DEVELOPMENT AND CHARACTERIZATION OF NOVEL *TOXOPLASMA GONDII* INHIBITORS

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PETER GRUNDT

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I would like to thank Dr. Peter Grundt and Bogdana Krivogorsky for their endless support and time. I would also like to thank my fellow lab workers Emily Kalinowski, Amber Nelson, Taylor Jacobsen, and many others.

Dedication

I dedicate this thesis to my wife and family. Thank you all for your love and support.

Table of Contents

List of Tables

List of Figures

List of Schemes

1. Introduction

Toxoplasma gondii (*T. gondii*) is an obligate intracellular, protozoan parasite which lives in the gut of cats, requiring the feline host to complete the sexual portion of its life cycle.[1] Feline infection occurs by consumption of a previously infected animal. After the parasite has matured within the cat (generally 2-3 weeks), it is shed in the feces.[2] The feces of the cat are then contacted by another organism, commonly a rodent or other mammal, but *T. gondii* has been found in many animals including fish and birds. If the secondary host is a rodent, a peculiar behavioral change occurs: the rodent loses the instinctive fear of cat scents and open areas.[3] This is thought to help the parasite infect another cat, thus continuing its life cycle. Humans, however, are commonly unwitting hosts of the parasite. Human infection can occur from direct contact with contaminated cat feces, or by consumption of undercooked meat containing tissue cysts (Figure 1).

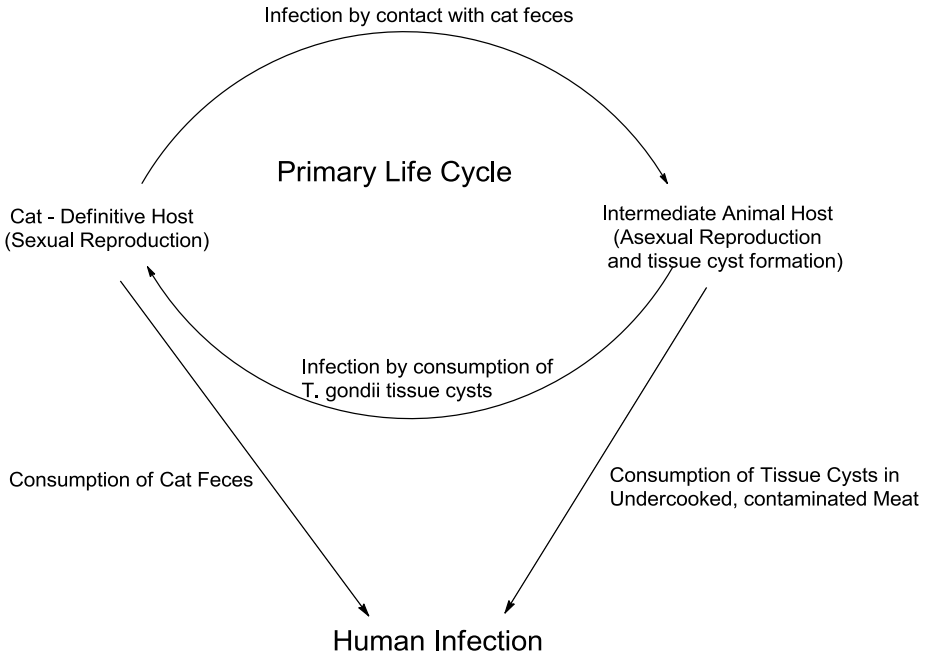


Figure 1: *T. gondii* infection

Human infection causes generally unnoticeable or non-specific symptoms in the immunocompetant host. After acute infection, tissue cysts form and remain dormant for many years. If the host becomes immunocompromised, such as by HIV/AIDS infection, the quiescent *T. gondii* tissue cysts can become active again, and the tachyzoites become aggressive, causing encephalitis or abscess in the central nervous system (CNS).[4] Acute *T. gondii* infection presenting in pregnant women is known to cause severe birth defects and even loss of the fetus.[5] Finally, latent *T. gondii* infection is believed to be a contributing factor in development of mental disorders, specifically schizophrenia.[6]

The current treatment for *T. gondii* infection in immunocompromised humans is the synergistic administration of sulfadiazine and pyrimethamine (with folinic acid to reduce the occurrence of thrombocytopenia).[4] These treatments, however, are limited by toxicity (pyrimethamine causes bone marrow toxicity) and are limited in their effectiveness. This

treatment cannot be used in pregnant woman, for both compounds are known to be teratogenic. For this reason, the development of safer and more effective treatments for toxoplasmic encephalitis (TE) is necessary.[4]

The effect of *T. gondii* infection on the rodent brain may help explain the observation that humans who have schizophrenia have a statistically higher rate of *T. gondii* antibodies than those who do not suffer from the disease.[7] Other psychiatric disorders have also been associated with *T. gondii* seropositivity.[8] Additionally, it has been found that drugs historically used to treat mental illness are effective at killing *T. gondii* tachyzoites *in vitro.*[9] A recent study has shown that *T. gondii* is capable of synthesizing dopamine, a neurotransmitter involved in many psychiatric illnesses.[10]

In recent studies, a number of compounds have been found to have anti-Toxoplasma activity. Among these are the plant products artemisinin (**1**), berberine (**2**), and tryptanthrin (**3**).

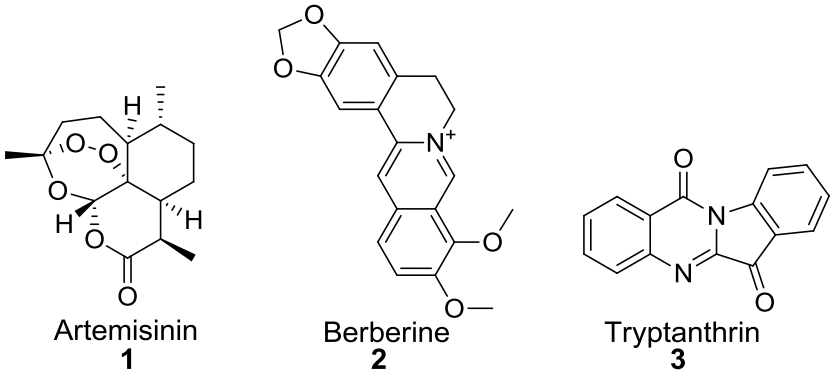


Figure 2: Active anti-*Toxoplasma gondii* compounds.

Artemisinin, known as Qinghaosu, and its derivatives are potent and fast-acting antimalarial drugs.[11] Due to the close evolutionary relationship between *Plasmodium falciparum* and *Toxoplasma gondii*, artemisinin and some of its analogues have been tested against *T.*

gondii tachyzoites and have shown high therapeutic indices (TIs) *in vitro.*[12] A problem that has been noted with artemisinin, however, is the discovery that *Plasmodium* species are readily developing resistance to the drug, and the World Health Organization has strongly discouraged its use as a monotherapy. It is foreseeable that a similar problem would be found if it were in widespread use against *T. gondii*.

Berberine, isolated from a number of plants from in the *Berberis* genus, has been used as an herbal supplement thought to be useful in fighting infection. Some berberine analogues have been shown to be quite active against *T. gondii* tachyzoites in vitro, with TI values near 4,000.[13]

A drug that has shown particular promise for combating toxoplasmic encephalitis is the plant product tryptanthrin (indolo[2,1-b]quinazoline-6,12-dione). Studies carried out *in vitro* show that the analogue 8-bromotryptanthrin has a high therapeutic index against *T. gondii* tachyzoites, indicating low toxicity and high potency.[14]

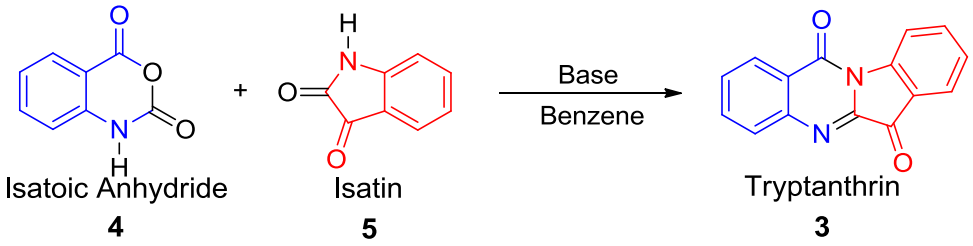
The topic of this thesis will be to explore tryptanthrin analogues. Included are synthesis of the starting materials, namely isatin and isatoic anhydride, and their analogues. Also, the construction of modified tryptanthrin by esterification and Suzuki coupling will be discussed.

Although tryptanthrin and its analogues have shown significant activity against Toxoplasma, plant studies were performed to help discover new active compounds. Plants that have been used in herbal medicine, or those used to treat other parasitic diseases such as malaria were subjected to solvent extraction and their extracts were tested *in vitro* against *T. gondii*.

Finally, a colorimetric method of testing compounds and extracts against *Toxoplasma gondii* will be discussed. This gives some insight into how dye-tagged sugars can be used to determine survival of a parasite, giving results that can be determined by use of a spectrophotometer. This method is applied to 96-well microplates, which allows fast scanning of a large number of samples and concentrations at once, decreasing labor and increasing safety relative to radiometric methods.

2. Synthesis of Starting Materials

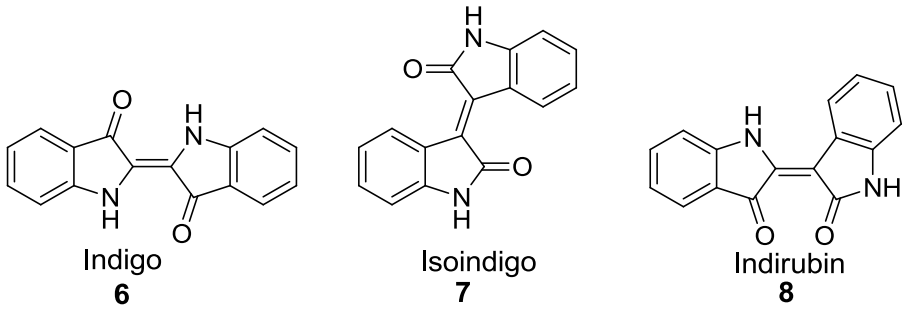
A relatively convenient synthesis of tryptanthrins has been developed using isatin and isatoic anhydride heated in benzene or toluene with a base such as triethylamine or morpholine (Scheme 1).[15] Many isatin derivatives are commercially available or easily synthesized. Isatoic anhydride derivatives are easily synthesized from commercially available substrates.

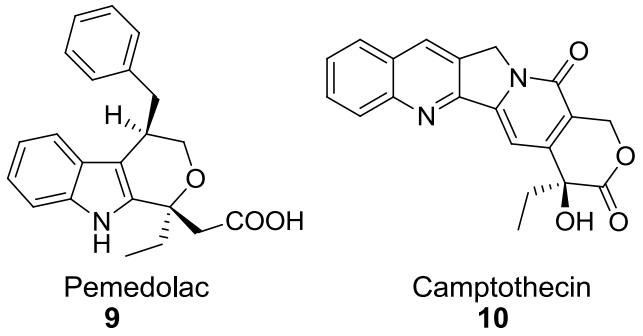


Scheme 1: Synthesis of tryptanthrin by reaction of isatin and isatoic anhydride, with colors representing the portions of the backbone contributed by each starting compound.

2.1 Synthesis of Isatin Derivatives

Isatin (1H-indole-2,3-dione, **5**) was first synthesized in 1841 by the oxidation of indigo using chromic and nitric acids.[16] It is found in nature in plants of the genus *Isatis* and some others.[17] Isatin is also a human metabolite of adrenaline.[18] Isatin is widely used as a precursor in heterocyclic synthesis, such as that of the plant pigments indigo (**6**), isoindigo (**7**), and indirubin (**8**)[19]; analgesic drug pemedolac (**9**)[20]; and topoisomerase I inhibitor camptothecin (**10**).[21]



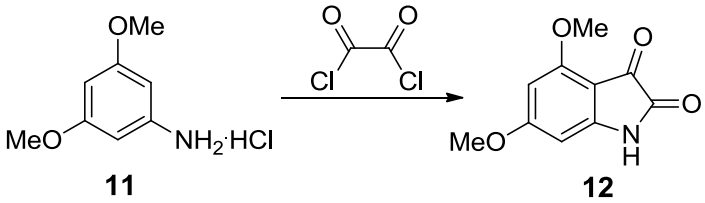


Pemedolac
9

Camptothecin
10

Figure 3: Several compounds synthesized from isatin

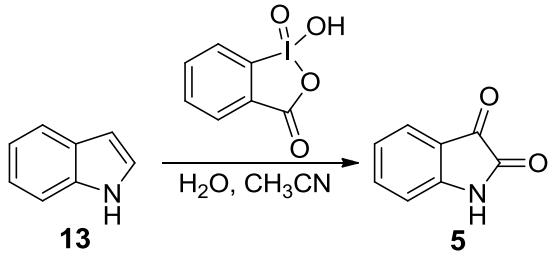
There are several known methods of isatin synthesis. An important method was developed by Stolle et al., reacting aniline with oxalyl chloride (Scheme 2). This method has been used in the formation of dimethoxyisatins[22] and polycyclic isatins.[16]



11 **12**

Scheme 2: Cyclization of 3,5-dimethoxyaniline to 4,6-dimethoxyisatin

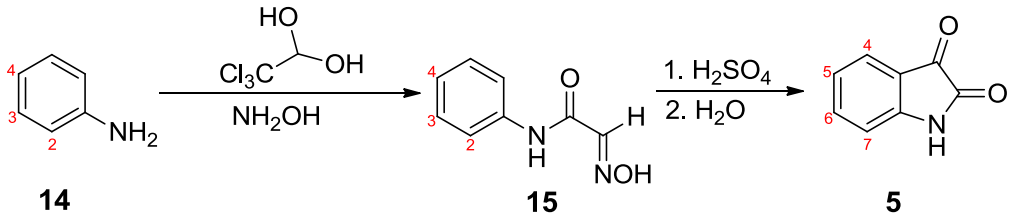
Oxidation of indoles can also give isatins as products, such as oxidation with the hypervalent iodine compound 2-iodoxybenzoic acid (IBX) as shown in Scheme 3.[23]



13 **5**

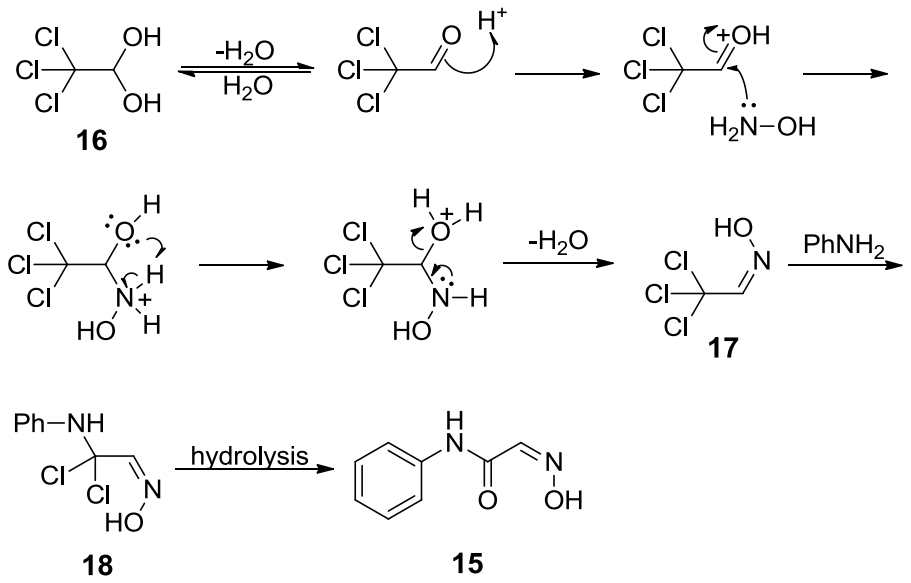
Scheme 3: Oxidation of indole to give isatin using IBX in water/acetonitrile

The most commonly used method is the Sandmeyer isonitrosoacetanilide synthesis. The reaction of aniline with chloral hydrate and hydroxylamine in aqueous hydrochloric acid produces an N-substituted aniline, which further reacts in hot sulfuric acid to produce isatin (Scheme 4). The process also works for ring-substituted anilines to produce the corresponding isatin.



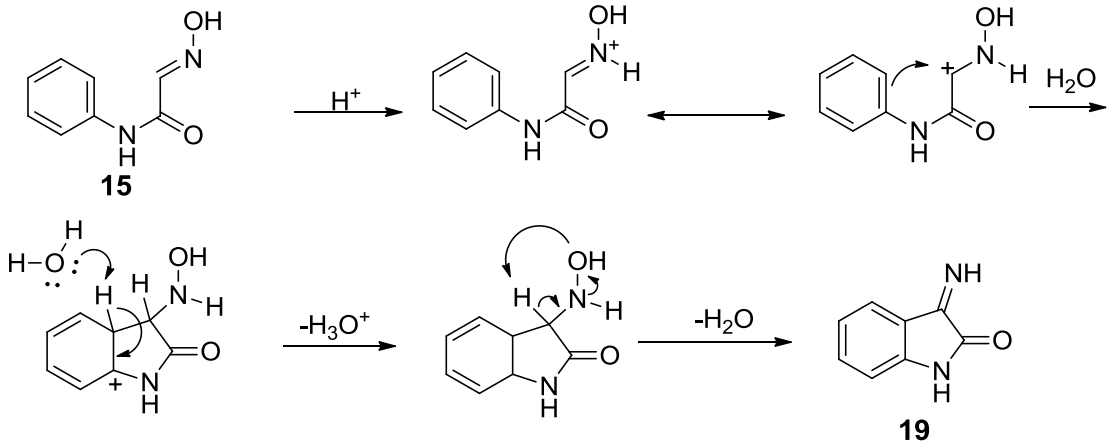
Scheme 4: Isatin synthesis by the Sandmeyer isonitrosoacetanilide method.

In the first step of the synthesis (Scheme 5), chloral hydrate reacts with hydroxylamine in solution, forming chloral oxime. Aniline then displaces one of the chlorines in a nucleophilic substitution, forming dichloroamine, which undergoes hydrolysis to yield the isonitrosoanilide **15**.



Scheme 5: Step one of the Sandmeyer synthesis.

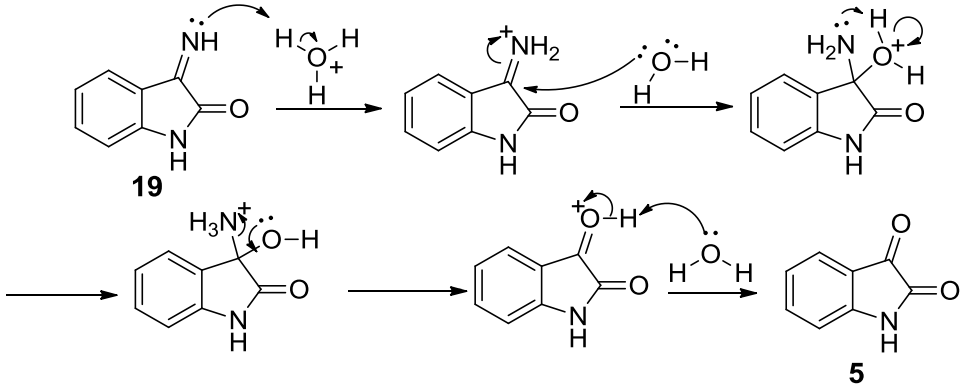
After isolation and drying of isonitrosoanilde **15**, cyclization is performed in hot sulfuric acid followed by hydrolysis in water. The mechanism of this cyclization step (Scheme 6) was recently supported by ESI-MS(/MS) monitoring of the reaction.[24]



Scheme 6: Cyclization mechanism as elucidated by Silva et al.[24]

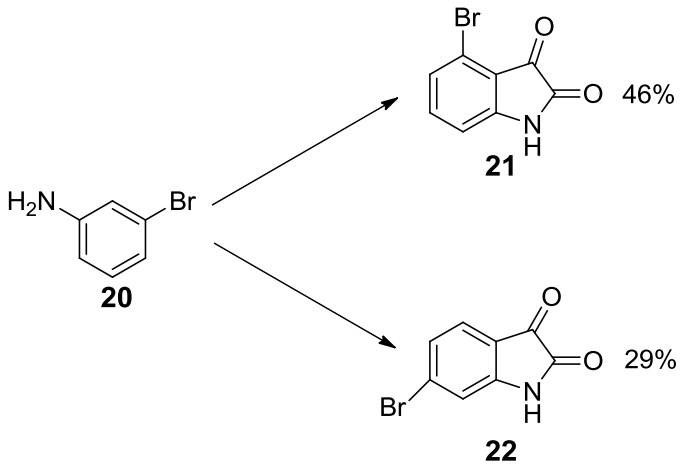
Isonitrosoanilide **15** undergoes a protonation in the acidic media. The electrons in the aromatic double bond then attack the carbocation, cyclizing the system. The proton on the previously aromatic ring is removed by water, restoring aromaticity. Water is then lost, leaving

an azo isatin. The final step in the isatin synthesis is hydrolysis of the C-N double bond, yielding the second isatin carbonyl (Scheme 7).



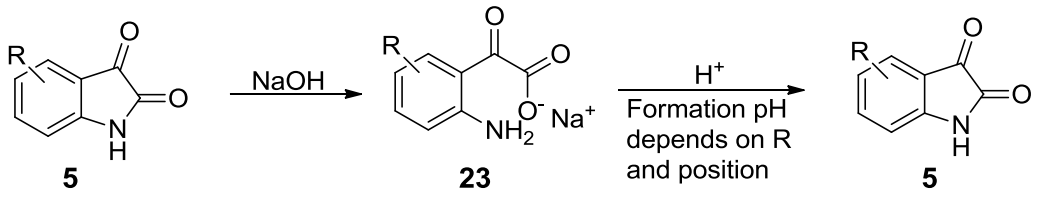
Scheme 7: Mechanism of amine hydrolysis to a ketone.

This method of synthesis works well with aniline. Meta-substituted anilines, however, give two isomers, as in the example of 3-bromoaniline (Figure 5).[25]



Scheme 8: Products of 3-bromoaniline after cyclization using the Sandmeyer isonitrosoacetanilide synthesis.[25]

The mixture of these isomers can be separated. This is done by treating the isatin mixture with aqueous NaOH, breaking the N-C2 bond, followed by careful recyclization by acidification, the 4-substituted isatin precipitating at higher pH than the 6-substituted.[26]



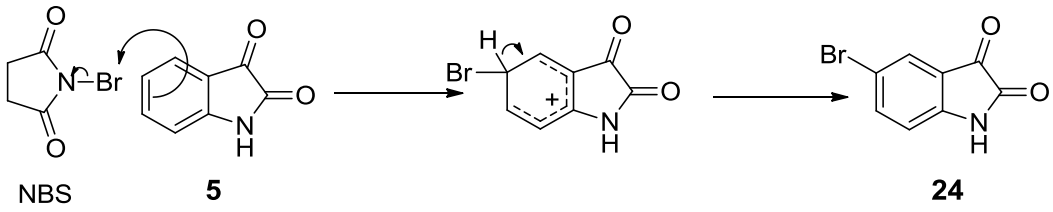
Scheme 9: Decyclization and recyclization of isatin derivatives allows for pH dependent separation of products

The optimal pH for isolation of pure product must be determined experimentally before application to large batches.

Table 1: Isatins synthesized by the Sandmeyer isonitrosoacetanilide method. Yields combined for two steps (from aniline).

Compound	Substituent	Yield	Melting Point (°C)
5a	5-fluoro	11%	225-227
5b	5-methoxy	18%	169-172
5c	5-isopropyl	40%	128-130
5d	6-fluoro	55%	194-196
5e	7-bromo	30%	193-195
5f	7-fluoro	27%	191-192

Isatin itself can also be modified by electrophilic aromatic substitution. This process works well with the Br^+ ion, as provided by N-bromosuccinimide (Scheme 10).



Scheme 10: Electrophilic aromatic addition of Br+ to isatin using N-bromosuccinimide (NBS).

Addition of bromine at the 5-position, para- to the amine and meta- to the carbonyl occurs, but the 7-position (ortho- to the amine) is also a possible reaction. The author suspects

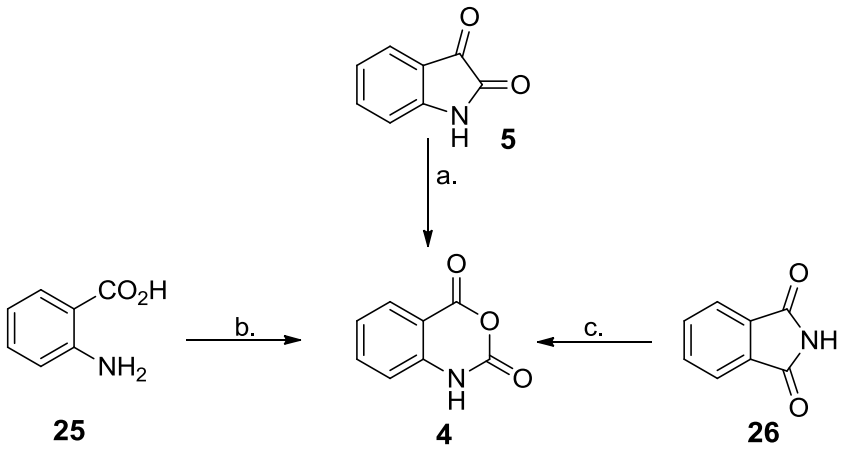
that the reason that the 5-position substitution is so heavily favored is simply due to sterics. This is logical with a large atom such as bromine, but it has been shown that chlorine and nitro groups also add with high selectivity.[27]

Table 2: Isatin bromination products

Compound	Substituent	Yield	Melting Point (°C)
5g	5,6-dibromo	49%	>250
5h	5,7-dibromo	65%	>250

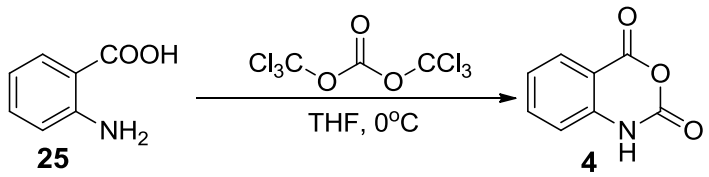
2.2 Synthesis of Isatoic Anhydride Derivatives

Isatoic anhydride (**4**) was synthesized in 1883 by the reaction of 2,1-benzisoxozole with ethyl chloroformate by P. Friedländer and W. Wiëugel.[28] The synthesis of isatoic anhydride by oxidation of isatin with CrO_3 was then carried out in 1884,[29] and the correct formula was determined by Niementowski and Rozanski.[30]



Scheme 11: Most common synthesis of isatoic anhydride, adapted from a previous review.[31] a. CrO_3, 50°C. b. Triphosgene, THF, 0°C to RT. c. 1. NaOCl 2. H^+.

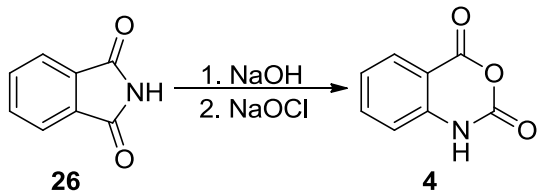
Isatoic anhydride synthesis is commonly carried out from three starting moieties. The most common synthetic method is the reaction of phosgene (or safer triphosgene) with an anthranilic acid derivative, giving the corresponding anhydride.[32]



25 4

Scheme 12: Synthesis of isatoic anhydride from anthranilic acid and triphosgene

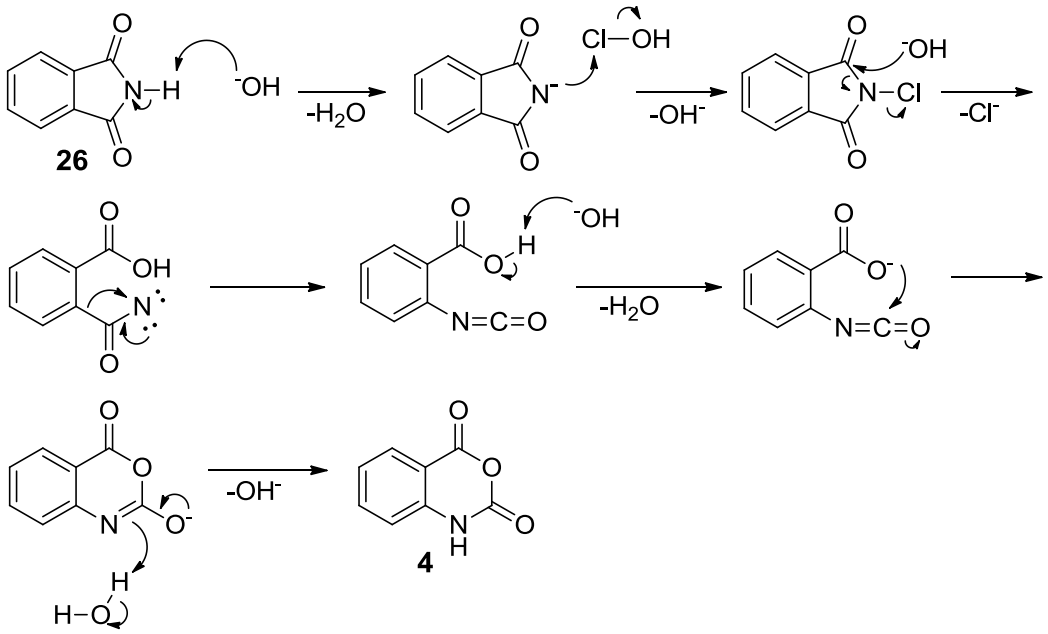
Phthalimide, or salts thereof, can also be used as a starting material, with rearrangement by NaOCl (Scheme 13).[33]



26 4

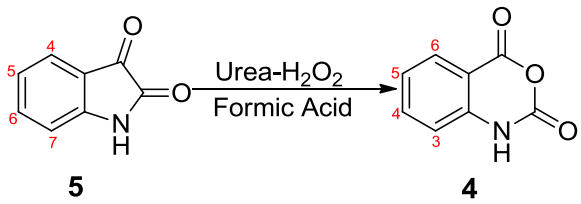
Scheme 13: Synthesis of isatoic anhydride from phthalimide

This reaction is interesting, as the rearrangement takes place through an isocyanate intermediate, as can be seen in Scheme 14.



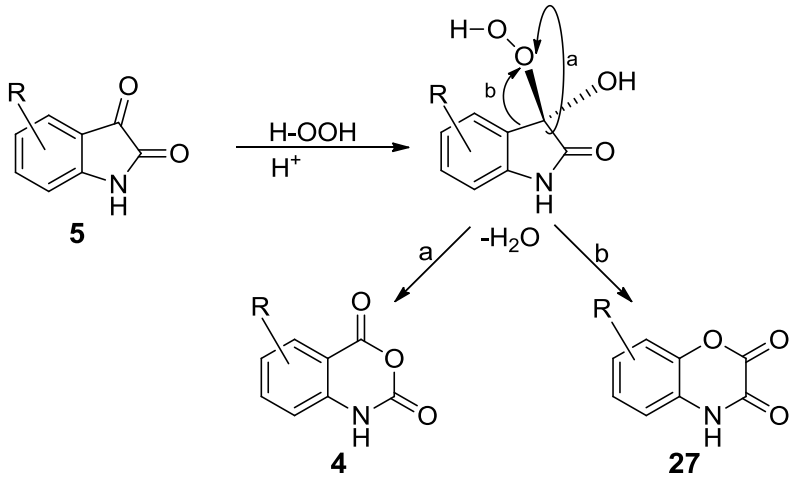
Scheme 14: Mechanism of phthalimide to isatoic anhydride transformation

Isatoic anhydrides for this experiment were synthesized from the corresponding isatin in a Baeyer-Villiger oxidation. Though this method is not common and gives poorer yields than the above methods, it was convenient because substituted isatins had been synthesized already. One reaction scheme involves use of a urea-hydrogen peroxide complex in formic acid (Scheme 15).[34]



Scheme 15: Oxidation of isatin to isatoic anhydride. Common numbering for the aromatic carbons is included.

This oxidation, especially with an electron-withdrawing group in the 6-position has a possibility of two major products: isatoic anhydride (**5**) or 2,3-dioxo,1-4-benzoxazine (**27**).[35]



Scheme 16: Possible products of Baeyer-Villiger oxidation of isatin.

Rearrangement of the Criegee intermediate formed after addition of the peroxide to C-8 leads to either isomer (Scheme 16), but can be carried out selectively by use of varying reaction conditions, as was studied previously by Reissenweber et al.[35]

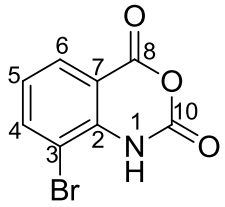
Electrophilic aromatic substitution of isatoic anhydride also gives the 5-substituted product, similar to the way this reaction occurs with isatin. This allows synthesis of 5-bromoisatoic anhydride from commercially available isatoic anhydride and N-bromosuccinimide at high yield.

Table 3: Isatoic anhydrides synthesized by oxidation of the corresponding isatin.

Compound	Substituent	Yield	Melting Point (°C)
4a	3-bromo	30%	197-199
4b	3,5-dibromo	53%	>250
4c	3-fluoro	33%	214-217
4d	4-fluoro	30%	>250
4e	4,5-dibromo	83%	>250
4f	4,5-difluoro	55%	229-232
4g	5-fluoro	63%	239-243
4h	5-isopropyl	42%	230-234
4i	6-bromo	35%	>250

2.3 NMR Spectroscopic characterization of 3- and 6-bromoisatoic anhydride

3-bromoisatoic anhydride



Carbon #	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**10**
Shift (ppm)	140.0	108.4	140.6	125.0	129.1	113.4	159.6	147.0
Proton shift (ppm)			8.01	7.19	7.94			

Figure 4: Assignment of carbon and proton signals in 3-bromoisatoic anhydride

The ^{1}H NMR spectrum of 3-bromoisatoic anhydride in DMSO-d_6 shows four signals: Two doublets at 7.14ppm and 8.01ppm, a triplet at 7.19ppm and broad singlet at 11.07ppm. All ^{3}J coupling constants are found to be equivalent and are observed to be 7.9Hz. This splitting pattern allows the assignment of the signals corresponding to the **5-H** hydrogen atom on the aromatic ring and the **1-H** hydrogen atom bound to the nitrogen atom. Thus the triplet at 7.19ppm is assigned to the **5-H** hydrogen atom, while the singlet at 11.07ppm belongs to the **1-H** hydrogen atom. The remaining hydrogen atoms **4-H** and **6-H** cannot be assigned solely on the ^{1}H NMR spectrum.

The ^{13}C NMR spectrum reveals eight signals. The chemical shifts of these peaks ranges from 108.4 to 159.6ppm. The secondary aromatic carbon atoms can be easily identified with help of the HMQC spectrum (Figure 5). The **C-5** carbon atom is observed at 125.0ppm. The signals at 129.1ppm and 140.6ppm belong to the proton doublet signals at 8.01ppm and 7.14ppm respectively. Discerning between these two signals required an HMBC spectrum (Figure 6). Three-bond coupling is expected to the **C-8** carbonyl, and this coupling was found

between the doublet **6-H** signal at 7.94ppm and **C-8** carbon signal at 159.6ppm. The **4-H** proton then was deduced to result in the signal at 8.01ppm. Carbon **C-2** shows coupling to both doublet proton signals, leading to its assignment as 140.0ppm. **C-10** is five bonds away from the nearest proton, and does not couple, and must result in the uncoupled signal at 147.0ppm. Two-bond coupling between proton **4-H** and carbon **C-3** allow assignment of **C-3** as the signal at 108.4ppm. The triplet proton signal at 7.19ppm couples not only to the carbon **C-3**, but also **C-7**, which can be assigned to 113.4ppm.

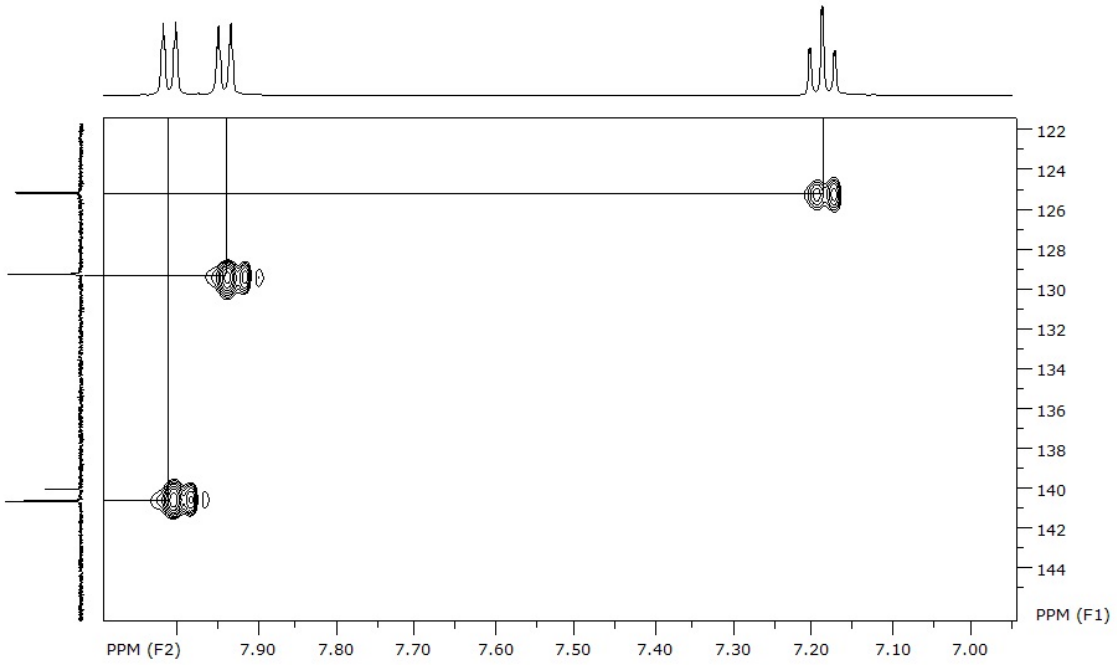


Figure 5: HMQC spectrum of 3-bromoisatoic anhydride

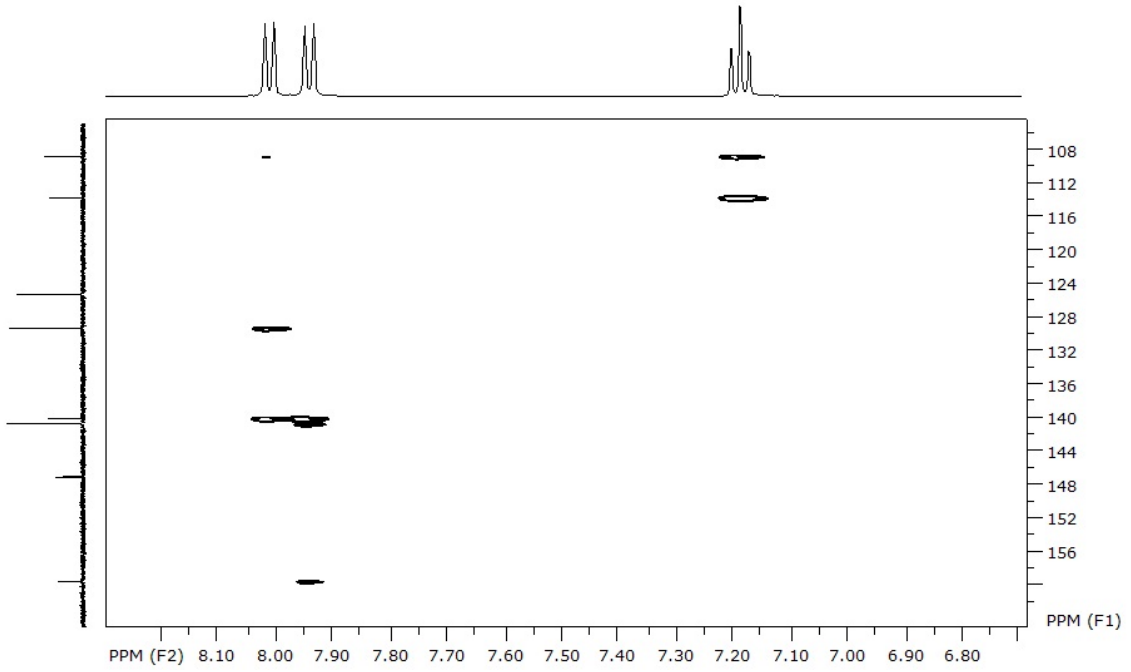
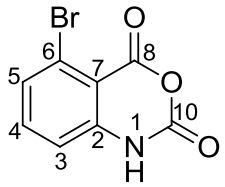


Figure 6: HMBC spectrum of 3-bromoisatoic anhydride

6-bromoisatoic anhydride



Carbon #	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**10**
Shift (ppm)	144.0	115.2	136.7	129.4	123.3	109.3	156.9	146.5
Proton shift (ppm)		7.14	7.56	7.51				

Figure 7: Assignment of carbon and proton signals in 6-bromoisatoic anhydride

The ^{1}H NMR spectrum of 3-bromoisatoic anhydride in DMSO-d6 shows four signals: Two doublets at 7.14ppm and 7.51ppm, a triplet at 7.56ppm and broad singlet at 11.84ppm. All ^{3}J coupling constants are found to be equivalent and are calculated to be 8.0Hz. This splitting

pattern allows the assignment of the signals corresponding to the **4-H** hydrogen atom on the aromatic ring and the **1-H** hydrogen atom bound to the nitrogen atom. The triplet signal at 7.56ppm corresponds to the proton **4-H** and the broad singlet at 11.84ppm to the proton **1-H**. The remaining two doublet proton signals for the **3-H** and **5-H** protons cannot be assigned by one dimensional proton NMR alone. A ROESY spectrum (Figure 8) allows assignment of the **3-H** peak by coupling to the proton **1-H**. The **3-H** proton was found to give the signal at 7.14ppm. The remaining doublet signal found at 7.51ppm therefore arises from the **5-H** proton.

The ^{13}C NMR shows eight signals from 109.3ppm to 156.9ppm. The secondary aromatic carbon atoms **3-H**, **4-H** and **5-H** can easily be assigned by HSQC (Figure 9), as the signals 115.2ppm, 136.7ppm, and 129.4ppm respectively. The carbon atom **C-7** can also be assigned, by HMBC coupling (Figure 10), to the **3-H** and **5-H** protons as 109.3ppm. The remaining carbons cannot be assigned by NMR directly, since **C-2** and **C-6** both couple to the **4-H** proton signal, as 123.3ppm and 144.0ppm. Similarly, the **C-8** and **C-10** carbon signals do not couple to any protons due to the distance being too great. Assignments of these must be done by comparison to the spectrum of 3-bromoisatoic anhydride. By comparison, the **C-2** carbon signal as assigned as 144.0ppm and the **C-6** signal is assigned to 123.3ppm. The carbonyls **C-8** and **C-10** were assigned by comparison to the same spectrum and were found to be 156.9ppm and 146.5ppm. All of these assignments could be done with confidence, as the carbon signals of **C-2** and **C-6** in each case were separated by approximately 20ppm; the **C-8** and **C-10** signals were separated by approximately 10ppm. The signal difference between each carbon in the 3-bromoisatoic anhydride and 6-bromoisatoic anhydride was much less than the difference between the unassigned carbons within each molecule.

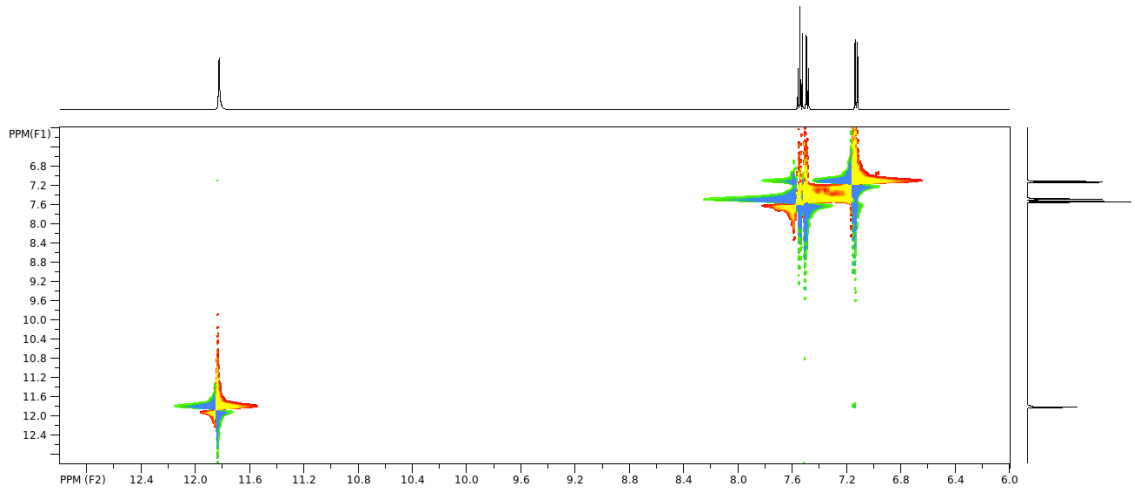


Figure 8: ROESY spectrum of 6-bromoisatoic anhydride

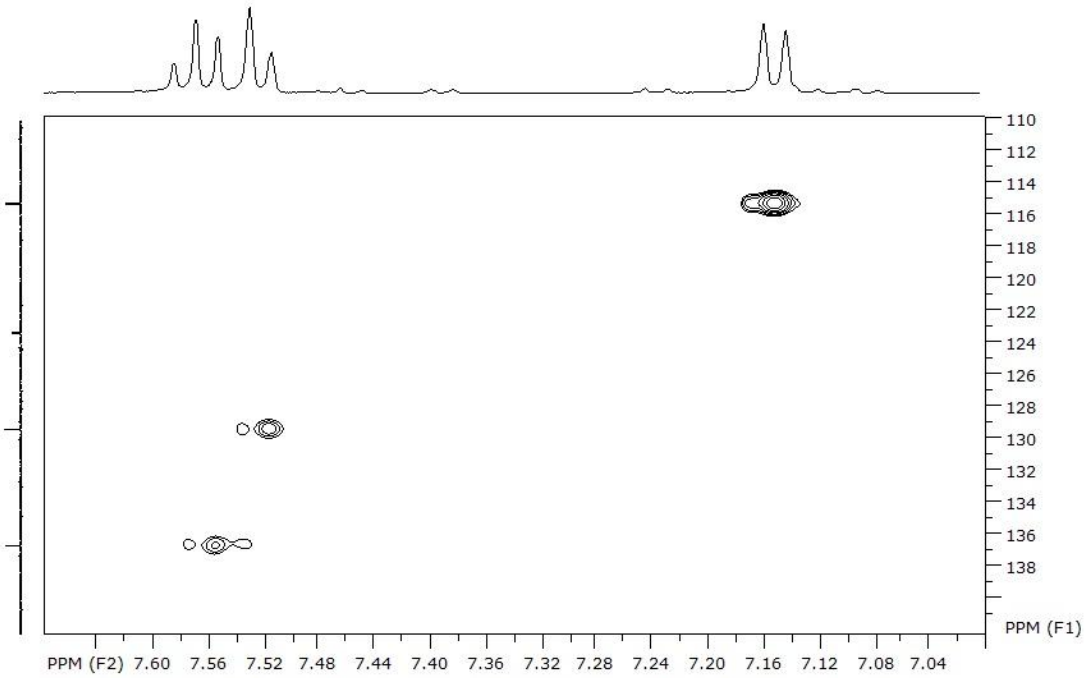


Figure 9: HSQC spectrum of 6-bromoisatoic anhydride

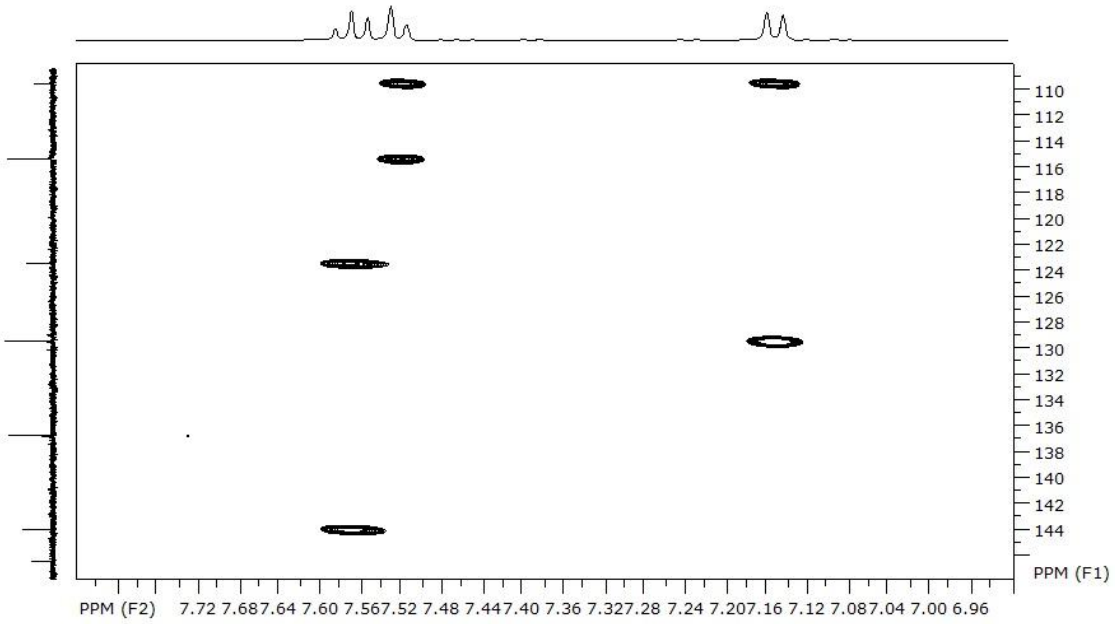


Figure 10: HMBC spectrum of 6-bromoisatoic anhydride

2.4 Crystal Structures of 3-bromoisatoic anhydride (4a) and 6-bromoisatoic anhydride (4i)

The anhydrides **4a** and **4i** were crystallized from ethyl acetate and their structures were confirmed by X-diffraction anaylsis.

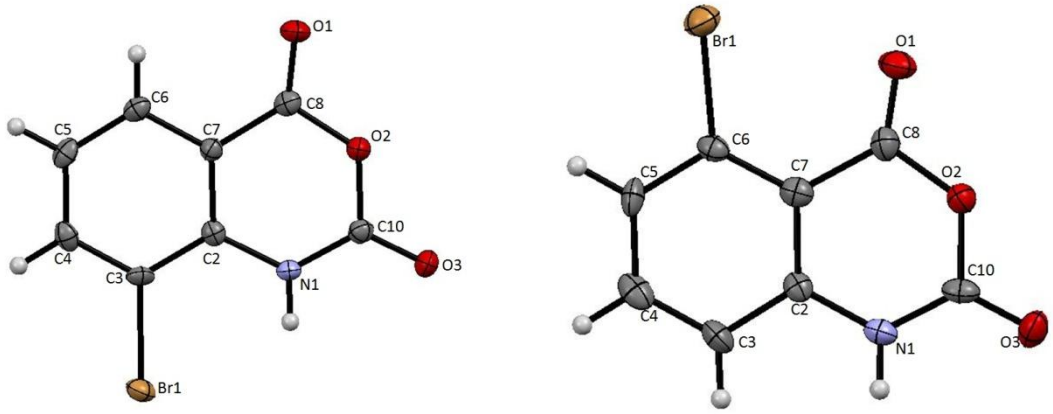


Figure 11: ORTEP plot of the anhydrides 4a and 4i.

The anhydride **4a** crystallizes in a monoclinic crystal system (space group $P_{21/c}$, Table 5), while the crystal of **4i** was found to be orthorhombic (space group P_{212121}). The unit cells of both compounds have similar volume. There are four crystallographically independent molecules per unit cell. Both isatoic anhydrides are essentially planar. Due to the proximity of the bromine atom and the carbonyl group, the 6-bromo compound **4i** displays the largest angle between the plane of the benzene ring and the plane of the oxazine system (3.83°).

