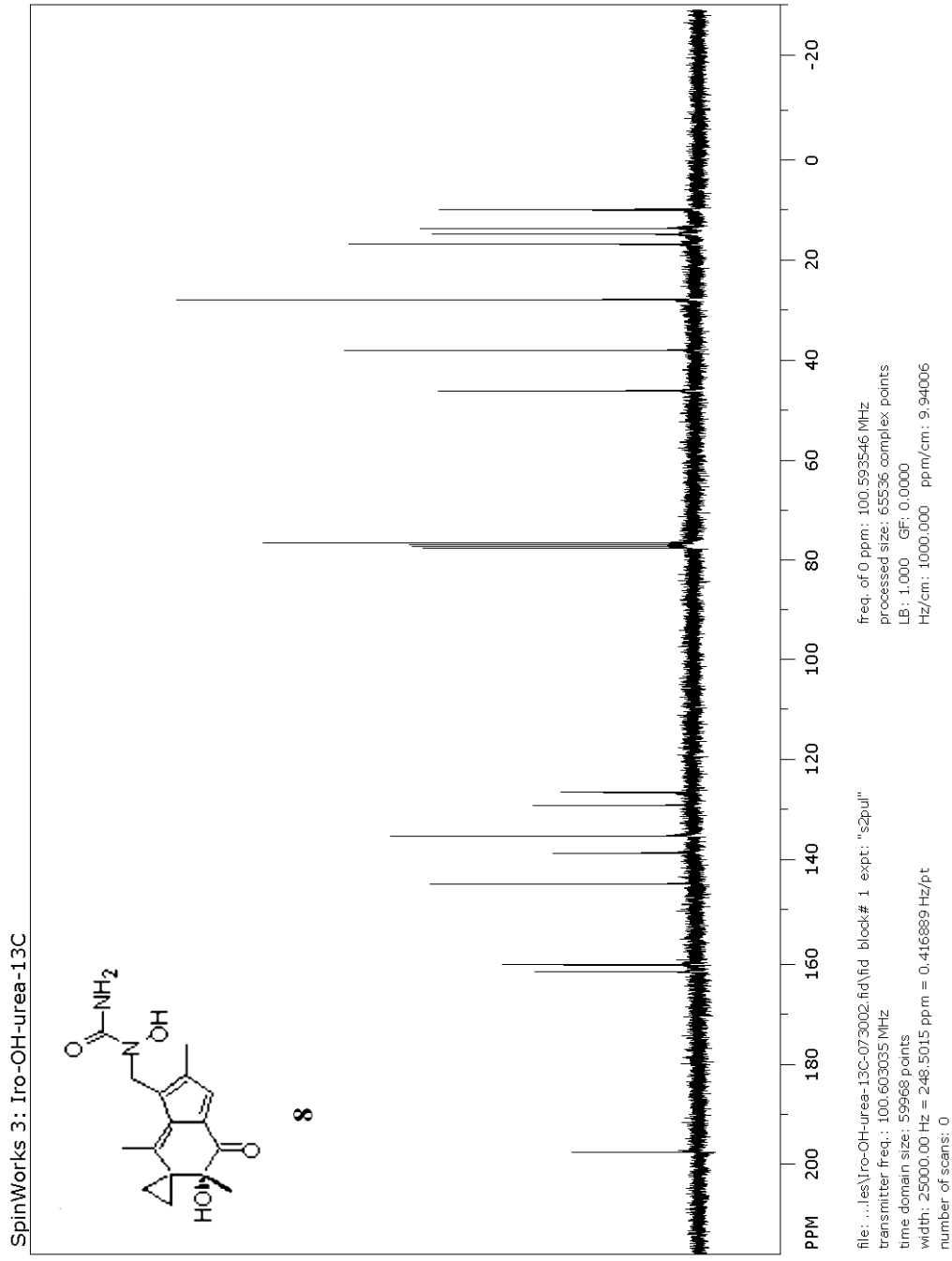
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