Table 4: Data and structure refinement details for the isatoic anhydrides 4a and 4i

	4a	4i
Crystal System	Monoclinic	Orthorhombic
Space group	$P_{21/c}$	P_{212121}
Unit cell dimensions		
a	11.1479(8) Å	5.0832(1) Å
b	4.3804(2) Å	6.0412(2) Å
c	16.4901(12) Å	25.1364(17) Å
alpha	90°	90°
beta	104.959(7)°	90°
gamma	90°	90°
volume	777.96(9) $Å^3$	771.90(6) $Å^3$
Z	4	4
Goodness-of-fit on F2	0.9799	1.0508
R indices [I > 2s(I)]	R_1 = 0.0296, wR2 = 0.0532	R_1 = 0.0402, wR2 = 0.0942
R indices (all data)	R_1 = 0.0425, wR2 = 0.0646	R_1 = 0.0433, wR2 = 0.1005

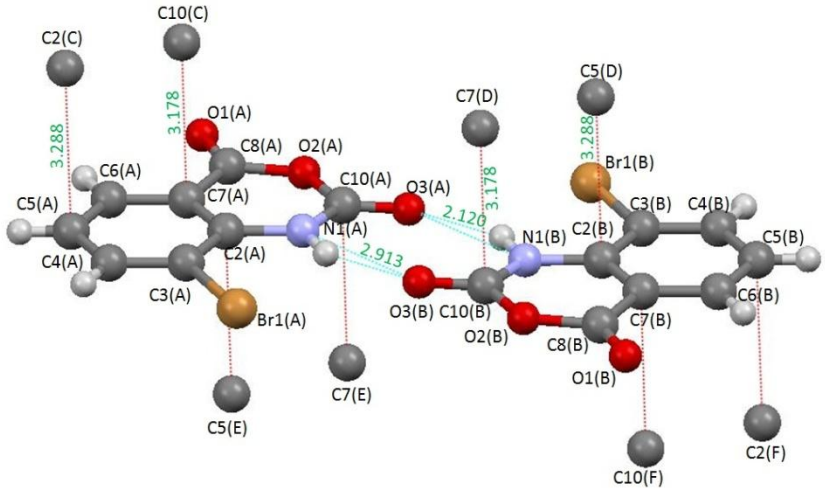


Figure 12: Intermolecular bonding in 3-bromoisatoic anhydride crystals

As can be seen in Figure 12, the packing and intermolecular bonding include edge-on contacts with hydrogen bonding between the nitrogen-bound hydrogen and the nearby carbonyl. There appear to be π-π interactions between stacked molecule aromatic rings. This is

not found in the packing of the crystal structure of 6-bromoisatoic anhydride (**4i**). Interestingly the packing of **4i** is quite dissimilar to that of the 3-bromoisatoic anhydride. The hydrogen bonding between the N-H proton and carbonyl is not edge-on and shared between only two molecules. This stacking is not in planes, as seen in 3-bromoisatoic anhydride.

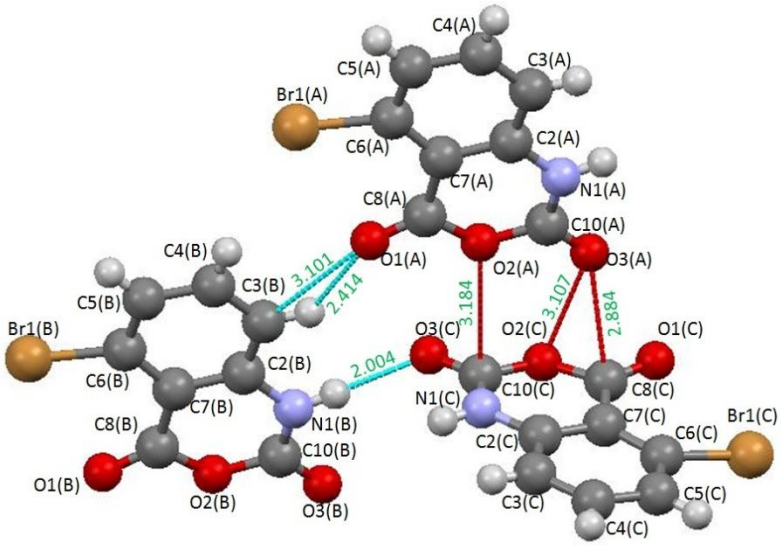
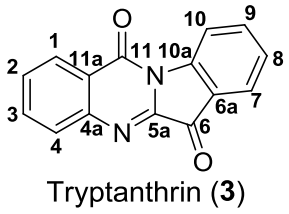


Figure 13: Intermolecular bonding in 6-bromoisatoic anhydride

3. Synthesis of Tryptanthrins

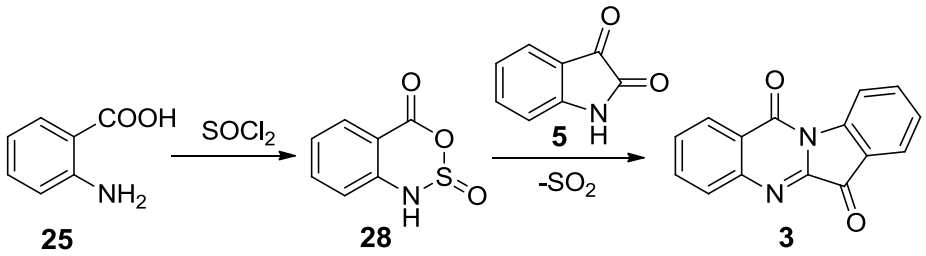
In 1892, Charles O'Neil published that he had reacted indigo with glacial acetic acid and potassium permanganate, producing a, "…new substance… of a very pale yellow color," in small crystals.[36] The structure was elucidated in 1915[37], and the x-ray structure was solved in 1971.[38] Tryptanthrin has been subsequently found in numerous natural sources. Tryptanthrin-containing dye plants include: *Isatis tinctoria*[39], *Polygonium tinctorum*[39-40], and *Wrightia tincoria.*[41] Tryptanthrin has even be found in urine from the Asian elephant (*Elephant maximus*)[42] and wing sac contents of the bat *Saccopteryx bilineata.*[43]



Tryptanthrin (**3**)

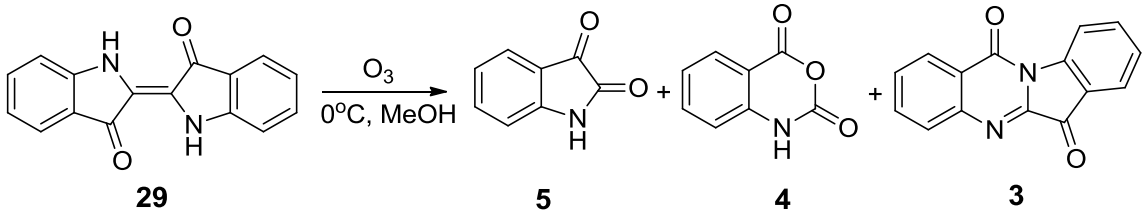
Figure 14: Tryptanthrin with carbon atoms labeled

Since its successful synthesis, tryptanthrin has been found to possess antibiotic,[44] antineoplastic,[45] and antiparasitic[14, 46] properties. Many synthetic methods have been explored, the most common of which involve the reaction of isatoic anhydride and an oxindole or isatin.[15] Another reaction scheme involves the reaction of anthranilic acid with thionyl chloride (Scheme 17).[47]



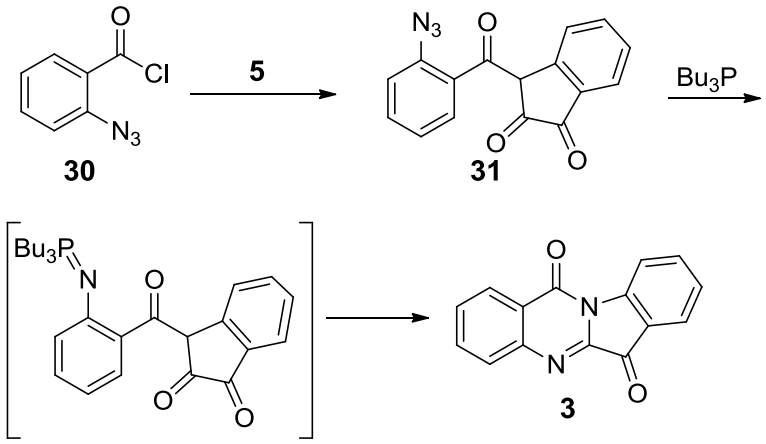
Scheme 17: Tryptanthrin synthesis by reaction of thionyl chloride and anthranilic acid, follwed by isatin. Note that the product of the first reaction is a thio derivative of isatoic anhydride.

Ozonation of indigo produces tryptanthrin, as well as isatin and isatoic anhydride(Scheme 18).[48]



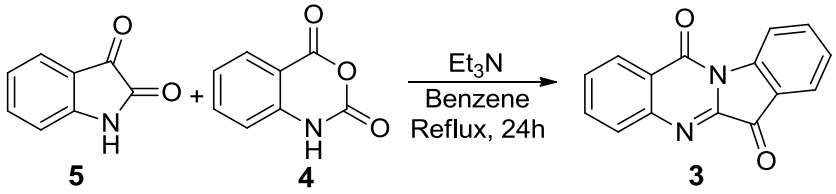
Scheme 18: Ozonolysis of indigo gives isatin and isatoic anhydride, leading to the formation of tryptanthrin

Reaction of 2-azidobenzoyl chloride (**30**) with isatin in dioxane with triethylamine and DMAP gives tryptanthrin as a product in an aza-Wittig type reaction(Scheme 19).[49]



Scheme 19: Tryptanthrin synthesis by reaction of 2-azidobenzoyl chloride with isatin, followed by tributylphosphine.

The most common and practical synthesis of tryptanthrin is the reaction of isatin (**5**) and isatoic anyhydride (**4**). Reaction conditions conducive to high yield are a solvent such as benzene and an organic base such as triethylamine (Scheme 20). Toluene may be substituted for benzene as it is safer, but the higher reflux temperature can lead to undesirable side products and decomposition.



Scheme 20: The most common tryptanthrin synthetic method, with isatin and isatoic anhydride

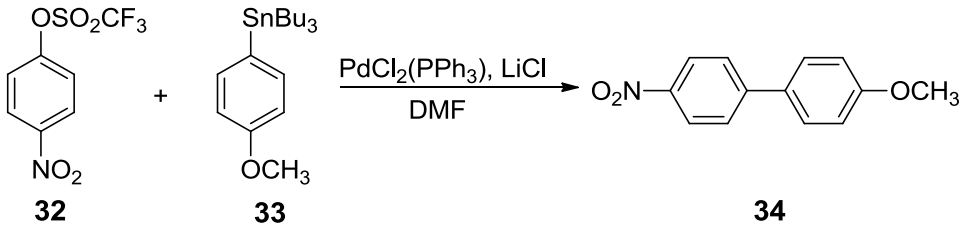
This reaction has been shown to work with a wide variety of substituted isatins and isatoic anhydrides, some of which can be found in Table 5.

Table 5: Substituted tryptanthrins synthesized

Compound	Substituent	Yield	Melting Point (°C)
3a	1-bromo	23%	>250
3b	2-bromo	42%	>250
3c	3-bromo	26%	>250
3d	4-bromo	28%	>250
3e	2,3-dibromo	56%	>250
3f	2,4-dibromo	18%	>250
3g	2,8-dibromo	36%	>250
3h	2-fluoro	47%	>250
3i	3-fluoro	38%	>250
3j	4-fluoro	36%	>250
3k	2,3-difluoro	44%	>250
3l	2,8-difluoro	36%	>250
3m	8-bromo-2-fluoro	33%	>250
3n	8-bromo-2,3-difluoro	32%	>250
3o	8-isopropyl	53%	181-183
3p	8-methoxy	34%	>250

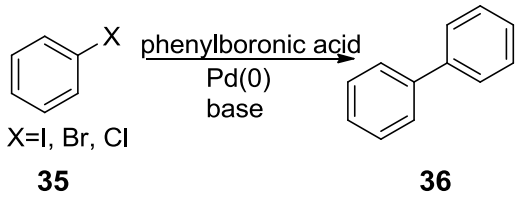
3.1 Aromatic Substitution

There are multiple methods to form aromatic rings with phenyl substituents. One such procedure, developed by Stille, requires a aryl halide (or triflate), organotin reagent (such as a tributylstannyl compound), and a palladium catalyst.[50] The net reaction is formation of a new carbon-carbon bond, as shown in Scheme 21.



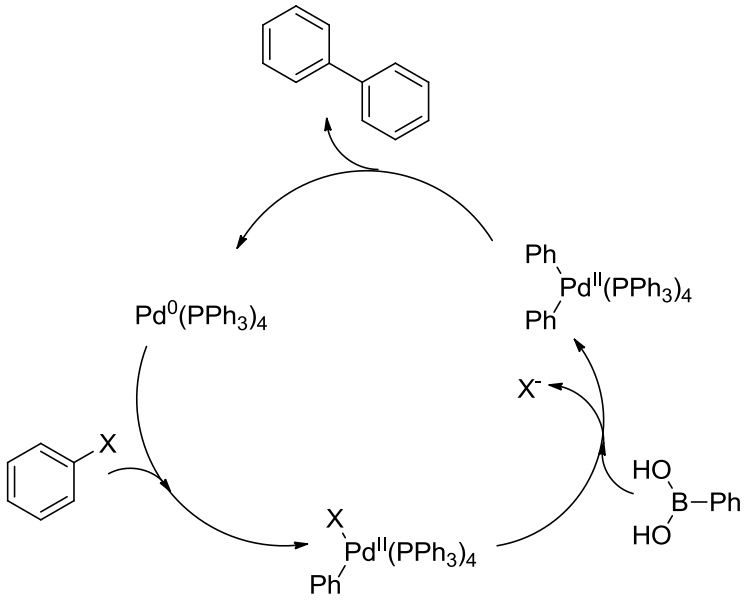
Scheme 21: Stille-coupling to form a biphenyl compound.[50]

Another common palladium-catalyzed procedure is a Suzuki-coupling reaction using a halogenated alkene or aromatic system and phenylboronic acid (Scheme 22).



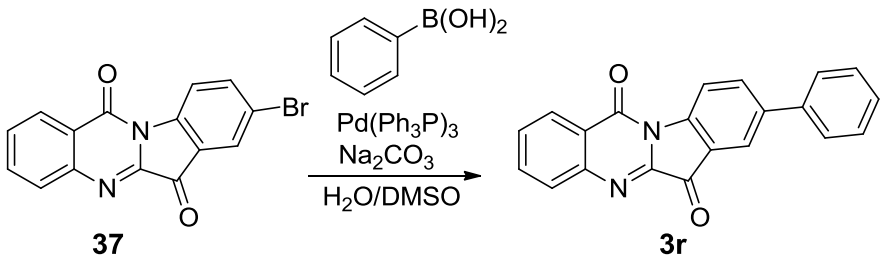
Scheme 22: Aromatic phenyl substitution via Suzuki coupling.

This reaction takes place in three steps at the palladium metal center: Oxidative addition of the ligand with loss of the leaving group (typically a halogen), transmetallation of the boronic acid, and reductive elimination of the newly coupled product (Scheme 23).



Scheme 23: Mechanism of palladium catalyzed aromatic phenyl coupling.

In this way, a bond between an sp^2 hybridized carbon and the group attached to the boronic acid can be formed. In the case of 8- and 2-bromotryptanthrin, this reaction ran to completion with 60-90% product isolated.



Scheme 24: Aromatic phenyl addition by Suzuki coupling to 8-bromotryptanthrin

NMR spectroscopic characterization of 8-phenyltryptanthrin (3r)

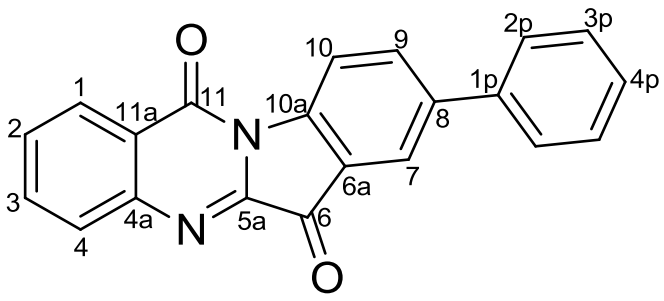


Figure 15: 8-phenyltryptanthrin, with carbons labeled for NMR identification

The ^{1}H NMR spectrum of 8-phenyltryptanthrin (**3r**) in deuterated chloroform shows ten signals: five doublets at δ 8.67, 8.45, 8.05, 8.01, and 7.62ppm (integration two); three triplets at δ 7.86, 7.69, 7.50 (integration two), and 7.43ppm; and a singlet at δ 8.13ppm. Protons **2p-H** and **3p-H** are identified by their integration of two each and their splitting pattern, allowing their assignment as signals at 7.62ppm and 7.50ppm respectively. The signal due to proton **7-H** is identifiable as the only singlet signal in the spectrum, found at 8.13ppm. Using gCOSY (Figure 16), proton **4p-H** can be assigned to the triplet signal at 7.43ppm, as it couples to the signal belonging to proton **3p-H**.

Table 6: 8-phenyltryptanthrin NMR identified carbon and proton signals

Carbon letter	Carbon peak (ppm)	Proton peak (ppm)
1	127.7	8.45
2	130.5	7.69
3	135.3	7.86
4	131.0	8.05
4a	146.8	
5a	144.7	
6	182.8	
6a	122.7	
7	123.8	8.13
8	140.8	
9	137.0	8.01
10	118.4	8.67
10a	145.4	
11	158.1	
11a	123.9	
1p	138.8	
2p	127.0	7.63
3p	129.3	7.5
4p	128.6	7.43

The ^{13}C NMR reveals 19 signals, with chemical shifts ranging from 182.8 to 118.4ppm. Assignment of these carbons and attached protons requires analysis of HSQC (Figure 17) and HMBC (Figure 18) spectra. Carbons associated with the phenyl protons were identified by HSQC as 127.0ppm, 129.3ppm and 128.6ppm for carbons **C-2p**, **C-3p**, and **C-4p** respectively. The carbon signal 138.8ppm can be assigned to carbon **C-1p** using HMBC coupling to the signal **3p-H**. Also, HMBC allows assignment of the two carbonyls, **C-6** and **C-11**. Coupling to the singlet proton peak **7-H** shows that the carbon **C-6** signal is 182.8ppm. The carbonyl **C-11** should couple to doublet **1-H** in HMBC, and this fits for the signal at 158.1ppm coupled to the doublet signal at 8.45ppm. Having assigned the proton **1-H**, assignments of the protons **2-H**, **3-H** and **4-H** can be

easily accomplished using the gCOSY spectrum. Proton **1-H** couples to the triplet 7.69ppm, which is therefore proton **2-H**. Proton **2-H** couples both to proton **1-H** and the signal at 7.86ppm, which is then proton **3-H**. Finally proton **3-H** couples to proton **2-H** and the doublet signal at 8.05ppm, allowing this signal to be assigned to proton **4-H**. The ^{3}J coupling constants are consistent with this assessment. The carbon atoms associated with protons **2-H**, **3-H** and **4-H** are assigned using HSQC to be 130.5ppm, 135.3ppm, and 131.0ppm respectively.

The final two unassigned protons are the doublets at 8.01ppm and 8.67ppm, with ^{3}J coupling constants equal to 8.4Hz. These are assigned, using HMBC, by coupling to carbon **C-1p**, which couples to protons **3p-H**, **7-H** and the proton doublet signal at 8.01ppm, corresponding to proton **9-H**. The signal for proton **10-H** is then found at 8.67ppm. Assignment of the attached carbons **C-9** and **C-10** are then made by HSQC and are found to be the signals 137.0ppm and 118.4ppm respectively. The remaining carbons are tertiary and will be assigned by their coupling to the known protons, except carbon **C-5a** which will not couple to any proton. Carbon **C-4a** will couple over three bonds to protons **1-H** and **3-H**, and was found to result in signal 146.8ppm. The carbon **C-11a** will theoretically couple to both protons **2-H** and **4-H**, and this corresponds to signal 123.9ppm. Carbon **C-10a** couples to protons **9-H** and **7-H**, corresponding to the carbon signal 145.4ppm. The carbon **C-8** should couple with protons **10-H** and **2p-H**, and is found to have the signal 140.8ppm. The final signal assignable by HMBC is the signal for **C-6a**, which only couples to proton **10-H**, and was therefore found to be the signal 122.7ppm. The signal for **C-5a** is then the remaining, uncoupled ^{13}C signal, which is 144.7ppm.

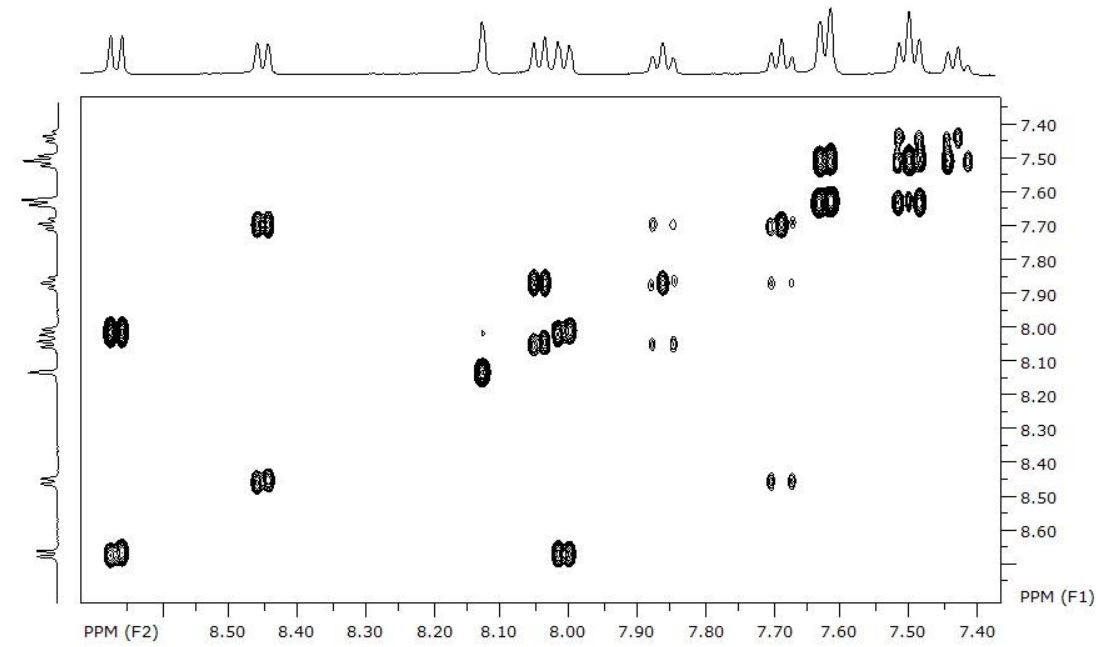


Figure 16: gCOSY spectrum of 8-phenyltryptanthrin in chloroform-d

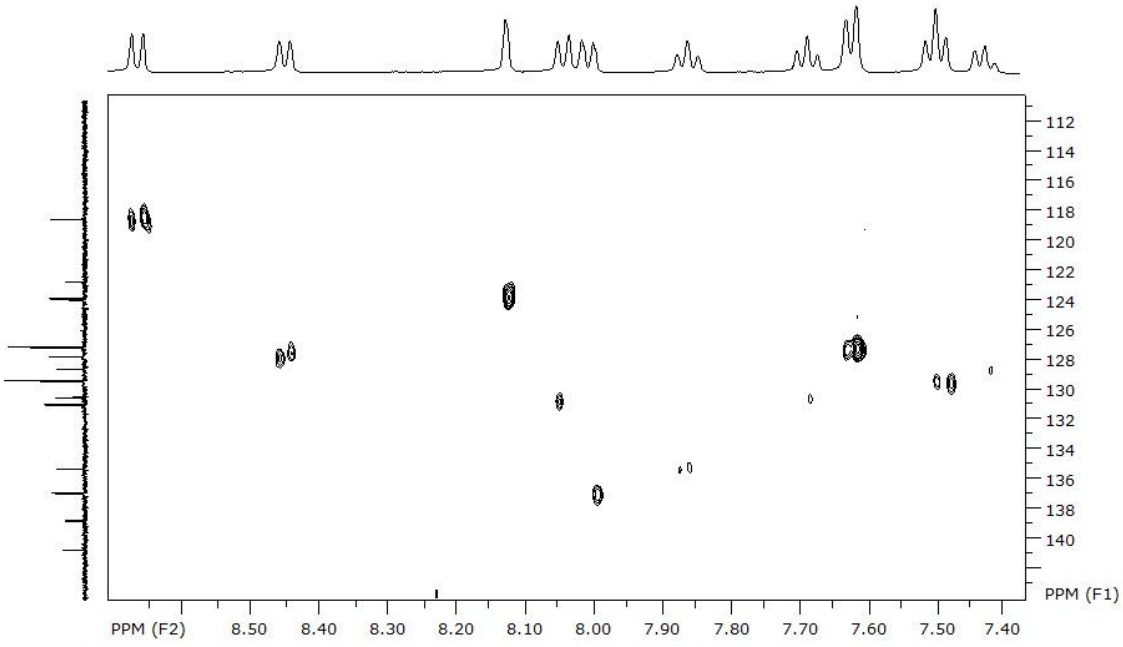


Figure 17: HSQC NMR spectra of 8-phenyltryptanthrin in chloroform-d.

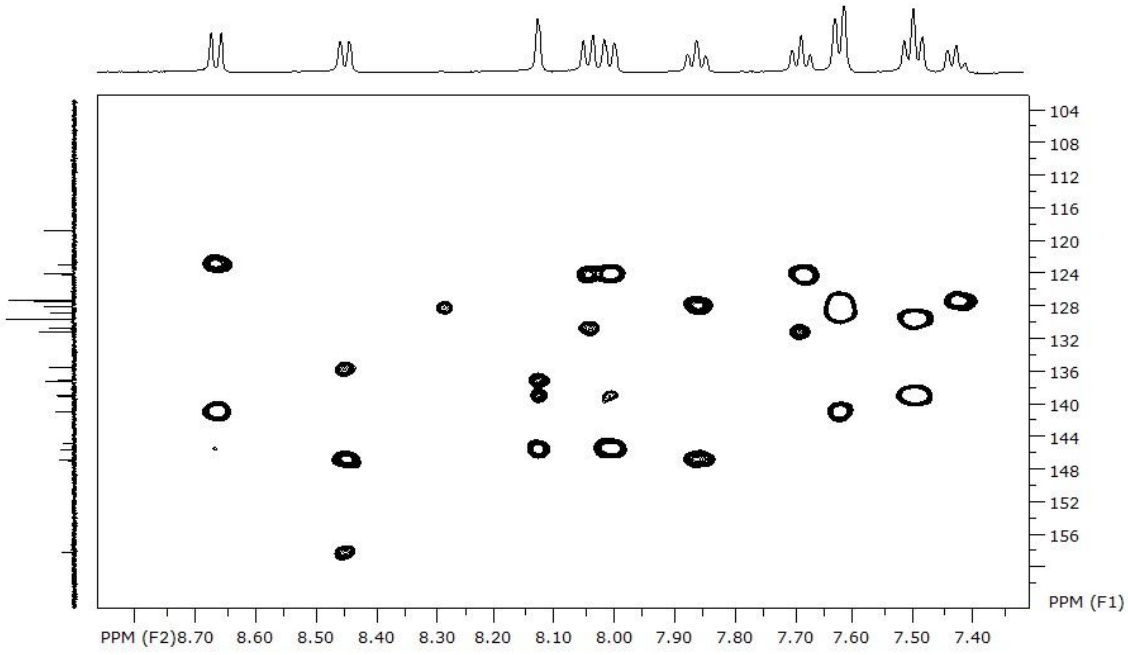
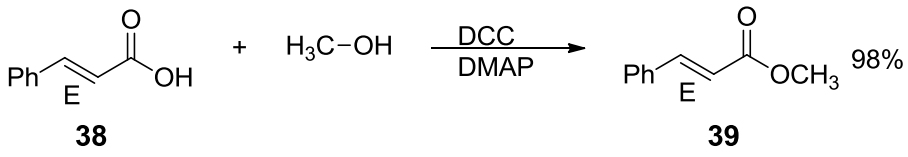


Figure 18: HMBC NMR spectrum of 8-phenyltryptanthrin in chloroform-d

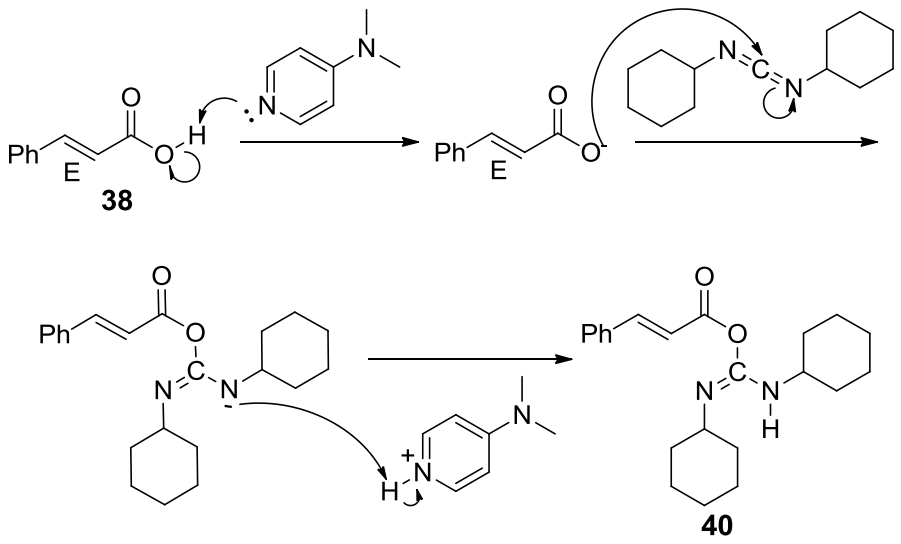
3.2 Esterification Reactions

Formation of esters can be accomplished from multiple starting materials. The classic method of esterification is the Fischer method.[51] In this reaction, a carboxylic acid and an alcohol are coupled in a dehydration reaction with a catalyst such as sulfuric acid. This method was improved upon by use of coupling agents such as dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP), as in the Steglich method (Scheme 25).[52]



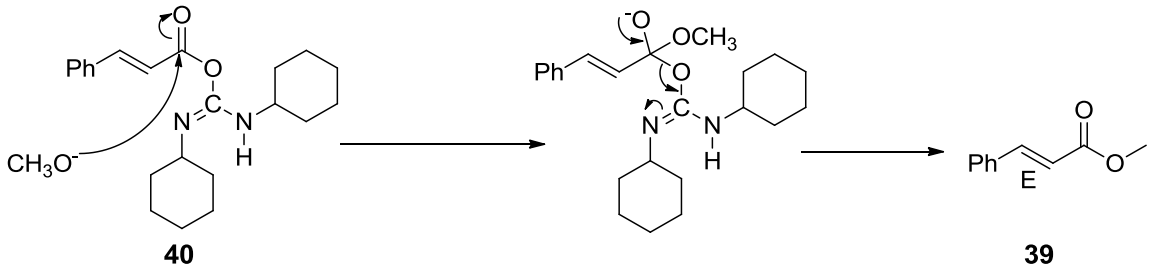
Scheme 25: Steglich esterification

The use of DCC and DMAP have greatly improved yields, as the o-acyl isourea formed by the reaction of the acid with DCC is more reactive than the free acid toward esterification (Scheme 26).



Scheme 26: Mechanism of Steglich esterification, Part 1.

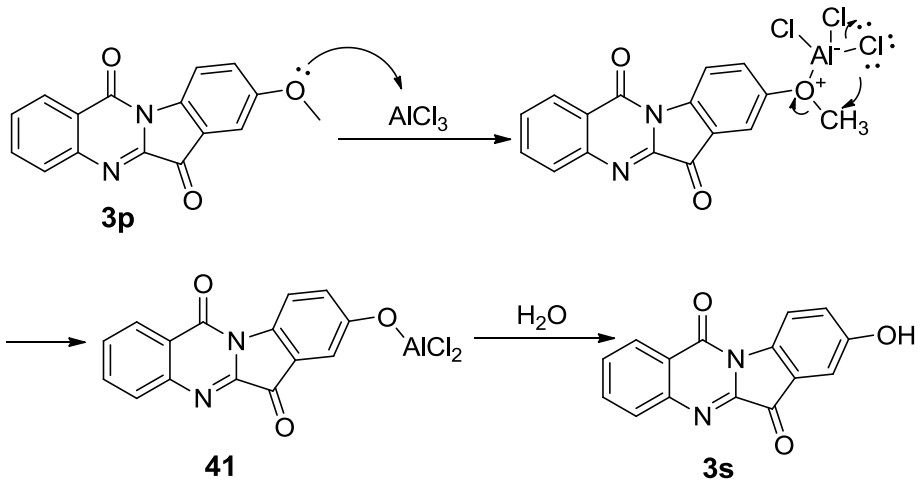
The alcohol is then able to attack the ester, producing the dicycohexylurea leaving group in a transesterification reaction (Scheme 27).



Scheme 27: Mechanism of Steglich esterification, Part 2.

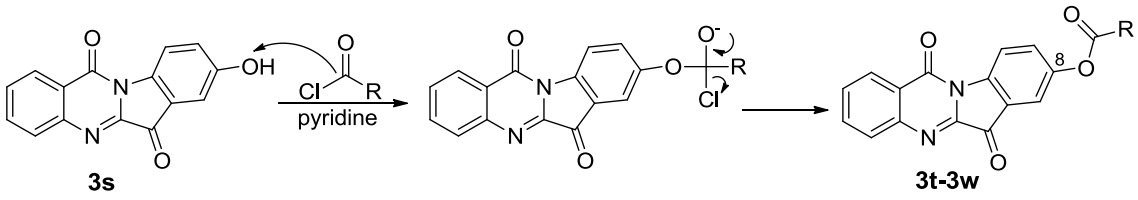
Another straightforward method of esterification involves reaction of an alcohol with an acid chloride. This method is simple as it is irreversible and can be worked up by addition of water and filtration. The hydroxytryptanthrin must first be synthesized, and this is done by

demethylation of 8-methoxytryptanthrin (**3p**). A method of o-demethylation for tryptanthrin was first carried out by Lee et al.[53] This method employed freshly distilled dichloromethane solvent (in large excess due to low solubility methoxytryptanthrin) and the Lewis acid aluminum chloride ($AlCl_3$). Aluminum chloride is also used in great excess.



Scheme 28: Demethylation of 8-methoxytryptanthrin using $AlCl_3$.

Esterification is then carried out on the purified alcohol using a solvent, base and the corresponding acid chloride (Scheme 29).



Scheme 29: Esterification of 8-hydroxytryptanthrin with acid chlorides.

Table 7: 8-esters of tryptanthrin

Compound	Substituent	Molar Mass	Yield
3t	R=CH_3	306	79%
3u	R=$CH_2CH_2CH_3$	334	23%
3v	R=$(CH_2)_4CH_3$	362	33%
3w	R=C_6H_5	368	59%

This reaction was first attempted with DMF as a solvent with triethylamine as a base, but the difficulty involved in removing DMF led to a change in solvent. Pyridine was much easier to remove by washing with water and was basic enough for the reaction to proceed without additional base.

4. Plant Material Extraction and Fractionation for Activity against *T. gondii*

Development of new antiparasitic drugs can be difficult without a starting point. One approach is to seek medicinal plants traditionally used as herbal medicine. An extraction of dried plant material in methanol can give a large number of compounds to be tested at once. If a particular extraction shows potency and low toxicity, then the plant material can be fractionated and the potent compound(s) isolated and synthesized in the lab.

Plants for this study were chosen because they have been used to treat malaria (caused by *Plasmodium falciparum*) or other related ailments. Chaparral (*Larrea tridentata*) has been shown to have antiprotozoal activity.[54] Sweet wormwood (*Artemisia annua*) contains the compound artemisinin, an antimalarial and antineoplastic agent.[55] *Bupleurum falcatum* has been used for thousands of years to treat fever, influenza, and malaria.[56] Da Qing Ye (*Isatis indigotica* leaf) is known to contain the compound tryptanthrin, and has been used in traditional Chinese medicine for many infections.[57] The remaining plants were chosen in a similar manner.

Once an active compound has been isolated and synthesized in high purity, modification of the chemical follows. These modifications can alter solubility, protein binding, biostability and many other factors in drug metabolism and action. As these new synthetic compounds are synthesized and tested, the modifications which improve efficacy are preserved in an evolutionary fashion, leading to a range of drugs being tested *in vivo*.

Table 8: Plant materials extracted and relative potencies against *T. gondii*

Common Name	Species	ID_{50} (µg/mL)	TD_{50} (µg/mL)	TI
Quassia	Picrasma exelsa	11	12	1.2
Dong Quai	Angelica sinensis	9.6	4.8	0.50
Agrimony	Agrimonia eupatoria	>320	>320	ND
Pau D'Arco	Tabebuia impetigirosa	4.4	15	3.4
Uva Ursi	Arctostaphylos uva-ursi	200	>320	>1.6
Wild Indigo	Baptista tinctoria	86	40	0.46
Sheep Sorrel	Rumex acetosella	11	66	6.0
Oregon Grape	Mahonia aquifolia	3.0	0.30	0.10
Sweet Wormwood	Artemisia annua	>320	>320	ND
Chaparral	Larrea tridentata	94	>320	>3.4
Da Qing Ye	Isatis indigotica	>320	>320	ND

The results in Table 8 include ID_{50} and TD_{50} values, which indicate the concentrations that inhibit *T. gondii* and human cells, respectively, as described in greater detail in the Biological Testing Chapter. The therapeutic index (TI) is the ratio of the ID_{50} to the TD_{50}, and larger values of TI indicate higher efficacy.

From preliminary biological testing, it is likely that there is a compound in *Larrea tridentata* which is active against *T. gondii* and it appears that the extract was non-toxic. This fits with the previous findings of antiprotozoal activity against the related organisms, *Plasmodium falciparum* (causative agent of malaria), and *Trypanosoma brucei rhodesiense*.[54] The activity of this plant extract has been attributed to meso-nordihydroguaiaretic acid. The methanolic extract of Sheep Sorrell was found to be even more active, however more toxic as well.

A fractionation was carried out on dried plant material from the chinese thoroughwax plant (*Bupleurum falcatum*). The progression of solvents used and the ID_{50} values for each extract are listed below.

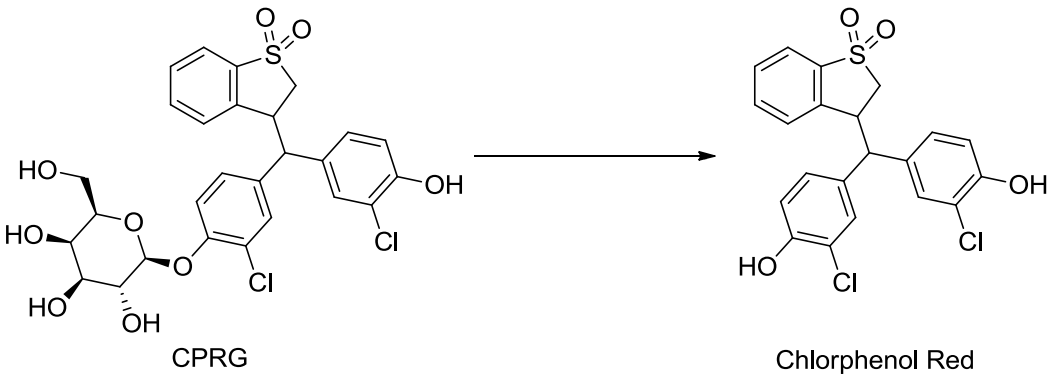
Table 9: Fractionation of *Bupleurum falcatum* and ID_{50} values of extracts

Extract	ID_{50} µg/mL
hexane	12
benzene	21
dichloromethane	1.76
tetrahydrofuran	4
acetone	65
acetonitrile	106
ethanol	98
methanol	220

The dichloromethane and tetrahydrofuran extracts of the plant were found to be quite active relative to other plant extracts, and further investigation into the toxicity of these extracts is warranted.

5. Biological Testing of Anti-*Toxoplasma* Agents

In many studies, the first step in development of new antimicrobial drug is design of an *in vitro* system that allows quantitative testing of new compounds against the microbe. In this case, the colorimetric assay was first developed by McFadden et al. and replaced the [5,6-^{3}H]uracil assay, relieving the need for radioactive tags in anti-*Toxoplasma* assays.[58] This new assay used a strain of *T. gondii* capable of digesting the chlorphenol red-β-D-galactopyranoside (CPRG) molecule to determine survival of *T. gondii* tachyzoites.



Scheme 30: Digestion of CPRG by β-galactosidase expressing *T. gondii*. CPRG is red or purple, while chlorphenol red is yellow at media pH.

The digestion of CPRG by *T. gondii* (strain RHβ1) changes the solution color from yellow to red, which can be read on a microplate reader as a change in absorbance at 570nm. From this simple test, the viability of *T. gondii* tachyzoites after treatment with a drug at varying concentrations can be displayed, giving an inhibitory dose (ID_{50}) value, representing the dose at which 50% of the *T. gondii* has been rendered inviable. A similar procedure has been developed to determine the toxic dose (TD_{50}) value for a given drug, that which kills 50% of host cells using a commercially available cell viability reagent which can also be measured colorimetrically.

In practice, this involves a few basic steps. First, a human cell line is used as a host. Human foreskin fibroblast cells (HFFs) form a monolayer when grown to confluence in 96-well, flat bottomed, plates. The cells are then washed to remove waste products, while the cells remain attached to the bottom of the wells. Fresh growth medium is then added to the plates, and drug is added to the wells and serially diluted across the plate. Finally, RHβ1 *T. gondii* tachyzoites are added to each well of the plate and the plates are incubated for 72 hours. In addition to these, a set of wells is reserved to test the same drug concentrations with only HFF cells present for cytotoxicity measurement.

After incubation, CPRG is added to the *T. gondii*-containing wells and the plates are incubated for 24 additional hours prior to colorimetric determination of *T. gondii* viability. To the HFF-only wells, a commercially available cell viability reagent is added and these are incubated for 3 hours prior to scanning.

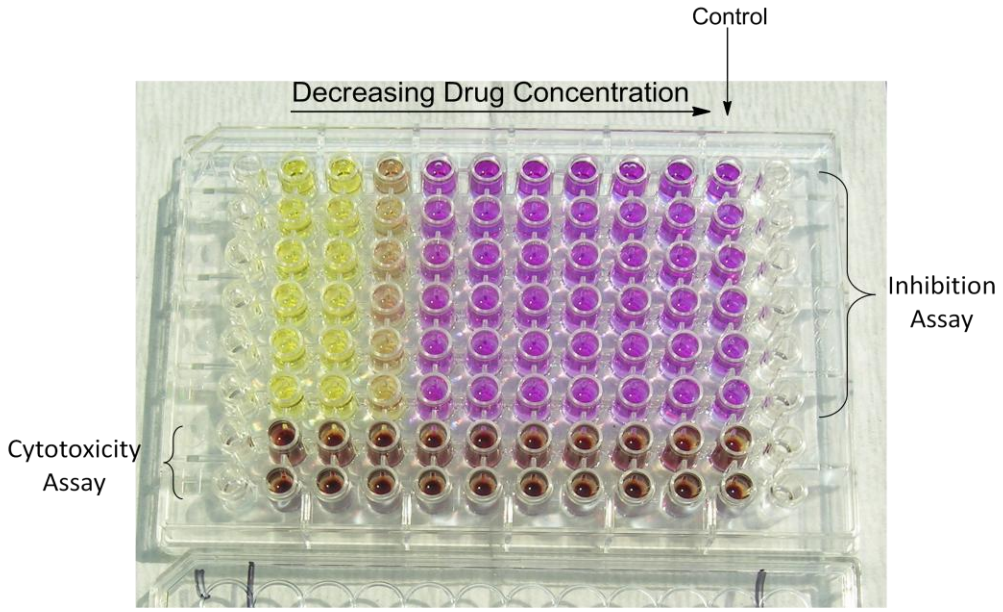


Figure 19: *T. gondii* inhibition assay and cytotoxicity assay.

The absorbance for the inhibition assay wells is measured from 570-650nm, while absorbance for the cytotoxicity wells was measured from 490-650nm. A graph of absorbance versus –log (concentration) was then made for each compound, giving a curve for determination of ID_{50} and TD_{50} (Figure 20).

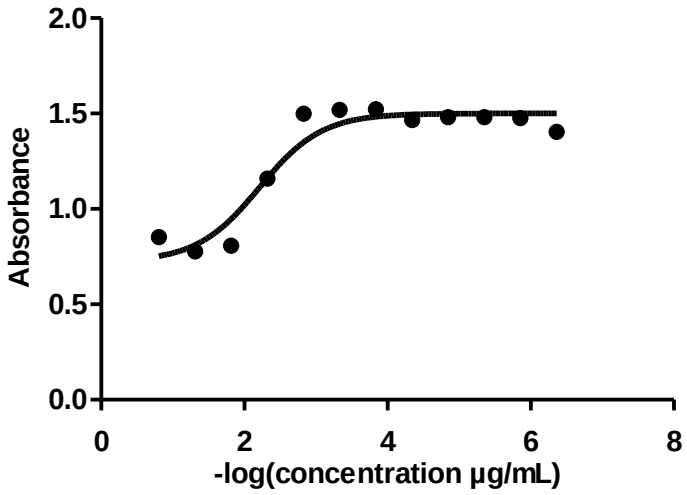


Figure 20: Inhibition curve for *T. gondii*, the ID_{50} value occurs at the inflection point of the graph.

Table 10: Relative activity (ID_{50}) of certain tryptanthrin derivatives

Compound	Name	ID_{50} (µg/ml)
3a	1-bromotryptanthrin	0.13
3b	2-bromotryptanthrin	0.051
3c	3-bromotryptanthrin	0.073
3d	4-bromotryptanthrin	0.73
3e	2,3-dibromotryptanthrin	0.12
3f	2,4-dibromotryptanthrin	0.016
3g	2,8-dibromotryptanthrin	0.015
3h	2-fluorotryptanthrin	0.063
3i	3-fluorotryptanthrin	0.17
3j	4-fluorotryptanthrin	0.19
3k	2,3-difluorotryptanthrin	0.1
3m	8-bromo-2-fluorotryptanthrin	0.08
3n	8-bromo-2,3-difluorotryptanthrin	0.051
3s	8-hydroxytryptanthrin	0.14
3t	Tryptanthrin-8-ethanoate	0.032
	Trimethoprim	0.082

In agreement with previous studies, those compounds containing the 8-bromo moiety are active against *T. gondii* in vitro. Further studies will need to be conducted to determine the therapeutic index of each substance (by determination of the TD_{50} in each case), but these compounds already show high activity relative to the trimethoprim control. Additionally, tryptanthrins generally have higher TD_{50} values than trimethoprim, which would suggest high TIs for these compounds. To determine activity of these compounds more accurately, these studies must be repeated multiple times. This did not occur due to limitations in our ability to procure additional RHβ1 *T. gondii*.

Due to the success of tryptanthrins as anti-*Toxoplasma* agents in vitro, further studies are warranted, particularly those with modifications at the 8-position.

6. Experimental

General Methods

^{1}H NMR (500MHz) and ^{13}C (125MHz) spectra were obtained using a Varian-500 spectrometer and TMS as an internal standard. All NMR shifts are given in ppm, and coupling constants are given in Hz. GC-MS spectra were recorded on an HP 5890 GC-MS spectrometer. Melting points were determined using a Thermo Scientific Mel-Temp melting point apparatus. All reagents were ACS grade and used without purification unless prepared in a previous step. All solvents were used without purification or drying unless otherwise noted.

General Procedure 1: Preparation of substituted isatins from aniline derivatives:

The aniline derivative (74mmol) was dissolved dropwise in 500mL 0.1M HCl solution at 50^{o}C. Then hydroxylamine HCl (259mmol, 18.5g) followed by sodium sulfate (600mmol, 84g) were added. Chloral hydrate (88.8mmol, 14.8g) was dissolved in the solution, which was then stirred overnight at 50^{o}C. The suspension was filtered and the filtrate was discarded. The solid was washed with water allowed to air dry for at least 24 hours.

Once dry, the resulting 2-(hydroxyimino)-*N*-phenyl-acetamide derivative was then slowly added to concentrated sulfuric acid (20mL) at 70^{o}C, taking care to maintain temperature between 70^{o}C and 80^{o}C. After all of the solid was added, the temperature was raised to 80^{o}C and the reaction was stirred. The reaction was monitored by NMR and when complete, the solution was poured over ice, and the precipitate was filtered and washed with water until neutral. Reported yields are over both steps.

5a. 5-fluoroisatin. Prepared from 4-fluoroanline using General Procedure 1. Yield: 11%. Yellow solid. Mp.: 225-227^{o}C. ^{1}H NMR (DMSO-d_6): δ 11.04 (br., 1H), 7.42 (t, J 8.7, 1H), 7.35 (d, J 7.0, 1H), 6.90 (dd, J 3.9, 8.5, 1H). ^{13}C NMR (DMSO-d_6): δ 184.0 (J_{CF} 2), 159.6 (J_{CF} 2), 158.1 (J_{CF} 241), 147.0 (J_{CF} 2), 124.6 (J_{CF} 242), 118.5 (J_{CF} 72), 113.6 (J_{CF} 7), 111.4 (J_{CF} 24).

5b. 5-methoxyisatin. Prepared from p-anisidine using General Procedure 1. Yield: 25% yield. Brown-purple solid. Mp.: 169-172^{o}C. ^{1}H NMR (DMSO-d_6): δ 10.81 (br., 1H), 7.13 (dd, J 2.8, 8.5, 1H), 7.00 (d, J 2.7, 1H), 6.80 (d, J 8.5, 1H), 3.71 (s, 3H). ^{13}C NMR (DMSO-d_6): δ 184.7, 159.6, 155.3, 144.7, 124.8, 118.1, 113.2, 108.8, 55.7. Mass (rel. intensity): 177 (100), 176 (50.8).

5c. 5-isopropylisatin. Prepared from 4-isopropylaniline using General Procedure 1. Yield: 53%. Orange solid. Mp.: 128-130^{o}C. ^{1}H NMR ($CDCl_3$): δ 8.94 (br., 1H), 7.47 (s, 1H), 7.42 (d, J 8.1, 1H), 6.90 (d, 8.0, 1H), 2.88 (sep, J 6.8, 1H), 1.23 (dd, J 0.9, 6.9, 1H). ^{13}C NMR ($CDCl_3$): δ 183.8, 160.2, 147.6, 145.2, 137.3, 123.6, 118.2, 112.6, 33.7, 23.9. Mass (rel. intensity):189 (100).

5d. 6-fluoroisatin. Prepared from 3-fluoroaniline using General Procedure 1. Yield: 55%. Yellow solid. Mp.: 194-196^{o}C. ^{1}H NMR (DMSO-d_6): δ 11.05 (br., 1H), 7.61 (dd, J 5.7, 8.3, 1H). 6.87 (dt, J 2.3, 10.1, 1H), 5.73 (dd, J 2.3, 9.3, 1H). ^{13}C NMR (DMSO-d_6): δ 182.8, 168.2 (J_{CF} 255.7), 159.8, 153. 4 (J_{CF} 14.3), 127.9 (J_{CF} 11.8), 114.8, 109.9 (J_{CF} 23.4), 100.5 (J_{CF} 27.1).

5e. 7-bromoisatin. Prepared from 2-bromoaniline using General Procedure 1. Yield: 30%. Orange solid. Mp.: 193-195^{o}C. ^{1}H NMR (DMSO-d_6): δ 11.29 (br., 1H), 7.71 (dd, J 1.1, 8.1, 1H), 7.46 (dt, J 0.9, 7.3, 1H), 6.98 (dd, J 0.7, 8.1, 1H). ^{13}C NMR (DMSO-d_6): δ 183.8, 159.7, 149.5, 140.5, 124.3, 123.7, 120.1, 104.8. Mass (rel. intensity):199 (100), 225 (23.5), 227 (32.8).

5f. 7-fluoroisatin. Prepared from 2-fluoroanline using General Procedure 1. Yield: 27%. Yellow solid. Mp.: 191-192^{o}C. ^{1}H NMR (DMSO-d_6): δ 11.55 (br., 1H), 7.49 (qd, J 0.5, 8.3, 1H), 7.33 (d, J 7.4, 1H), 7.05 (qd, J 0.5, 7.6, 1H). ^{13}C NMR (DMSO-d_6): δ 183.4 (J_{CF} 4), 159.0, 147.4 (J_{CF} 245), 137.6 (J_{CF} 13), 125.0 (J_{CF} 17), 123.7 (J_{CF} 6), 120.8 (J_{CF} 3), 120.6 (J_{CF} 4). Mass (rel. intensity):165.

General Procedure 2: Bromination of isatins.

N-bromosuccinamide (4.5mmol, 0.8g) was added, under argon, to a solution of isatin (4mmol) in DMF (dry, 10mL) and the reaction was stirred at room temperature for 3 days. After that time, water (10mL) was added and the solid was collected by filtration. The solid was washed with water to remove DMF.

5g. 5,6-dibromoisatin.Prepared from 6-bromisatin isatin using General Procedure 2. Yield: 49%. Orange solid. Mp.: >250^{o}C. ^{1}H NMR (DMSO-d_6): δ 11.22 (br., 1H), 7.83 (d, J 0.6, 1H), 7.25 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 182.3, 159.0, 149.9, 133.2, 128.7, 118.9, 117.0, 116.8.

5h. 5,7-dibromoisatin.Prepared from 7-bromoisatin isatin using General Procedure 2. Yield: 65%. Orange solid. Mp.:239-242^{o}C. ^{1}H NMR (DMSO-d_6): δ 11.44 (br., 1H), 8.02 (d, J 1.88, 1H), 7.66 (dd, J 0.6, 1.9, 1H). ^{13}C NMR (DMSO-d_6): δ 182.5, 159.4, 148.6, 141.2, 126.0, 121.3, 114.7, 105.8. Mass (rel. intensity): 277 (100), 303 (16.7), 305 (32.8), 307 (17.8).

General Procedure 3: Oxidation of isatins to isatoic anhydrides.

To a solution of formic acid (4mL) and sulfuric acid (4 drops) was added substituted isatin (2mmol) and urea-hydrogen peroxide complex (2.5mmol, 235mg). The resulting solution was sonicated for 30 minutes at room temperature and stirred overnight at room temperature. The

resulting solid was filtered and washed with 1mL ethyl acetate and three times with 5mL hexane. The solid was then air dried.

4a. 3-bromoisatoic anhydride. Prepared from 7-bromoisatin using General Procedure 3. Yield: 30%. Pale yellow solid. Mp.: 213-215^{o}C. ^{1}H NMR (DMSO-d_6): δ 11.07 (br., 1H), 8.01 (d, J 7.9, 1H), 7.94 (d, J 7.9, 8.0, 1H), 7.19 (t, J 7.9, 1H). ^{13}C NMR (DMSO-d_6): δ 159.6, 147.0, 140.6, 140.0, 129.1, 125.0, 113.4, 108.4.

4b. 3,5-dibromoisatoic anhydride. Prepared from 5,7-dibromoisatin using General Procedure 3. Yield: 27%. Pale yellow solid. Mp.: >250^{o}C. ^{1}H NMR (DMSO-d_6): δ 11.26 (br., 1H), 8.25 (d, J 2.2, 1H), 8.00 (d, J 2.2, 1H). ^{13}C NMR (DMSO-d_6): δ 158.2, 146.3, 141.5, 1.39.1, 130.4, 114.8, 114.4, 109.4. Mass (rel. intensity): 277 (100), 319 (11.0), 321 (20.9), 323 (10.1).

4c. 3-fluoroisatoic anhydride. Prepared from 7-fluoroisatin using General Procedure 3. Yield: 33%. Pale yellow solid. Mp.: 214-217^{o}C. ^{1}H NMR (DMSO-d_6): δ 11.86 (br., 1H), 7.76 (dt, J 1.0, 8.0, 1H), 7.67 (qd, J 1.2, 8.13, 1H), 7.24 (dt, J 3.3, 4.6, 1H).

4d. 4-fluoroisatoic anhydride. Prepared from 6-fluoroisatin using General Procedure 3. Yield: 30%. Pale yellow solid. Mp.: >250^{o}C. ^{1}H NMR (DMSO-d_6): δ 11.87 (br., 1H), 8.00 (ddd, J 0.8, 6.0, 8.8, 1H), 7.11 (tdd, J 1.1, 2.5, 8.8, 1H), 6.88 (ddd, J 0.8, 2.5, 9.6, 1H). ^{13}C NMR (DMSO-d_6): δ 166.8 (J_{CF} 254), 158.9, 147.0, 143.7 (J_{CF} 13), 132.4 (J_{CF} 12), 111.5 (J_{CF} 24), 107.4 (J_{CF} 2), 101.8 (J_{CF} 26).

4e. 4,5-dibromoisatoic anhydride. Prepared from 5,6-difluoroisatin using General Procedure 3. Yield: 83%. Pale yellow solid. Mp: >250^{o}C. ^{1}H NMR (DMSO-d_6): δ 11.90 (br., 1H), 8.13 (s, 1H), 7.44 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 158.3, 146.6, 141.1, 132.7, 132.5, 120.0, 117.3, 111.8.

4f. 4,5-difluoroisatoic anhydride. Prepared from 5,6-difluoroisatin using General Procedure 3. Yield: 55%. Pale yellow solid. Mp.: 229-232^{o}C. ^{1}H NMR (DMSO-d_6): δ 11.91 (br., 1H), 7.99 (t, J 9.0, 1H), 7.11 (dd, J 3.9, 10.6, 1H).

4g. 5-fluoroisatoic anhydride. Prepared from 5-fluoroisatin using General Procedure 3. Yield: 63%. Pale yellow solid. Mp.: 239-243^{o}C. ^{1}H NMR (DMSO-d_6): δ 11.19 (br., 1H), 7.36 (dd, J 2.6, 8.4, 1H), 6.87 (dt, J 2.2, 8.4, 1H), 6.74 (dd, J 2.2, 9.3, 1H). ^{13}C NMR (DMSO-d_6): δ 158.9 (J_{CF} 92), 156.6, 146.8, 138.1 (J_{CF} 2), 124.8 (J_{CF} 24), 117.6 (J_{CF} 8), 114.0 (J_{CF} 24), 111.5 (J_{CF} 8).

4h. 5-isopropylisatoic anhydride. Prepared from 5-isopropylisatin. Yield: 42%, Pale yellow solid. Mp.: 230-234^{o}C. ^{1}H NMR (DMSO-d_6): δ 9.53 (br., 1H), 7.86 (d, J 8.3, 1H), 6.87 (d, J 1.7, 1H), 6.70 (dd, J 1.6, 8.3, 1H). ^{13}C NMR (DMSO-d_6): δ 162.0, 155.3, 147.4, 146.1, 122.7, 120.5, 117.1, 113.0, 33.2, 23.9. Mass (rel. intensity): 162 (100), 205 (27.2).

4i. 6-bromoisatoic anhydride. Prepared from 4-bromoisatin using General Procedure 3. Yield: 35%. Pale yellow solid. Mp.: >250^{o}C. ^{1}H NMR (DMSO-d_6): δ 11.82 (br., 1H), 7.56 (t, J 8.0, 1H), 7.51 (d, J 7.8, 1H), 7.14 (d, J 8.0, 1H). ^{13}C NMR (DMSO-d_6): δ 156.9, 146.5, 144.0, 136.7, 129.4, 123.3, 115.2, 109.3. Mass (rel. intensity): 197 (100), 243 (29.9), 245 (36.7).

Bromination of Isatoic anhydride.

Isatoic anhydride (10mmol, 1.63g) was dissolved in dry DMF. N-bromosuccinamide (20mmol, 3.56g) was added under argon. The resulting solution was stirred at 50^{o}C for 30 minutes, and then stirred at room temperature overnight. To the solution was added 10mL water, and the resulting solid was filtered and washed with water to remove DMF.

4j. 5-bromoisatoic anhydride. Yield: 93%, pale white solid.

^{1}H NMR (DMSO-d_6): δ 11.85 (br., 1H), 7.96 (dd, J 0.8, 8.7, 1H), 7.87 (dd, J 2.3, 8.7, 1H), 7.09 (dd, J 0.8, 8.8, 1H). ^{13}C NMR (DMSO-d_6): δ 158.9, 146.8, 140.9, 139.4, 130.7, 117.7, 114.7, 112.5.

X-ray analysis of Isatoic Anhydrides

Suitable crystals for X-ray analysis for 3-bromo isatoic anhydride (**4a**) and 6-bromo isatoic anhydride (**4i**) were obtained by slow evaporation of ethyl acetate solutions. X-ray diffraction data were collected on a Rigaku RAPID II diffractometer using graphite-monochromated MoKα radiation (λ = 0.71073 Å) at 123 K. Multiscan absorption correction was applied to the data using the CrystalClear 2.0 program (Rigaku Inc. 2010). The structure was solved by the Patterson method (PATTY) using the Crystal-Structure 4.0 program and refined by full-matrix least-squares refinement on F2 using the Crystals for Windows program.

Crystallographic data for the structural analysis are deposited in the Cambridge Crystallographic Data Centre; **4a** (CCDC 906032), **4i** (CCDC 903035). Copies of this information may be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge, CB2 1FZ, UK (email:deposit@ccdc.cam.ac.uk or http:// www.ccdc.cam.ac.uk).

General Procedure 4: Synthesis of Substituted Tryptanthrin:

Isatin (2mmol) and isatoic anhydride (2.5mmol) were dissolved in benzene (5mL) and triethylamine (0.6mL). The resulting solution was refluxed at 80^{o}C for 24 hours and the precipitate was filtered and recrystalized from ethanol.

3a. 1-bromotryptanthrin. Prepared from isatin and 6-bromoisatoic anhydride using General Procedure 4. Yield: 23%. Yellow solid. Mp.: >250^{o}C. ^{1}H NMR (DMSO-d_6): δ 8.48 (d, J 8.1, 1H), 7.95 (m, 2H), 7.88 (m, 2H), 7.76 (t, J 8.0, 1H), 7.49 (t, J 7.5, 1H). ^{13}C NMR (DMSO-d_6): δ 156.8, 155.6,

149.7, 146.7, 145.9, 138.5, 136.8, 138.9, 130.9, 127.7, 125.5, 122.9, 122.2, 121.9, 117.9. Mass (rel. intensity): 326 (100), 328 (98.6).

3b. 2-bromotryptanthrin. Prepared from isatin and 5-bromoisatoic anhydride using General Procedure 4. Yield: 42%. Yellow solid. Mp.: >250^{o}C. ^{1}H NMR ($CDCl_3$): δ 8.26 (d, J 8.1, 1H), 8.57 (d, J 2.3, 1H), 7.92 (m, 3H), 7.81 (t, J 7.6 1H), 7.45 (t, J 7.6, 1H). ^{13}C NMR ($CDCl_3$): δ 182.2, 157.0, 146.2, 145.6, 144.6, 138.6, 138.5, 132.3, 130.5, 127.7, 125.7, 125.2, 124.8, 122.1, 118.2. Mass (rel. intensity): 326 (100) and 328 (98.7).

3c. 3-bromotryptanthrin. Prepared from isatin and 4-bromoisatoic anhydride using General Procedure 4. Yield: 26%. Yellow solid. Mp.: >250^{o}C. ^{1}H NMR (DMSO-d_6): δ 8.46 (d, J 8.0, 1H), 8.21 (m, 8.21), 7.89 (m, 3H), 7.49 (t, J 7.5, 1H). Mass (rel. intensity): 326 (100), 328 (99.6).

3d. 4-bromotryptanthrin. Prepared from isatin and 3-bromoisatoic anhydride using General Procedure 4. 38%. Yellow solid. Mp.: >250^{o}C. ^{1}H NMR (DMSO-d_6): δ 8.47 (d, J 8.4, 1H), 8.33 (d, J 7.8, 1H), 8.27 (d, J 7.8, 1H), 7.90 (m, 2H), 7.64 (t, J 8.1, 1H), 7.51 (t, J 7.7, 1H). ^{13}C NMR (DMSO-d_6): δ 182.2, 157.2, 145.7, 145.6, 144.3, 138.7, 137.8, 130.6, 127.2, 126.7, 125.3, 124.9, 124.6, 122.2, 117.0. Mass (rel. intensity): 326 (100), 328 (99.3).

3e. 2,3-dibromotryptanthrin. Prepared from isatin and 4,5-dibromoisatoic anhydride using General Procedure 4. Yield: 56%. Yellow solid. Mp.: >250^{o}C. ^{1}H NMR (DMSO-d_6): δ 8.52 (s, 1H), 8.44 (d, J 8.04, 1H), 8.40 (s, 1H), 7.89 (m, 2H), 7.51 (dt, J 0.6, 7.5, 1H). Mass (rel. intensity): 404 (49.2), 406 (100), 408 (49.8).

3f. 2,4-dibromotryptanthrin. Prepared from isatin and 3,5-dibromoisatoic anhydride using General Procedure 4. Yield: 18%. Yellow solid. Mp.: >250^{o}C. ^{1}H NMR (DMSO-d_6): δ 8.53 (d, J 2.3,

1H), 8.45 (d, J 8.0, 1H), 8.40 (d, J 2.3, 1H), 7.91 (m, 2H), 7.52 (t, J 7.4, 1H). Mass (rel. intensity): 404 (49.7), 406 (100), 408 (49.8).

3g. 2,8-dibromotryptanthrin. Prepared from 5-bromoisatin and 5-bromoisatoic anhydride using General Procedure 4. Yield: 36%. Yellow solid. Mp.: >250^{o}C. ^{1}H NMR (DMSO-d_6): δ 8.36 (d, J 8.8, 2H), 8.12 (dd, J 2.3, 8.7, 1H), 8.05 (dt, 1.7, 8.5, 2H), 7.90 (d, J 8.5, 1H).

3h. 2-fluorotryptanthrin. Prepared from isatin and 5-fluoroisatoic anhydride using General Procedure 4. Yield: 47%. Yellow solid. Mp.: >250^{o}C. ^{1}H NMR (DMSO-d_6): δ 8.48 (d, 1H, 7.8), 8.04 (m, 2H), 7.89 (m, 2H), 7.83 (dt, J 3.1, 8.6, 1H), 7.52 (t, J 7.6, 1H). Mass (rel. intensity): 266 (100).

3i. 3-fluorotryptanthrin. Prepared from isatin and 4-fluoroisatoic anhydride using General Procedure 4. Yield: ?%. yellow solid. Mp.: >250^{o}C. ^{1}H NMR (DMSO-d_6): δ 8.47 (d, J 8.0, 1H), 8.38 (dd, J 6.2, 8.9, 1H), 7.88 (m, 2H), 7.82 (dd, J 2.6, 9.7, 1H), 7.61 (dt, J 2.5, 8.6, 1H), 7.49 (t, J 7.6, 1H). Mass (rel. intensity): 266 (100).

3j. 4-fluorotryptanthrin. Prepared from isatin and 3-fluoroisatoic anhydride using General Procedure 4. Yield: 36%. Yellow solid. Mp.: >250^{o}C. ^{1}H NMR (DMSO-d_6): δ 8.46 (d, J 8.0, 1H), 8.21 (m, 2H), 7.90 (m, 3H), 7.49 (t, J 7.6, 1H). Mass (rel. intensity): 266 (100).

3k. 2,3-difluorotryptanthrin. Prepared from isatin and 4,5-difluoroisatoic anhydride using General Procedure 4. Yield: 44%. Yellow solid. Mp.: >250^{o}C. ^{1}H NMR (DMSO-d_6): δ 8.44 (d, J 8.0, 1H), 8.28 (t, J 9.7, 1H), 8.13 (dd, J 7.4, 10.9, 1H), 7.89 (m, 2H), 7.50 (t, J 7.6, 1H). Mass (rel. intensity): 284 (100).

3l. 2,8-difluorotryptanthrin. Prepared from 5-fluoroisatin and 5-fluoroisatoic anhydride using General Procedure 4. Yield: 13%.Yellow-green solid. Mp.: >250^{o}C. ^{1}H NMR (DMSO-d_6): δ 8.48

(dd, J 4.1, 8.8, 1H), 8.07-8.01 (m, 2H), 7.85 (dt, J 3.0, 8.6, 1H), 7.80 (dd, J 2.6, 7.0, 1H), 7.74 (dt, J 2.6, 8.9, 1H). ^{13}C NMR (DMSO-d_6): δ 180.1 (J_{CF} 2), 161.1 (J_{CF} 223), 159.2 (J_{CF} 219), 155.6 (J_{CF} 3), 143.7 (J_{CF} 2), 142.1 (J_{CF} 2), 141 (J_{CF} 2), 131.7 (J_{CF} 9), 124.0 (J_{CF} 9), 122.9 (J_{CF} 25), 122.9 (J_{CF} 8), 122.2 (J_{CF} 24), 117.8 (J_{CF} 8), 111.2 (J_{CF} 24), 110.4 (J_{CF} 25). Mass (rel. intensity): 275 (100).

3m. 8-bromo-2-fluorotryptanthrin. Prepared from 5-bromoisatin and 5-fluoroisatoic anhydride using General Procedure 4. Yield: ?%. Yellow solid. Mp.: >250°C. ^{1}H NMR (DMSO-d_6): δ 8.38 (d, J 8.4, 1H), 8.05 (m, 4H), 7.85 (dt, J 3.1, 8.5, 1H). ^{13}C NMR (DMSO-d_6): δ 181.0, 162.1 (J_{CF} 250), 156.9 (J_{CF} 3), 144.6, 144.4 (J_{CF} 3), 143.3 (J_{CF} 2), 139.8, 132.8 (J_{CF} 9), 128.3, 127.2, 125.0 (J_{CF} 9), 124.3, 123.4 (J_{CF} 24), 119.2 (J_{CF} 50), 112.4 (J_{CF} 24).

3n. 8-bromo-2,3-difluorotryptanthrin. Prepared from 5-bromoisatin and 4,5-dibromoisatoic anhydride using General Procedure 4. Yield: 31%. Yellow solid. Mp.: >250°C. ^{1}H NMR (DMSO-d_6): δ 8.37 (d, J 8.5, 1H), 8.29 (dd, J 8.6, 10.2, 1H), 8.12 (dd, J 7.3, 10.9, 1H), 8.07 (dt, J 2.1, 10.1, 2H). Mass (rel. intensity): 362 (100), 364 (97.3).

3o. 8-isopropyltryptanthrin. Prepared from 5-isopropylisating and isatoic anhydride using General Procedure 4. Yield: 53%. Mp.: 181-183°C. Yellow Solid. ^{1}H NMR (DMSO-d_6): δ 8.38 (d, J 8.1, 1H), 8.31 (d, J 7.9, 1H), 7.94-7.90 (m, 2H), 7.75-7.72 (m, 3H), 3.04 (sept, J 6.9, 1H), 1.26 (d, J 6.9, 6H). ^{13}C NMR (DMSO-d_6): δ 182.5, 157.5, 147.6, 146.5, 145.3, 144.2, 136.0, 135.1, 129.9, 129.8, 126.9, 123.4, 122.4, 122.2, 117.0, 33.0, 23.6. Mass (rel. intensity): 275 (100), 290 (73.2), 291 (15.4).

3p. 8-methoxytryptanthrin. Prepared from 5-methoxyisatin and isatoic anhydride using General Procedure 4. Yield 34%. Brown solid. Mp.: >250°C. ^{1}H NMR (DMSO-d_6): δ 8.36 (d, J 8.5, 1H), 8.30 (d, J 7.8, 1H), 7.93 (d, J 3.9, 2H), 7.72 (5-let, J 3.9, 1H), 7.43-7.38 (m, 2H) 3.87 (s, 3H).

^{13}C NMR (DMSO-d_6): δ 182.4, 158.0, 157.3, 146.4, 145.4, 139.9, 135.0, 129.9, 129.8, 126.8, 123.8, 123.8, 123.4, 123.4, 118.3, 108.5. Mass (rel. intensity): 278 (100), 279 (18.7).

General Procedure 5: Phenyl-substituted tryptanthrins.

Bromotryptanthrin (0.6mmol), sodium carbonate (1.2mmol, 127mg), and phenylboronic acid (1mmol, 121.93mg) were dissolved in 12mL dimethylsulfoxide and 3mL water. The solution was flushed with argon, and then 15mg tetrakis(triphenylphosphine)palladium(0) was added. The reaction tube was sealed and heated, stirring, to 100^{o}C and reacted overnight. The solution was cooled to room temperature and neutralized with HCl, then filtered and air dried. The solid was then recrystalized from ethanol.

3q. 2-phenyltryptanthrin. Prepared from 2-bromotryptanthrin using General Procedure 5. Yield: 62%. Yellow solid. Mp.: 243-246^{o}C. ^{1}H NMR ($CDCl_3$): δ 8.66-8.63 (m, 2H), 8.10-8.07 (m, 2H), 7.92 (d, J 7.6, 1H), 7.80 (t, J 7.6, 1H), 7.73 (d, J 7.4, 2H), 7.52 (t, J 6.0, 2H), 7.48-7.41 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 182.5, 158.2, 146.3, 145.7, 144.2, 143.3, 138.8, 138.2, 133.8, 131.2, 129.2, 128.7, 127.3, 127.2, 125.5, 125.4, 124.1, 122.1, 118.0. Mass (rel. intensity): 324 (100), 325 (24.6).

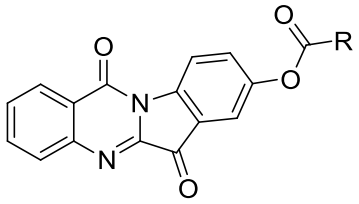
3r. 8-phenyltryptanthrin. Prepared from 8-bromotryptanthrin using General Procedure 5. Yield: 90%. Yellow solid. Mp.: 244-246^{o}C. ^{1}H NMR ($CDCl_3$): δ 8.67 (d, J 8.4, 1H), 8.45 (d, J 7.9, 1H), 8.13 (s, 1H), 8.05 (d, J 8.2, 1H), 8.01 (d, J 8.4, 1H), 7.86 (t, 7.7, 1H), 7.69 (t, J 7.9, 1H), 7.62 (d, J 7.7 2H), 7.50 (t, J 7.6, 2H), 7.43 (t, J 7.6, 1H). ^{13}C NMR ($CDCl_3$): δ 182.8, 158.2, 146.8, 145.4, 144.7, 140.8, 138.8, 137.0, 135.3, 130.9, 130.6, 129.3, 128.6, 127.7, 127.1, 123.9, 123.8, 122.7, 118.4. Mass (rel. intensity): 324 (100), 325 (22.7).

General Procedure 6: Tryptanthrin 8-esters.

8-methoxytryptanthrin (5.6g, 20mmol) was reacted with AlCl3 (13.8g, 104mmol) in dry dichloromethane (300mL) at reflux. After 48 hours, water was carefully added to the reaction, and the resulting solid was filtered.

3s. 8-hydroxytryptanthrin. Yield: 82%. Brown solid. Mp.: >250^{o}C. ^{1}H NMR (DMSO-d_6): δ 10.23 (br., 1H), 8.27 (d, J 8.9, 2H), 7.91 (d, J 3.9, 1H), 7.71 (sept, J 4.0, 1H), 7.22 (dd, J 2.6, 8.6, 1H), 7.15 (d, J 2.4, 1H).

8-hydroxytryptanthrin (0.8mmol , 0.211g) was reacted with acid chloride (1.2mmol) in pyridine (5mL) at room temperature for 12 hours. Water (10mL) was added and the solid was filtered and washed with water in small portions.



3t. Tryptanthrin-8-ethanoate (R=CH_3). Prepared from 8-hydroxytryptanthrin and acetyl chloride per General Procedure 6. Yield: 79%. Yellow solid. Mp.: 238-240^{o}C. ^{1}H NMR ($CDCl_3$): δ 8.64 (d, J 8.7, 1H), 8.43 (dd, J 1.2, 7.9, 1H), 8.03 (d, J 8.0, 1H), 7.85 (dt, J 1.4, 7.6, 1H), 7.68 (t, J 7.7, 1H), 7.65 (d, J 2.4, 1H), 7.49 (dd, 2.5, 8.7, 1H), 2.36 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 197.0, 181.9, 169.0, 158.1, 149.5, 146.7, 143.8, 135.4, 131.3, 131.0, 130.6, 127.7, 123.8, 123.0, 119.1, 118.8, 21.1.

3u. Tryptanthrin-8-butanoate (R=$CH_2CH_2CH_3$). Prepared from 8-hydroxytryptanthrin and buteryl chloride per General Procedure 6. Yield: 23%. Yellow solid. Mp.: >250^{o}C. ^{1}H NMR ($CDCl_3$):

δ 8.64 (d, J 8.7, 1H), 8.44 (d, J 8.0, 1H), 8.03 (d, J 8.1, 1H), 7.86 (t, J 7.7, 1H), 7.69 (t, J 7.7, 1H), 7.65 (d, J 2.5, 1H), 7.49 (dd, J 2.5, 8.8, 1H), 2.59 (t, J 7.5, 2H), 1.81 (sex, J 7.5, 2H), 1.07 (t, J 7.4, 3H). ^{13}C NMR ($CDCl_3$): δ 181.8, 171.7, 157.9, 149.4, 146.6, 144.4, 143.6, 135.2, 131.3, 130.9, 130.4, 127.6, 123.7, 122.9, 118.9, 118.7, 36.2, 18.5, 13.8.

3v. Tryptanthrin-8-hexanoate (R=$CH_2CH_2CH_2CH_2CH_3$). Prepared from 8-hydroxytryptanthrin and hexanoyl chloride per General Procedure 6. Yield: 33%. Yellow solid. Mp.: 222-224^{o}C. ^{1}H NMR ($CDCl_3$): δ 8.64 (d, J 8.7, 1H), 8.43 (dd, J 1.5, 7.9, 1H), 8.03 (d, J 8.1, 1H) 8.03 (d, J 8.1, 1H), 7.86 (dt, J 1.5, 7.4, 1H), 7.68 (t, J 7.4, 1H), 7.64 (d, J 2.4, 1H), 7.48 (dd, J 2.5, 8.7, 1H), 2.60 (t, J 7.4, 2H), 1.78 (5let, J 7.3, 2H), 1.43-1.36 (m, 4H), 0.95 (t, J 7.1, 3H). ^{13}C NMR ($CDCl_3$): δ 182.0, 172.0, 158.1, 149.6, 146.7, 144.6, 143.7, 135.3, 131.4, 131.0, 130.6, 127.7, 123.9, 123.0, 119.1, 118.8, 42.3, 31.4, 24.6, 22.4, 14.0.

3w. Tryptanthrin-8-benzoate (R=C_6H_5). Prepared from 8-hydroxytryptanthrin and benzoyl chloride per General Procedure 6. Yield: 33%. Yellow solid. Mp.: >250^{o}C. ^{1}H NMR ($CDCl_3$): δ 8.70 (dd, J 1.6, 8.6, 1H), 8.45 (dd, J 7.7, 1H), 8.21 (d, J 7.8, 2H), 8.04 (d, 7.8, 1H), 7.86 (t, J 7.9, 1H), 7.79 (t, 2.3, 1H), 7.69 (t, J 7.7, 2H), 7.63 (dt, J 2.3, 8.6, 1H), 7.55 (t, J 7.8, 2H). ^{13}C NMR ($CDCl_3$): δ 181.8, 164.8, 158.0, 149.7, 146.6, 144.4, 143.7, 135.3, 134.2, 131.4, 130.9, 130.5, 130.4, 128.8, 128.6, 127.6, 123.7, 123.0, 119.0, 118.9.

General Procedure 7: Preparation of plant extracts: Plant material (2.5-3.0g) was submerged in 50mL methanol. The suspension was stirred at room temperature for 5 days. The suspensions were vacuum filtered through Whatman 1 filter papers. The solutions were rotorevaporated to dryness and resuspended in a small amount of methanol (5-10mL), filtered through 0.7µm

syringe filter, then 0.2µm filter. The solution was then evaporated to dryness and 20mg/mL samples of the extracts were prepared in dimethyl sulfoxide for biological testing.

Fractionation of *Bupleurum falcatum:* Bupleurum falcatum (B. falcatum) root powder (16.8g) was extracted using a soxhlet extractor for 24 hours. Each solvent (50mL) was introduced in sequence, and between solvents the extractor and reflux condenser were allowed to dry. In sequence, B. falcatum was extracted with hexane, benzene, dichloromethane, acetone, tetrahydrofuran, acetonitrile, ethanol, and methanol. The extracts were each evaporated, redissolved in methanol, the solutions filtered through 0.7µm and 0.2µm syringe filters, and evaporated. Samples were prepared for biological testing as for crude methanolic extracts, above.

Biological Testing Procedure

Compounds were tested for in vitro efficacy against *T. gondii* tachyzoites, using methods which have been described in detail in previous publications.[14, 58] Briefly, test and control drugs (320 to 0.32 µg/ml) were added to normal human fibroblasts (ATCC, Manassas, VA) plated in 96 well plates. Directly following this, 50 tachyzoites of a β-galactosidase (β-Gal)-producing strain of *T. gondii*, RH (ATCC 50839), was added to 6/8 wells in each column. After 4 days at 37°C in 5% CO2, the β-Gal substrate chlorophenol red–β-D-galactopyranoside was added to these same wells for 1 additional day. Cytotoxicity was measured in the remaining 2/8 wells using a commercially available cell viability reagent (One Solution; Promega). Color reactions in all wells were read in a Vmax Microplate reader (Molecular Devices, CA). The 50% inhibitory dose (ID_{50}) and the 50% toxic dose (TD_{50}) were calculated by applying the Reed and Muench formula[59] using a minimum of 12 determinations for the ID_{50} and 6 determinations for the TD_{50}. GraphPad Prism

5 was used for data analysis. The therapeutic index (TI) was calculated by the equation $TI=TD_{50}/ID_{50}$.

7. References

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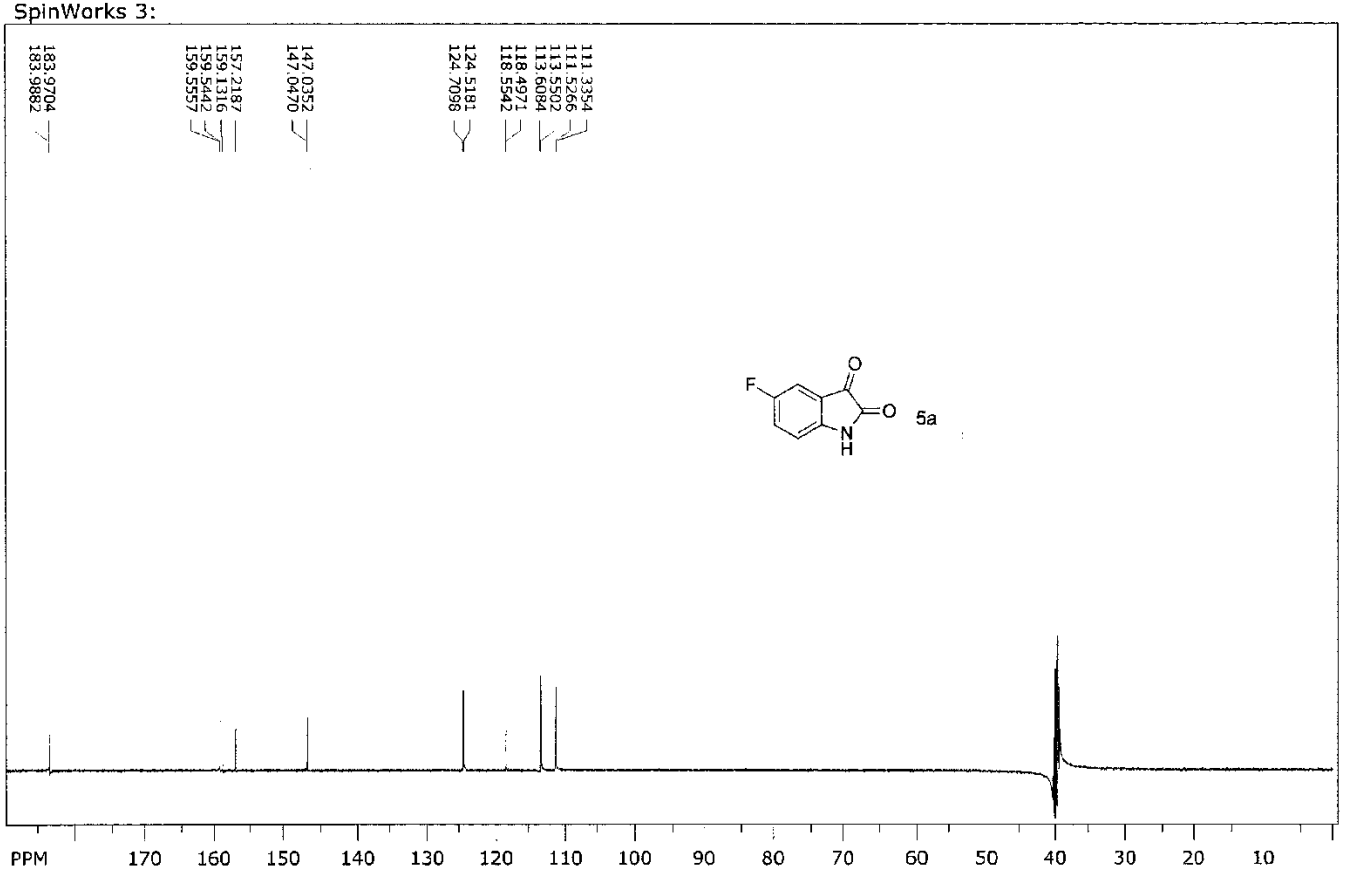
Appendix I: NMR spectra

SpinWorks 3:



file: ... NMRS\Isatins\5-fluoroisatin C\fid block# 1 expt: "s2pul"
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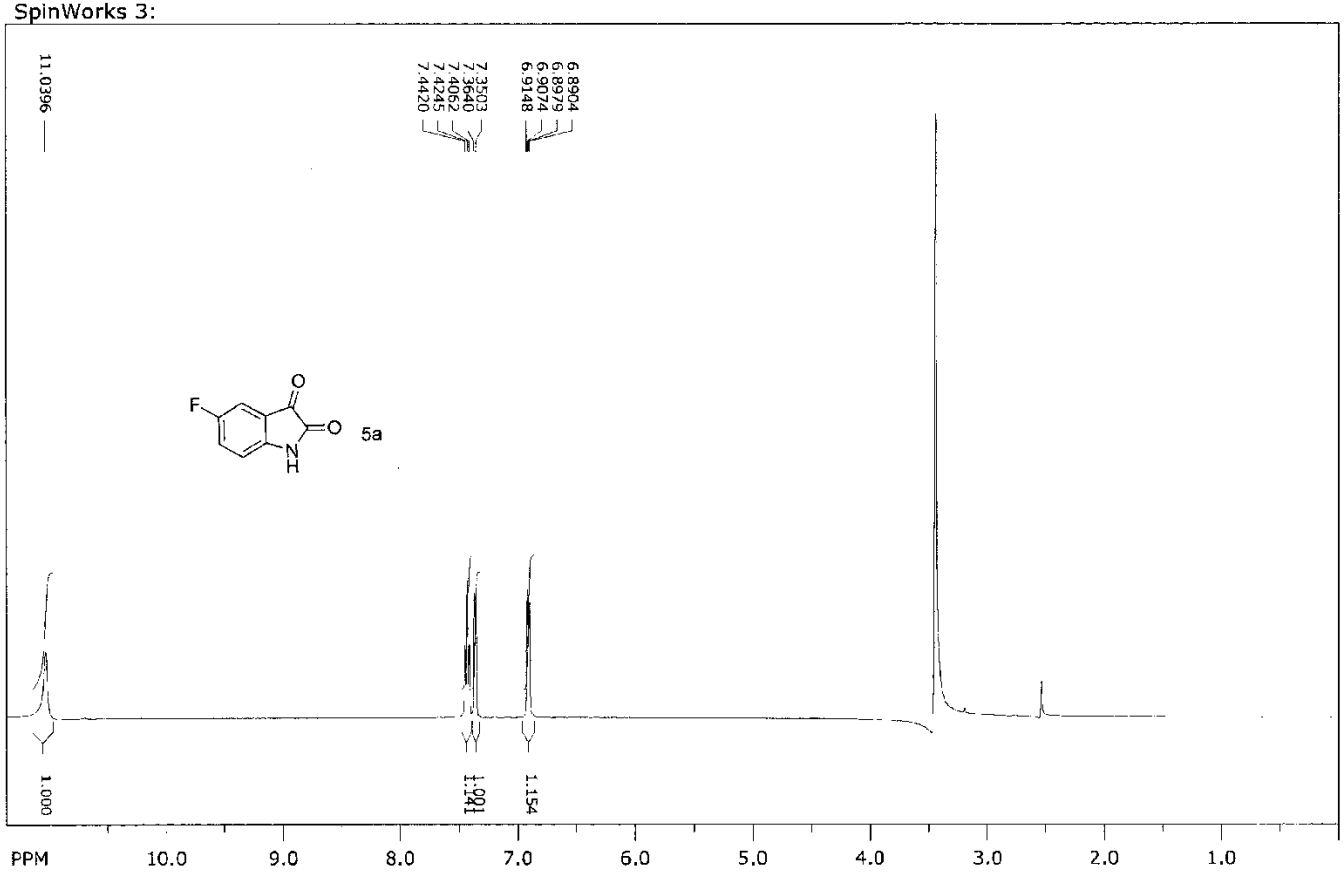
time domain size: 78430 points
width: 30154.54 Hz = 239.9485 ppm = 0.384477 Hz/pt
number of scans: 256

freq. of 0 ppm: 125.657727 MHz
processed size: 131072 complex points
LB: 0.500 GF: 0.0000
Hz/cm: 956.814 ppm/cm: 7.61365

SpinWorks 3:



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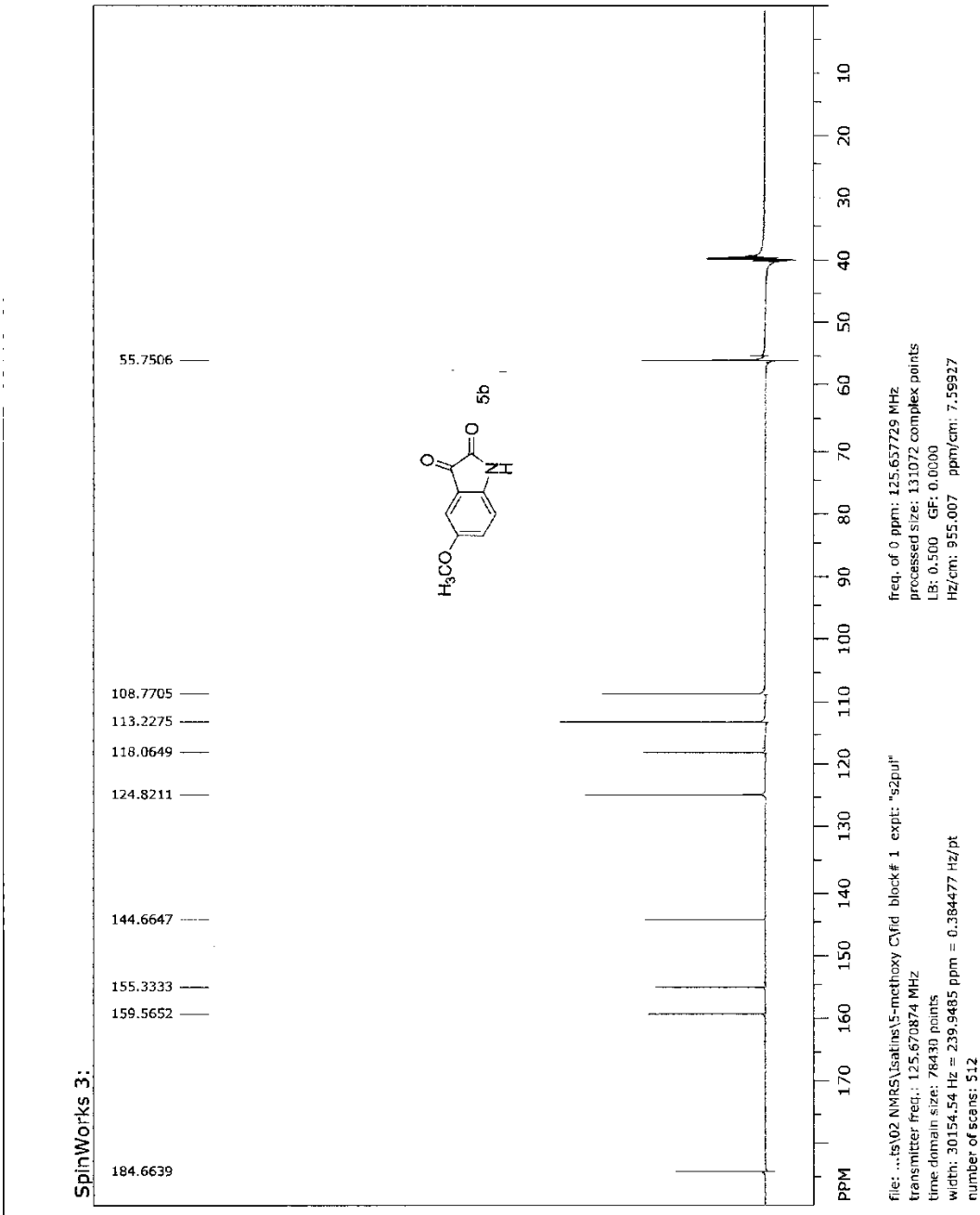
time domain size: 32762 points
width: 7996.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 8

freq. of 0 ppm: 499.731883 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 227.362 ppm/cm: 0.45497


SpinWorks 3:
55.7506
108.7705
113.2275
118.0649
124.8211
144.6647
155.3333
159.5652
184.6639
PPM
5b
H3CO
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time domain size: 78430 points
width: 30154.54 Hz = 239.9485 ppm = 0.384477 Hz/pt
number of scans: 512
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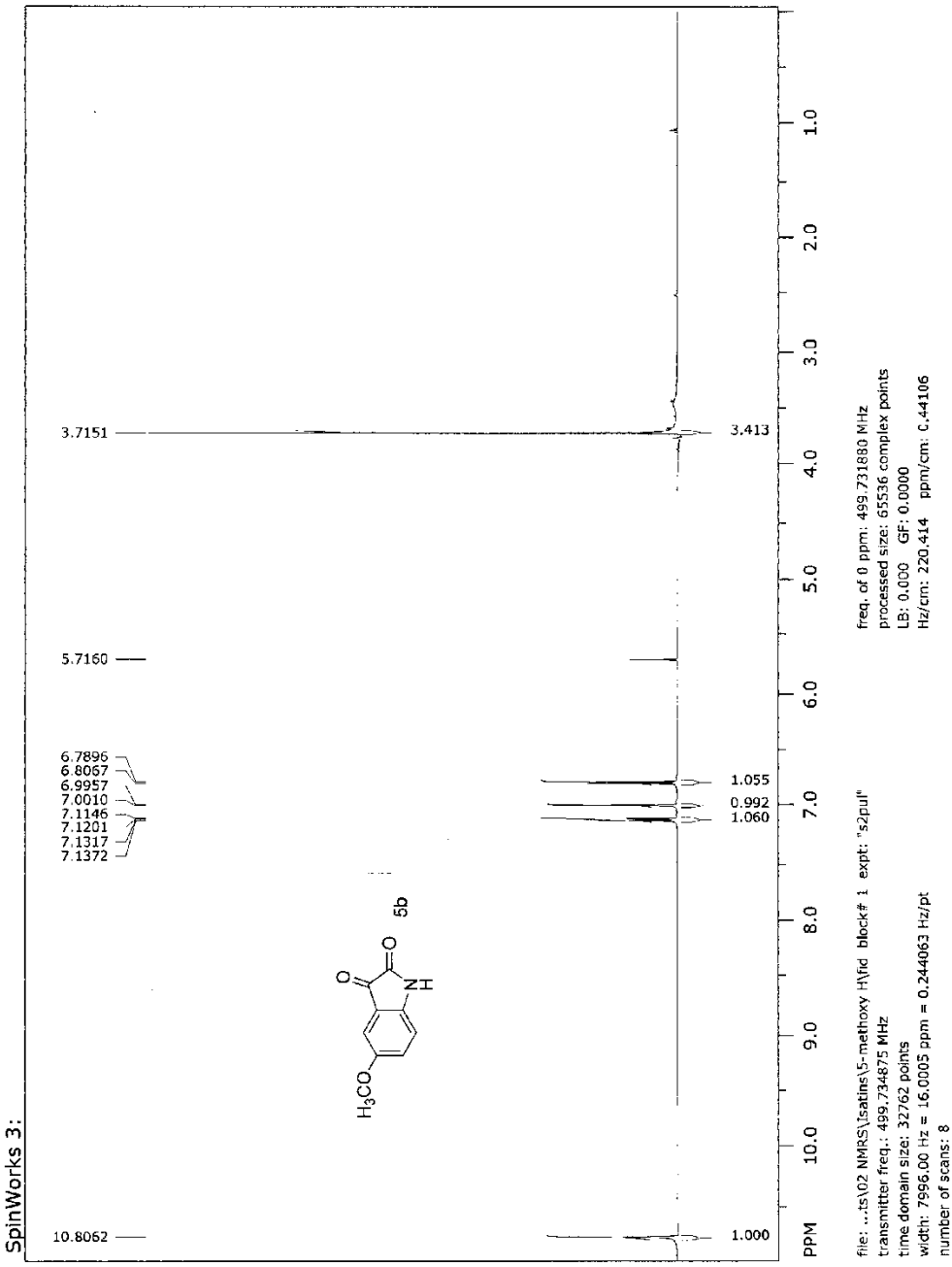
processed size: 131072 complex points
LB: 0.500 GF: 0.0000
Hz/cm: 955.007 ppm/cm: 7.59927


SpinWorks 3:
3.7151
5.7160
6.7896
6.8067
6.9957
7.0010
7.1146
7.1201
7.1317
7.1372
10.8062
3.413
1.055
0.992
1.060
1.000
5b
PPM
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
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transmitter freq.: 499.734875 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 8
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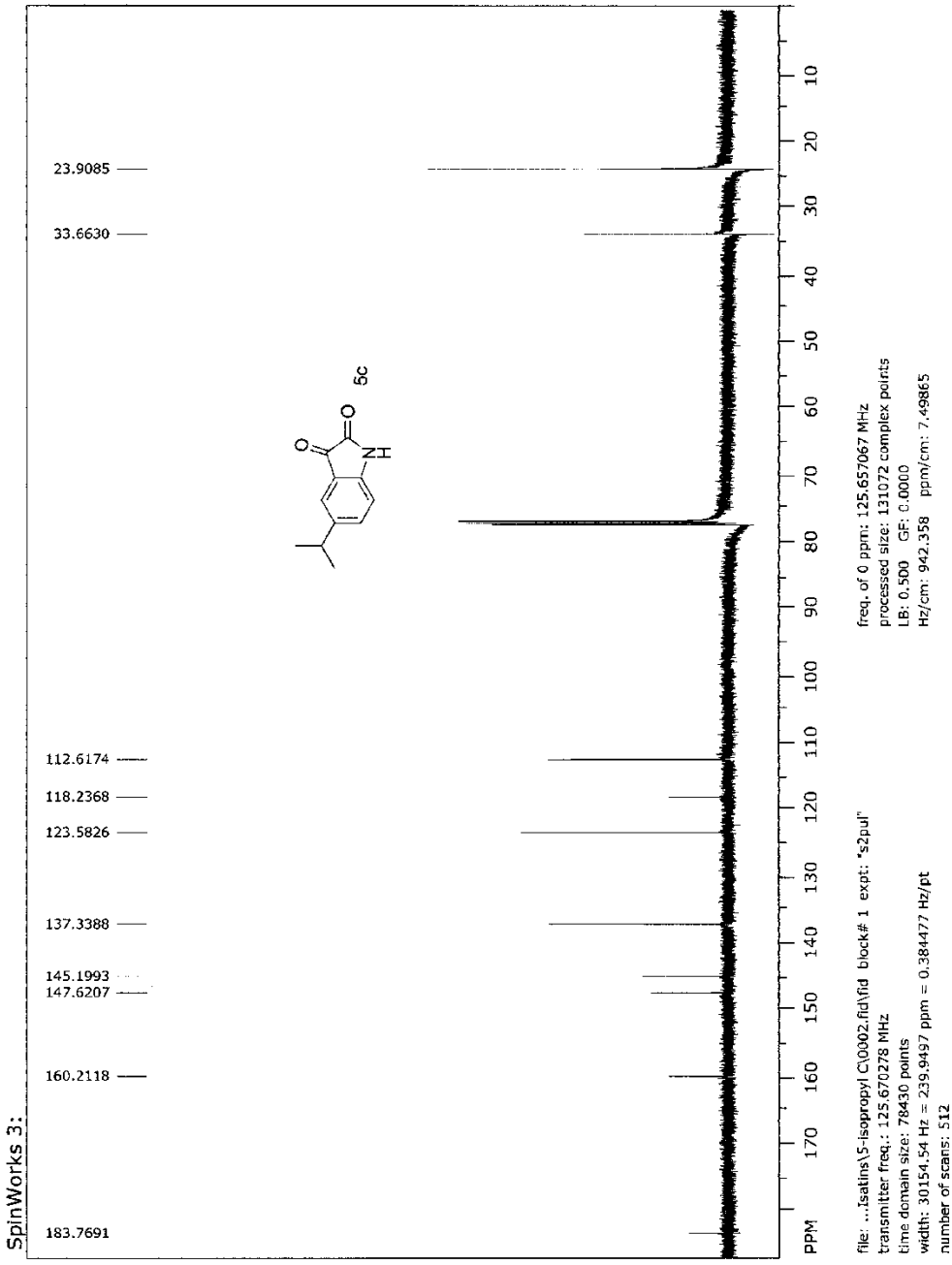
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 220.414 ppm/cm: 0.44106