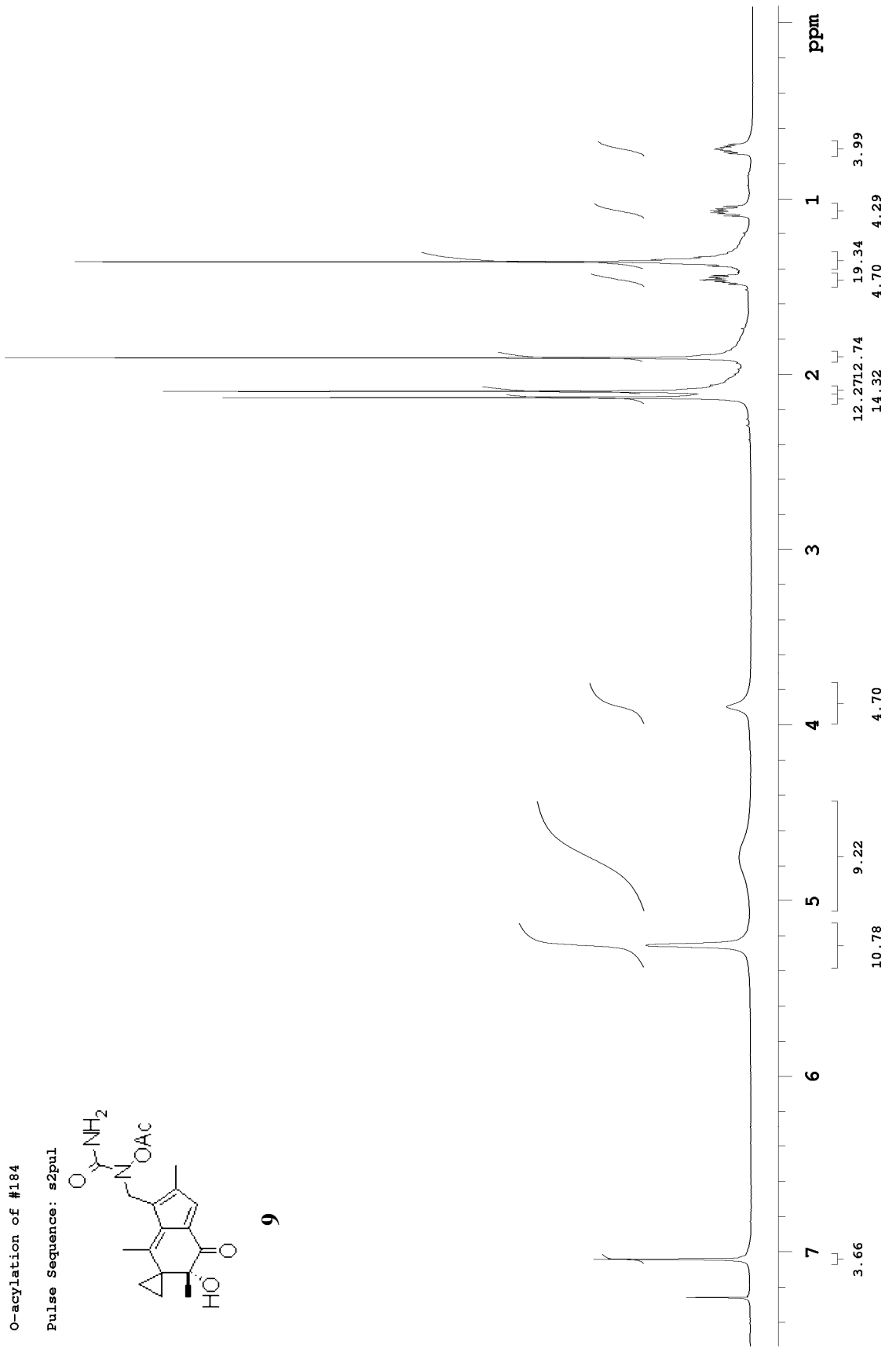
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