SpinWorks 3:
23.9085
33.6630
112.6174
118.2368
123.5826
137.3388
145.1993
147.6207
160.2118
183.7691
PPM
5c
file: ...\satins\5-isopropyl C\0002.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 125.670278 MHz
time domain size: 78430 points
width: 30154.54 Hz = 239.9497 ppm = 0.384477 Hz/pt
number of scans: 512
freq. of 0 ppm: 125.657067 MHz
processed size: 131072 complex points
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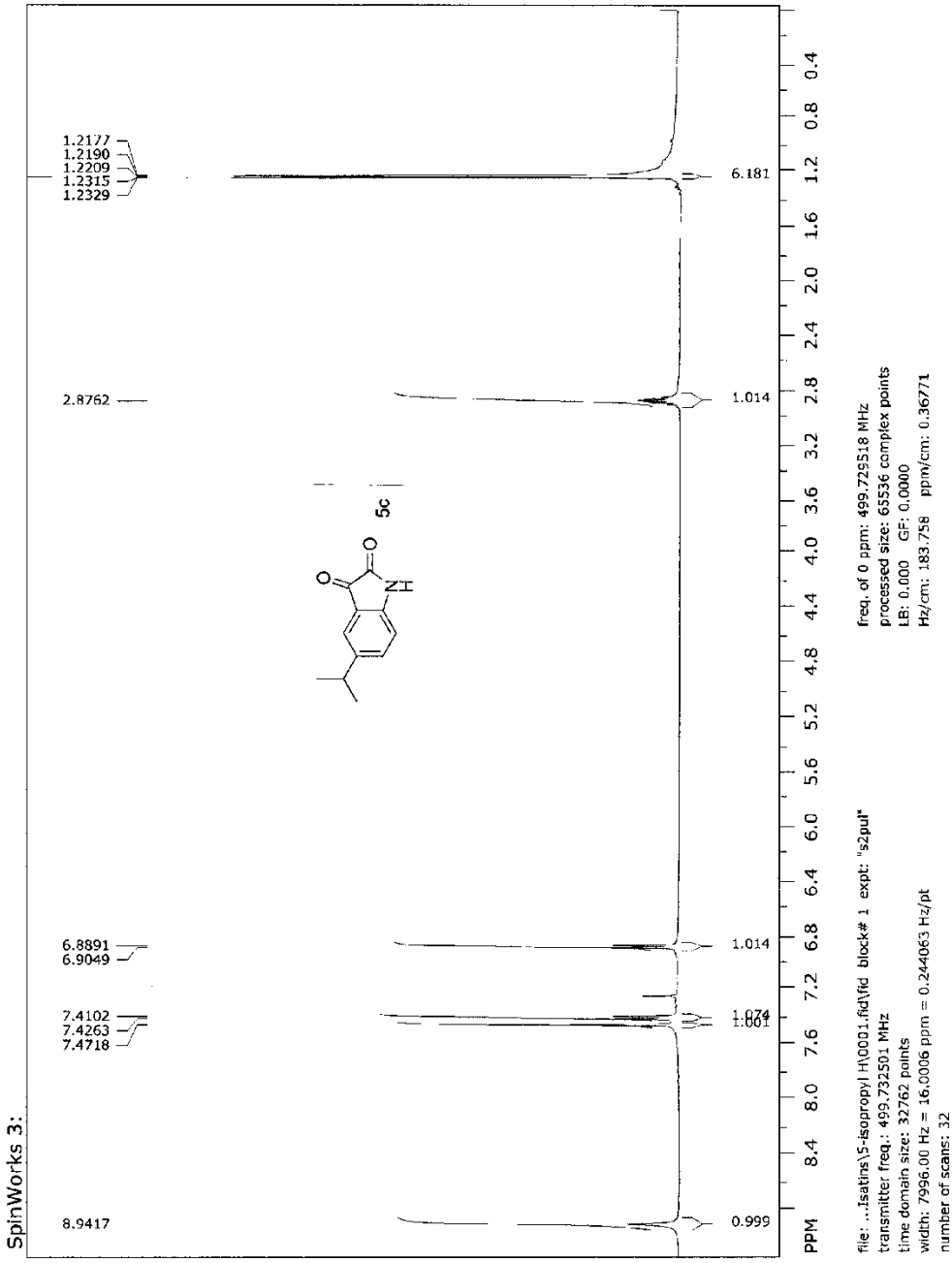
Hz/cm: 942.358 ppm/cm: 7.49865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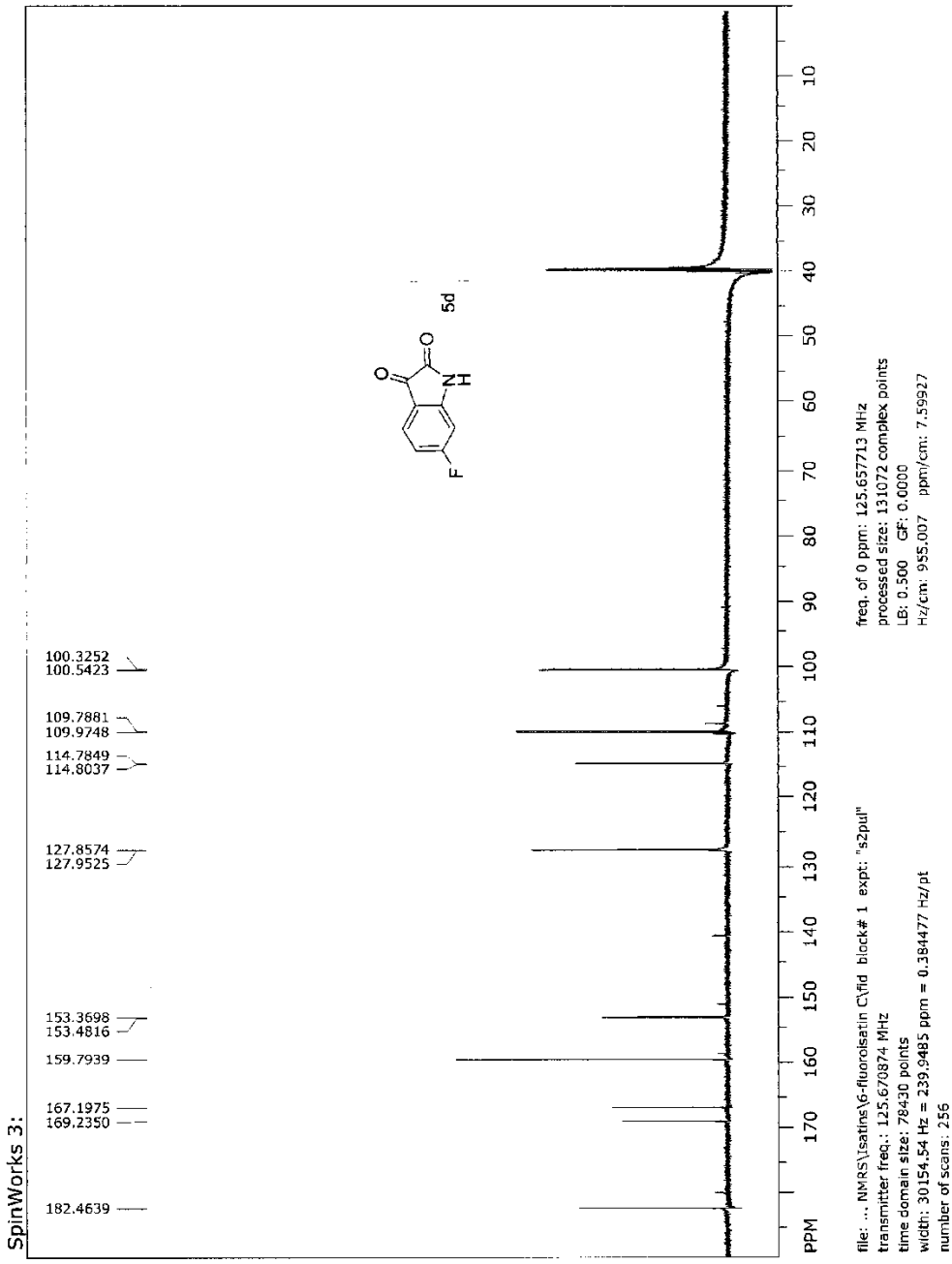
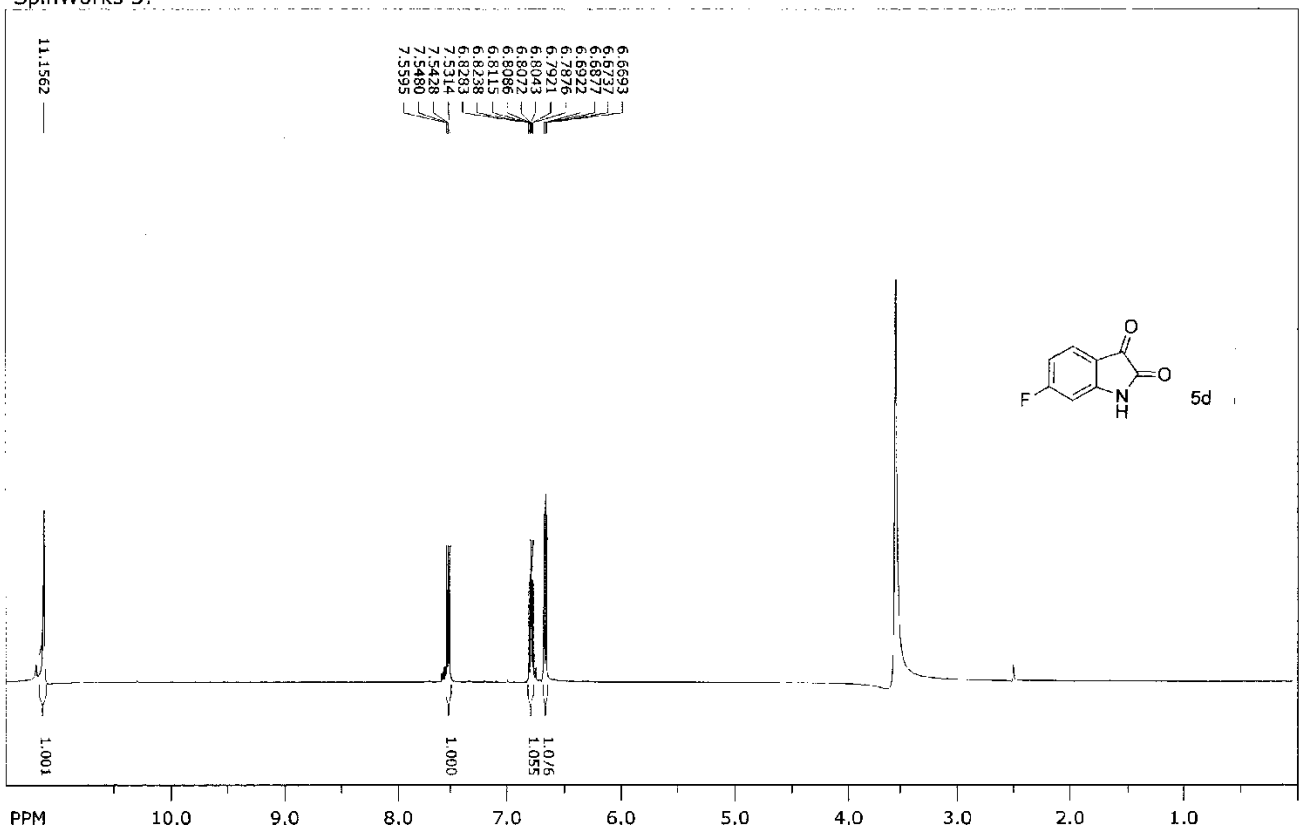
1.2190
1.2209
1.2315
1.2329
2.8762
6.8891
6.9049
7.4102
7.4263
7.4718
8.9417
6.181
1.014
1.014
1.074
1.001
0.999
5c
PPM
8.4 8.0 7.6 7.2 6.8 6.4 6.0 5.6 5.2 4.8 4.4 4.0 3.6 3.2 2.8 2.4 2.0 1.6 1.2 0.8 0.4
file: ...\satins\5-isopropyl H\0001.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 499.732501 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0006 ppm = 0.244063 Hz/pt
number of scans: 32
freq. of 0 ppm: 499.729518 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.000
Hz/cm: 183.758 ppm/cm: 0.36771


SpinWorks 3:
100.3252
100.5423
109.7881
109.9748
114.7849
114.8037
127.8574
127.9525
153.3698
153.4816
159.7939
167.1975
169.2350
182.4639
PPM
5d
file: ... NMRS\Isatins\6-fluoroisatin CYfid block# 1 expt: "s2pul"
transmitter freq.: 125.670874 MHz
time domain size: 78430 points
width: 30154.54 Hz = 239.9485 ppm = 0.384477 Hz/pt
number of scans: 256
freq. of 0 ppm: 125.657713 MHz
processed size: 131072 complex points
LB: 0.500 GF: 0.0000
Hz/cm: 955.007 ppm/cm: 7.59927

SpinWorks 3:



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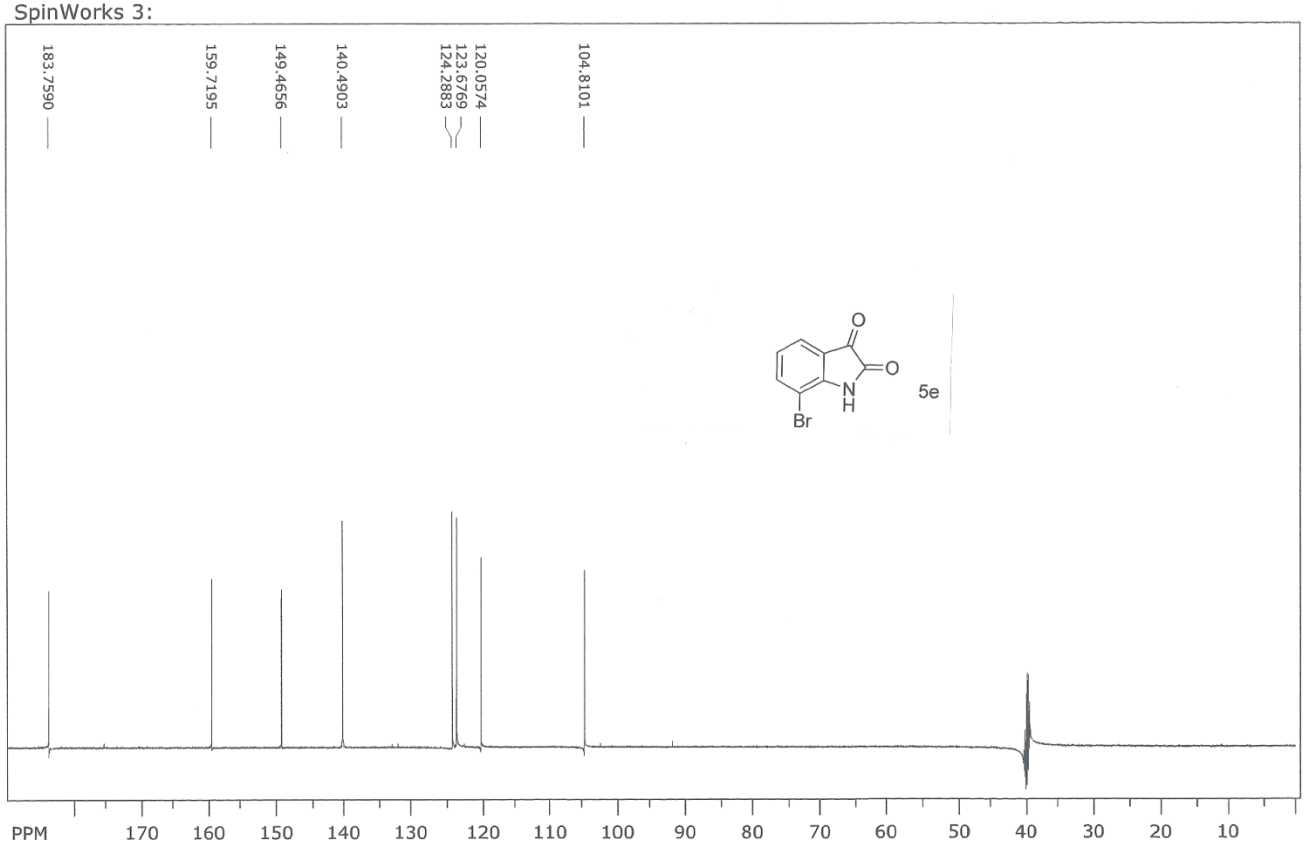
time domain size: 32762 points
width: 7996.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 8

freq. of 0 ppm: 499.731881 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 229.758 ppm/cm: 0.45976

SpinWorks 3:



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number of scans: 256

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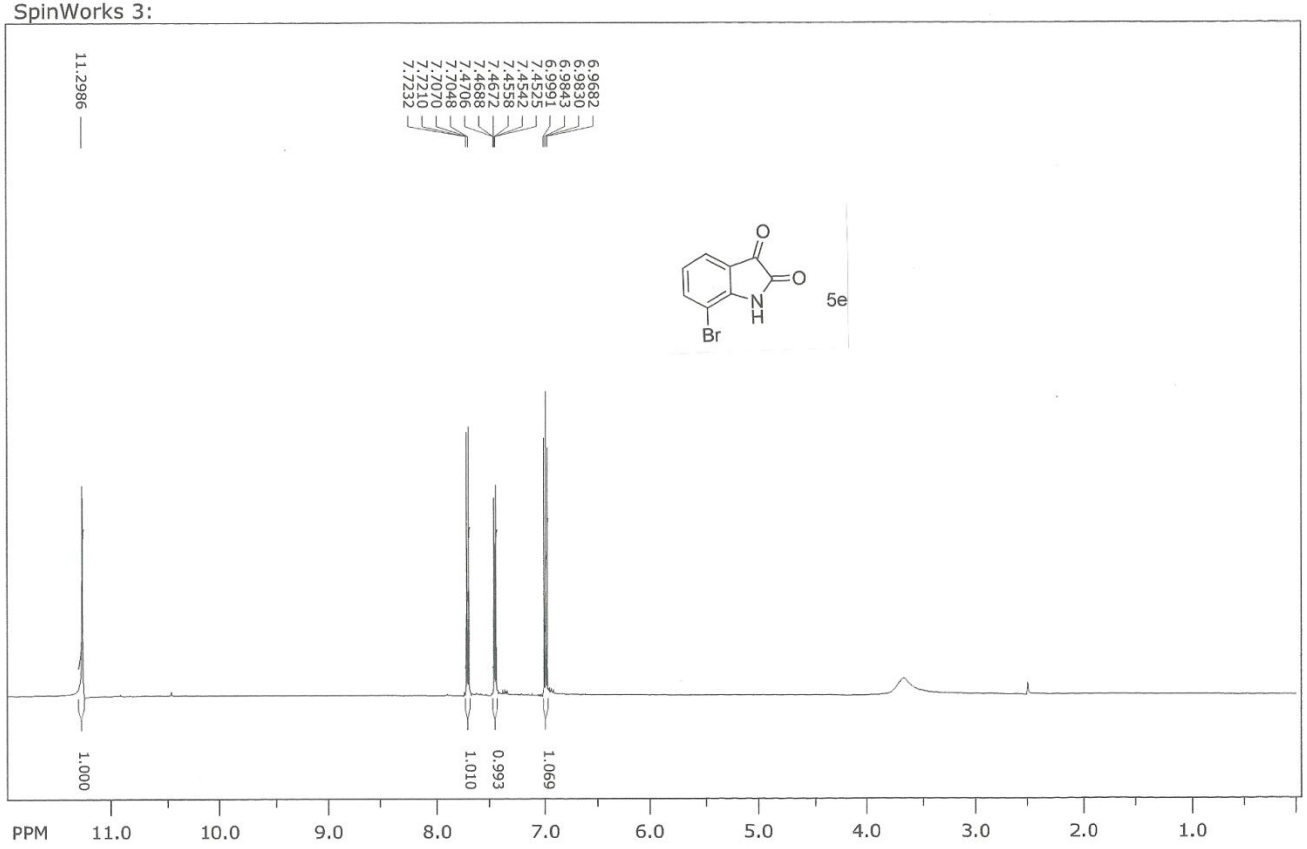
processed size: 131072 complex points
LB: 0.500 GF: 0.0000
Hz/cm: 955.007 ppm/cm: 7.59927

SpinWorks 3:



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time domain size: 32762 points
width: 7996.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 8

freq. of 0 ppm: 499.731884 MHz
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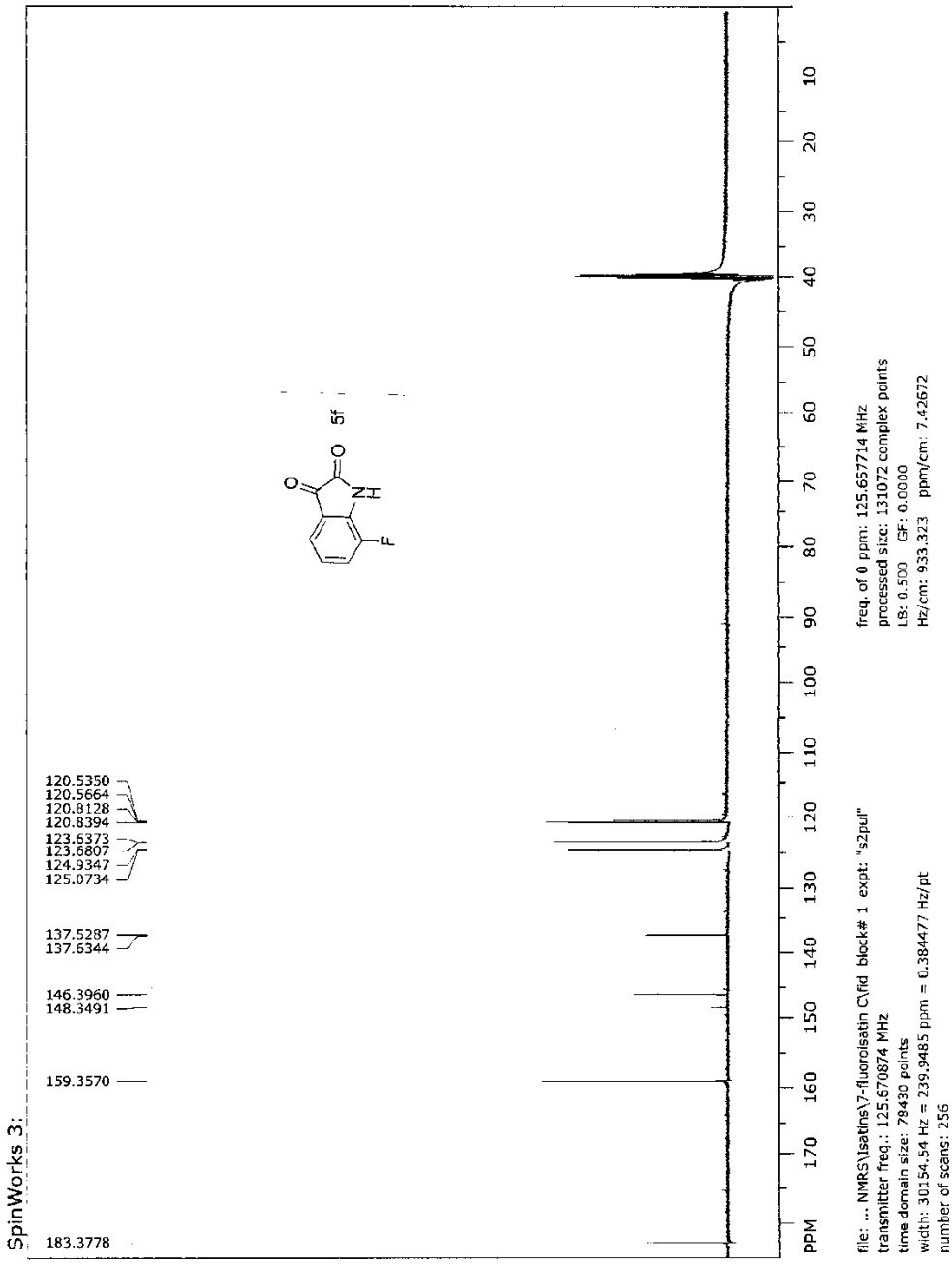
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123.6373
123.6807
124.9347
125.0734
137.5287
137.6344
146.3960
148.3491
159.3570
183.3778
PPM
file: ... NMRS\Isatins\7-fluoroisatin CYfid block# 1 expt: "s2pul"
transmitter freq.: 125.670874 MHz
time domain size: 78430 points
width: 30154.54 Hz = 239.9485 ppm = 0.384477 Hz/pt
number of scans: 256
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processed size: 131072 complex points
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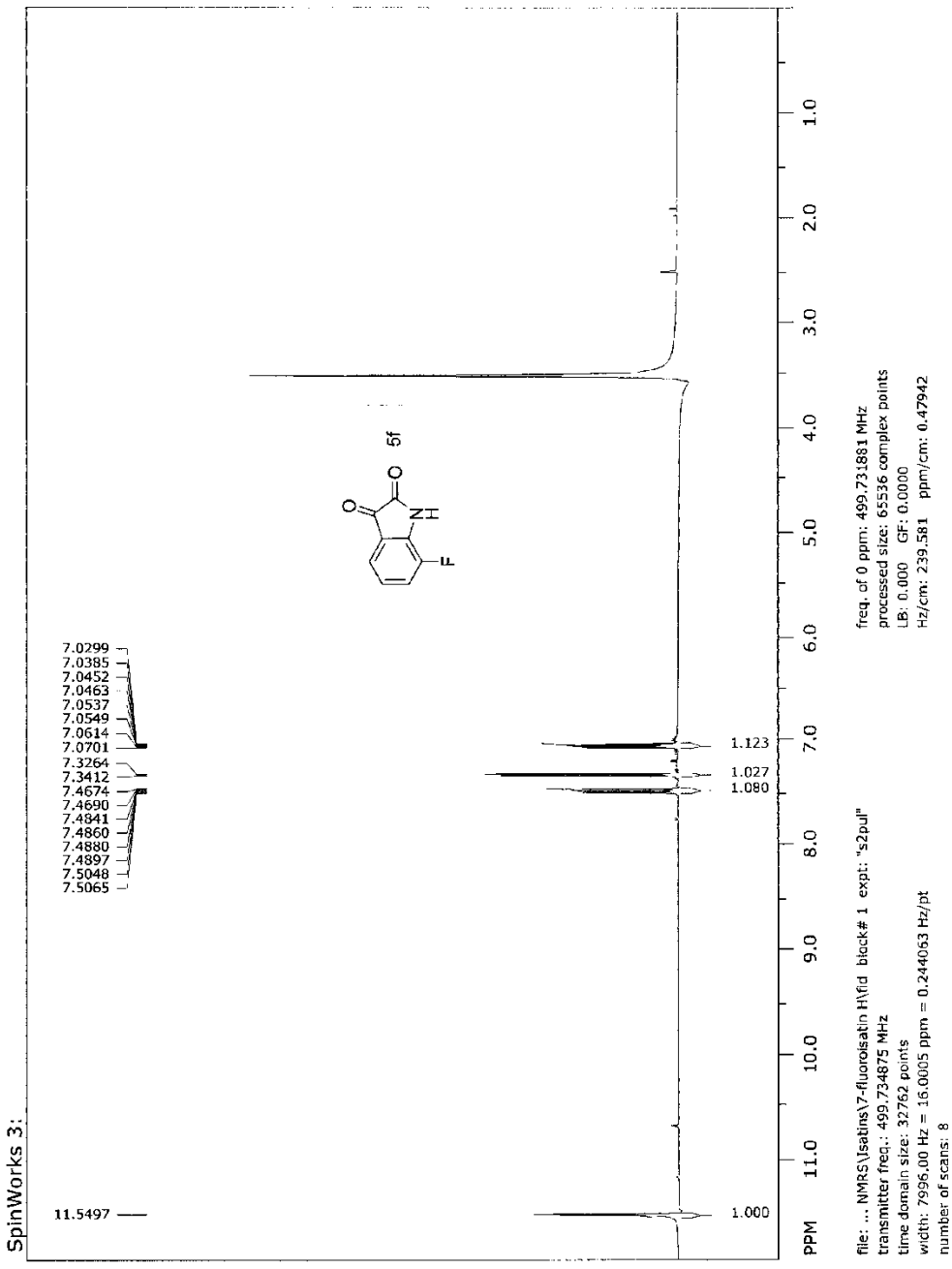
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5f
11.5497
7.0299
7.0385
7.0452
7.0463
7.0537
7.0549
7.0614
7.0701
7.3264
7.3412
7.4674
7.4690
7.4841
7.4860
7.4880
7.4897
7.5048
7.5065
1.123
1.027
1.080
1.000
PPM
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
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transmitter freq.: 499.734875 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 8
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processed size: 65536 complex points
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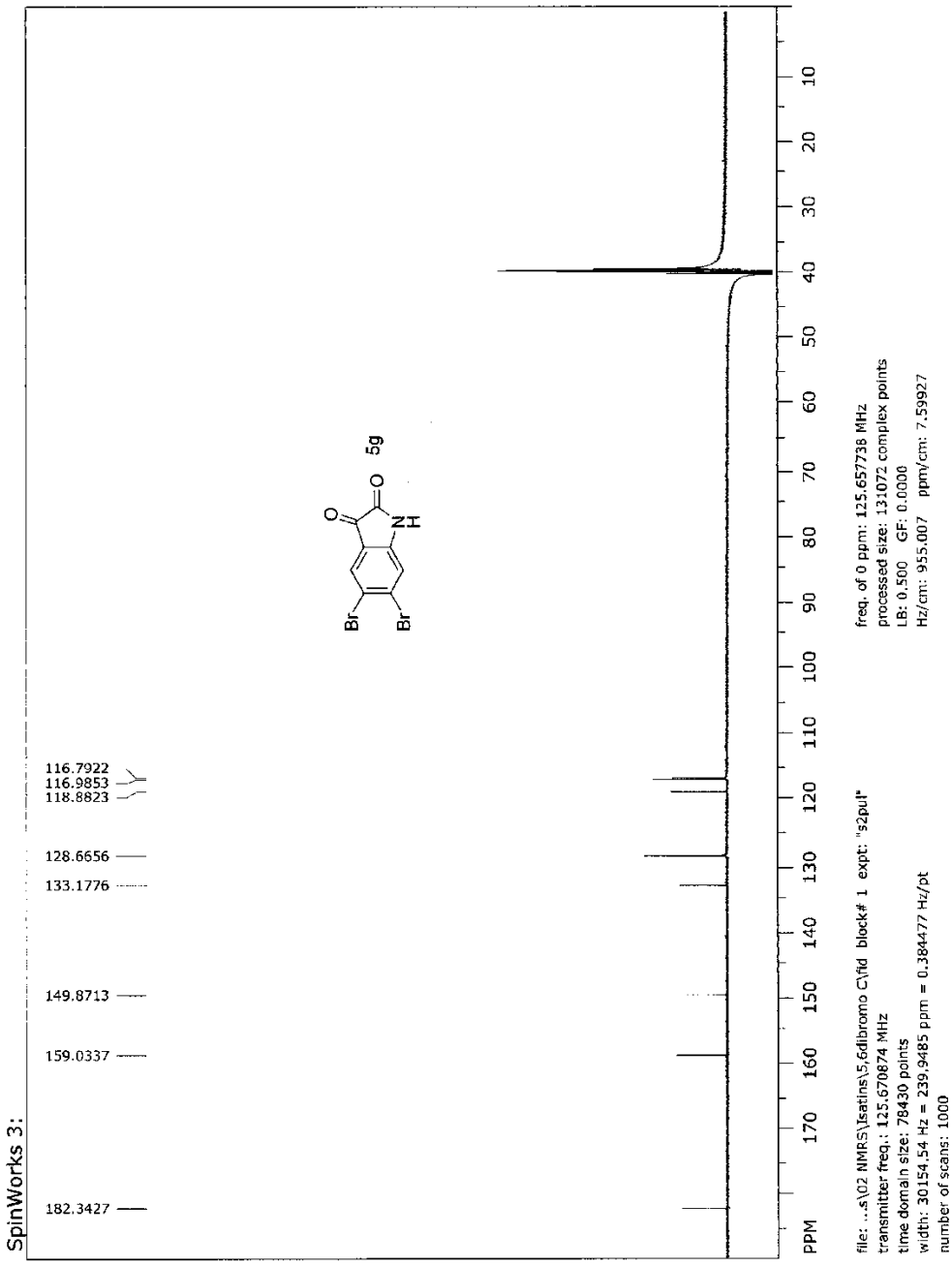
Hz/cm: 239.581 ppm/cm: 0.47942


SpinWorks 3:
116.7922
116.9853
118.8823
128.6656
133.1776
149.8713
159.0337
182.3427
5g
Br
Br
O
O
N
H
PPM
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
file: ...s\02 NMRS\Isatins\5,6dibromo C\fid block# 1 expt: "s2pul"
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time domain size: 78430 points
width: 30154.54 Hz = 239.9485 ppm = 0.384477 Hz/pt
number of scans: 1000
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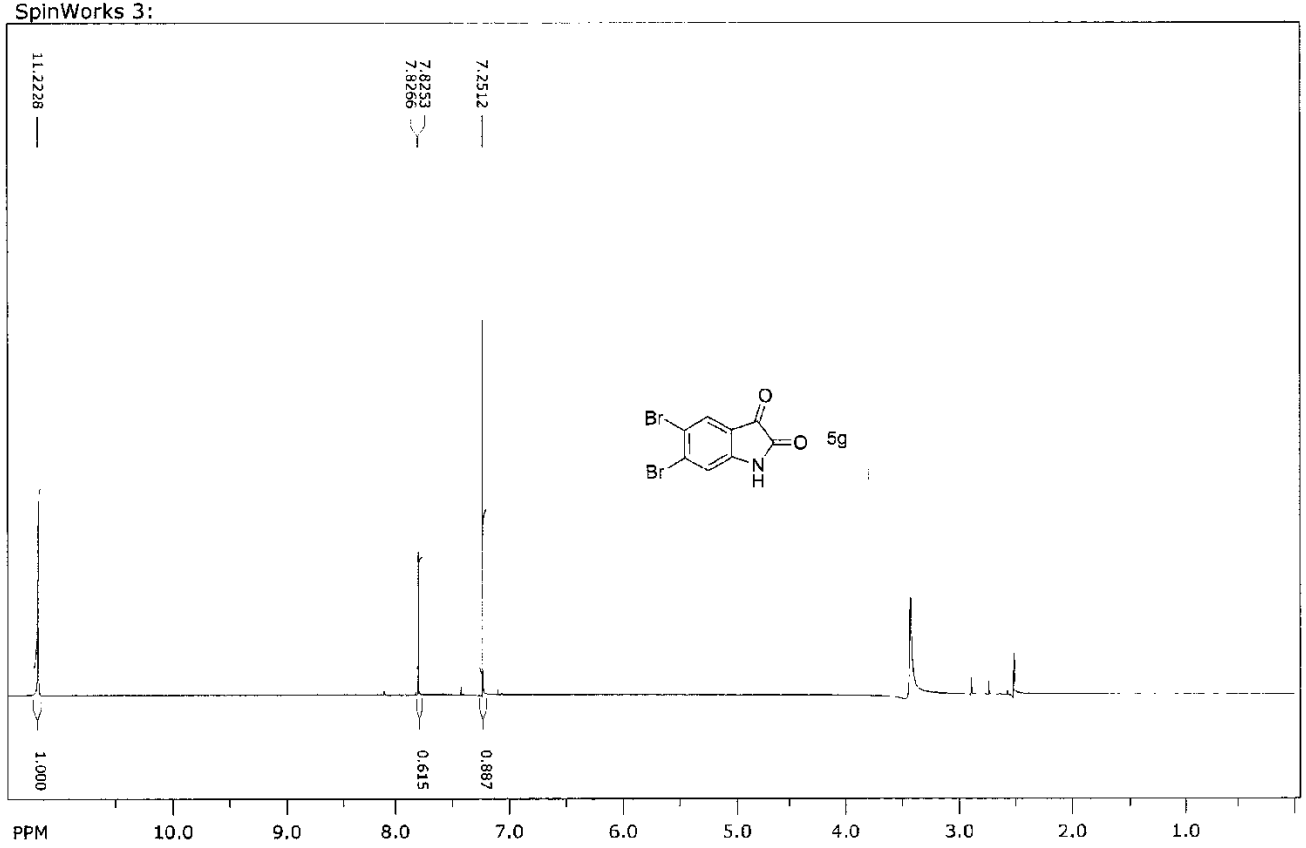
processed size: 131072 complex points
LB: 0.500 GF: 0.0000
Hz/cm: 955.007 ppm/cm: 7.59927

SpinWorks 3:



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time domain size: 32762 points
width: 7996.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 8

freq. of 0 ppm: 499.731883 MHz
processed size: 65536 complex points
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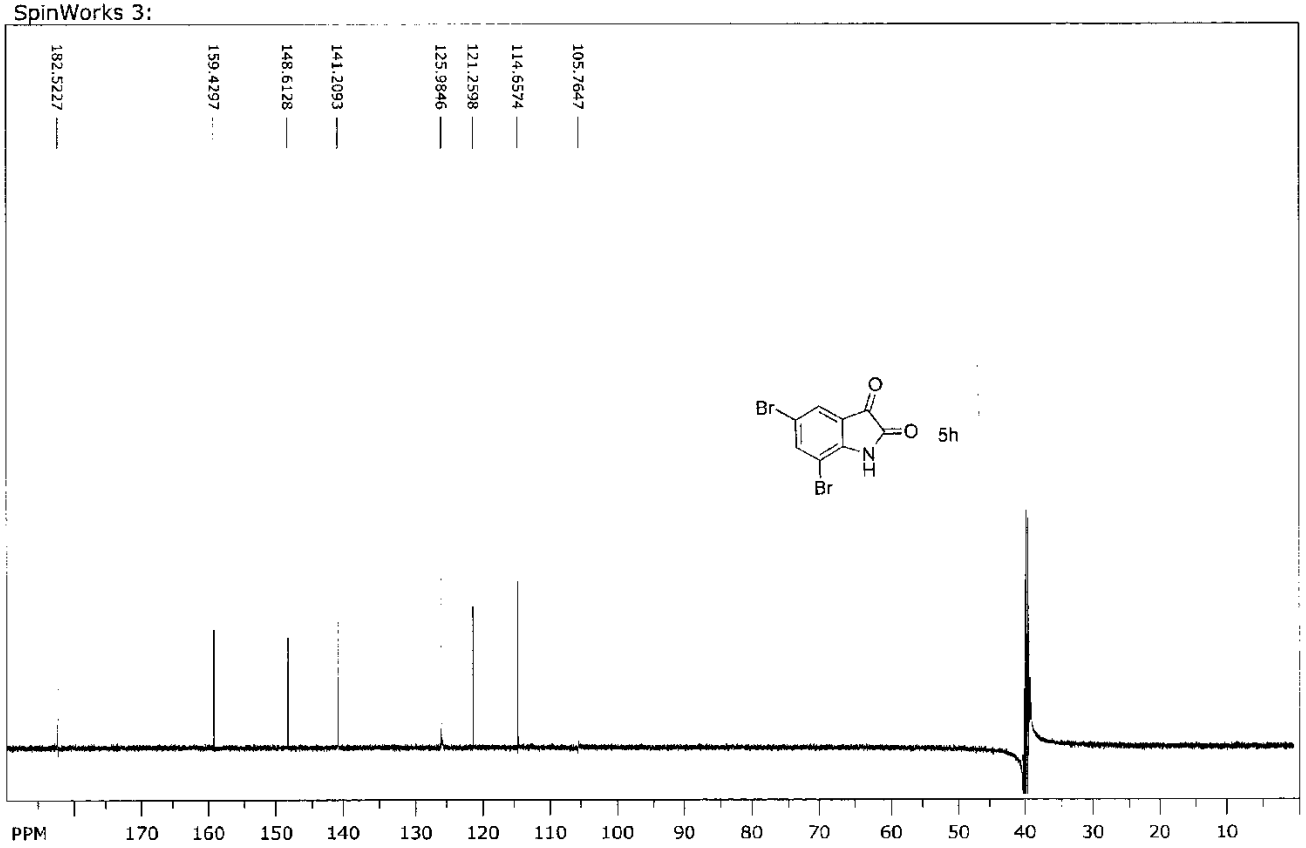
Hz/cm: 229.997 ppm/cm: 0.46024

SpinWorks 3:



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time domain size: 78430 points
width: 30154.54 Hz = 239.9485 ppm = 0.384477 Hz/pt
number of scans: 256

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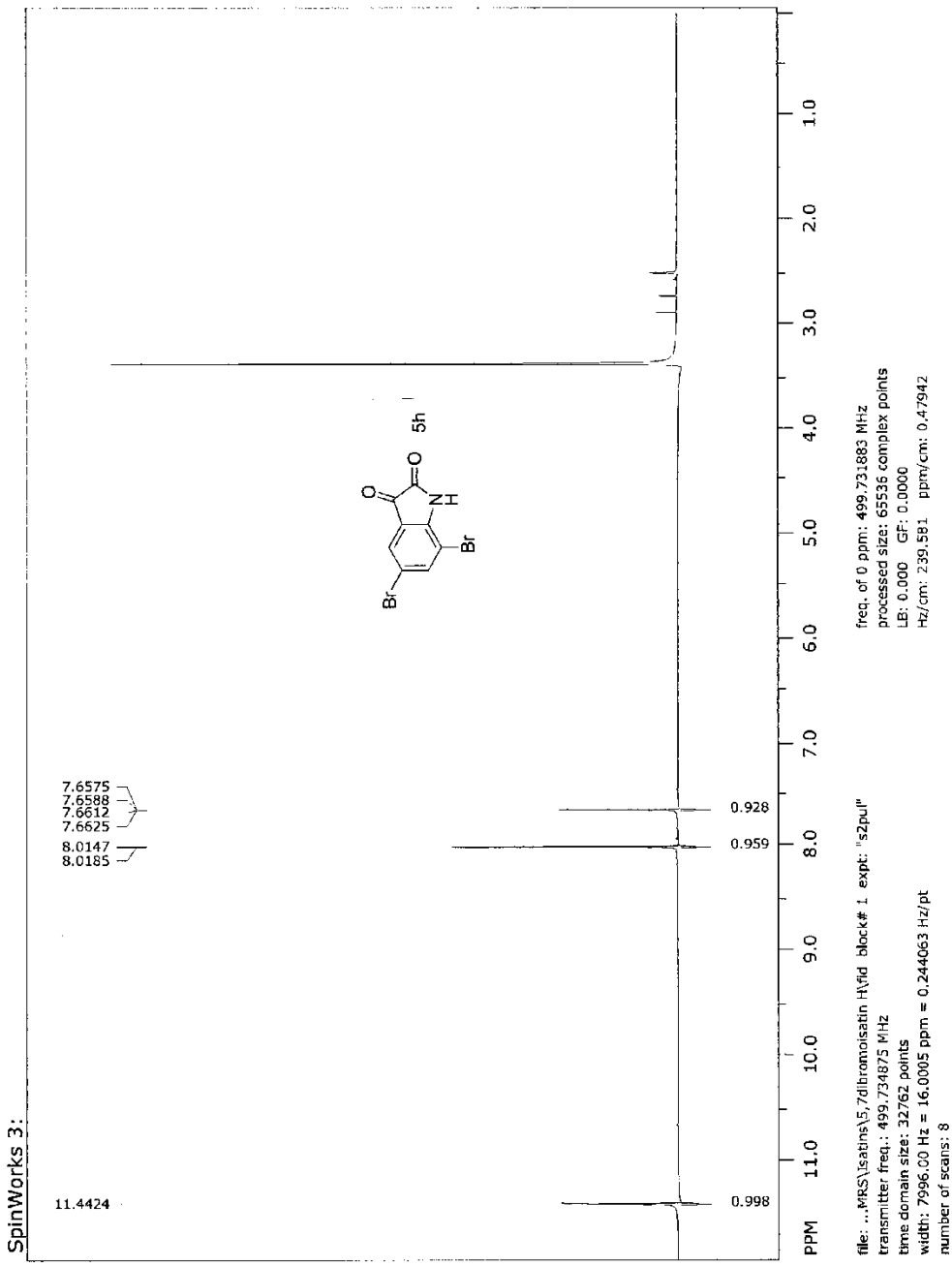
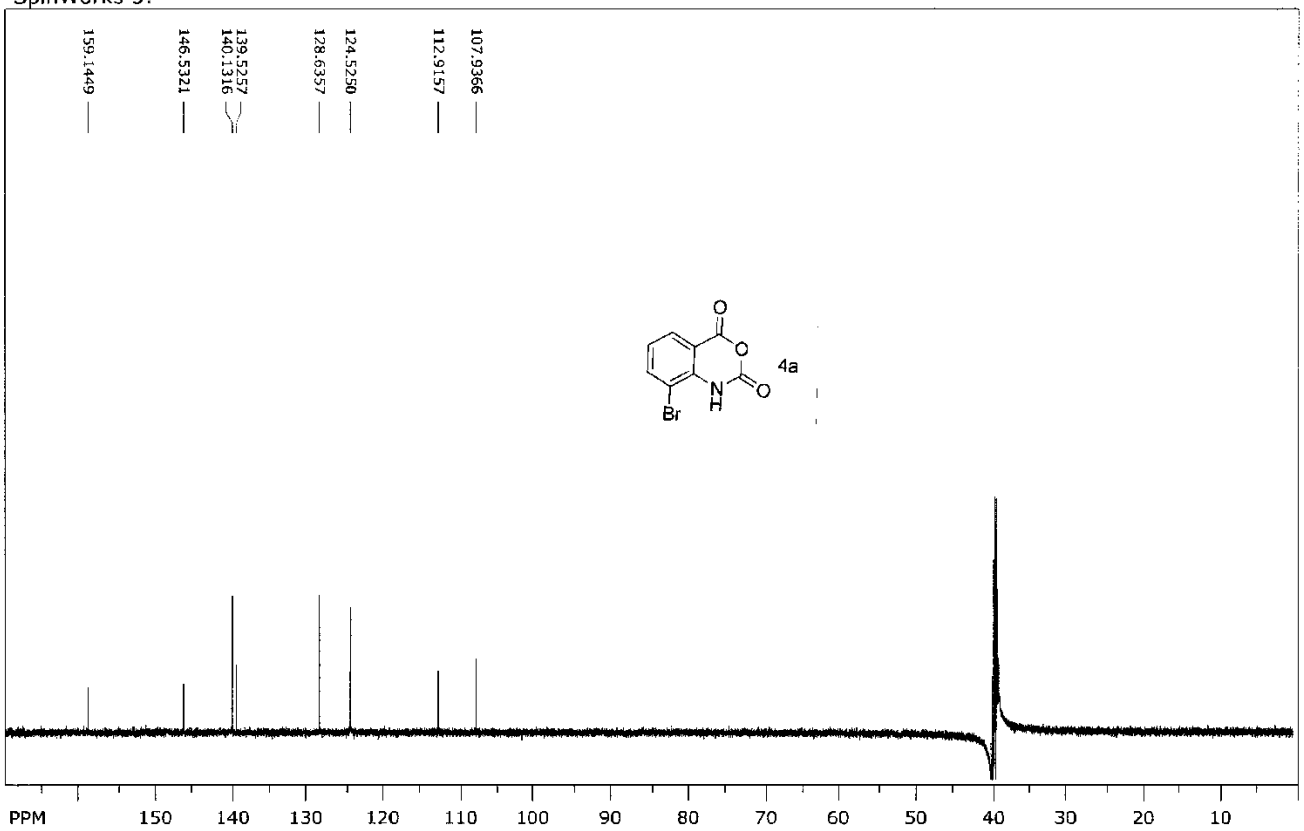
Hz/cm: 955.007 ppm/cm: 7.59927

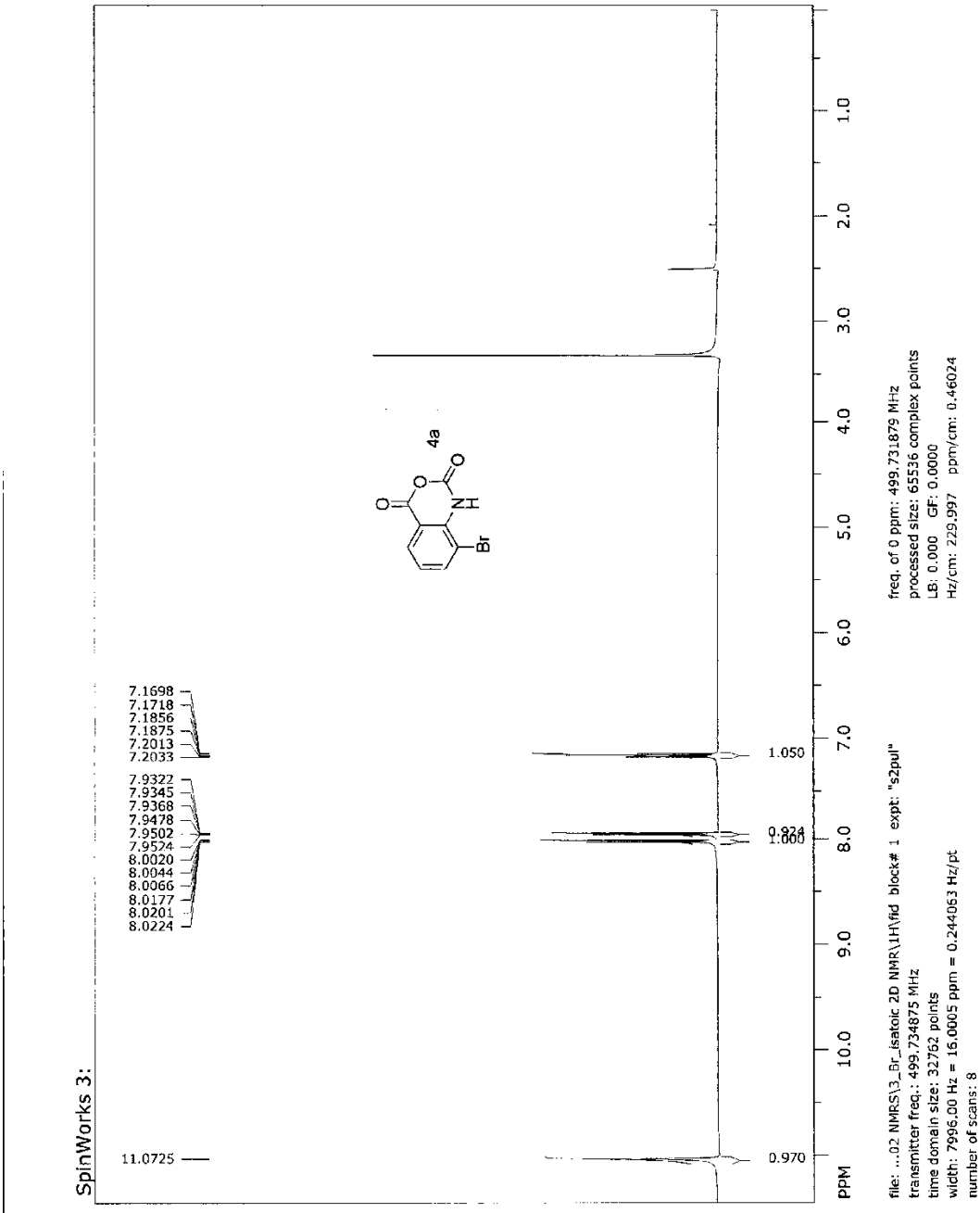

SpinWorks 3:
5h
Br
Br
O
O
N
H
11.4424
7.6575
7.6588
7.6612
7.6625
8.0147
8.0185
0.998
0.959
0.928
PPM
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
file: ...MRS\1satins\5,7dibromoisatin H\fid block# 1 expt: "s2pul"
transmitter freq.: 499.734875 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 8
freq. of 0 ppm: 499.731883 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 239.581 ppm/cm: 0.47942

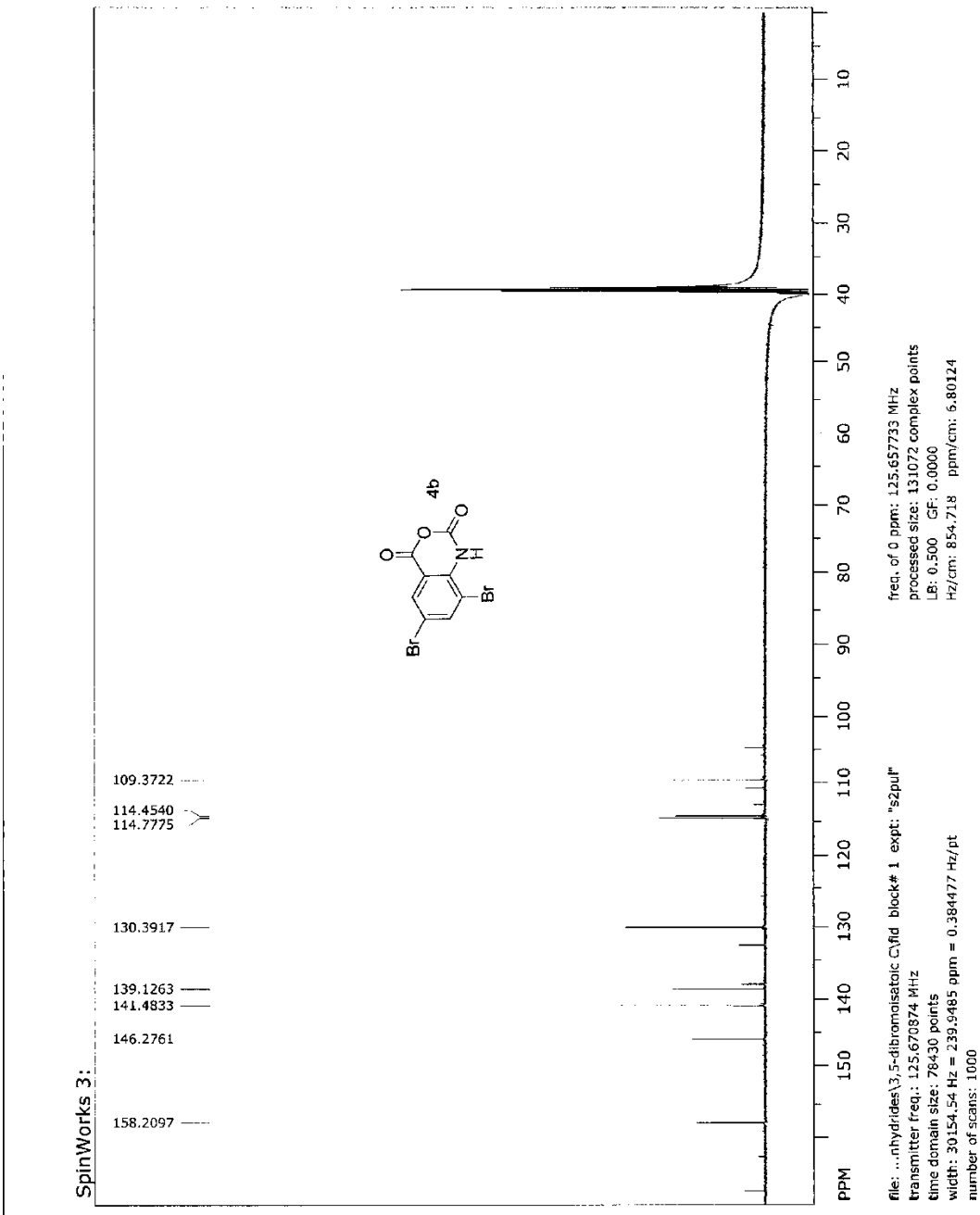
SpinWorks 3:



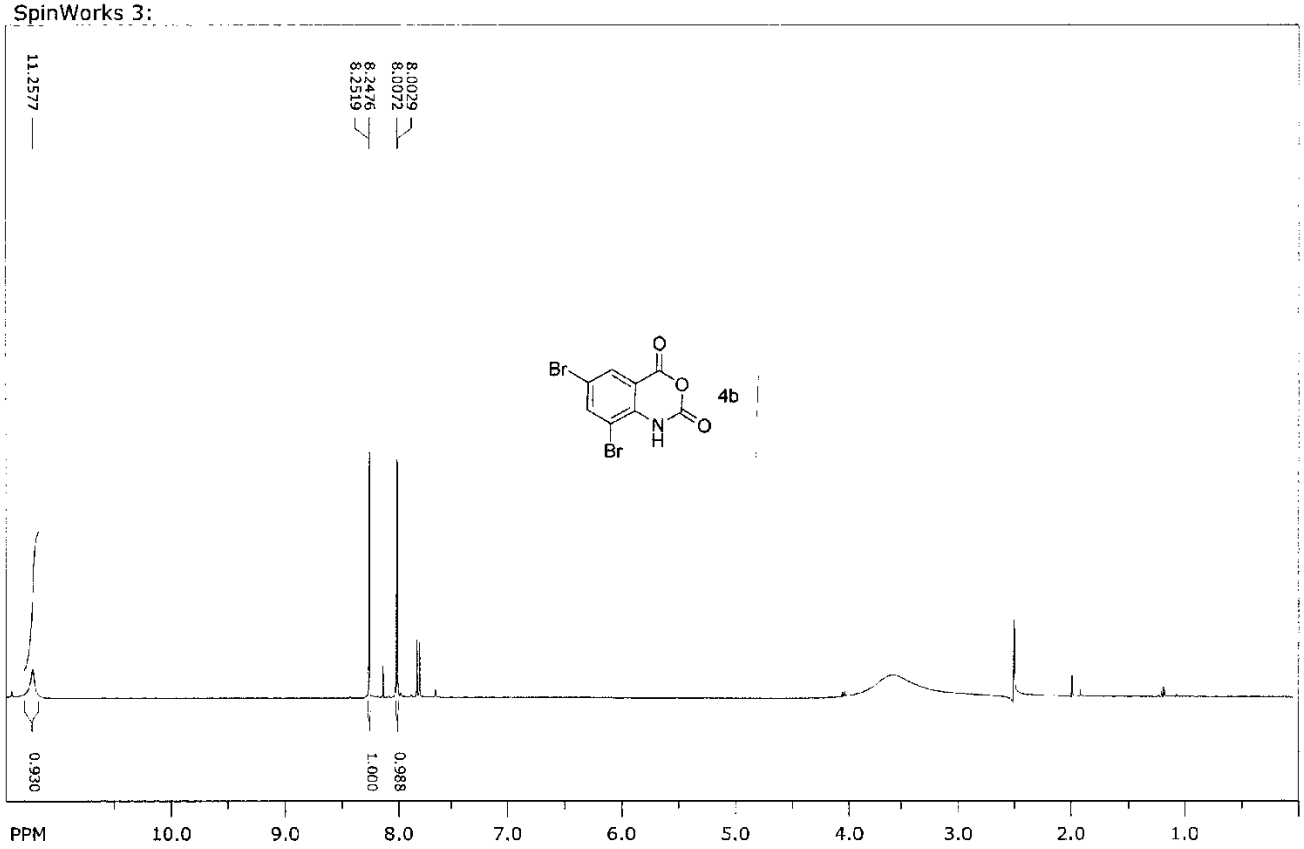
file: ...MRS\3_Br_Isatoic 2D NMR\Carbon\fid block# 1 expt: "s2pul"
transmitter freq.: 125.670874 MHz
time domain size: 78430 points
width: 30154.54 Hz = 239.9485 ppm = 0.384477 Hz/pt
number of scans: 128

freq. of 0 ppm: 125.657737 MHz
processed size: 65536 complex points
LB: 0.500 GF: 0.0000
Hz/cm: 854.718 ppm/cm: 6.80124


SpinWorks 3:
4a
Br
11.0725
7.1698
7.1718
7.1856
7.1875
7.2013
7.2033
7.9322
7.9345
7.9368
7.9478
7.9502
7.9524
8.0020
8.0044
8.0066
8.0177
8.0201
8.0224
1.050
0.924
1.000
0.970
PPM
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
file: ...02 NMRS\13_Br_isatoic 2D NMR\1H\fid block# 1 expt: "s2pul"
transmitter freq.: 499.734875 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 8
freq. of 0 ppm: 499.731879 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 229.997 ppm/cm: 0.46024

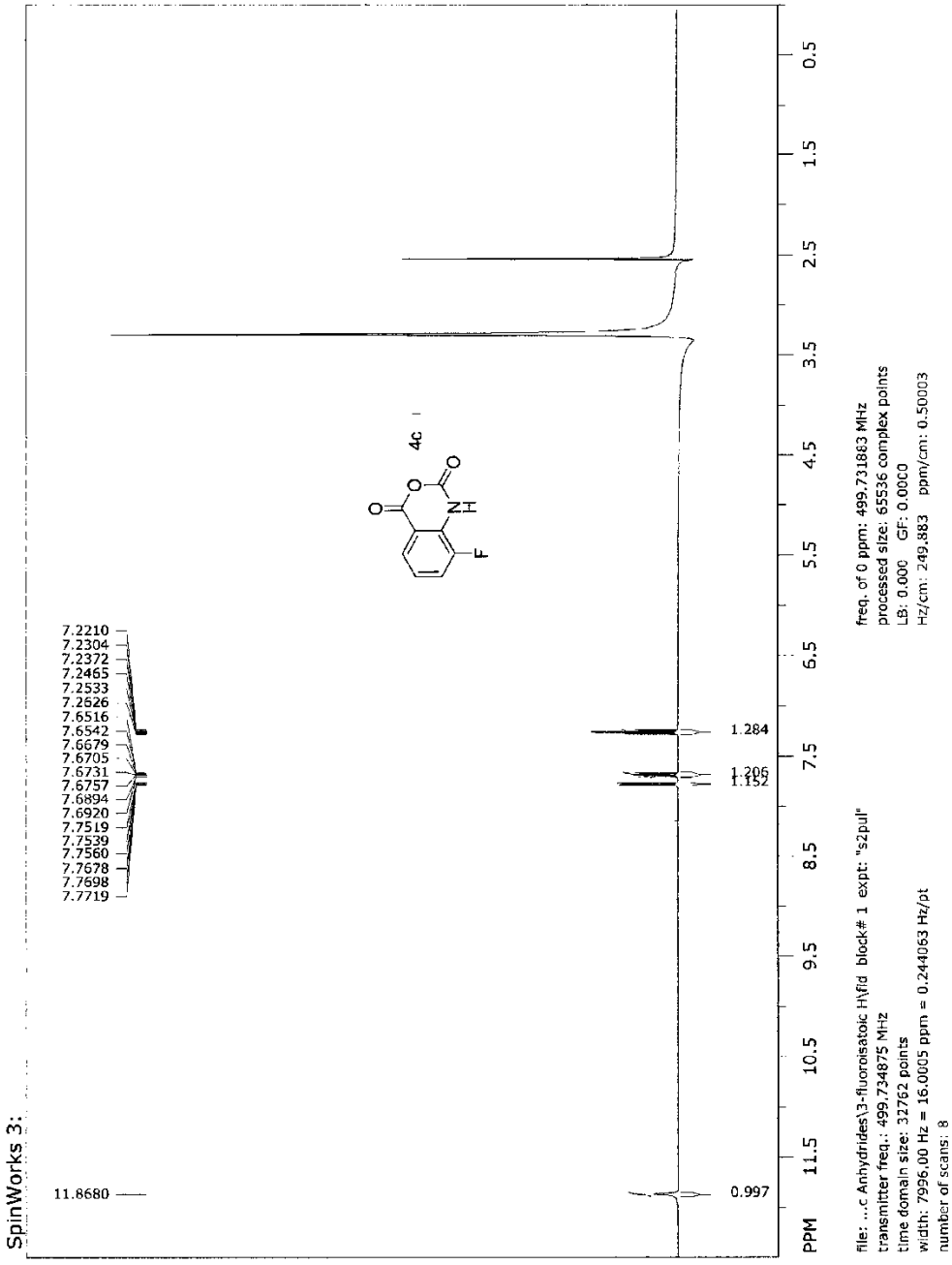

SpinWorks 3:
4b
109.3722
114.4540
114.7775
130.3917
139.1263
141.4833
146.2761
158.2097
PPM
file: ...nhydrides\3,5-dibromoisatoic C\fid block# 1 expt: "s2pul"
transmitter freq.: 125.670874 MHz
time domain size: 78430 points
width: 30154.54 Hz = 239.9485 ppm = 0.384477 Hz/pt
number of scans: 1000
freq. of 0 ppm: 125.657733 MHz
processed size: 131072 complex points
LB: 0.500 GF: 0.0000
Hz/cm: 854.718 ppm/cm: 6.80124

SpinWorks 3:

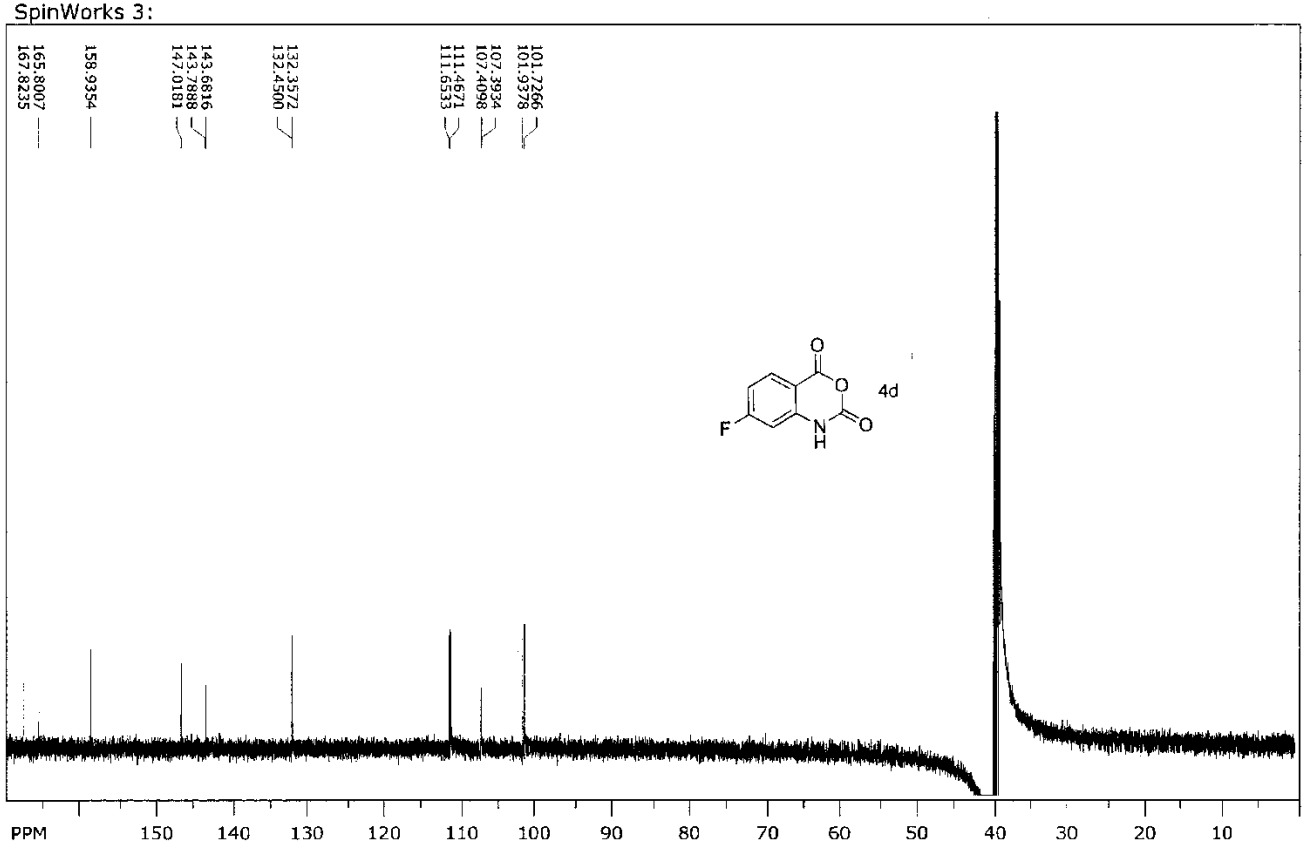


file: ...nhydrides\3,5-dibromoisatoic H\fid block# 1 expt: "s2pul"
transmitter freq.: 499.734875 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 8

freq. of 0 ppm: 499.731882 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 229.758 ppm/cm: 0.45976

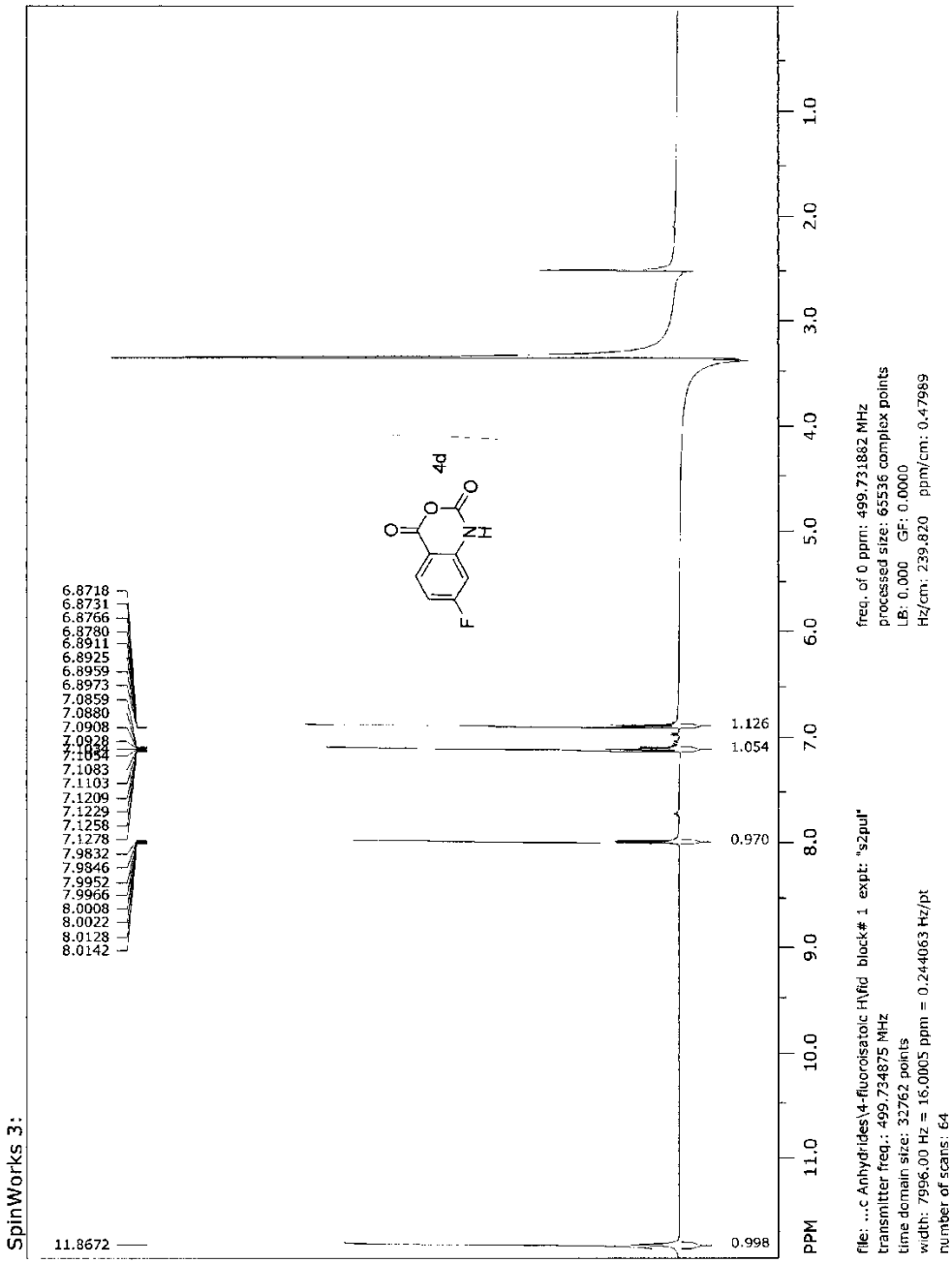

SpinWorks 3:
4c
7.2210
7.2304
7.2372
7.2465
7.2533
7.2626
7.6516
7.6542
7.6679
7.6705
7.6731
7.6757
7.6894
7.6920
7.7519
7.7539
7.7560
7.7678
7.7698
7.7719
11.8680
1.284
1.206
1.152
0.997
PPM
freq. of 0 ppm: 499.731883 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 249.883 ppm/cm: 0.50003
file: ...c Anhydrides\3-fluoroisatoic H\fid block# 1 expt: "s2pul"
transmitter freq.: 499.734875 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 8

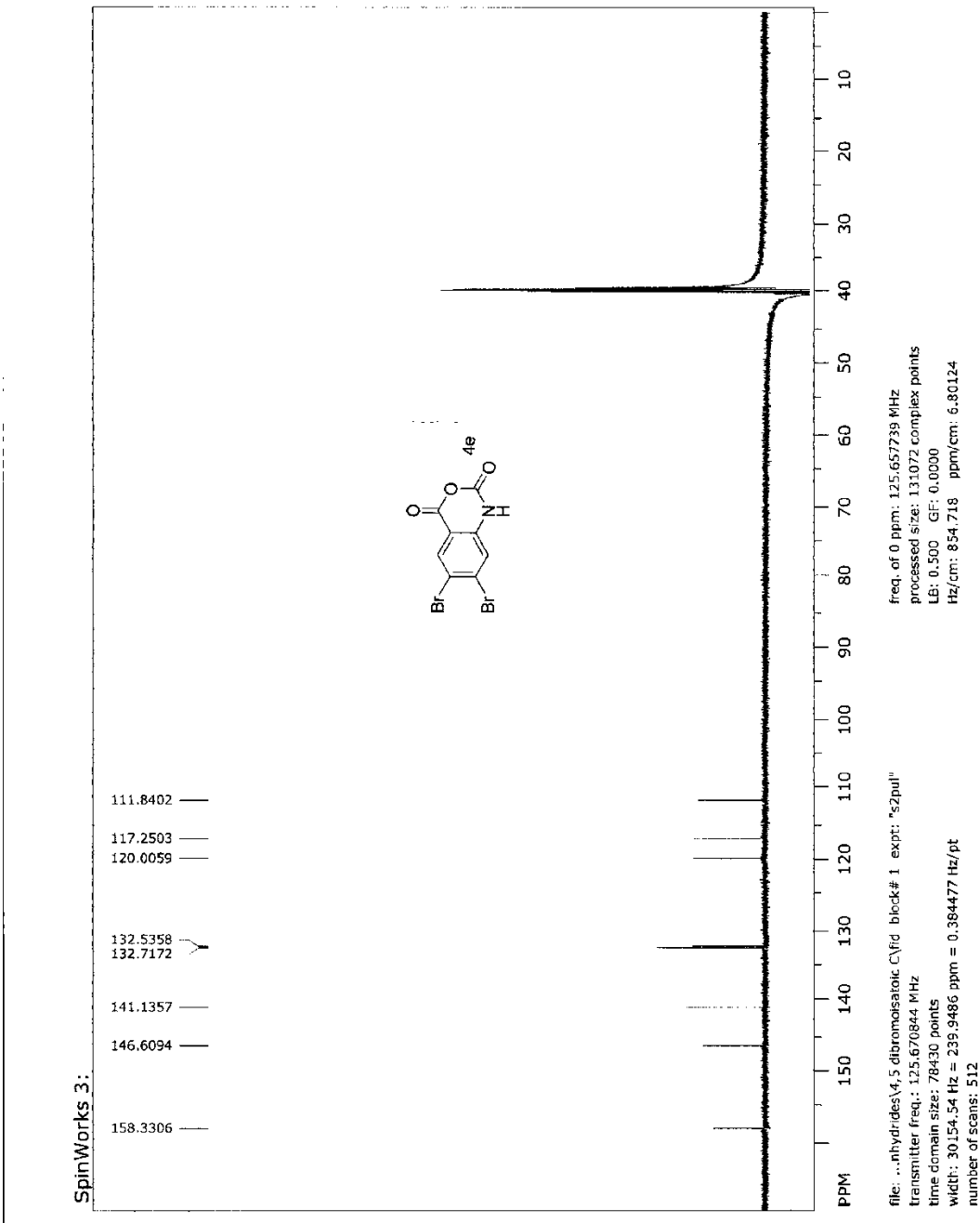
SpinWorks 3:



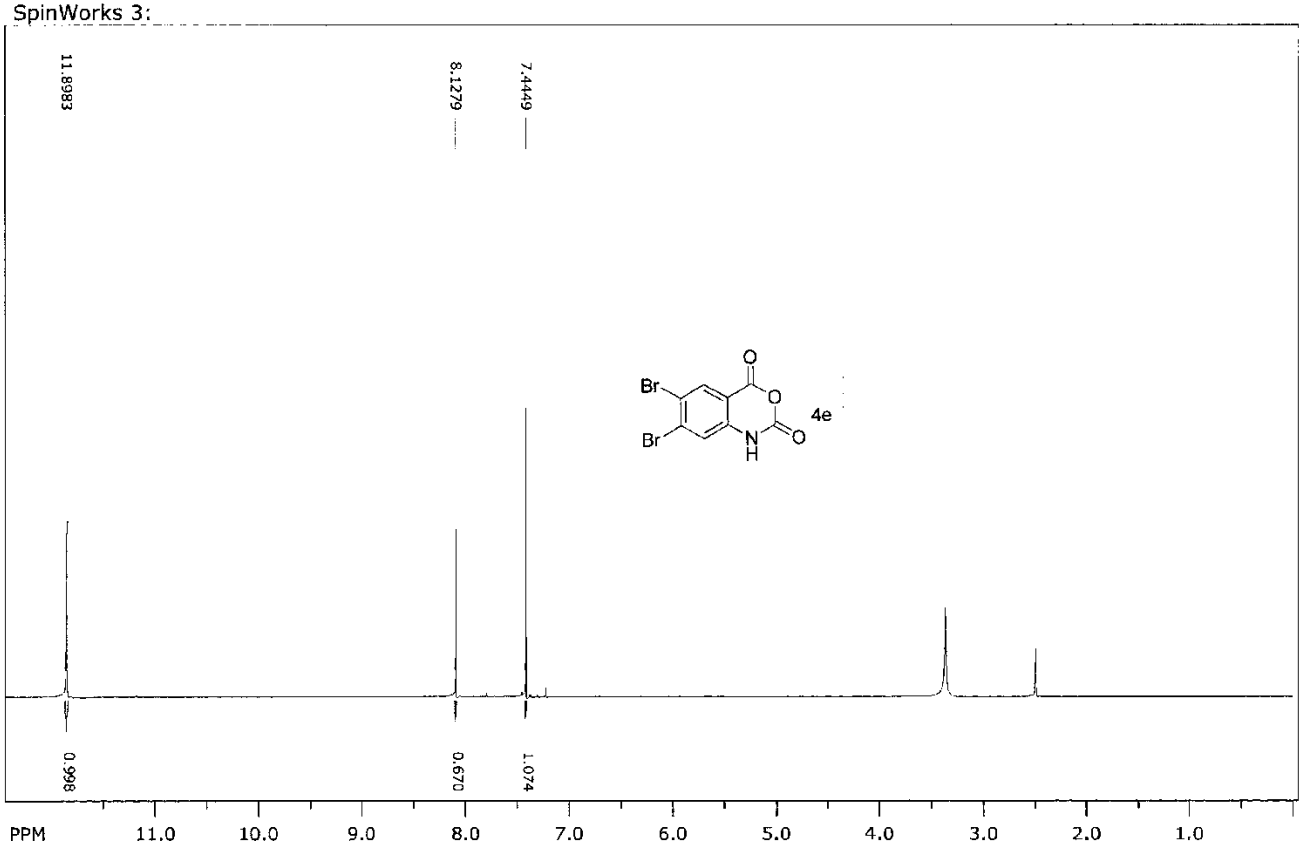
file: ...c Anhydrides\4-fluoroisatoic C\fid block# 1 expt: "s2pul"
transmitter freq.: 125.670874 MHz
time domain size: 78430 points
width: 30154.54 Hz = 239.9485 ppm = 0.384477 Hz/pt
number of scans: 1000

freq. of 0 ppm: 125.657737 MHz
processed size: 131072 complex points
LB: 0.500 GF: 0.0000
Hz/cm: 854.718 ppm/cm: 6.80124


SpinWorks 3:
4d
F
11.8672
6.8718
6.8731
6.8766
6.8780
6.8911
6.8925
6.8959
6.8973
7.0859
7.0880
7.0908
7.0928
7.1083
7.1103
7.1209
7.1229
7.1258
7.1278
7.9832
7.9846
7.9952
7.9966
8.0008
8.0022
8.0128
8.0142
1.126
1.054
0.970
0.998
PPM
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
file: ...c Anhydrides\4-fluoroisatoic Hyfid block# 1 expt: "s2pul"
transmitter freq.: 499.734875 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 64
freq. of 0 ppm: 499.731882 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 239.820 ppm/cm: 0.47989

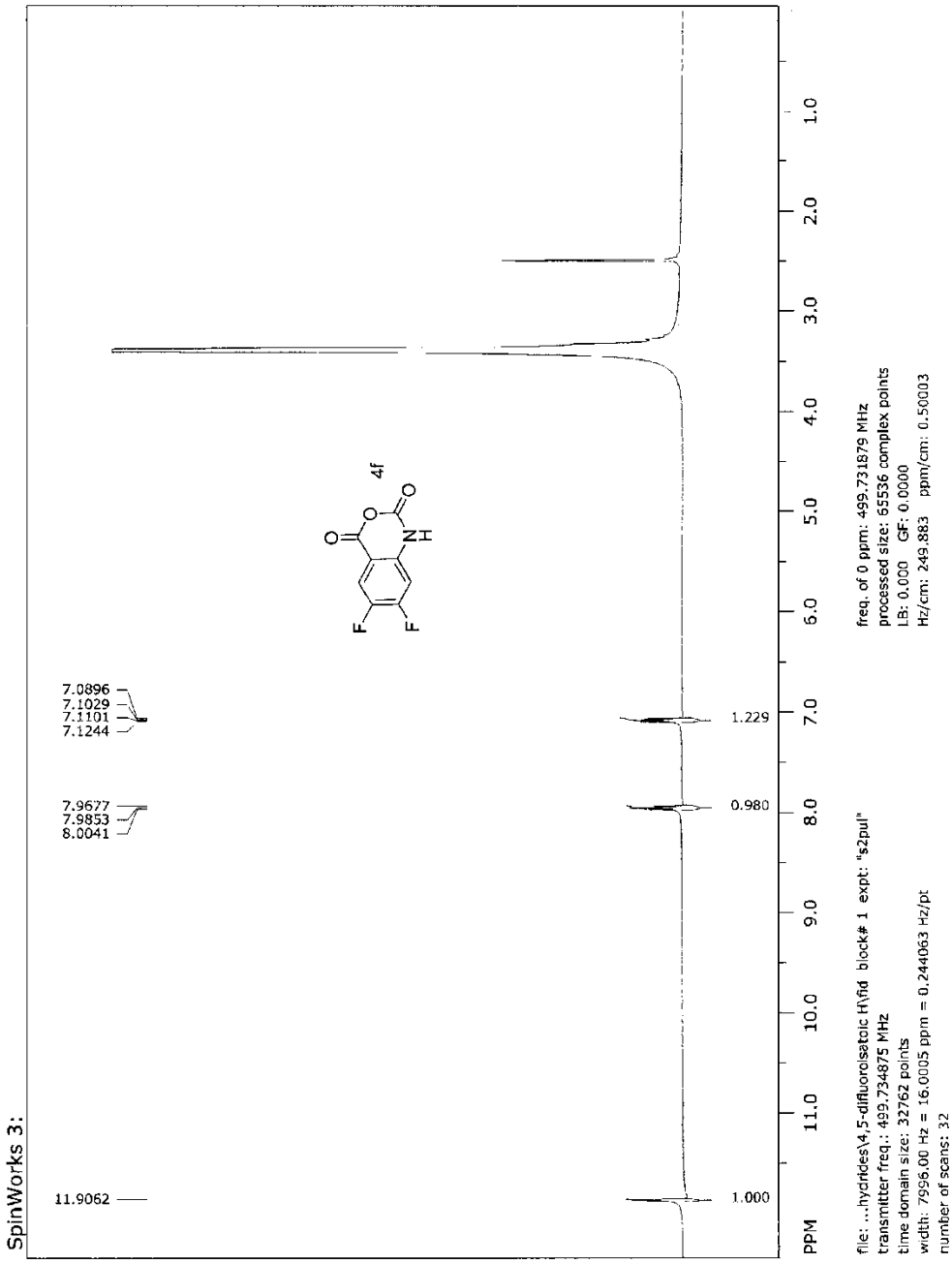

SpinWorks 3:
111.8402
117.2503
120.0059
132.5358
132.7172
141.1357
146.6094
158.3306
4e
Br
Br
O
O
N
H
O
PPM
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
file: ...nhydrides\4,5 dibromoisatoic C\fid block# 1 expt: "s2pul"
transmitter freq.: 125.670844 MHz
time domain size: 78430 points
width: 30154.54 Hz = 239.9486 ppm = 0.384477 Hz/pt
number of scans: 512
freq. of 0 ppm: 125.657739 MHz
processed size: 131072 complex points
LB: 0.500 GF: 0.0000
Hz/cm: 854.718 ppm/cm: 6.80124

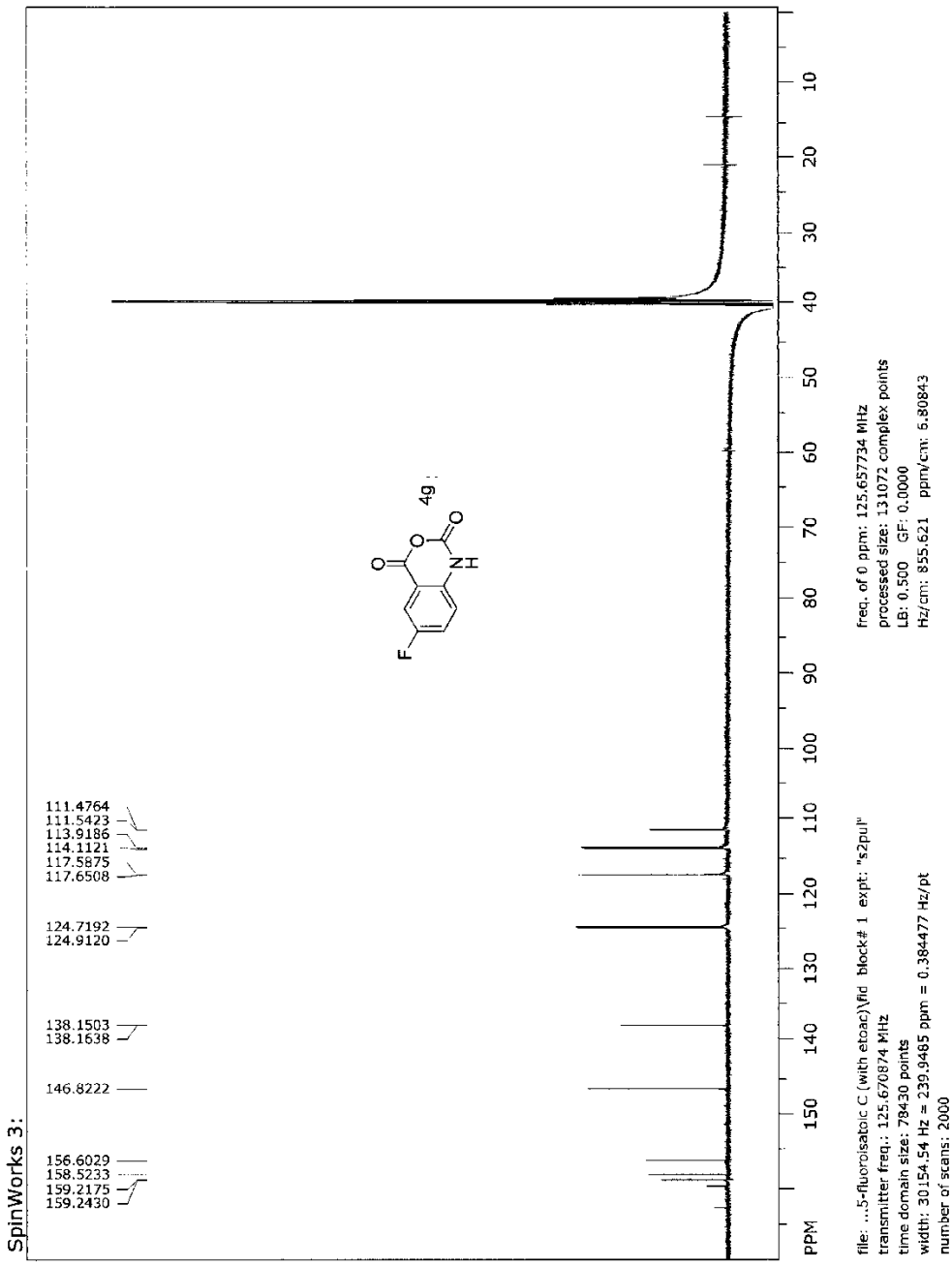
SpinWorks 3:

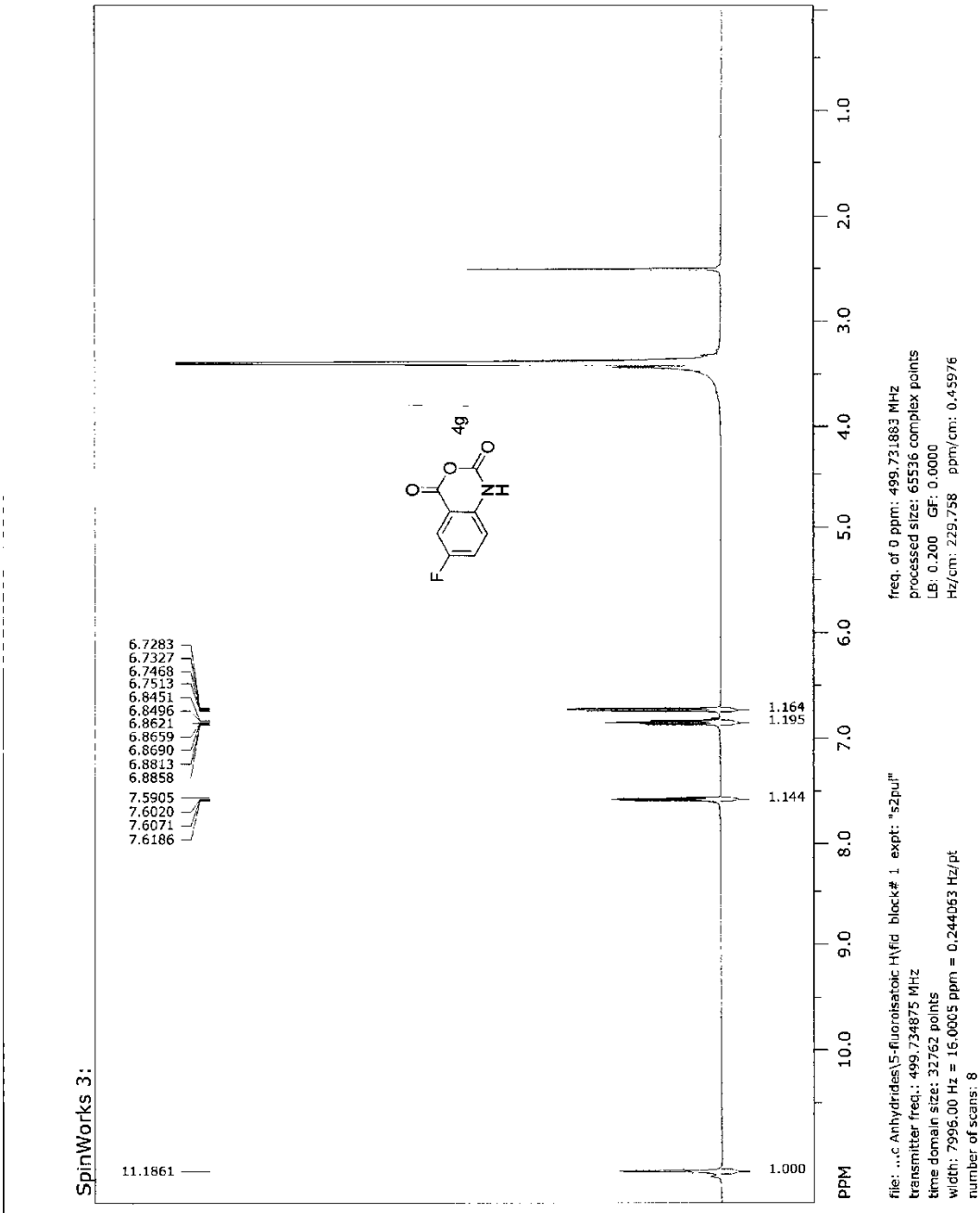


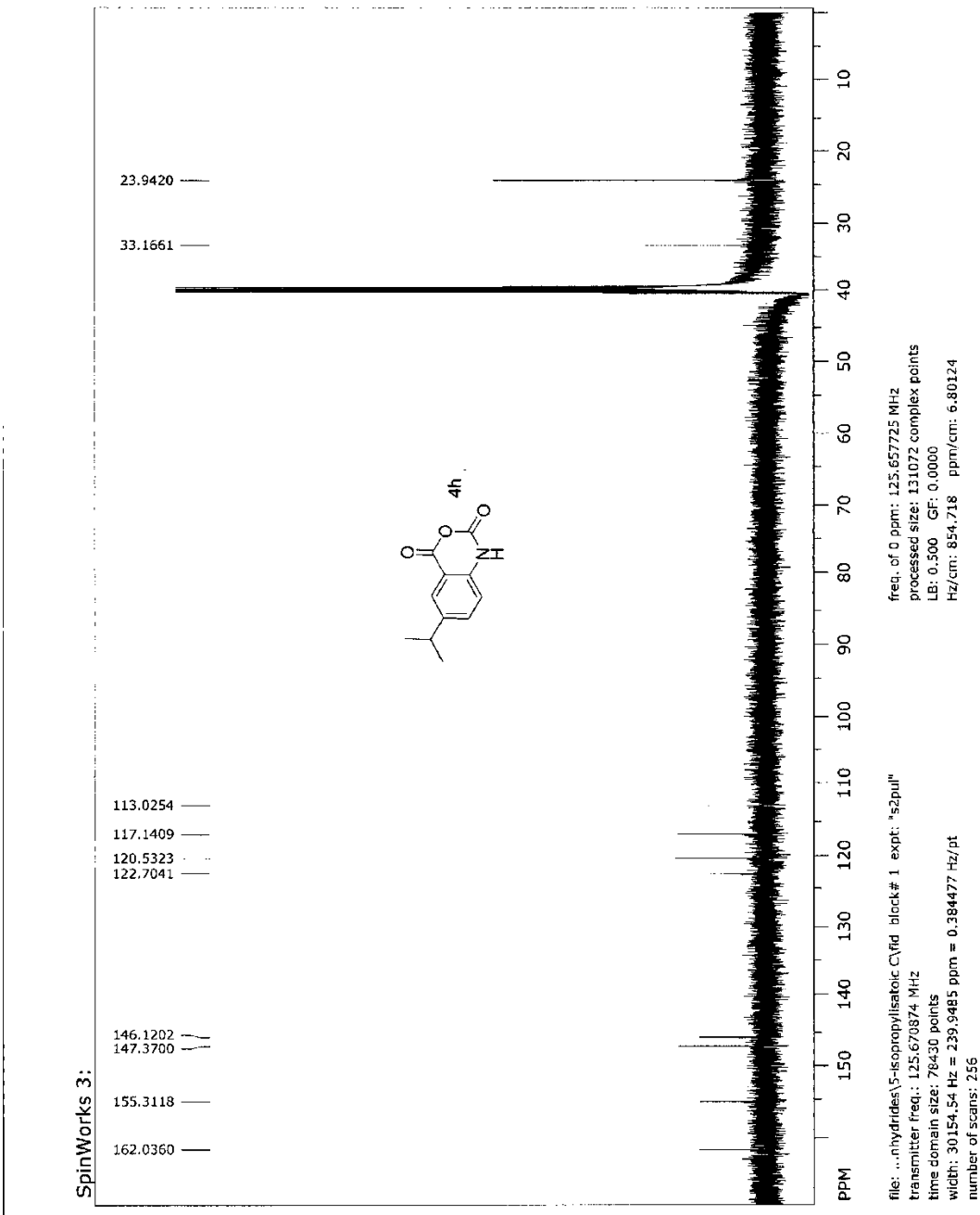
file: ...nhydrides\4,5 dibromoisatoic H\fid block# 1 expt: "s2pul"
transmitter freq.: 499.734875 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 8

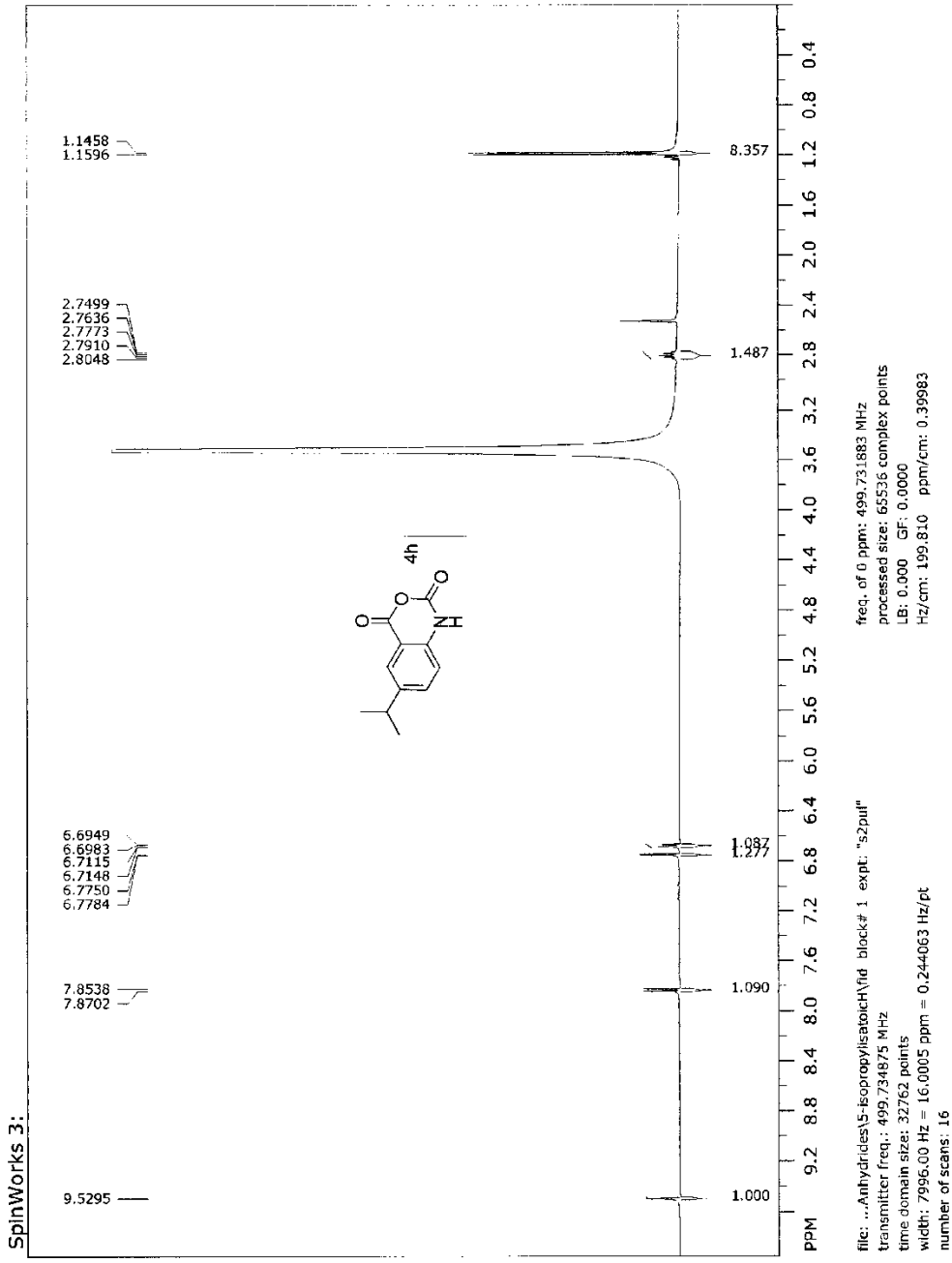
freq. of 0 ppm: 499.731884 MHz
processed size: 65536 complex points
LB: 0.200 GF: 0.0000
Hz/cm: 249.883 ppm/cm: 0.50003


SpinWorks 3:
4f
F
F
O
O
O
N
H
7.0896
7.1029
7.1101
7.1244
7.9677
7.9853
8.0041
11.9062
1.229
0.980
1.000
PPM
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
file: ...hydrides\4,5-difluoroisatoic H\fid block# 1 expt: "s2pul"
transmitter freq.: 499.734875 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 32
freq. of 0 ppm: 499.731879 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 249.883 ppm/cm: 0.50003

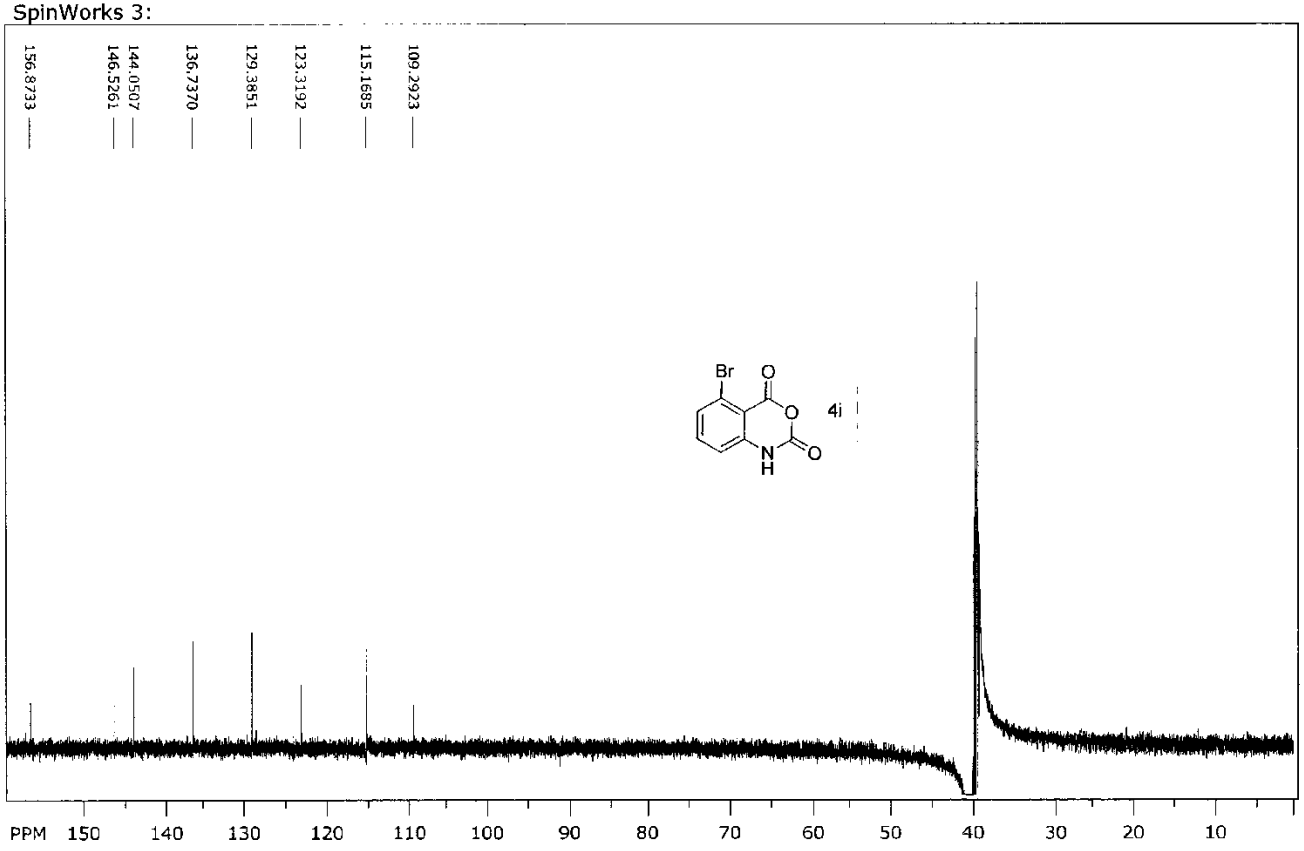

SpinWorks 3:
4g
111.4764
111.5423
113.9186
114.1121
117.5875
117.6508
124.7192
124.9120
138.1503
138.1638
146.8222
156.6029
158.5233
159.2175
159.2430
PPM
file: ...5-fluoroisatoic C (with etoac)\fid block# 1 expt: "s2pul"
transmitter freq.: 125.670874 MHz
time domain size: 78430 points
width: 30154.54 Hz = 239.9485 ppm = 0.384477 Hz/pt
number of scans: 2000
freq. of 0 ppm: 125.657734 MHz
processed size: 131072 complex points
LB: 0.500 GF: 0.0000
Hz/cm: 855.621 ppm/cm: 6.80843


SpinWorks 3:
4g
F
6.7283
6.7327
6.7468
6.7513
6.8451
6.8496
6.8621
6.8659
6.8690
6.8813
6.8858
7.5905
7.6020
7.6071
7.6186
11.1861
1.164
1.195
1.144
1.000
PPM
file: ...c Anhydrides\5-fluoroisatoic HYfid block# 1 expt: "s2pul"
transmitter freq.: 499.734875 MHz
time domain size: 32762 points
width: 7995.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 8
freq. of 0 ppm: 499.731883 MHz
processed size: 65536 complex points
LB: 0.200 GF: 0.0000
Hz/cm: 229.758 ppm/cm: 0.45976


SpinWorks 3:
23.9420
33.1661
113.0254
117.1409
120.5323
122.7041
146.1202
147.3700
155.3118
162.0360
4h
PPM
file: ...nhydrides\5-isopropylisatoic.CYfid block# 1 expt: "s2pul"
transmitter freq.: 125.670874 MHz
time domain size: 78430 points
width: 30154.54 Hz = 239.9485 ppm = 0.384477 Hz/pt
number of scans: 256
freq. of 0 ppm: 125.657725 MHz
processed size: 131072 complex points
LB: 0.500 GF: 0.0000
Hz/cm: 854.718 ppm/cm: 6.80124

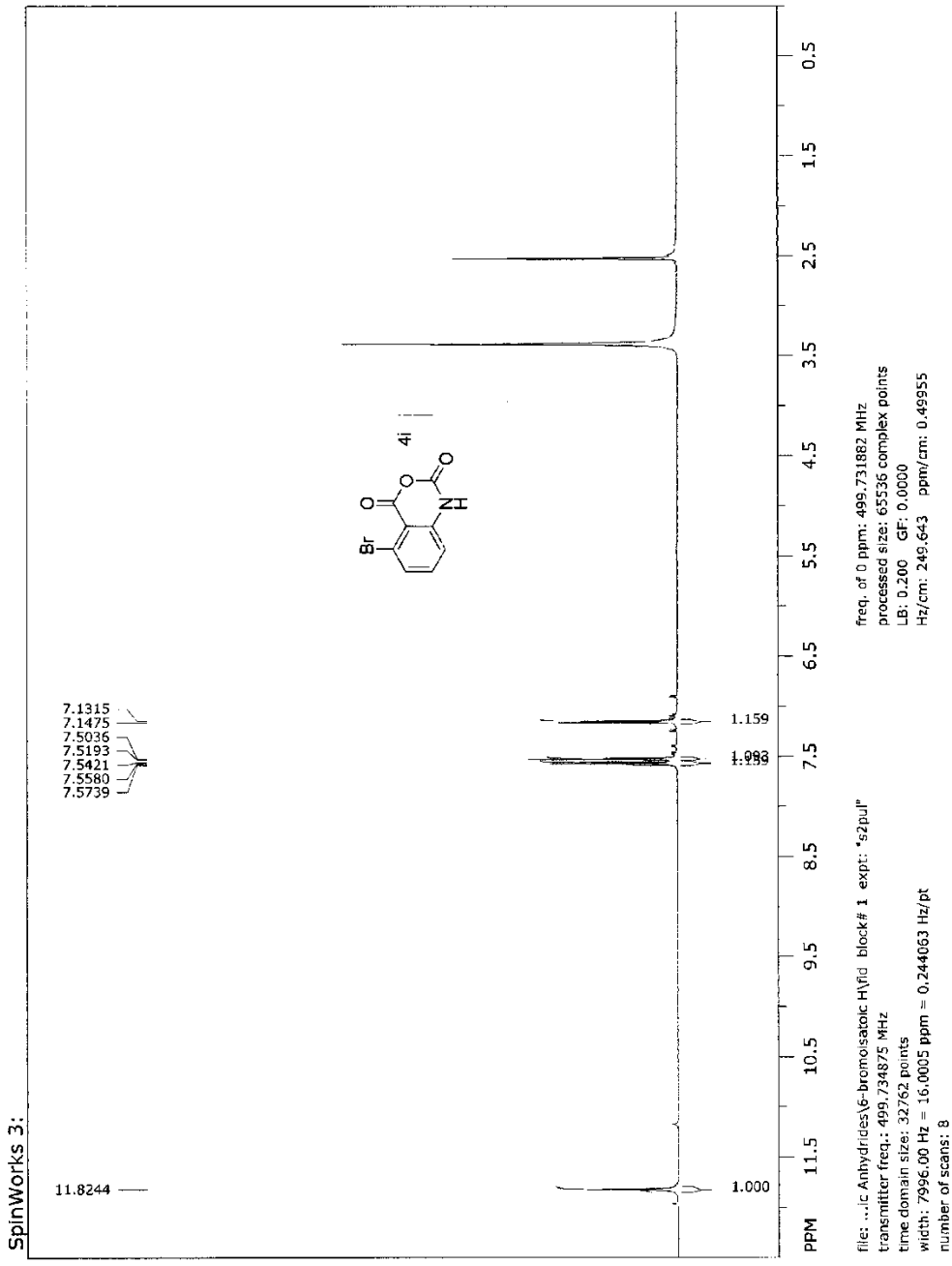

SpinWorks 3:
1.1458
1.1596
2.7499
2.7636
2.7773
2.7910
2.8048
6.6949
6.6983
6.7115
6.7148
6.7750
6.7784
7.8538
7.8702
9.5295
8.357
1.487
1.087
1.277
1.090
1.000
4h
PPM
9.2 8.8 8.4 8.0 7.6 7.2 6.8 6.4 6.0 5.6 5.2 4.8 4.4 4.0 3.6 3.2 2.8 2.4 2.0 1.6 1.2 0.8 0.4
file: ...Anhydrides\5-isopropylisatoicH\fid block# 1 expt: "s2pul"
transmitter freq.: 499.734875 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 16
freq. of 0 ppm: 499.731883 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 199.810 ppm/cm: 0.39983