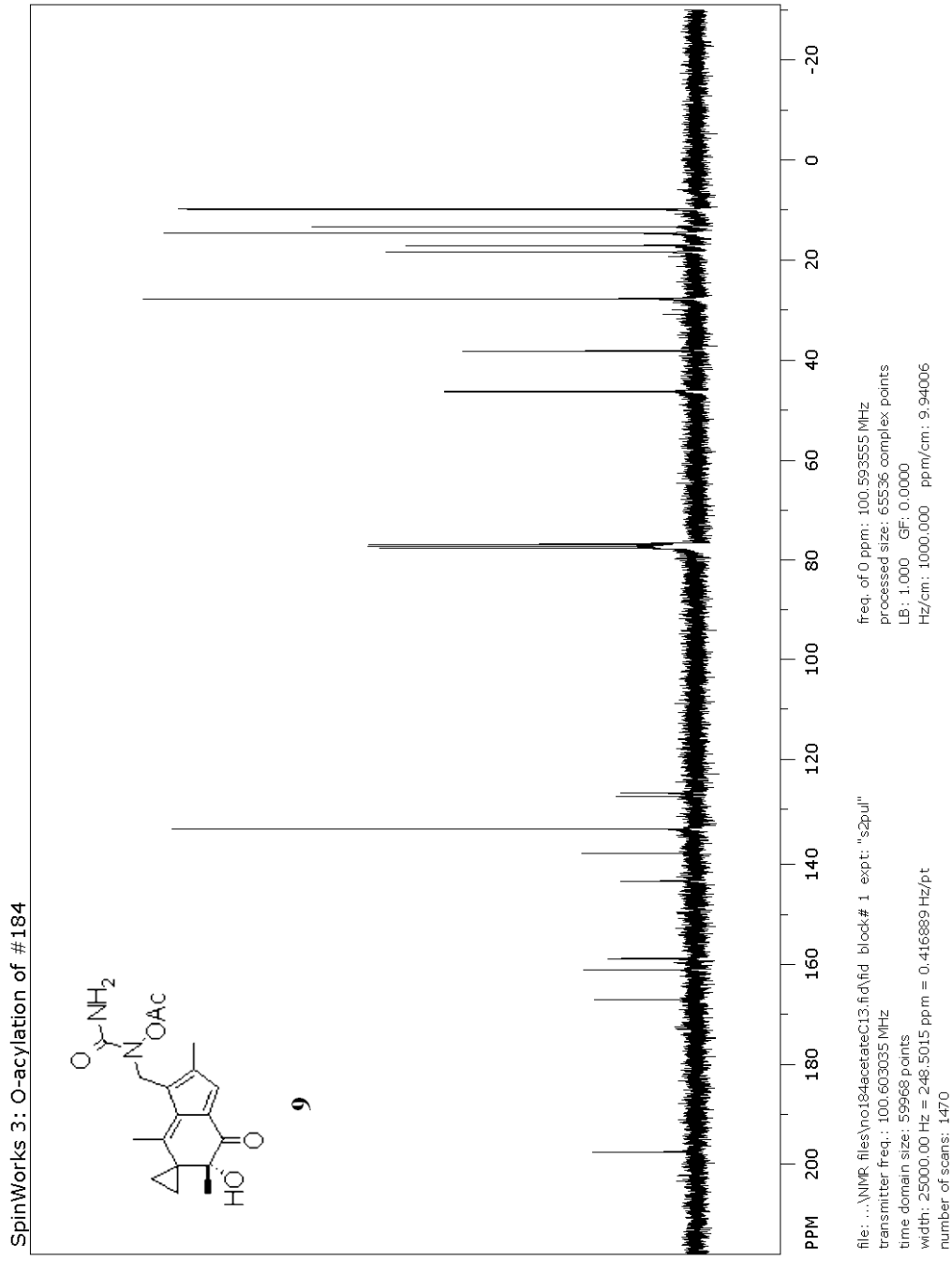
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