SpinWorks 3:

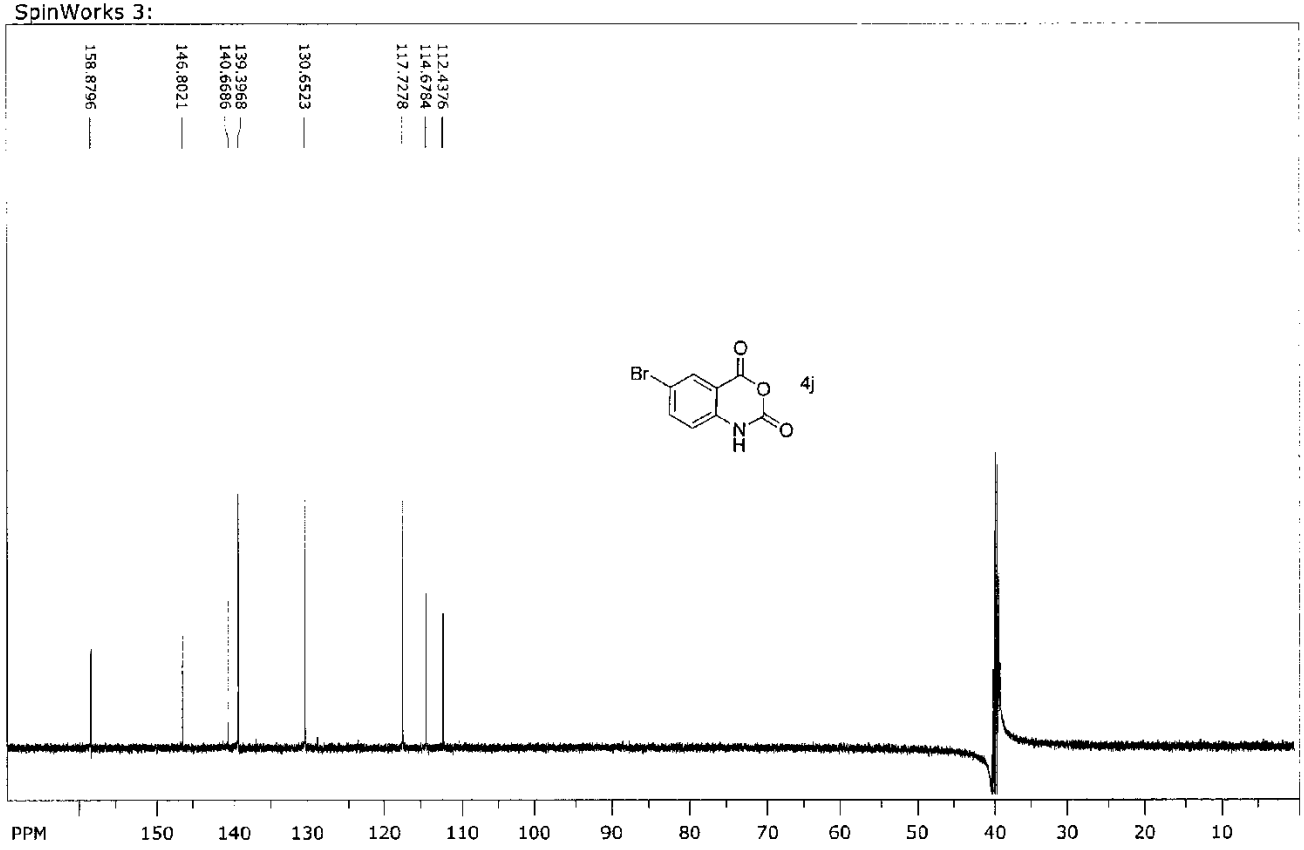


file: ...ic Anhydrides\6-bromoisatoic C\fid block# 1 expt: "s2pul"
transmitter freq.: 125.670844 MHz
time domain size: 78430 points
width: 30154.54 Hz = 239.9486 ppm = 0.384477 Hz/pt
number of scans: 512

freq. of 0 ppm: 125.657737 MHz
processed size: 131072 complex points
LB: 0.500 GF: 0.0000
Hz/cm: 804.121 ppm/cm: 6.39863


SpinWorks 3:
4i
Br
O
O
O
N
H
11.8244
7.1315
7.1475
7.5036
7.5193
7.5421
7.5580
7.5739
1.000
1.159
1.983
PPM
11.5
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
file: ...ic Anhydrides\6-bromoisatoic H\fid block# 1 expt: 's2pul'
transmitter freq.: 499.734875 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 8
freq. of 0 ppm: 499.731882 MHz
processed size: 65536 complex points
LB: 0.200 GF: 0.0000
Hz/cm: 249.643 ppm/cm: 0.49955

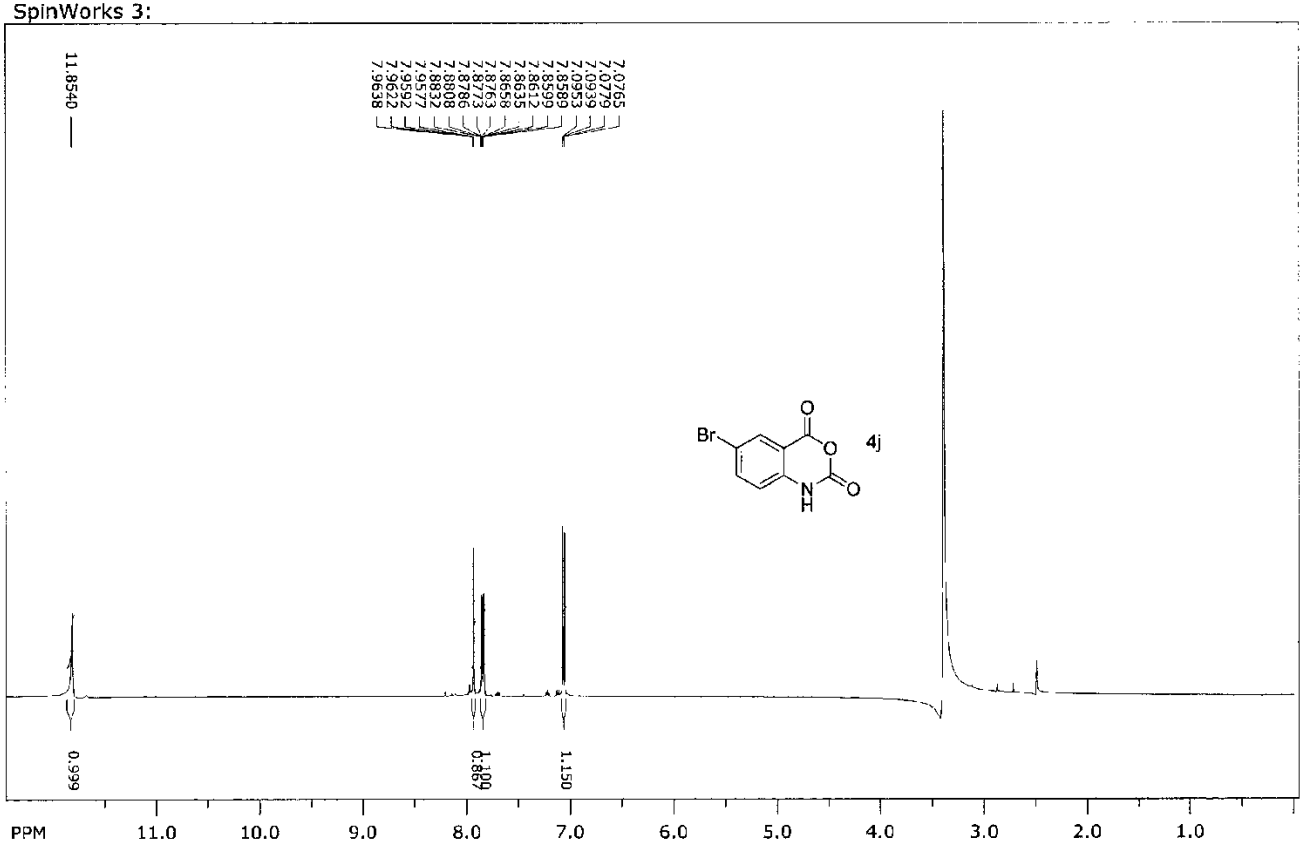
SpinWorks 3:



file: ...ic Anhydrides\5-bromoisatoic C\fid block# 1 expt: "s2pul"
transmitter freq.: 125.670874 MHz
time domain size: 78430 points
width: 30154.54 Hz = 239.9485 ppm = 0.384477 Hz/pt
number of scans: 256

freq. of 0 ppm: 125.657730 MHz
processed size: 131072 complex points
LB: 0.500 GF: 0.0000
Hz/cm: 853.814 ppm/cm: 6.79405

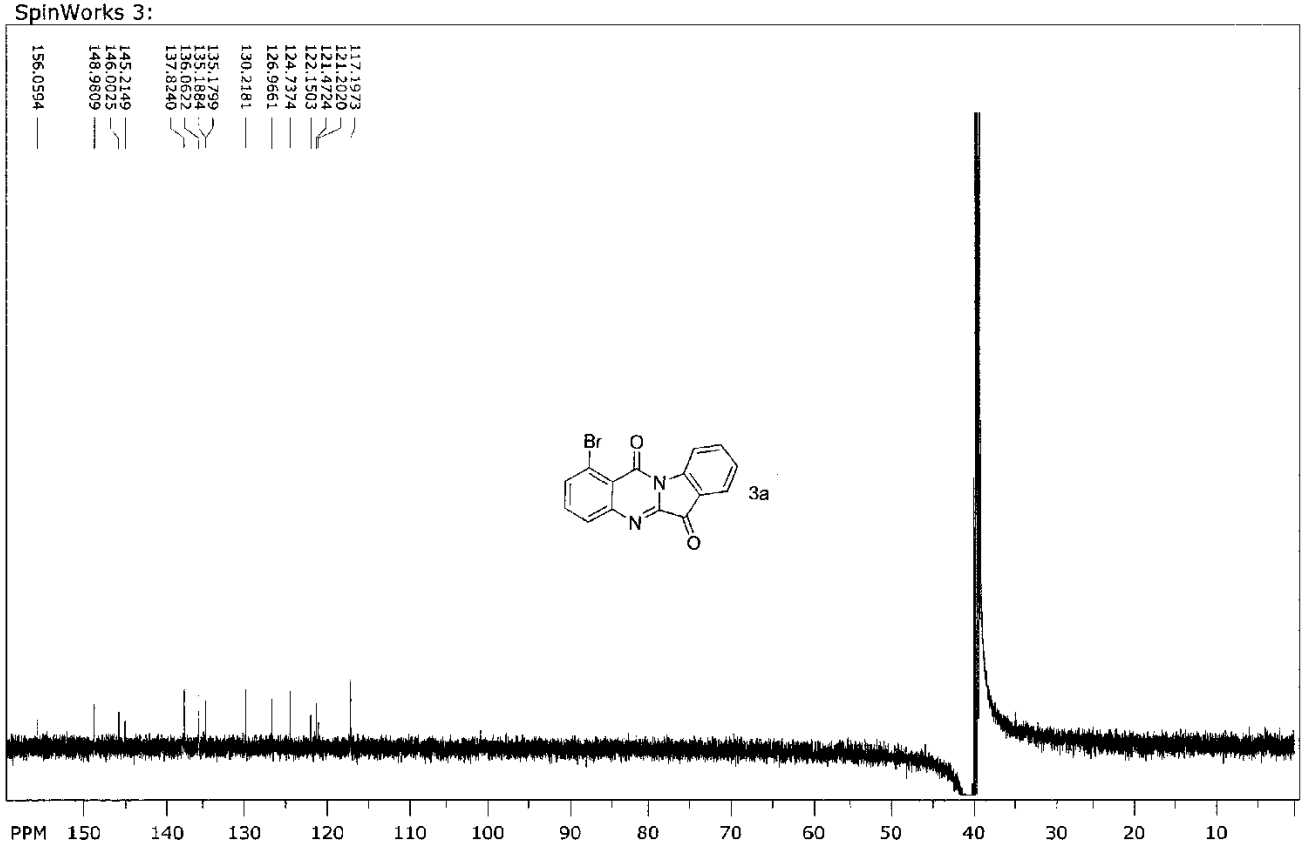
SpinWorks 3:



file: ...ic Anhydrides\5-bromoisatoic H\fid block# 1 expt: "s2pul"
transmitter freq.: 499.734875 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 8

freq. of 0 ppm: 499.731886 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 249.883 ppm/cm: 0.50003

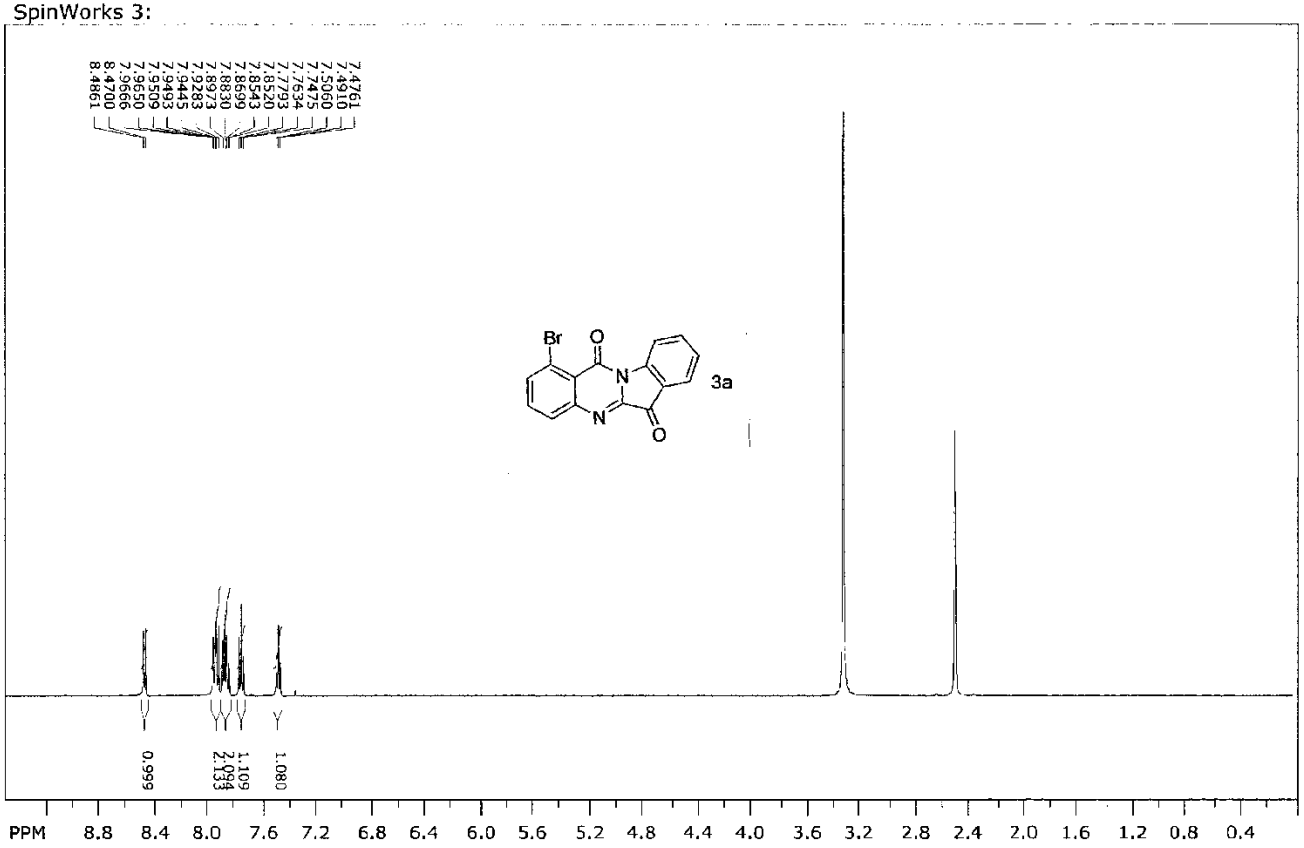
SpinWorks 3:



file: ...02 NMRS\Tryptanthrins\1-bromoC\fid block# 1 expt: "s2pul"
transmitter freq.: 125.670844 MHz
time domain size: 78430 points
width: 30154.54 Hz = 239.9486 ppm = 0.384477 Hz/pt
number of scans: 512

freq. of 0 ppm: 125.657738 MHz
processed size: 131072 complex points
LB: 0.500 GF: 0.0000
Hz/cm: 804.121 ppm/cm: 6.39863

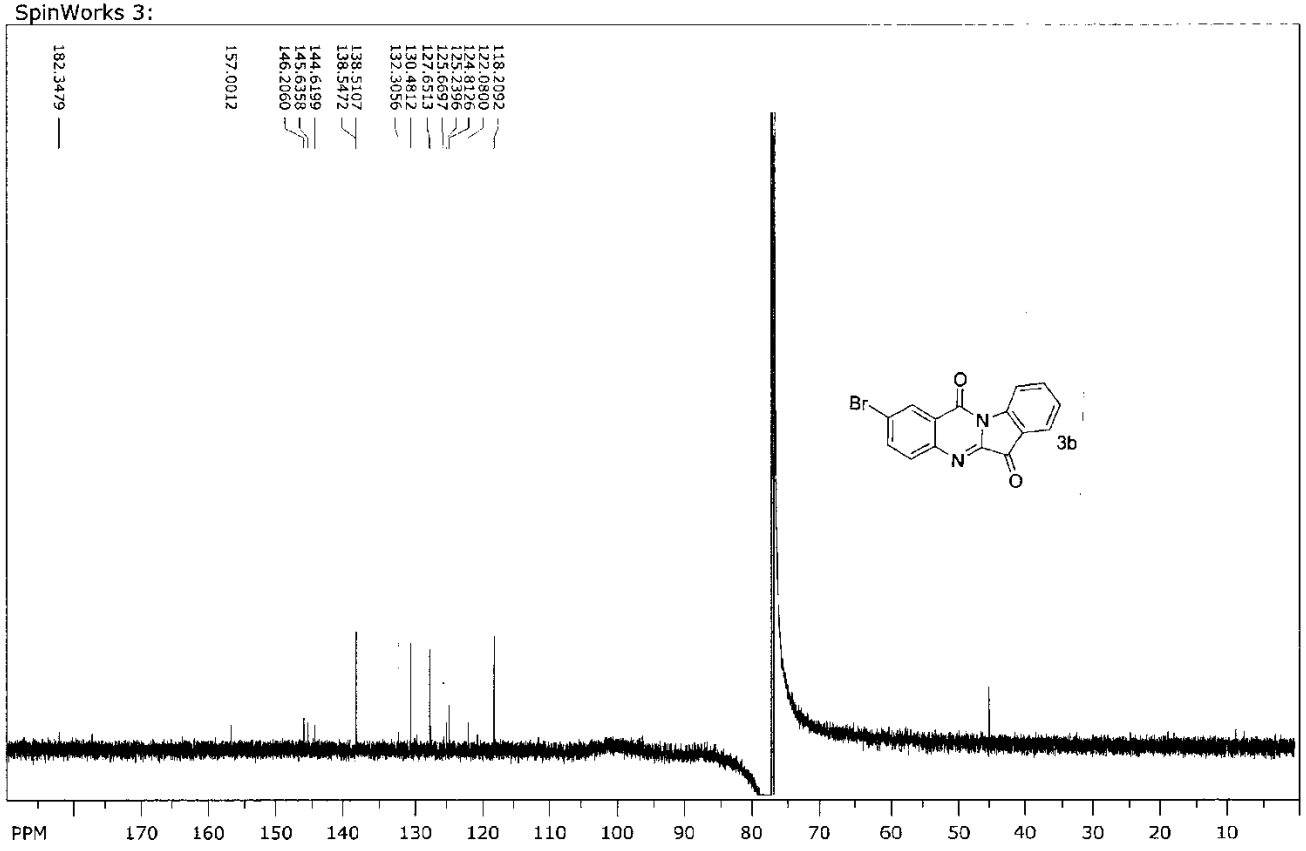
SpinWorks 3:



file: ...02 NMRS\Tryptanthrins\1-bromoH\fid block# 1 expt: "s2pul"
transmitter freq.: 499.734875 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 8

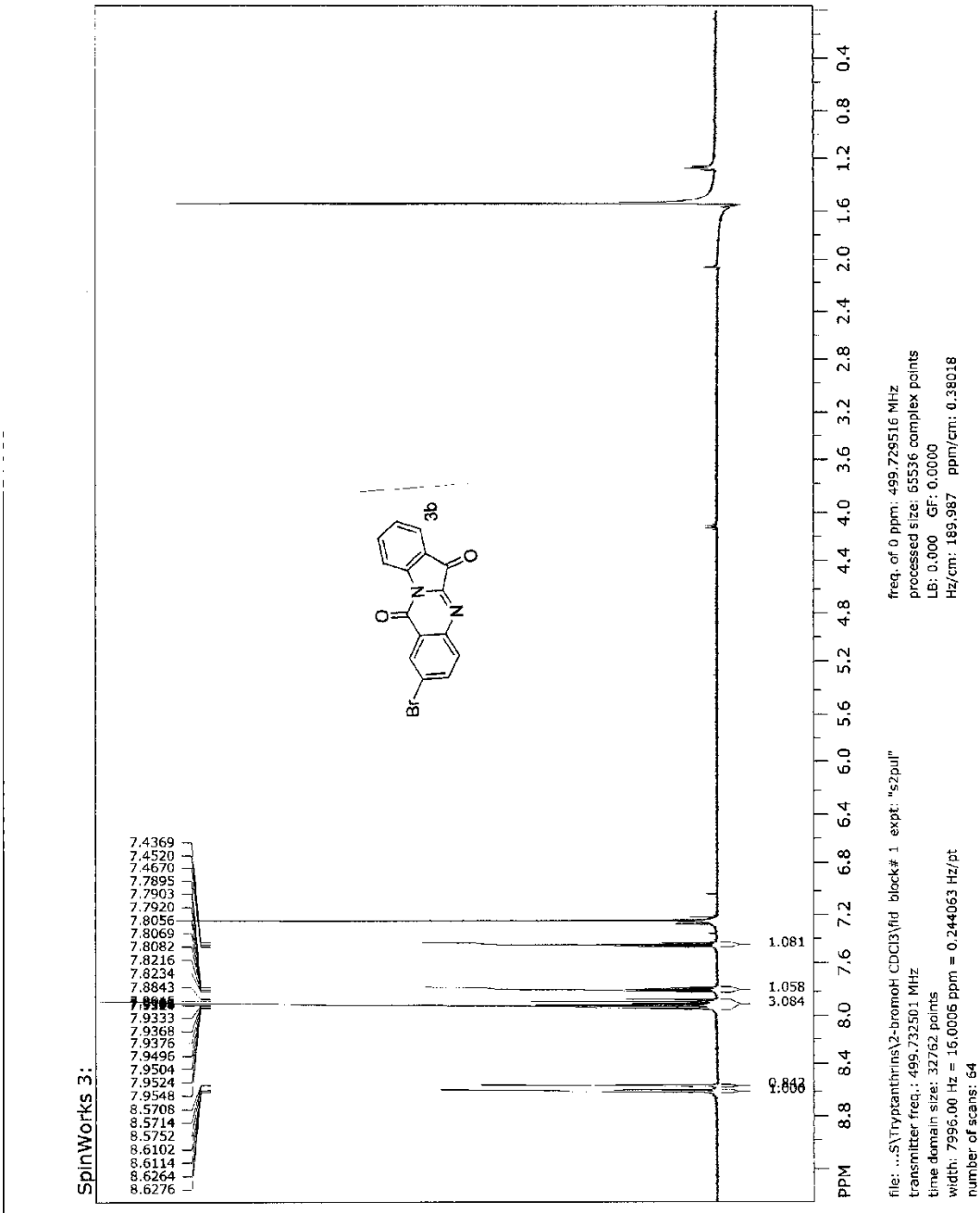
freq. of 0 ppm: 499.731883 MHz
processed size: 65536 complex points
LB: 0.200 GF: 0.0000
Hz/cm: 189.987 ppm/cm: 0.38018

SpinWorks 3:

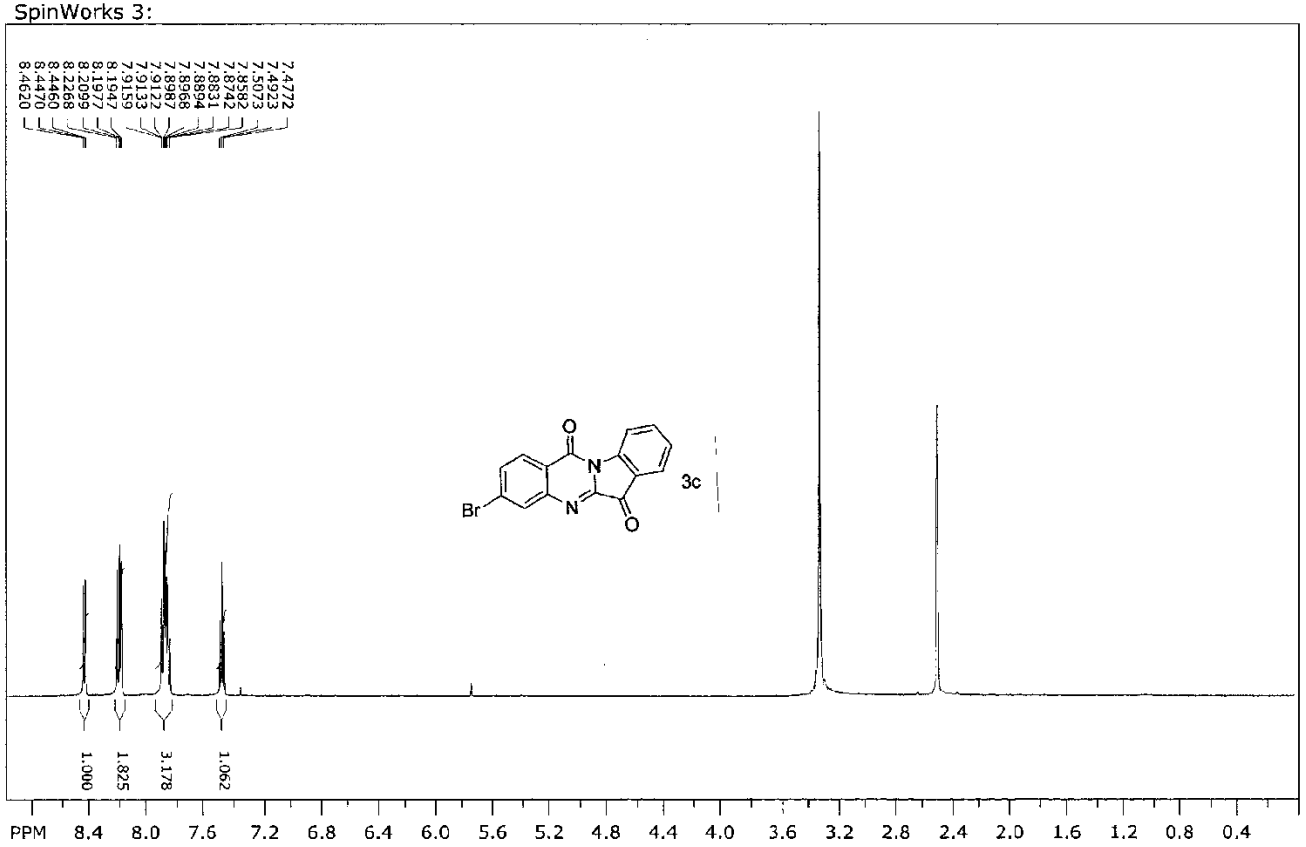


file: ...MRS\Tryptanthrins\2-Br C CDCl3\fid block# 1 expt: "s2pul"
transmitter freq.: 125.670278 MHz
time domain size: 78430 points
width: 30154.54 Hz = 239.9497 ppm = 0.384477 Hz/pt
number of scans: 17000

freq. of 0 ppm: 125.657064 MHz
processed size: 131072 complex points
LB: 0.500 GF: 0.0000
Hz/cm: 955.007 ppm/cm: 7.59931

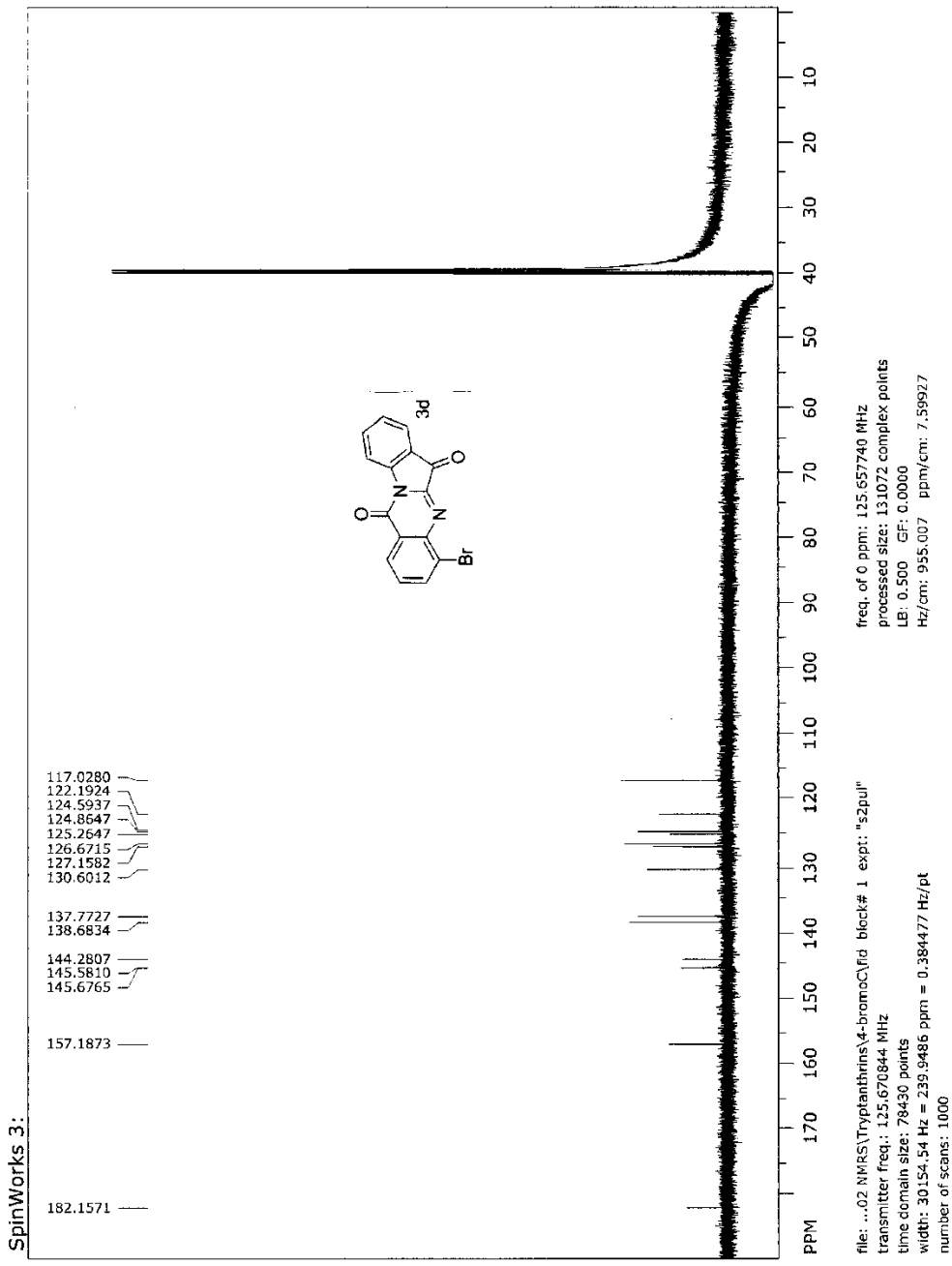

SpinWorks 3:
3b
Br
O
N
N
O
7.4369
7.4520
7.4670
7.7895
7.7903
7.7920
7.8056
7.8069
7.8082
7.8216
7.8234
7.8843
7.9333
7.9368
7.9376
7.9496
7.9504
7.9524
7.9548
8.5708
8.5714
8.5752
8.6102
8.6114
8.6264
8.6276
1.081
1.058
3.084
0.843
1.000
PPM
8.8 8.4 8.0 7.6 7.2 6.8 6.4 6.0 5.6 5.2 4.8 4.4 4.0 3.6 3.2 2.8 2.4 2.0 1.6 1.2 0.8 0.4
file: ...S\Tryptanthrins\2-bromoH CDCl3\fid block# 1 expt: "s2pul"
transmitter freq.: 499.732501 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0006 ppm = 0.244063 Hz/pt
number of scans: 64
freq. of 0 ppm: 499.729516 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 189.987 ppm/cm: 0.38018

SpinWorks 3:

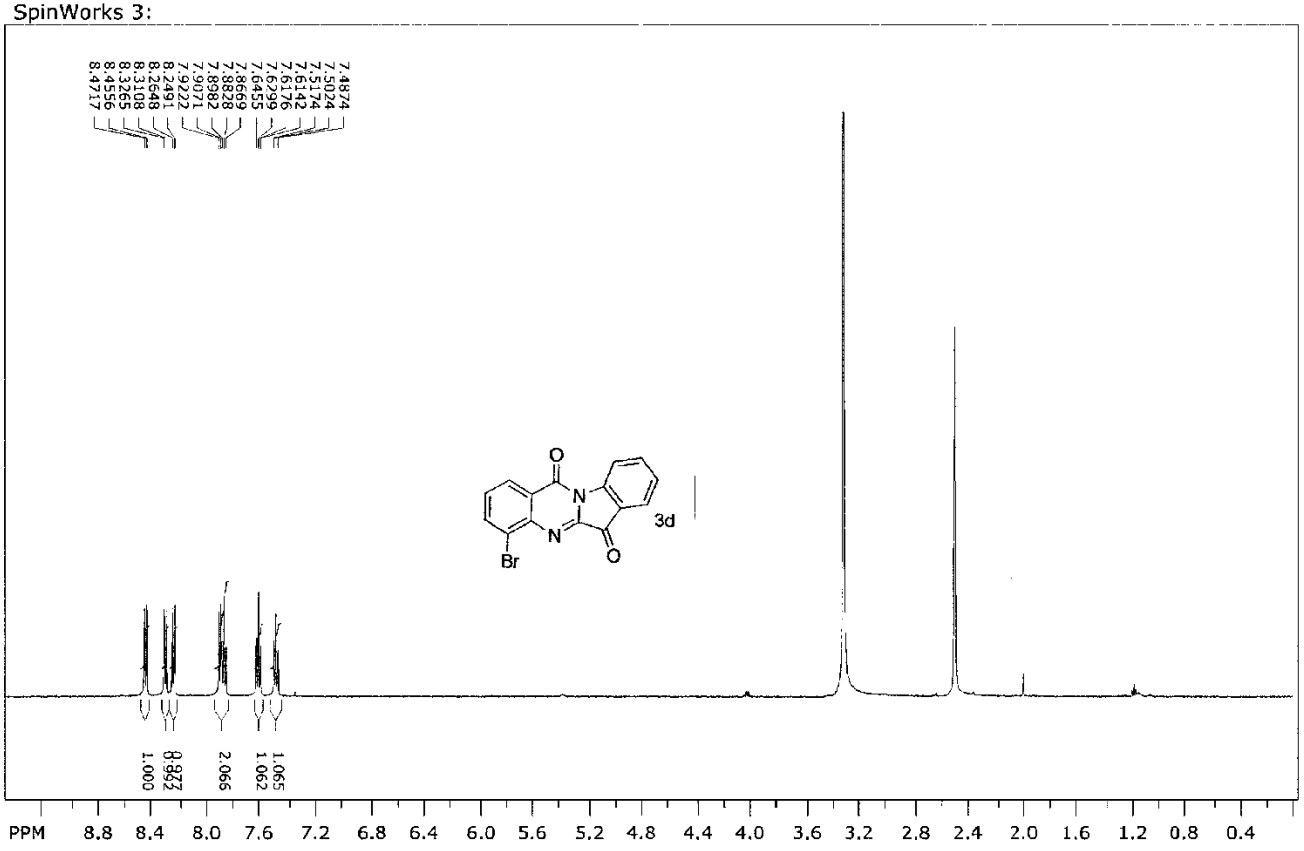


file: ...02 NMRS\Tryptanthrins\3-bromoH\fid block# 1 expt: "s2pul"
transmitter freq.: 499.734875 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 8

freq. of 0 ppm: 499.731883 MHz
processed size: 65536 complex points
LB: 0.200 GF: 0.0000
Hz/cm: 179.925 ppm/cm: 0.36004


SpinWorks 3:
117.0280
122.1924
124.5937
124.8647
125.2647
126.6715
127.1582
130.6012
137.7727
138.6834
144.2807
145.5810
145.6765
157.1873
182.1571
3d
Br
PPM
file: ...02 NMRS\Tryptanthrins\4-bromoCl\fid block# 1 expt: "s2pul"
transmitter freq.: 125.670844 MHz
time domain size: 78430 points
width: 30154.54 Hz = 239.9486 ppm = 0.384477 Hz/pt
number of scans: 1000
freq. of 0 ppm: 125.657740 MHz
processed size: 131072 complex points
LB: 0.500 GF: 0.0000
Hz/cm: 955.007 ppm/cm: 7.59927

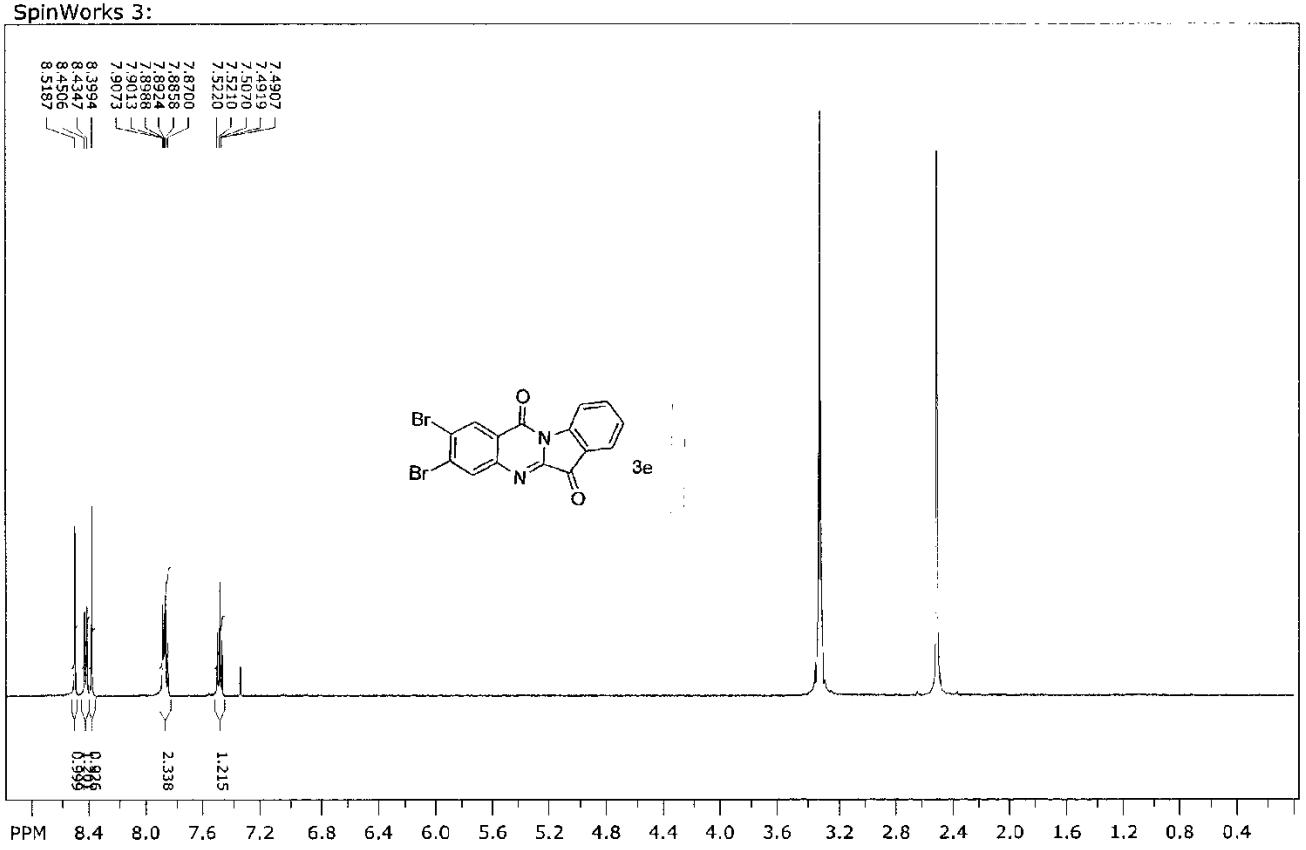
SpinWorks 3:



file: ...02 NMRS\Tryptanthrins\4-bromoH\fid block# 1 expt: "s2pul"
transmitter freq.: 499.734875 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 8

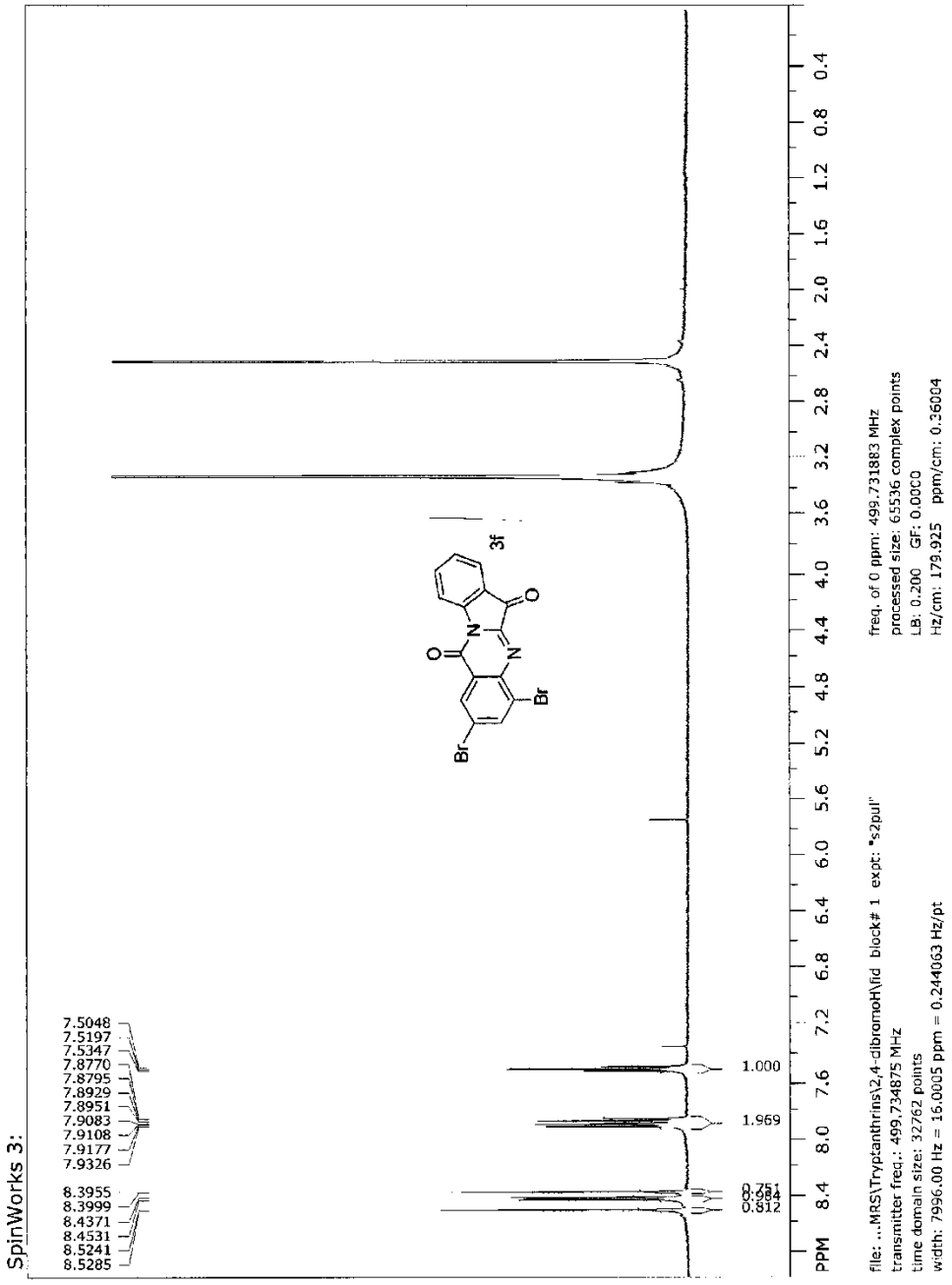
freq. of 0 ppm: 499.731886 MHz
processed size: 65536 complex points
LB: 0.200 GF: 0.0000
Hz/cm: 189.987 ppm/cm: 0.38018

SpinWorks 3:

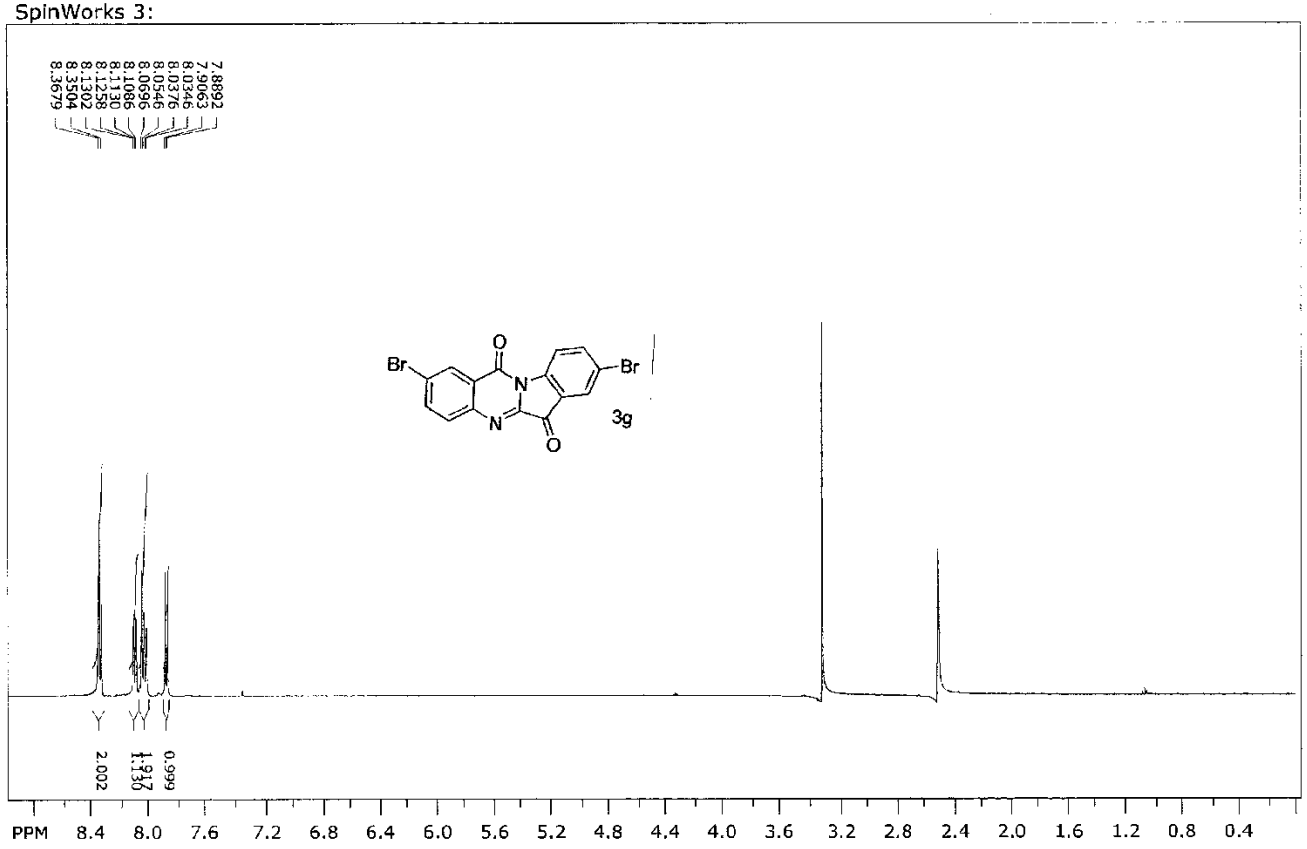


file: ...NMRS\Tryptanthrins\2,3-dibomoH\fid block# 1 expt: "s2pul"
transmitter freq.: 499.734875 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 8

freq. of 0 ppm: 499.731884 MHz
processed size: 65536 complex points
LB: 0.200 GF: 0.0000
Hz/cm: 179.925 ppm/cm: 0.36004


SpinWorks 3:
7.5048
7.5197
7.5347
7.8770
7.8795
7.8929
7.8951
7.9083
7.9108
7.9177
7.9326
8.3955
8.3999
8.4371
8.4531
8.5241
8.5285
1.000
1.969
0.751
0.984
0.812
3f
Br
Br
PPM
8.4 8.0 7.6 7.2 6.8 6.4 6.0 5.6 5.2 4.8 4.4 4.0 3.6 3.2 2.8 2.4 2.0 1.6 1.2 0.8 0.4
file: ...MRS\Tryptanthrins\2,4-dibromoH\fid block# 1 expt: "s2pul"
transmitter freq.: 499.734875 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 8
freq. of 0 ppm: 499.731883 MHz
processed size: 65536 complex points
LB: 0.200 GF: 0.0000
Hz/cm: 179.925 ppm/cm: 0.36004

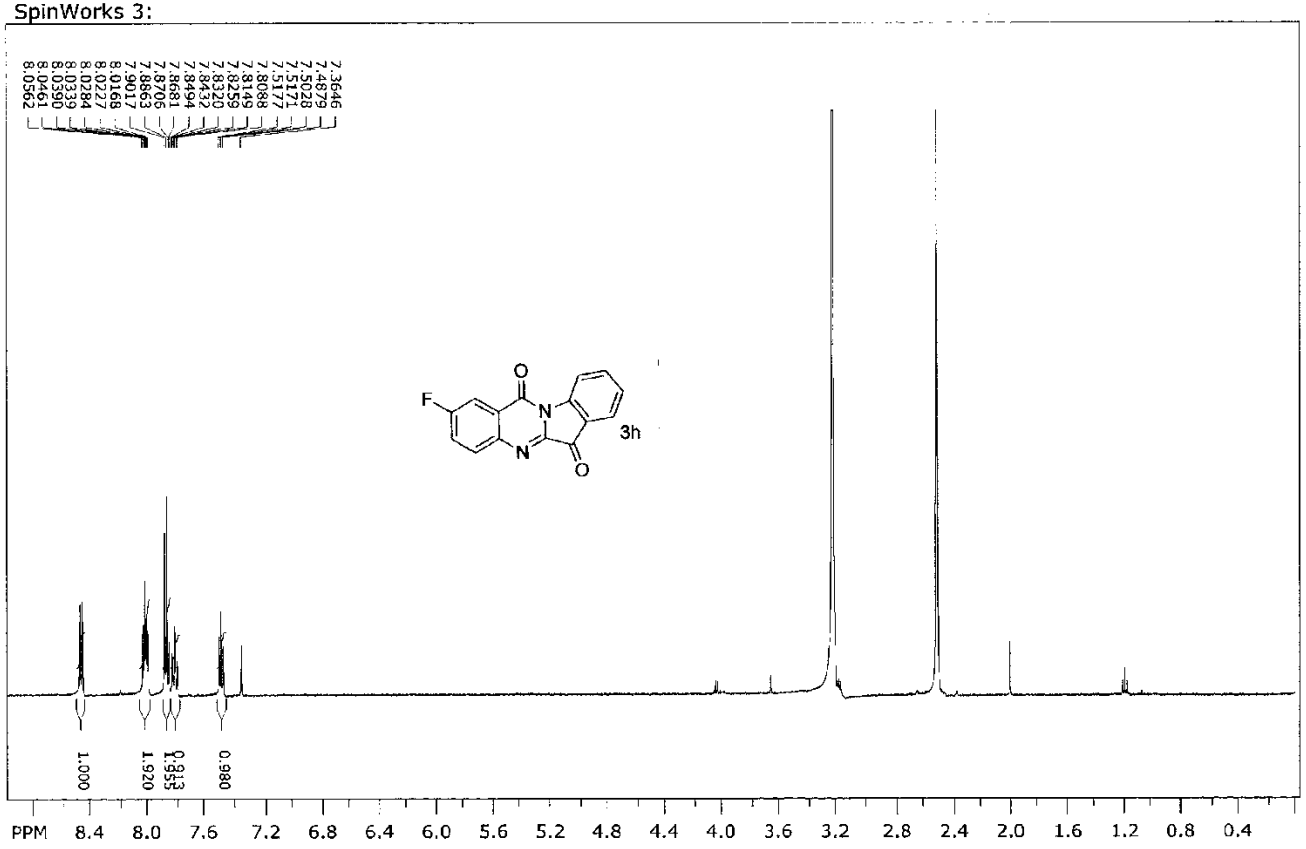
SpinWorks 3:



file: ...MRS\Tryptanthrins\2,8-dibromoH\fid block# 1 expt: "s2pul"
transmitter freq.: 499.734875 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 8

freq. of 0 ppm: 499.731887 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 180.165 ppm/cm: 0.36052

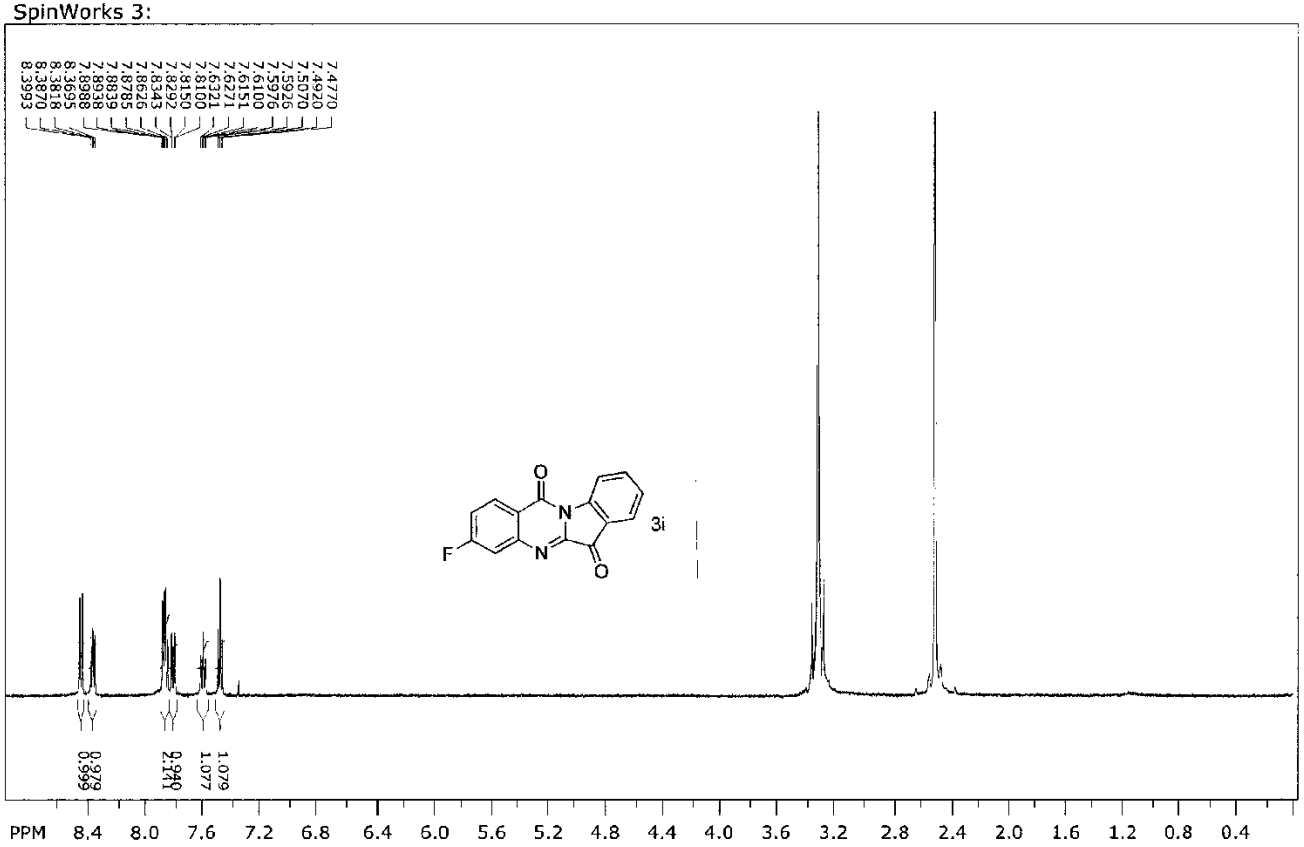
SpinWorks 3:



file: ...2 NMRS\Tryptanthrins\2-fluoroH\fid block# 1 expt: "s2pul"
transmitter freq.: 499.734875 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 8

freq. of 0 ppm: 499.731894 MHz
processed size: 65536 complex points
LB: 0.200 GF: 0.0000
Hz/cm: 180.168 ppm/cm: 0.36053

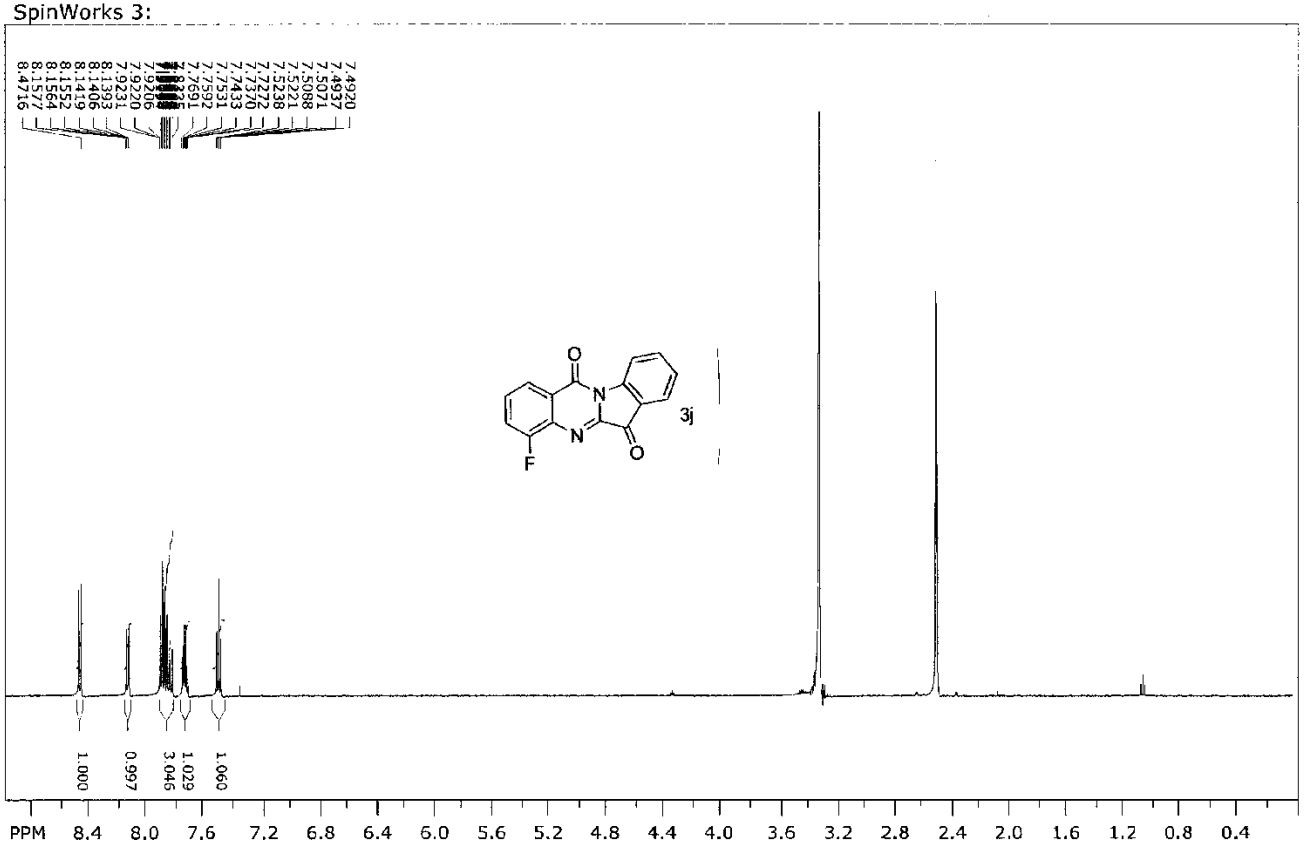
SpinWorks 3:



file: ...2 NMRS\Tryptanthrins\3-fluoroH\fid block# 1 expt: "s2pul"
transmitter freq.: 499.734875 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 8

freq. of 0 ppm: 499.731880 MHz
processed size: 65536 complex points
LB: 0.200 GF: 0.0000
Hz/cm: 179.925 ppm/cm: 0.36004

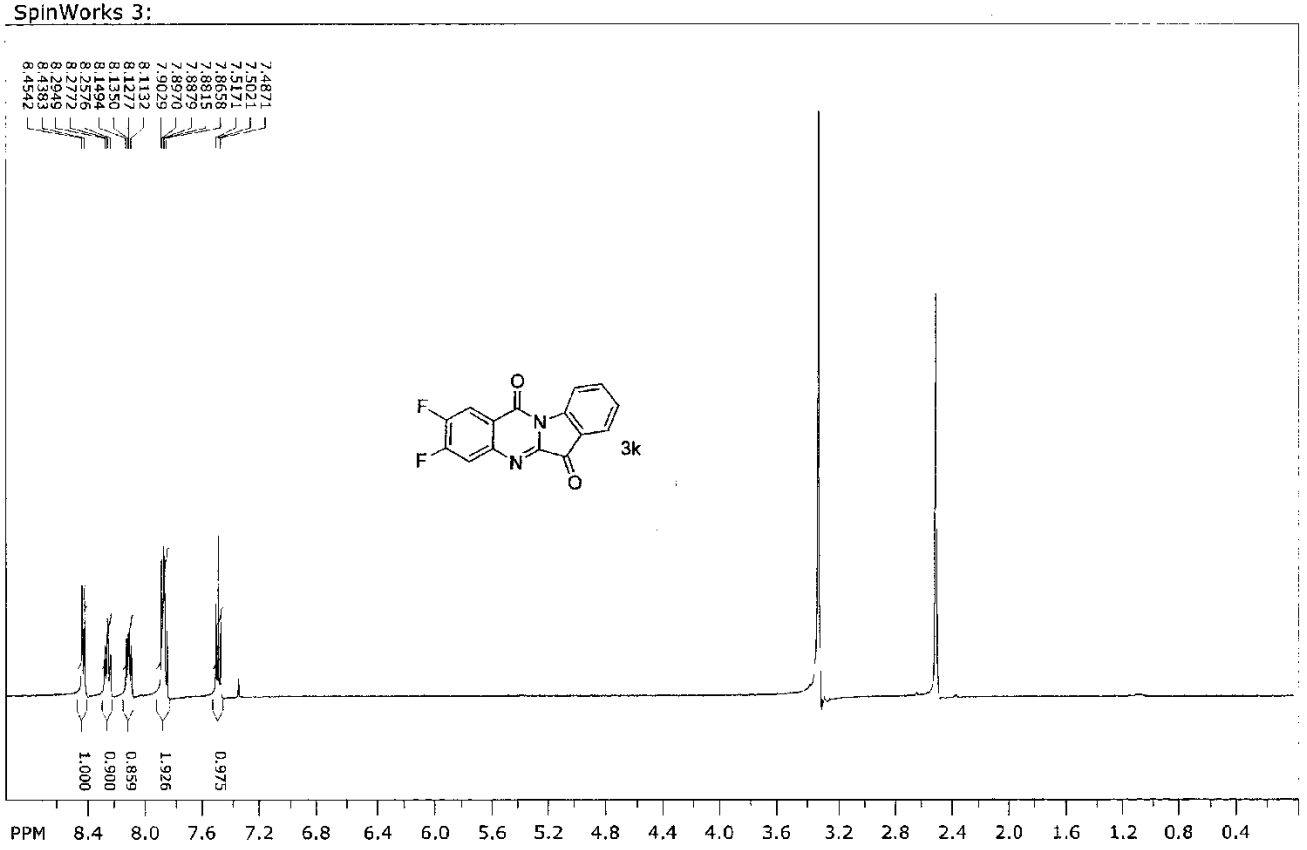
SpinWorks 3:



file: ...2 NMRS\Tryptanthrins\4-fluoroH\fid block# 1 expt: "s2pul"
transmitter freq.: 499.734875 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 8

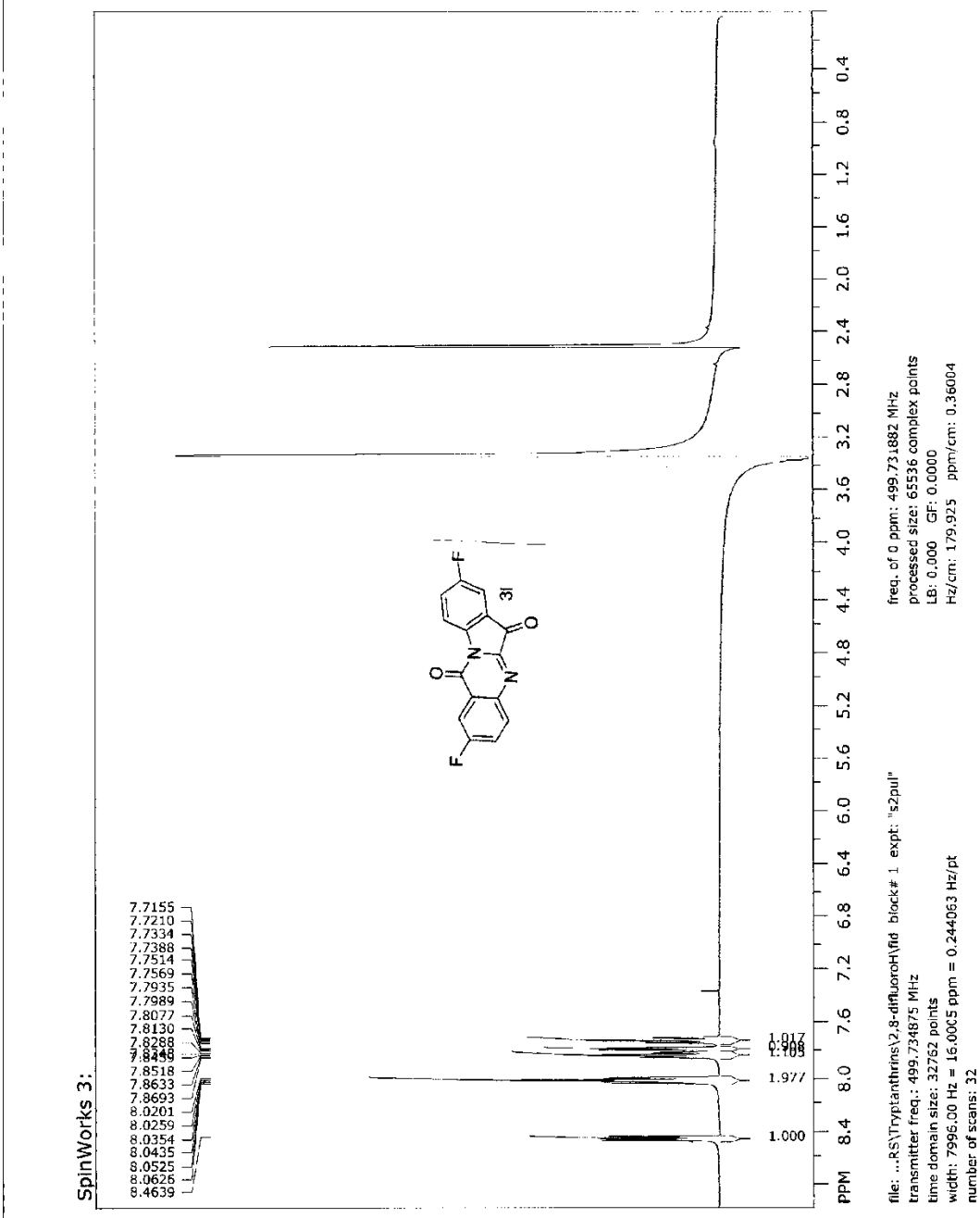
freq. of 0 ppm: 499.731882 MHz
processed size: 65536 complex points
LB: 0.200 GF: 0.0000
Hz/cm: 179.925 ppm/cm: 0.36004

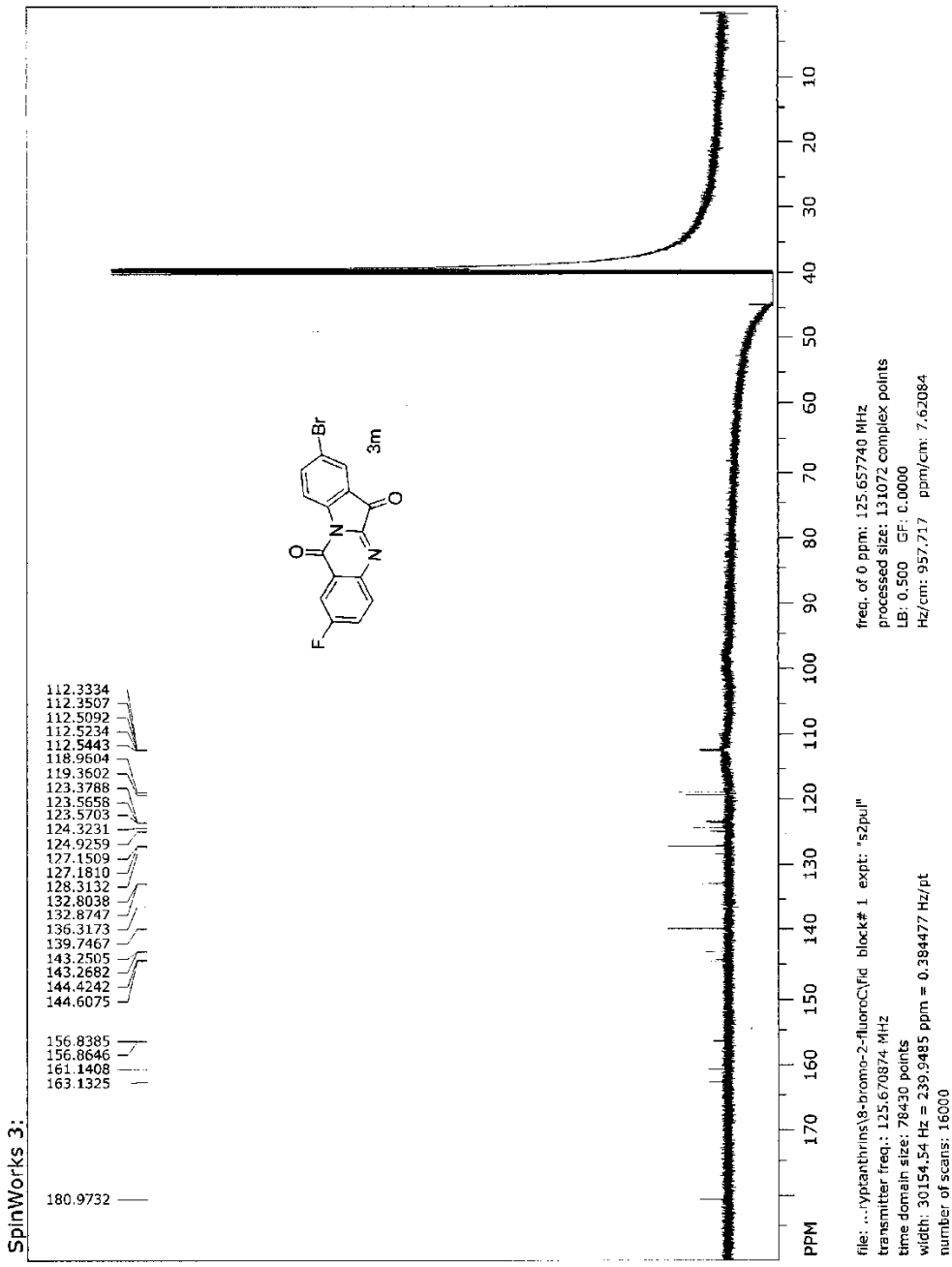
SpinWorks 3:



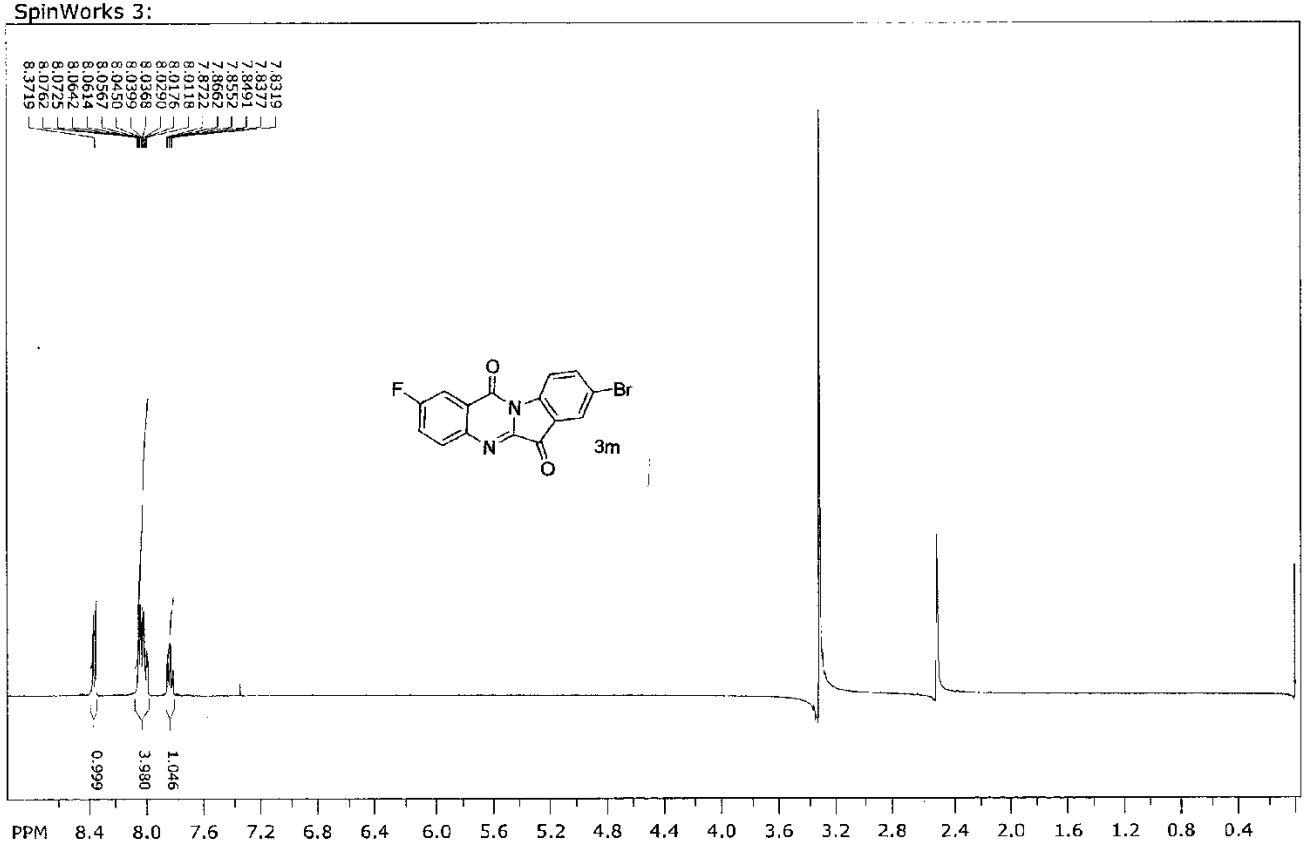
file: ...RS\Tryptanthrins\2,3-difluoroH\fid block# 1 expt: "s2pul"
transmitter freq.: 499.734875 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 8

freq. of 0 ppm: 499.731883 MHz
processed size: 65536 complex points
LB: 0.200 GF: 0.0000
Hz/cm: 179.685 ppm/cm: 0.35956


SpinWorks 3:
7.7155
7.7210
7.7334
7.7388
7.7514
7.7569
7.7935
7.7989
7.8077
7.8130
7.8288
7.8518
7.8633
7.8693
8.0201
8.0259
8.0354
8.0435
8.0525
8.0626
8.4639
1.917
1.977
1.000
PPM
freq. of 0 ppm: 499.731882 MHz
processed size: 65536 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 179.925 ppm/cm: 0.36004
file: ...RS\Tryptanthrins\2,8-difluoroH\fid block# 1 expt: "s2pul"
transmitter freq.: 499.734875 MHz
time domain size: 32762 points
width: 7995.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 32


SpinWorks 3:
112.3334
112.3507
112.5092
112.5234
112.5443
118.9604
119.3602
123.3788
123.5658
123.5703
124.3231
124.9259
127.1509
127.1810
128.3132
132.8038
132.8747
136.3173
139.7467
143.2505
143.2682
144.4242
144.6075
156.8385
156.8646
161.1408
163.1325
180.9732
Br
3m
O
O
N
N
F
PPM
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
file: ...ryptanthrins\8-bromo-2-fluoroC\fid block# 1 expt: "s2pul"
transmitter freq.: 125.670874 MHz
time domain size: 78430 points
width: 30154.54 Hz = 239.9485 ppm = 0.384477 Hz/pt
number of scans: 16000
freq. of 0 ppm: 125.657740 MHz
processed size: 131072 complex points
LB: 0.500 GF: 0.0000
Hz/cm: 957.717 ppm/cm: 7.62084

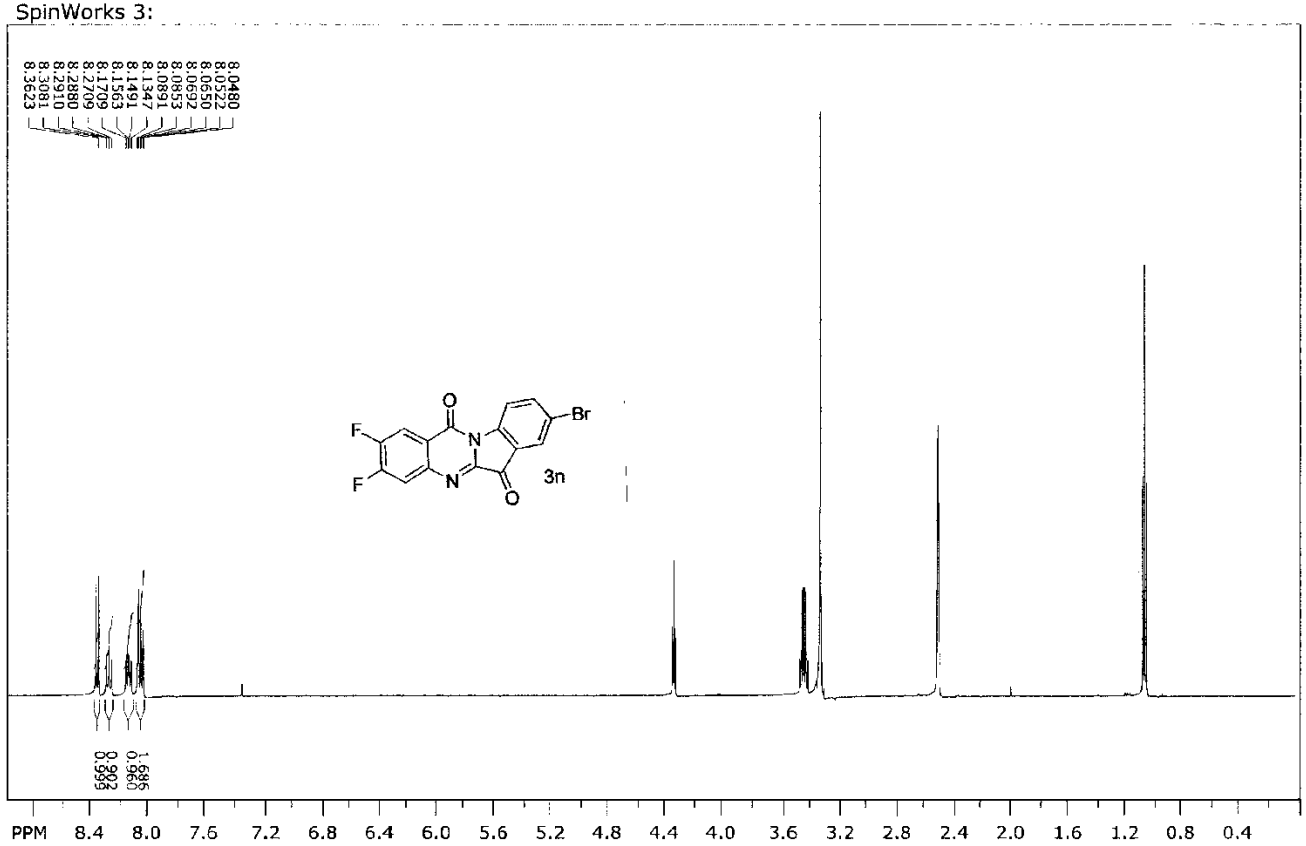
SpinWorks 3:



file: ...ryptanthrins\8-bromo-2-fluoroH\fid block# 1 expt: "s2pul"
transmitter freq.: 499.734875 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 8

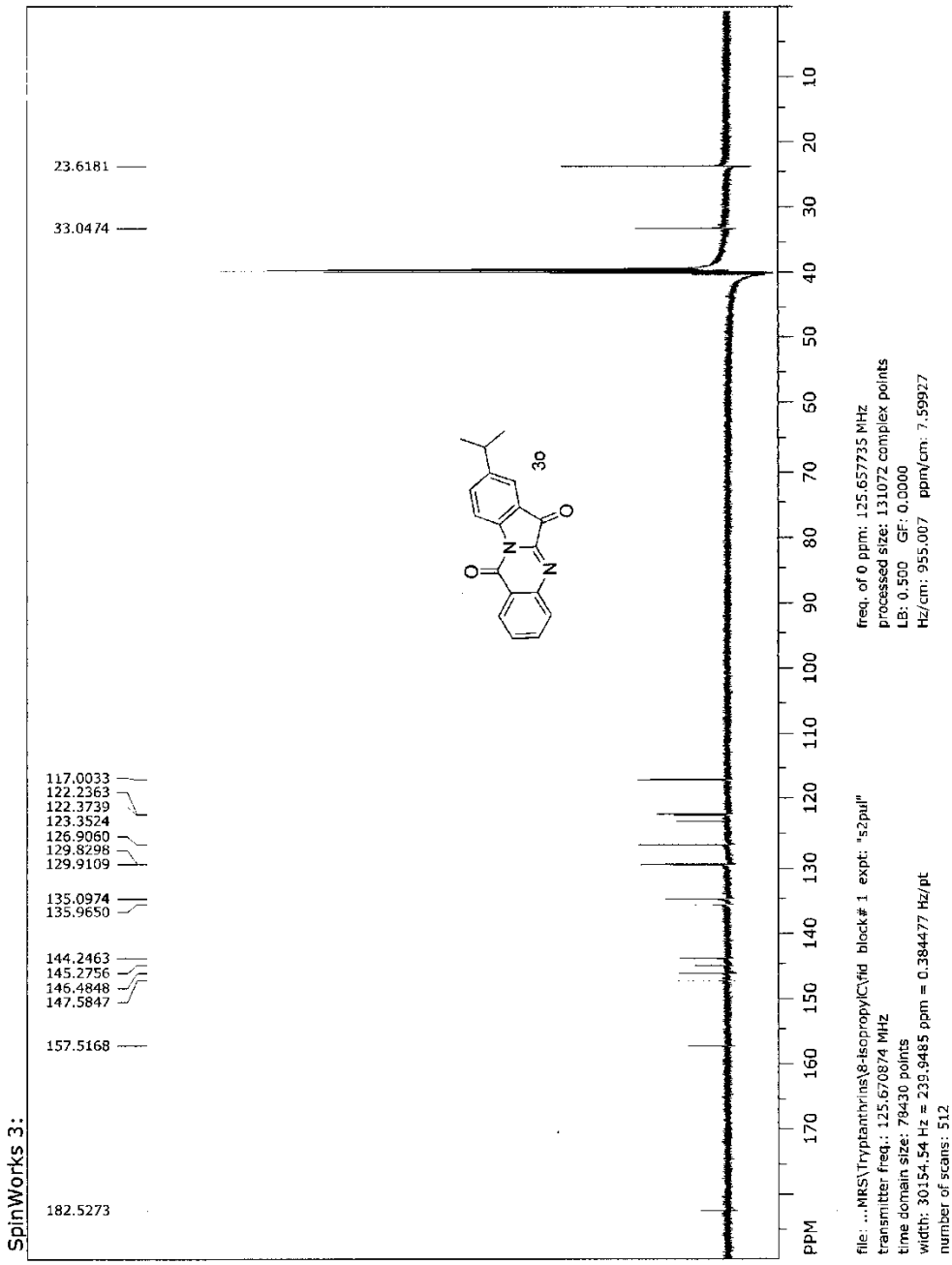
freq. of 0 ppm: 499.731882 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 179.925 ppm/cm: 0.36004

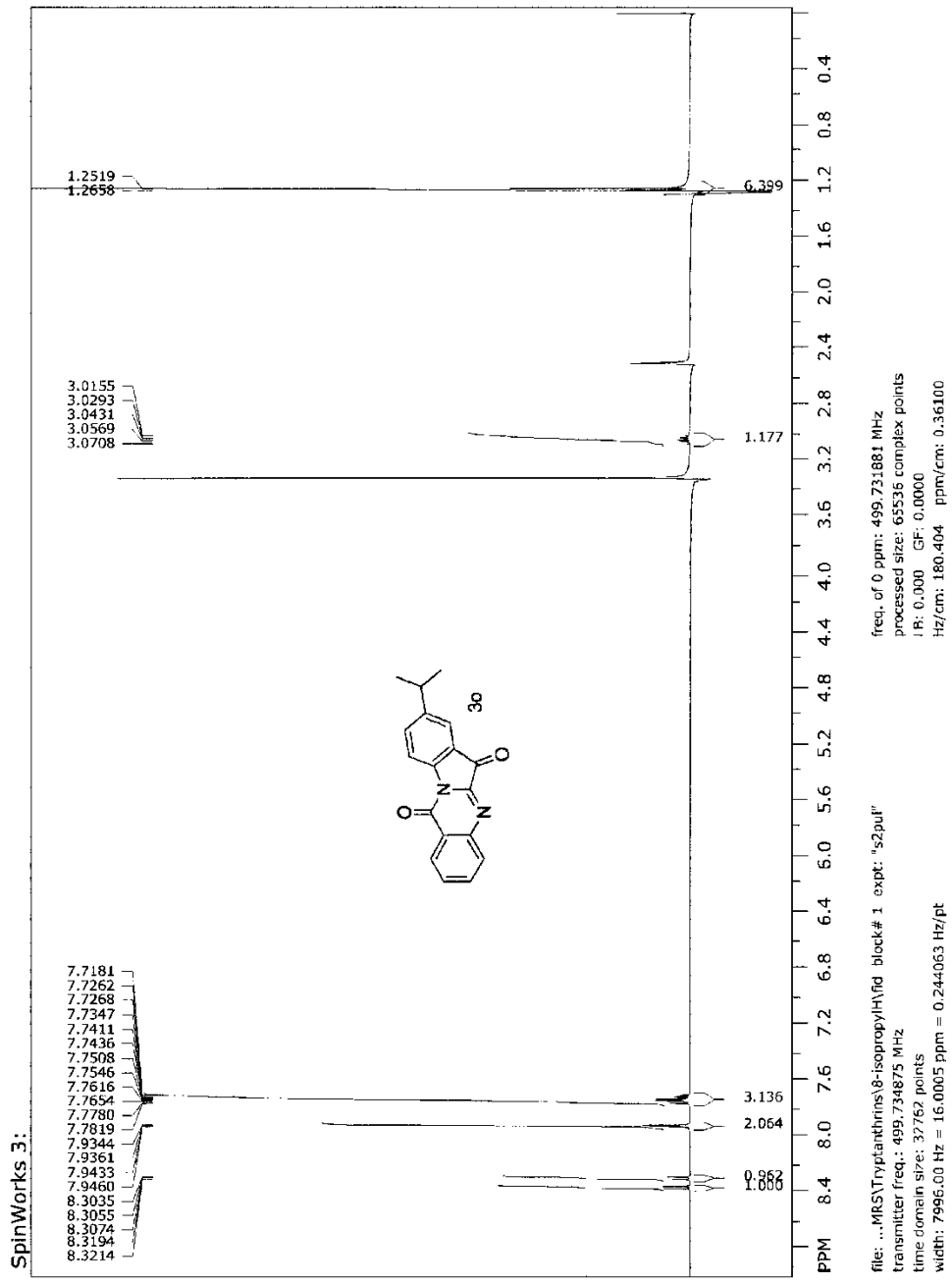
SpinWorks 3:

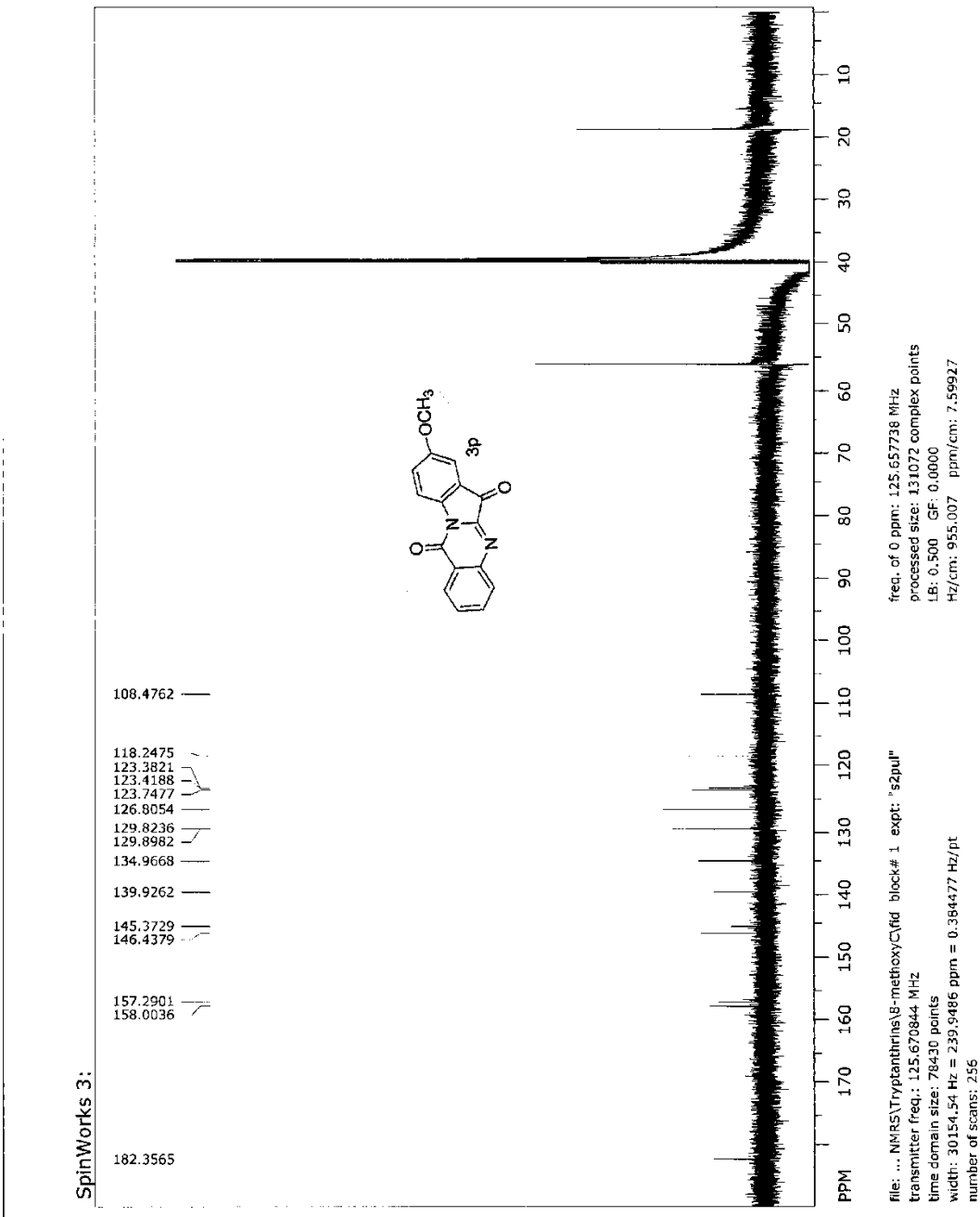


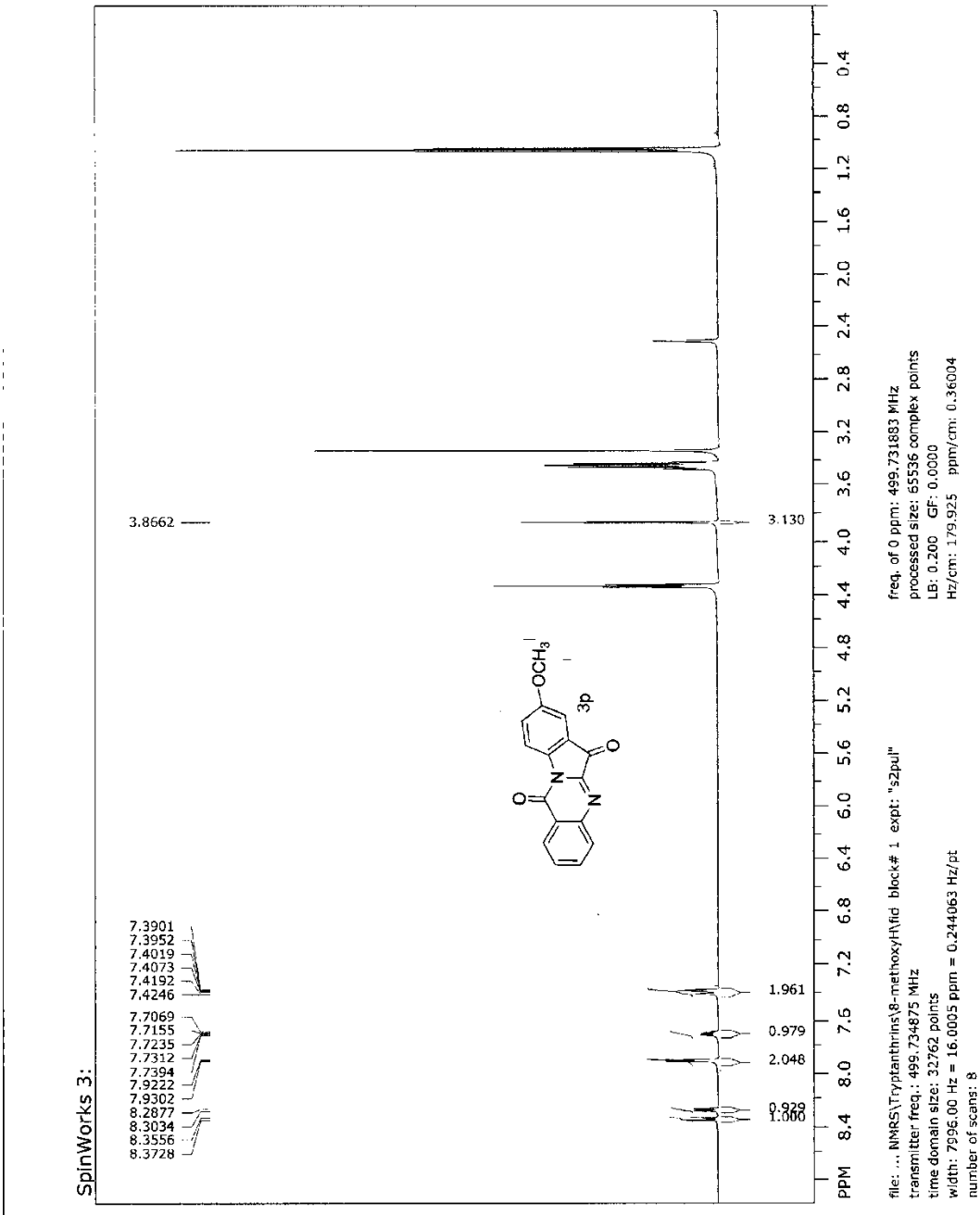
file: ...anthrins\8-bromo-2,3-difluoroH\fid block# 1 expt: "s2pul"
transmitter freq.: 499.734875 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 8

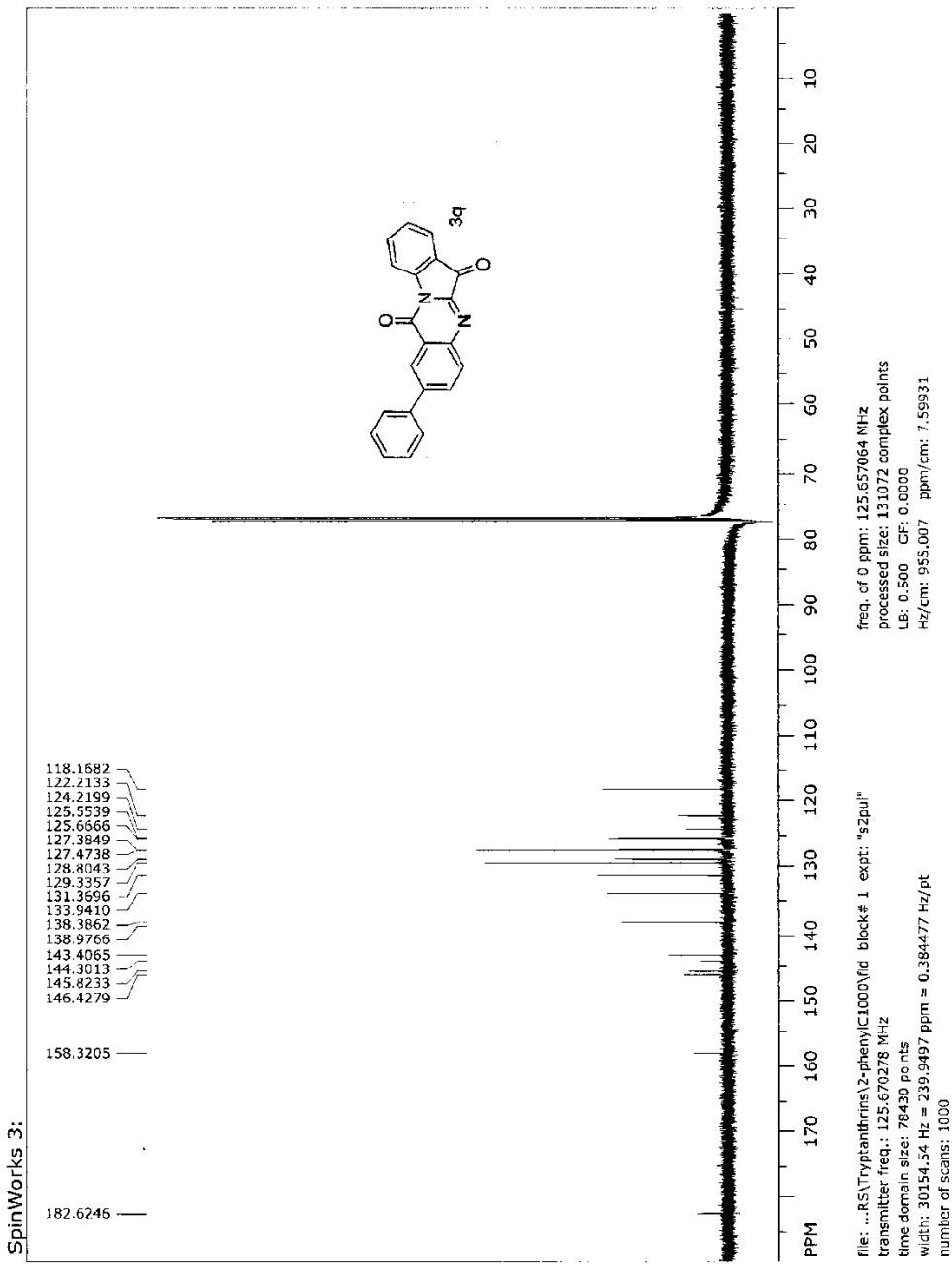
freq. of 0 ppm: 499.731883 MHz
processed size: 65536 complex points
LB: 0.200 GF: 0.0000
Hz/cm: 179.925 ppm/cm: 0.36004

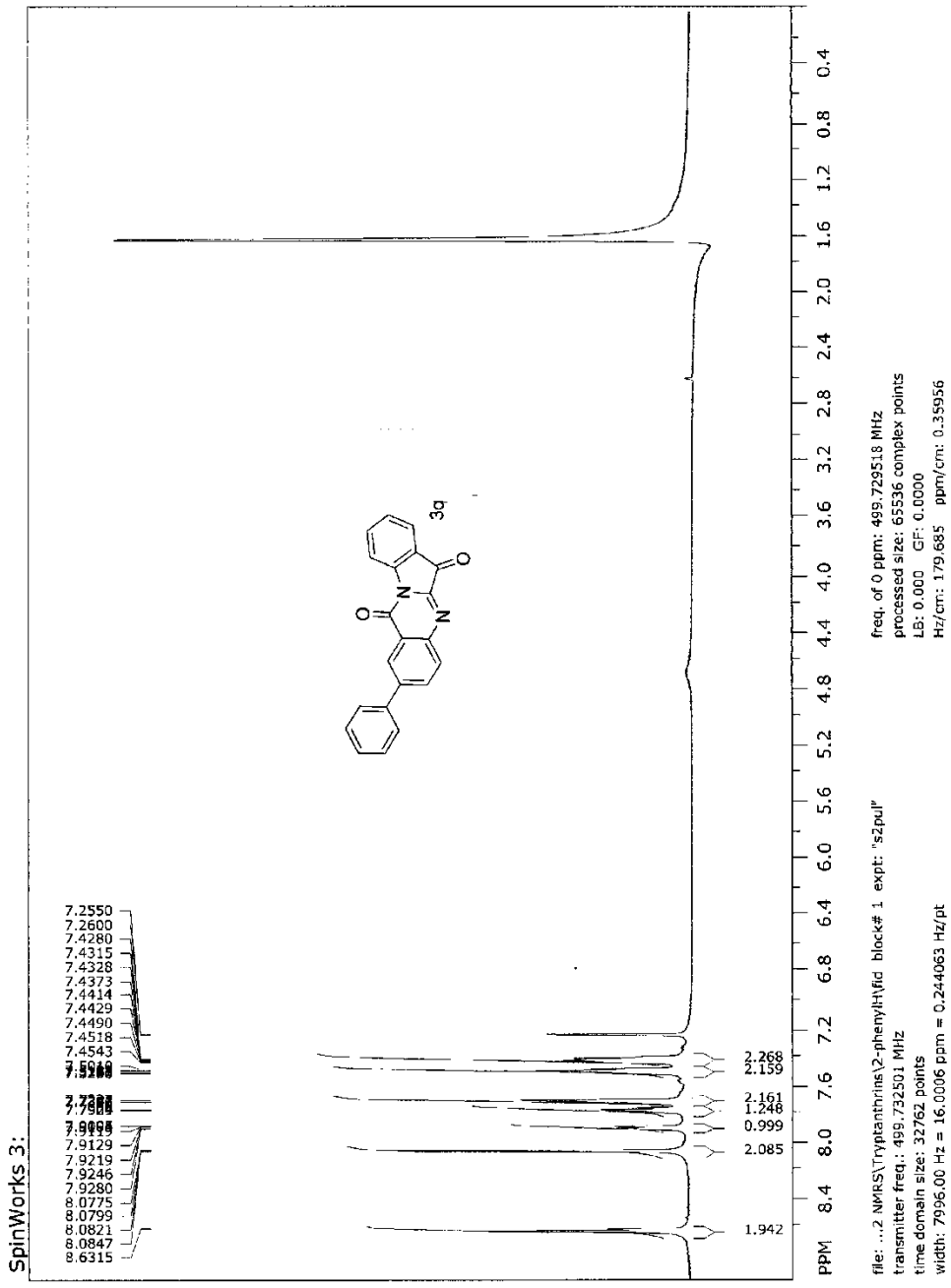

SpinWorks 3:
23.6181
33.0474
117.0033
122.2363
122.3739
123.3524
126.9060
129.8298
129.9109
135.0974
135.9650
144.2463
145.2756
146.4848
147.5847
157.5168
182.5273
PPM
3o
file: ...MRS\Tryptanthrins\8-isopropyl\C\fid block# 1 expt: "s2pul"
transmitter freq.: 125.670874 MHz
time domain size: 78430 points
width: 30154.54 Hz = 239.9485 ppm = 0.384477 Hz/pt
number of scans: 512
freq. of 0 ppm: 125.657735 MHz
processed size: 131072 complex points
LB: 0.500 GF: 0.0000
Hz/cm: 955.007 ppm/cm: 7.59927


SpinWorks 3:
1.2519
1.2658
6.399
3.0155
3.0293
3.0431
3.0569
3.0708
1.177
7.7181
7.7262
7.7268
7.7347
7.7411
7.7436
7.7508
7.7546
7.7616
7.7654
7.7780
7.7819
7.9344
7.9361
7.9433
7.9460
8.3035
8.3055
8.3074
8.3194
8.3214
3.136
2.064
0.962
1.000
3o
PPM
file: ...MRGVTryptanthrins\8-isopropylH\fid block# 1 expt: "s2pul"
transmitter freq.: 499.734875 MHz
time domain size: 37762 points
width: 7996.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 32
freq. of 0 ppm: 499.731881 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 180.404 ppm/cm: 0.36100

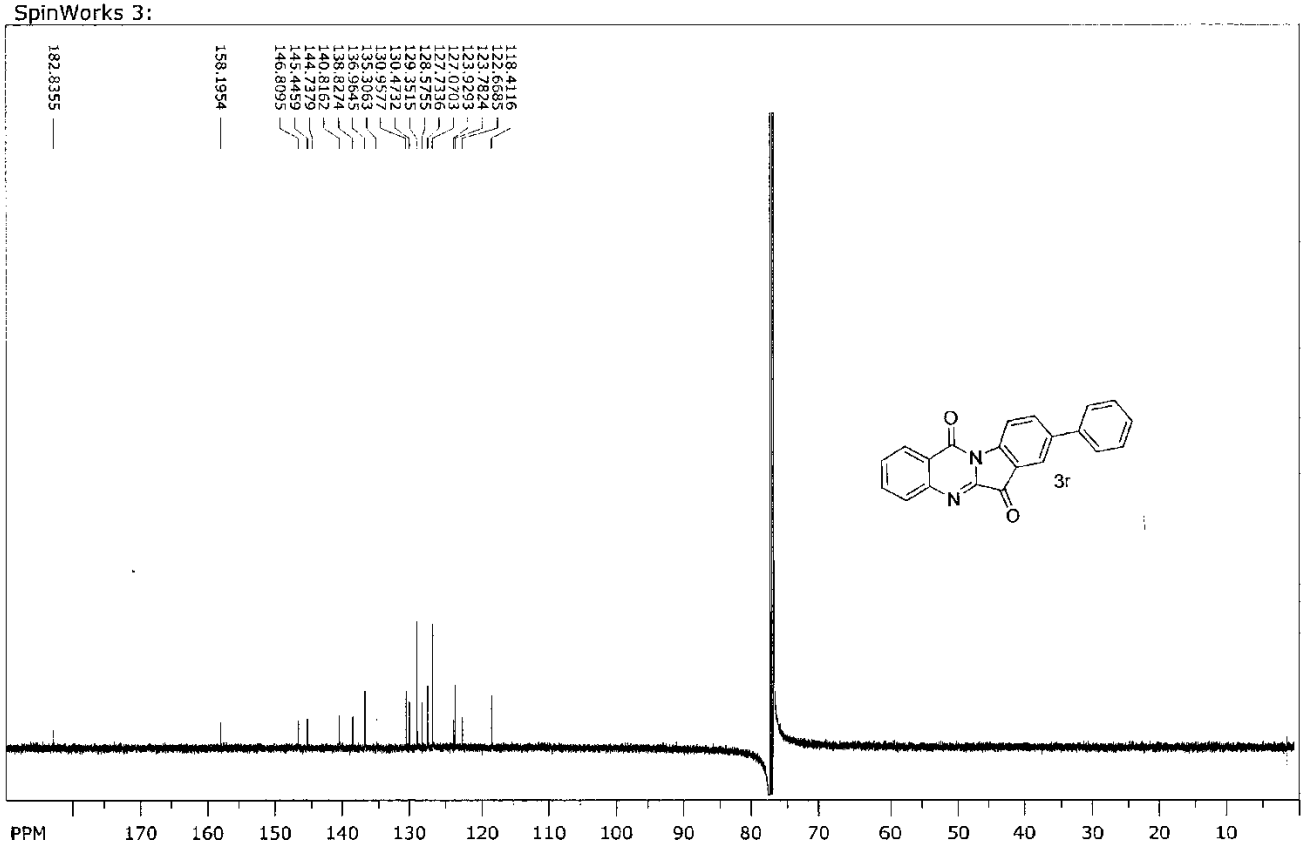

SpinWorks 3:
108.4762
118.2475
123.3821
123.4188
123.7477
126.8054
129.8236
129.8982
134.9668
139.9262
145.3729
146.4379
157.2901
158.0036
182.3565
OCH3
3p
O
N
N
O
PPM
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
file: ... NMRS\Tryptanthrins\8-methoxyCYfid block# 1 expt: 's2pul'
transmitter freq.: 125.670844 MHz
time domain size: 78430 points
width: 30154.54 Hz = 239.9486 ppm = 0.384477 Hz/pt
number of scans: 256
freq. of 0 ppm: 125.657738 MHz
processed size: 131072 complex points
LB: 0.500 GF: 0.0000
Hz/cm: 955.007 ppm/cm: 7.59927


SpinWorks 3:
3p
OCH3
3.8662
3.130
7.3901
7.3952
7.4019
7.4073
7.4192
7.4246
7.7069
7.7155
7.7235
7.7312
7.7394
7.9222
7.9302
8.2877
8.3034
8.3556
8.3728
1.961
0.979
2.048
0.929
1.000
PPM
file: ... NMRS\Tryptanthrins\8-methoxyH\fid block# 1 expt: "s2pul"
transmitter freq.: 499.734875 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 8
freq. of 0 ppm: 499.731883 MHz
processed size: 65536 complex points
LB: 0.200 GF: 0.0000
Hz/cm: 179.925 ppm/cm: 0.36004


SpinWorks 3:
118.1682
122.2133
124.2199
125.5539
125.6666
127.3849
127.4738
128.8043
129.3357
131.3696
133.9410
138.3862
138.9766
143.4065
144.3013
145.8233
146.4279
158.3205
182.6246
3q
PPM
file: ...RS\Tryptanthrins\2-phenyl\C1000\fid block# 1 expt: "s2pul"
transmitter freq.: 125.670278 MHz
time domain size: 78430 points
width: 30154.54 Hz = 239.9497 ppm = 0.384477 Hz/pt
number of scans: 1000
freq. of 0 ppm: 125.657064 MHz
processed size: 131072 complex points
LB: 0.500 GF: 0.0000
Hz/cm: 955.007 ppm/cm: 7.59931

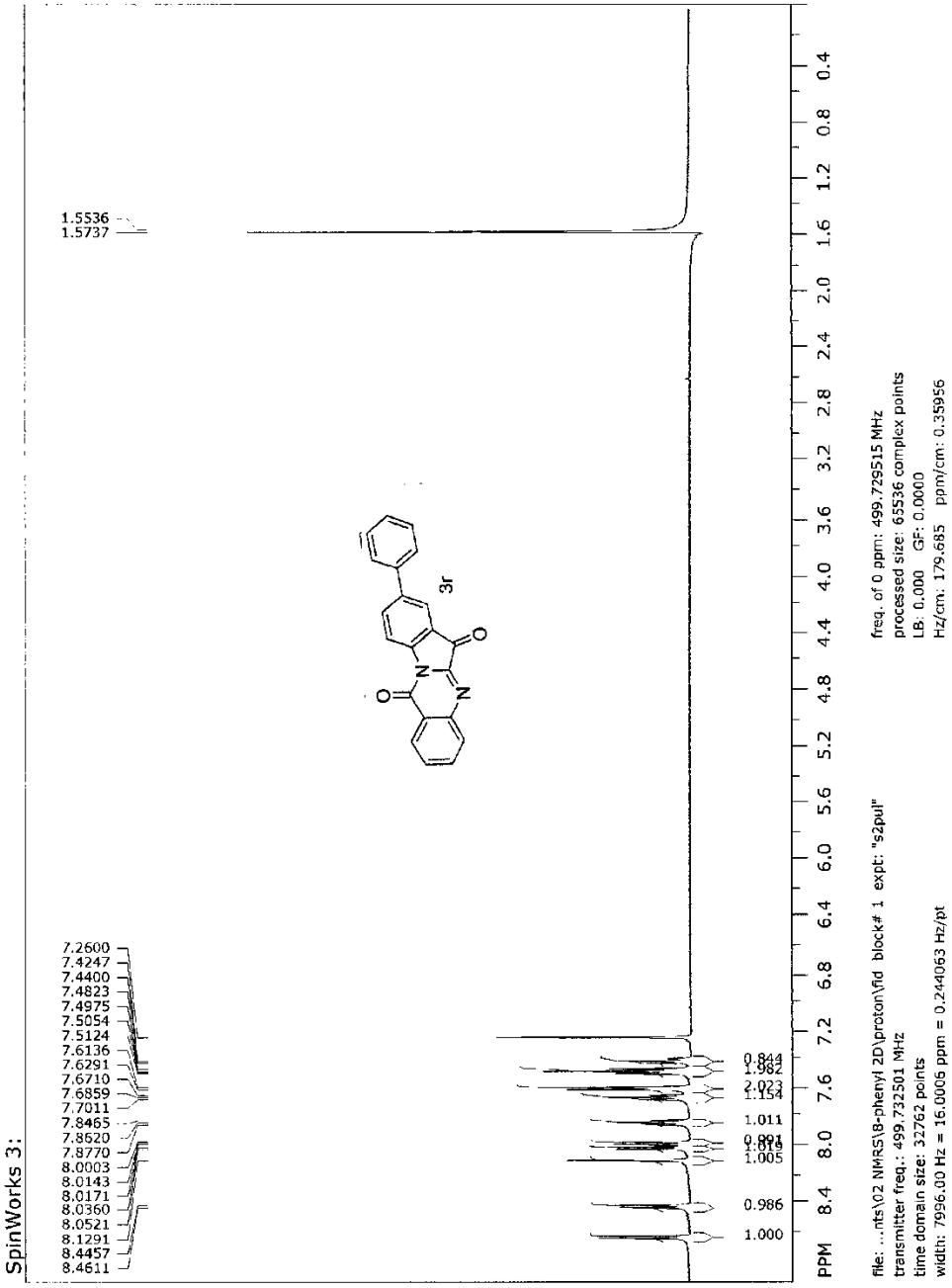

SpinWorks 3:
7.2550
7.2600
7.4280
7.4315
7.4328
7.4373
7.4414
7.4429
7.4490
7.4518
7.4543
7.9129
7.9219
7.9246
7.9280
8.0775
8.0799
8.0821
8.0847
8.6315
3q
2.268
2.159
2.161
1.248
0.999
2.085
1.942
PPM
8.4 8.0 7.6 7.2 6.8 6.4 6.0 5.6 5.2 4.8 4.4 4.0 3.6 3.2 2.8 2.4 2.0 1.6 1.2 0.8 0.4
file: ...2 NMRS\Tryptanthrins\2-phenylH\fid block# 1 expt: 's2pul'
transmitter freq.: 499.732501 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0006 ppm = 0.244063 Hz/pt
number of scans: 32
freq. of 0 ppm: 499.729518 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 179.685 ppm/cm: 0.35956

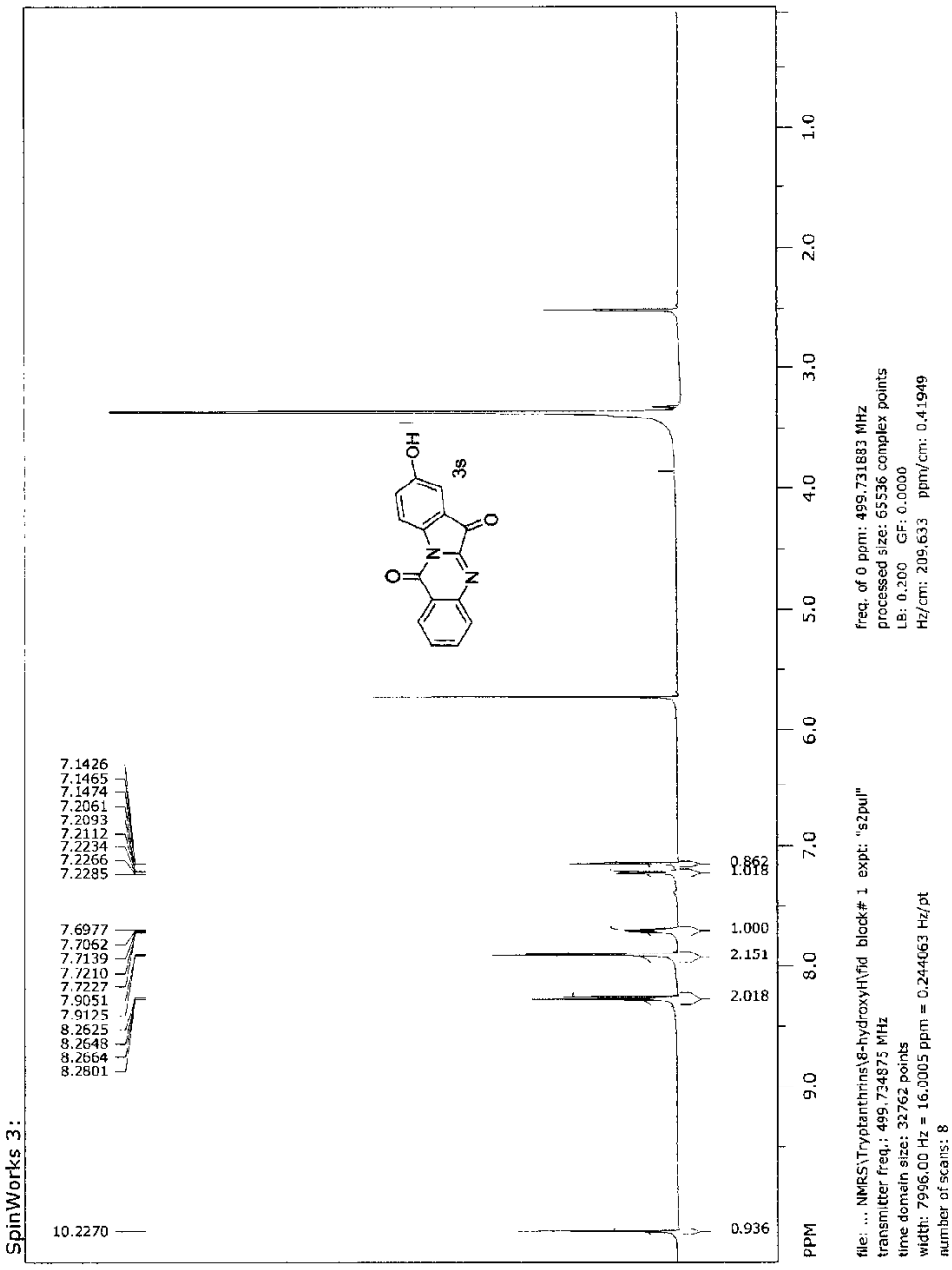
SpinWorks 3:

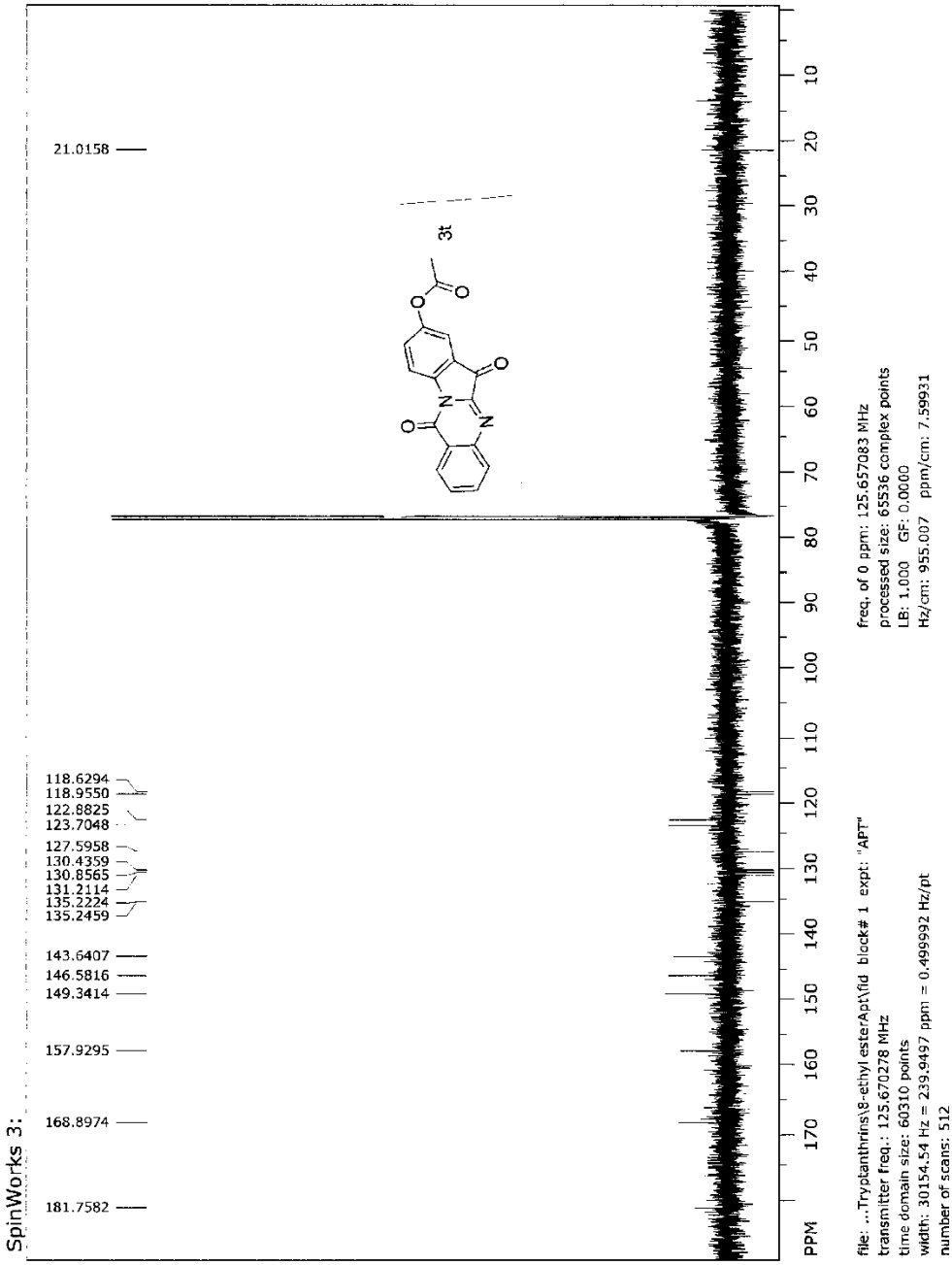


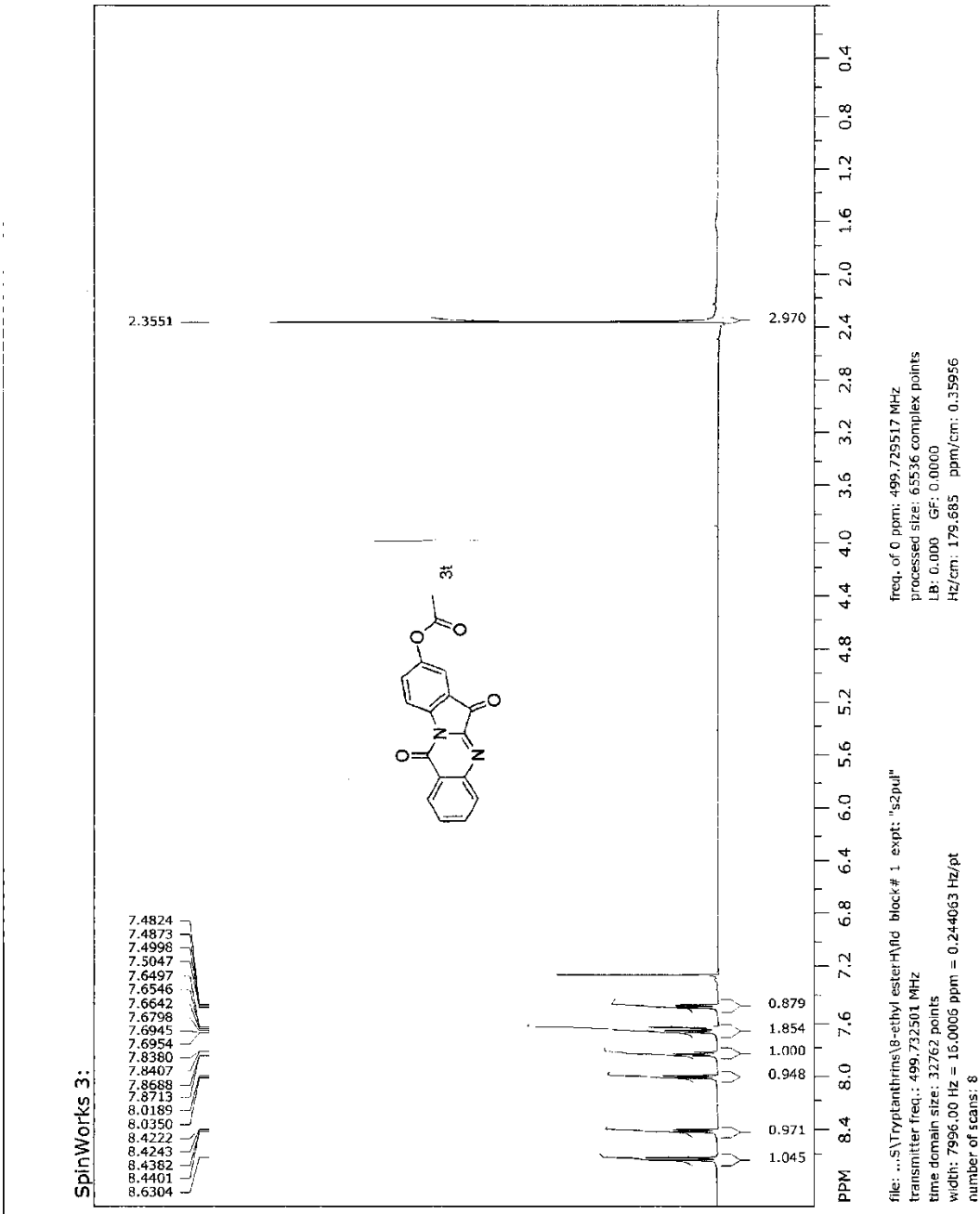
file: ...nts\02 NMRS\8-phenyl 2D\Carbon\fid block# 1 expt: "s2pul"
transmitter freq.: 125.670278 MHz
time domain size: 78430 points
width: 30154.54 Hz = 239.9497 ppm = 0.384477 Hz/pt
number of scans: 1216

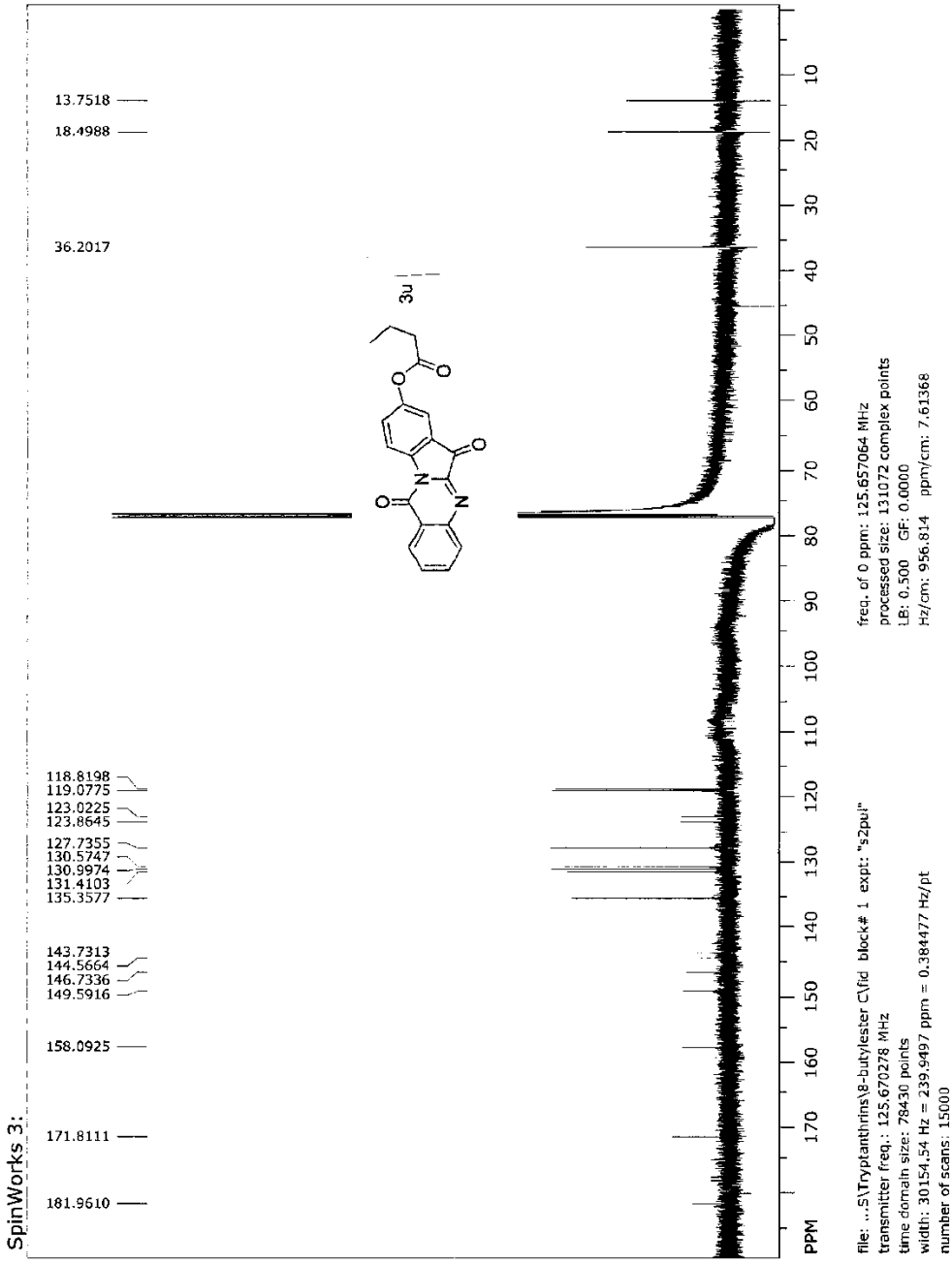
freq. of 0 ppm: 125.657063 MHz
processed size: 65536 complex points
LB: 0.500 GF: 0.0000
Hz/cm: 954.103 ppm/cm: 7.59212

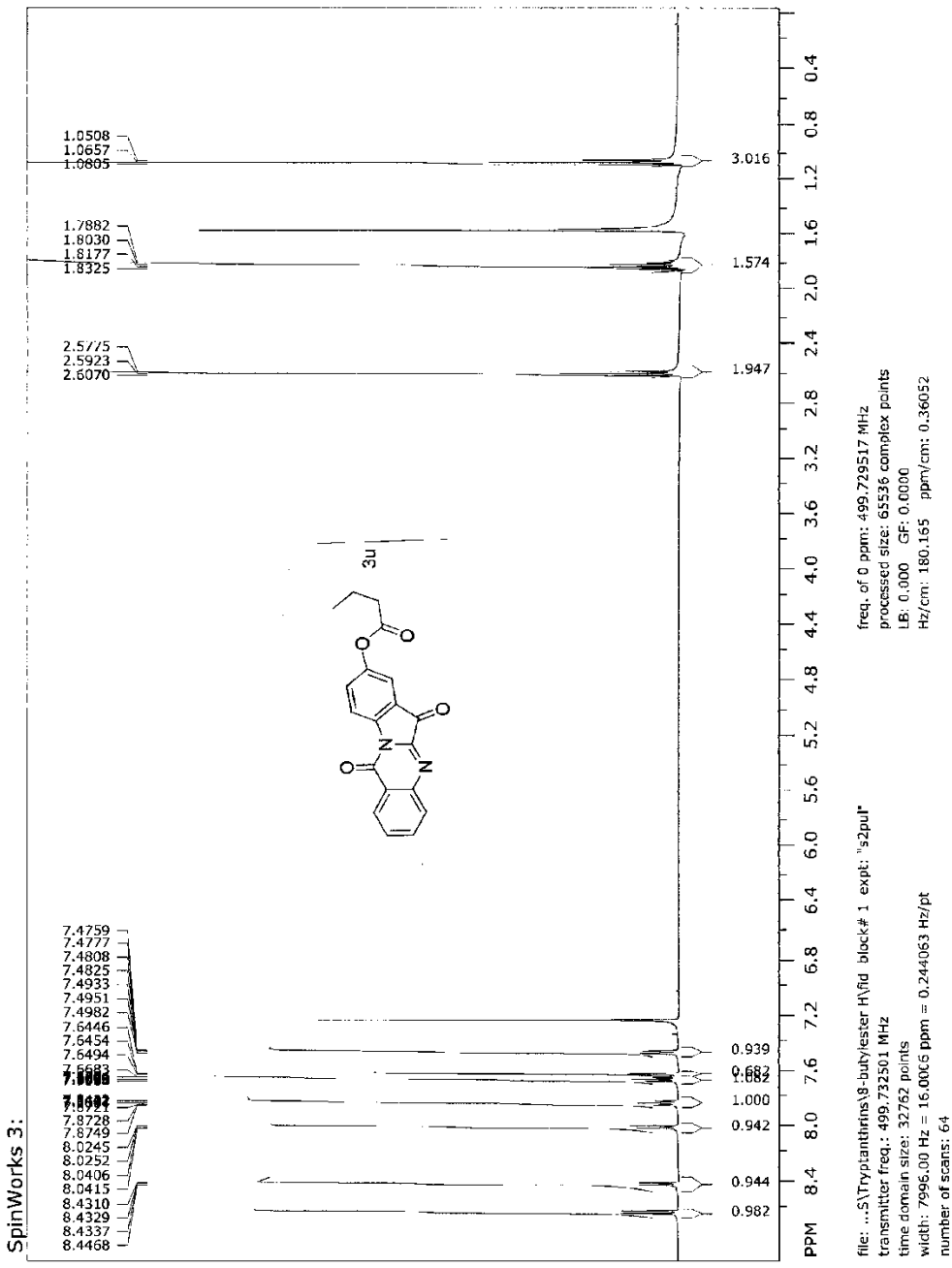

SpinWorks 3:
1.5536
1.5737
7.2600
7.4247
7.4400
7.4823
7.4975
7.5054
7.5124
7.6136
7.6291
7.6710
7.6859
7.7011
7.8465
7.8520
7.8770
8.0003
8.0143
8.0171
8.0360
8.0521
8.1291
8.4457
8.4611
3r
0.844
1.982
2.023
1.154
1.011
0.891
1.019
1.005
0.986
1.000
PPM
file: ...nts\02 NMRS\8-phenyl 2D\proton\fid block# 1 expt: "s2pul"
transmitter freq.: 499.732501 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0006 ppm = 0.244063 Hz/pt
number of scans: 8
freq. of 0 ppm: 499.729515 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 179.685 ppm/cm: 0.35956

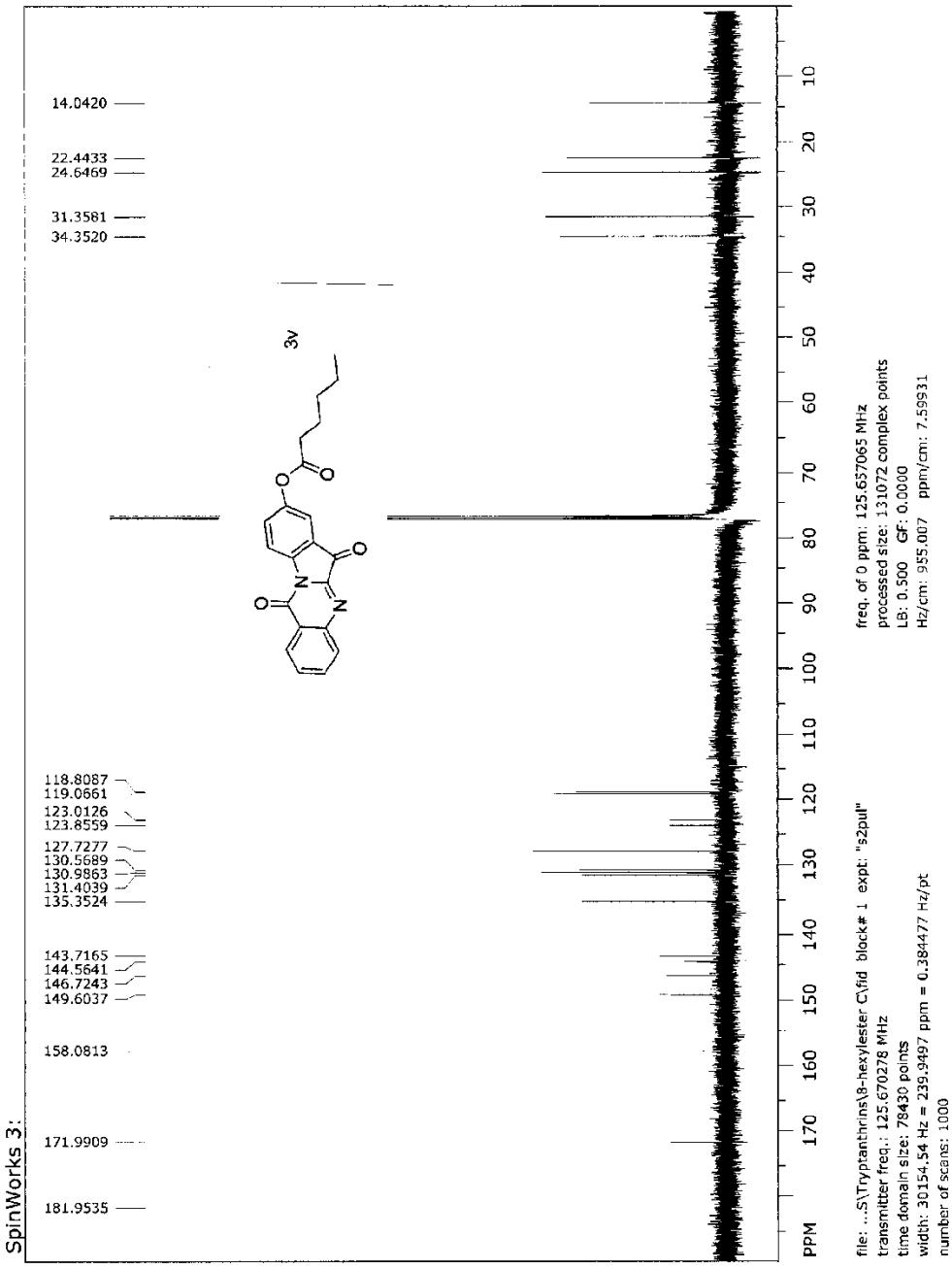

SpinWorks 3:
3s
OH
O
N
N
O
7.1426
7.1465
7.1474
7.2061
7.2093
7.2112
7.2234
7.2266
7.2285
7.6977
7.7062
7.7139
7.7210
7.7227
7.9051
7.9125
8.2625
8.2648
8.2664
8.2801
10.2270
0.862
1.018
1.000
2.151
2.018
0.936
PPM
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
file: ... NMRS\Tryptanthrins\8-hydroxyH\fid block# 1 expt: 's2pul'
transmitter freq.: 499.734875 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0005 ppm = 0.244063 Hz/pt
number of scans: 8
freq. of 0 ppm: 499.731883 MHz
processed size: 65536 complex points
LB: 0.200 GF: 0.0000
Hz/cm: 209.633 ppm/cm: 0.41949

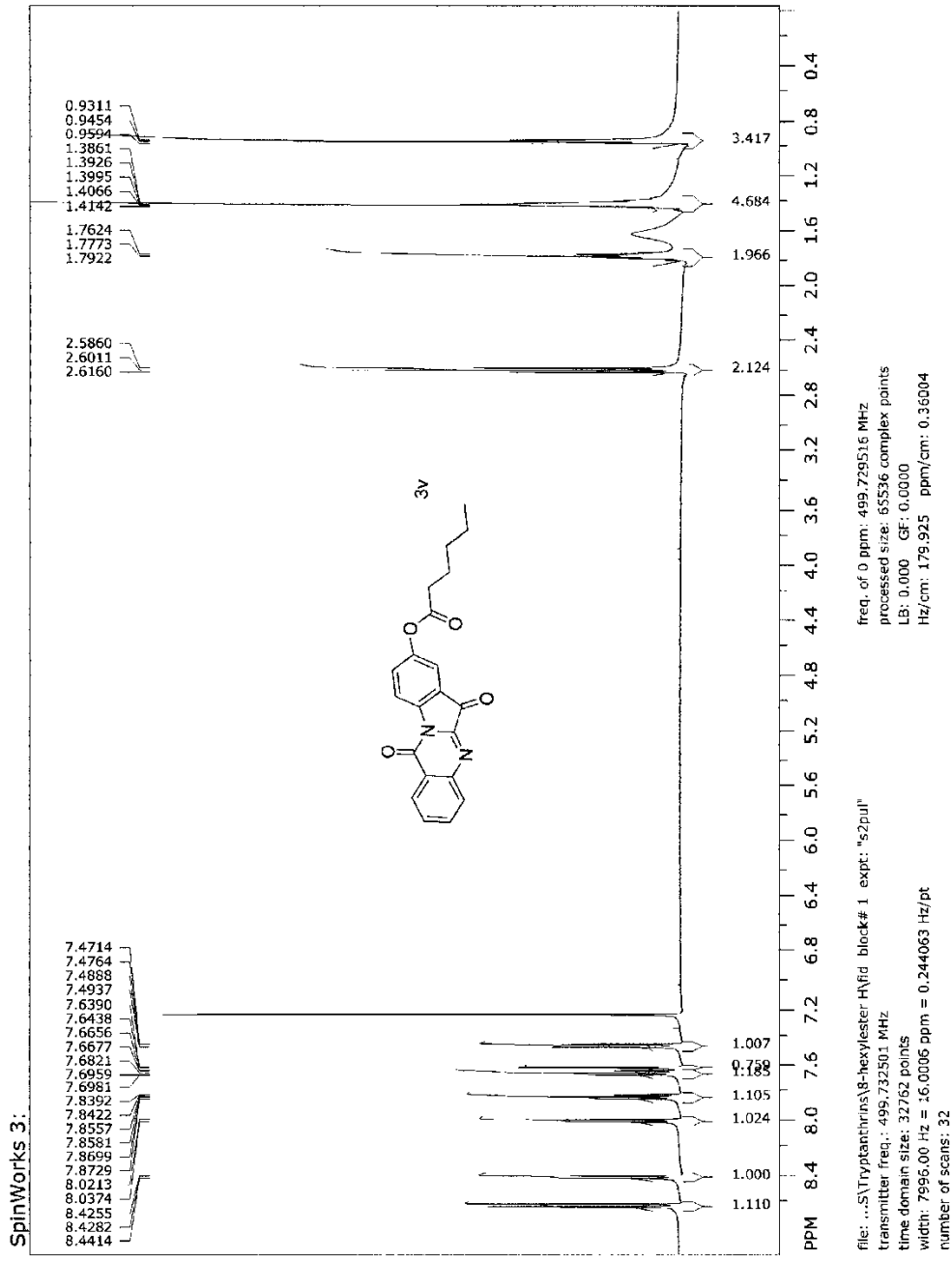

SpinWorks 3:
21.0158
118.6294
118.9550
122.8825
123.7048
127.5958
130.4359
130.8565
131.2114
135.2224
135.2459
143.6407
146.5816
149.3414
157.9295
168.8974
181.7582
3t
PPM
file: ...Tryptanthrins\8-ethylesterApt\fid block# 1 expt: "APT"
transmitter freq.: 125.670278 MHz
time domain size: 60310 points
width: 30154.54 Hz = 239.9497 ppm = 0.499992 Hz/pt
number of scans: 512
freq. of 0 ppm: 125.657083 MHz
processed size: 65536 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 955.007 ppm/cm: 7.59931

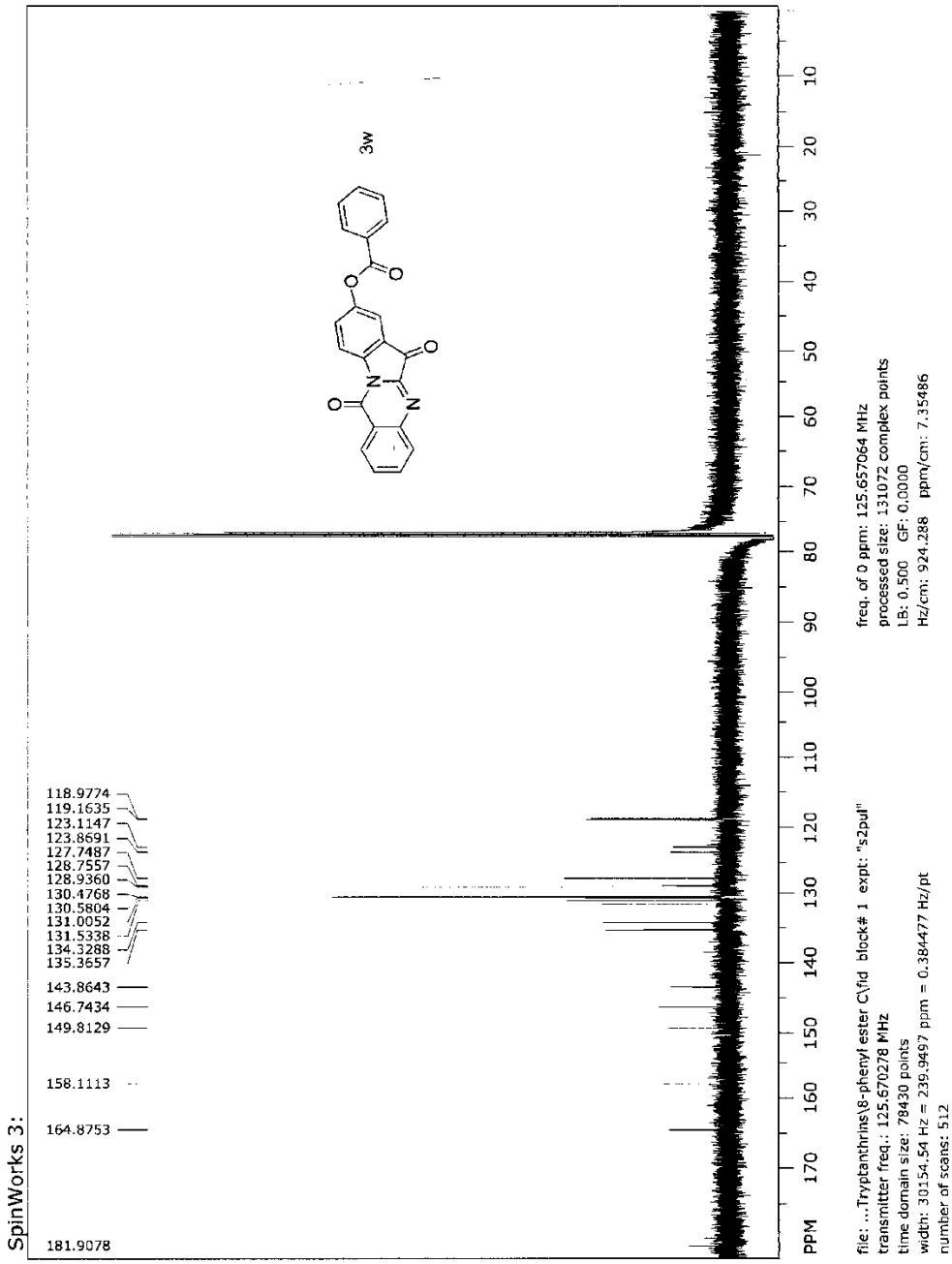

SpinWorks 3:
3t
7.4824
7.4873
7.4998
7.5047
7.6497
7.6546
7.6642
7.6798
7.6945
7.6954
7.8380
7.8407
7.8688
7.8713
8.0189
8.0350
8.4222
8.4243
8.4382
8.4401
8.6304
2.3551
2.970
0.879
1.854
1.000
0.948
0.971
1.045
PPM 8.4 8.0 7.6 7.2 6.8 6.4 6.0 5.6 5.2 4.8 4.4 4.0 3.6 3.2 2.8 2.4 2.0 1.6 1.2 0.8 0.4
file: ...S\Tryptanthrins\8-ethyl ester\fid block# 1 expt: 's2pul'
transmitter freq.: 499.732501 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0006 ppm = 0.244063 Hz/pt
number of scans: 8
freq. of 0 ppm: 499.729517 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 179.685 ppm/cm: 0.35956


SpinWorks 3:
13.7518
18.4988
36.2017
118.8198
119.0775
123.0225
123.8645
127.7355
130.5747
130.9974
131.4103
135.3577
143.7313
144.5664
146.7336
149.5916
158.0925
171.8111
181.9610
3u
PPM
file: ...S\Tryptanthrins\8-butylester C\fid block# 1 expt: "s2pul"
transmitter freq.: 125.670278 MHz
time domain size: 78430 points
width: 30154.54 Hz = 239.9497 ppm = 0.384477 Hz/pt
number of scans: 15000
freq. of 0 ppm: 125.657064 MHz
processed size: 131072 complex points
LB: 0.500 GF: 0.0000
Hz/cm: 956.814 ppm/cm: 7.61368

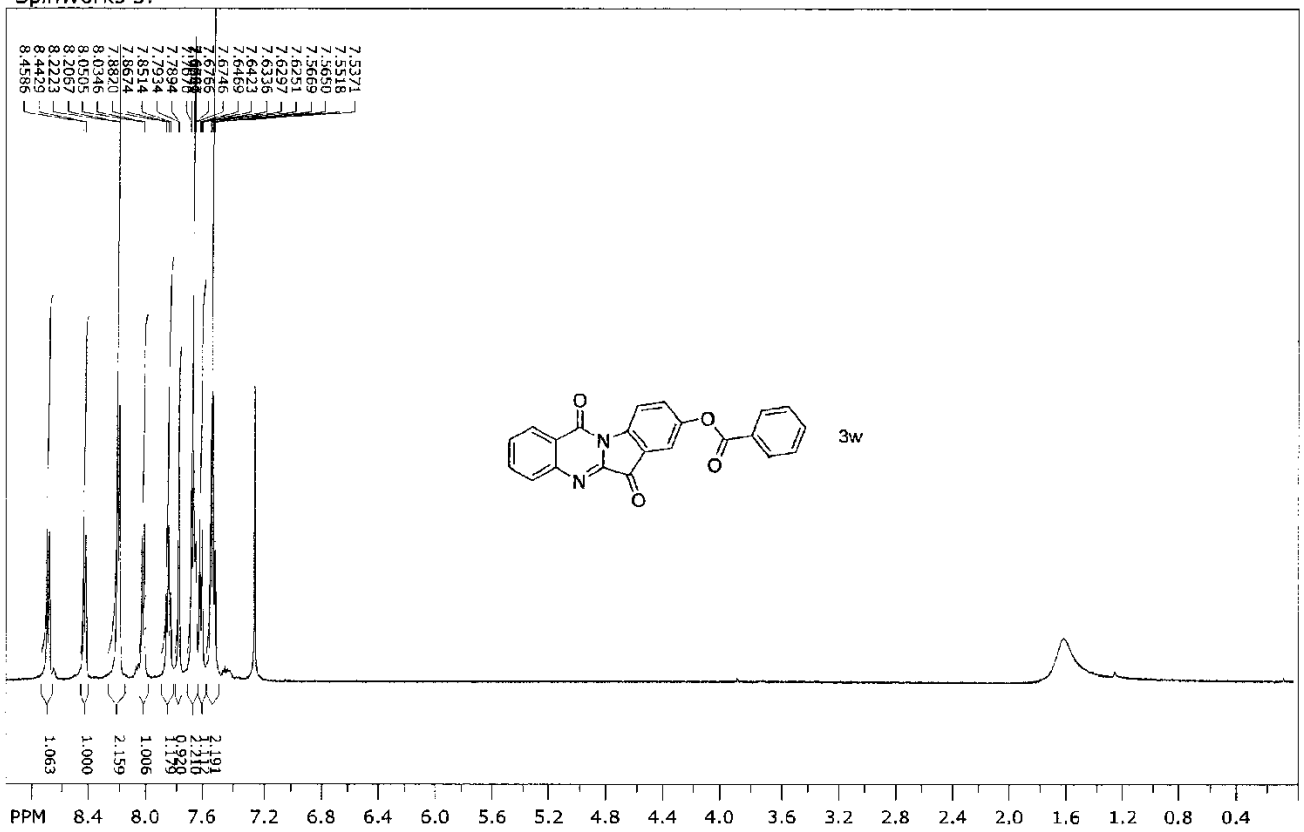

SpinWorks 3:
3u
1.0508
1.0657
1.0805
1.7882
1.8030
1.8177
1.8325
2.5775
2.5923
2.6070
7.4759
7.4777
7.4808
7.4825
7.4933
7.4951
7.4982
7.6446
7.6454
7.6494
7.8728
7.8749
8.0245
8.0252
8.0406
8.0415
8.4310
8.4329
8.4337
8.4468
3.016
1.574
1.947
0.939
0.682
1.082
1.000
0.942
0.944
0.982
PPM
file: ...S\Tryptanthrins\8-butylester H\fid block# 1 expt: "s2pul"
transmitter freq.: 499.732501 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0066 ppm = 0.244063 Hz/pt
number of scans: 64
freq. of 0 ppm: 499.729517 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 180.165 ppm/cm: 0.36052


SpinWorks 3:
14.0420
22.4433
24.6469
31.3581
34.3520
118.8087
119.0661
123.0126
123.8559
127.7277
130.5689
130.9863
131.4039
135.3524
143.7165
144.5641
146.7243
149.6037
158.0813
171.9909
181.9535
3v
PPM
file: ...S\Tryptanthrins\8-hexylester C\fid block# 1 expt: "s2pul"
transmitter freq.: 125.670278 MHz
time domain size: 78430 points
width: 30154.54 Hz = 239.9497 ppm = 0.384477 Hz/pt
number of scans: 1000
freq. of 0 ppm: 125.657065 MHz
processed size: 131072 complex points
LB: 0.500 GF: 0.000
Hz/cm: 955.007 ppm/cm: 7.59931


SpinWorks 3:
0.9311
0.9454
0.9594
1.3861
1.3926
1.3995
1.4066
1.4142
1.7624
1.7773
1.7922
2.5860
2.6011
2.6160
7.4714
7.4764
7.4888
7.4937
7.6390
7.6438
7.6656
7.6677
7.6821
7.6959
7.6981
7.8392
7.8422
7.8557
7.8581
7.8699
7.8729
8.0213
8.0374
8.4255
8.4282
8.4414
3.417
4.684
1.966
2.124
1.007
0.759
1.185
1.105
1.024
1.000
1.110
3v
PPM
file: ...S\Tryptanthrins\8-hexylester H\fid block# 1 expt: "s2pul"
transmitter freq.: 499.732501 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0006 ppm = 0.244063 Hz/pt
number of scans: 32
freq. of 0 ppm: 499.729516 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 179.925 ppm/cm: 0.36004


SpinWorks 3:
3w
118.9774
119.1635
123.1147
123.8691
127.7487
128.7557
128.9360
130.4768
130.5804
131.0052
131.5338
134.3288
135.3657
143.8643
146.7434
149.8129
158.1113
164.8753
181.9078
PPM
file: ...Tryptanthrins\8-phenyl ester C\fid block# 1 expt: "s2pul"
transmitter freq.: 125.670278 MHz
time domain size: 78430 points
width: 30154.54 Hz = 239.9497 ppm = 0.384477 Hz/pt
number of scans: 512
freq. of 0 ppm: 125.657064 MHz
processed size: 131072 complex points
LB: 0.500 GF: 0.0000
Hz/cm: 924.288 ppm/cm: 7.35486

SpinWorks 3:



3w

file: ...Tryptanthrins\8-phenyl ester H\fid block# 1 expt: "s2pul"
transmitter freq.: 499.732501 MHz
time domain size: 32762 points
width: 7996.00 Hz = 16.0006 ppm = 0.244063 Hz/pt
number of scans: 32

freq. of 0 ppm: 499.729515 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 179.925 ppm/cm: 0.36004