THE SYNTHESIS OF AN INTERNAL STANDARD FOR BICALUTAMIDE

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by

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SUMMARY

(*R*,*S*)-Bicalutamide [N-(4-cyano-3-trifluoromethylphenyl)-α-methyl-α-hydroxy-β-(4-fluorophenylsulfonyl)propanamide], sold as Casodex®, is the leading antiandrogen currently used to treat prostate cancer. It binds to androgen receptors and blocks cancer growth.

This work aims to develop internal standards for the bio-analytical component of clinical trials that are required to detect bicalutamide and derivatives. An internal standard is added to the body fluid sample (mostly blood) at the beginning of the sample work up at about the same concentration of the analyte to be quantified. An ideal internal standard has a similar extraction recovery and a similar retention time in HPLC. For quantification with mass spectrometry it should have a difference of at least 3 mass units from the analyte and a similar ionization response. The internal standard is used to calibrate the total ion current of the metabolite.

We did not have access to deuterium labeled starting materials and investigated structural analogues as an alternative strategy to obtain internal standards. *De novo* synthesis of structural analogues failed because we could not deprotonate the methyl sulfone in the presence of aromatic amides. We ascribed this to incomplete disclosure in the patented methods.

Treatment of bicalutamide with palladium on activated charcoal under the right conditions did not give the usually produced amide but gave smooth reduction of the C≡N group to a CH_3 group. This unusual reduction gave ready access to a good internal standard in good yield.

Elimination of the tertiary aliphatic hydroxy group of bicalutamide would give an alkene with similar polarity that could serve as an internal standard. Acid catalysis

using 1M HCl or p-toluene sulfonic acid failed, but treatment of bicalutamide with H_2SO_4 in benzene gave hydrolysis of the nitrile to an amide. This provides a second internal standard in good yield.

Bicalutamide did not react with weak base. Strong base such as LDA led to fission of the aliphatic moiety and isolation of aromatic sulfone and amide fragments.

Derivitization of the tertiary aliphatic hydroxy group of bicalutamide with 3-nitrobenzoyl chloride gave a benzoyl ester that allows facile thermal elimination of nitrobenzoic acid at 40 ºC to form an alkene. This represents a third potential internal standard. The NOESY experiment proves that the alkene has a Z-configuration. This indicates that the pro-R aliphatic hydrogen of bicalutamide was eliminated stereoselectively via a *syn*-periplanar cyclic transition state.

Efforts to eliminate the hydroxy group of bicalutamide photolytically at 300 nm in ethanol yielded an unexpected replacement of the aromatic CF_3 group with an ethoxy group. We could not find a similar transformation in the literature and believe it to be a novel reaction.

We used ^{19}F NMR to prove the absence or presence of fluorine and CF_3 moieties in the products. We also used the magnitude of ^{13}C-^{19}F coupling constants in proton decoupled ^{13}C spectra for accurate resonance assignment and structure elucidation.

We will test these novel analogues of bicalutamide in cancer bioassays and use them as internal standards for the quantification of bicalutamide and analogues in body fluids.

OPSOMMING

(*R,S*)-Bicalutamide [N-(4-siano-3-trifluorometielfeniel)-α-metiel-α-hydroksi-β-(4-fluorofenielsulfoniel)propaanamied], wat verkoop word onder die handelsnaam Casodex®, word beskou as een van die belangrikste anti-androgene middels wat tans gebruik word om prostraatkanker te behandel. Dit bind met androgene reseptore en inhibeer kanker groei.

Hierdie navorsing beoog om interne standaarde te ontwikkel vir die bio-analitiese komponent van kliniese studies wat vereis word om bikalutamied en derivate te registreer. ‘n Interne standard word aan die begin van die opwerk van die liggaamsvloeistofmonsters (meestal bloed) bygevoeg teen ongeveer dieselfde konsentrasie as die analiete wat gekwantifiseer moet word. Die ideale interne standaard ekstraëer teen ‘n soortgelyke konsentrasie en het ongeveer dieselfde retensietyd in HPLC as die analiet. Vir kwantifisering met massaspektrometrie moet daar ‘n verskil van ten minste 3 massa-eenhede en ‘n soortgelyke ionisasie reaksie in vergelyking met die analiet wees. Die interne standaard word gebruik om die totale ioon-vloei van die metaboliete te kalibreer.

Ons het nie toegang gehad tot gedeutereerde uitgangstowwe nie en het strukturele analoë ondersoek as ‘n alternatiewe strategie om interne standaarde daar te stel. De novo sintese van strukturele analoë het gefaal aangesien ons nie die metielsulfone kon deprotoneer in die teenwoordigheid van aromatiese amiede nie. Ons het dit toegeskryf aan onvolledige inligting in die gepatenteerde metodes.

Behandeling van bikalutamied met palladium op geaktiveerde koolstof onder die regte toestande het nie die gewone amied geproduseer nie, maar het geredelik die C≡N-groep na ‘n CH_3-groep gereduseer. Hierdie ongewone reduksie gee in ‘n goeie opbrengs gerieflike en maklike toegang tot ‘n interne standaard.

Eliminasie van die tersiêre alifatiese hidroksielgroep van bikalutamied behoort alkene met ‘n polariteit soortgelyk aan dié van die analiet te lewer wat as interne standaard kan dien. Suurkatalise (HCl (1 M) of *p*-tolueensulfoonsuur) het gefaal, maar behandeling van bikalutamied met H_2SO_4 in benseen het die nitriel na ‘n amied gehidroliseer, wat ‘n tweede interne standaard in ‘n goeie opbrengs lewer.

Bikalutamied het nie met ‘n swak basis reageer nie, maar sterk basisse soos LDA het tot splyting van die alifatiese moeïeteit en isolasie van aromatiese sulfone en amiedfragmente gelei.

Derivatisering van die tersiêre alifatiese hidroksielgroep van bikalutamied met 3-nitrobensoïelchloried het ‘n bensoïelester gelewer wat teen 40 ^{o}C fasiele termiese eliminasie van nitrobensoësuur toelaat om ‘n alkeen te vorm. Dit verteenwoordig ‘n derde potensiële interne standaard. NOESY eksperimente het bewys dat die alkeen ‘n Z-konfigurasie het, wat toon dat die pro-R alifatiese waterstof (en nie die pro-S alifatiese waterstof) van bikalutamied stereochemies geëlimineer is via ‘n syn-periplanêre sikliese transisie toestand.

Pogings om die hidroksielgroep van bikalutamied fotolities teen 300 nm in etanol te elimineer, het ‘n onverwagse vervanging van die aromatiese CF_3-groep met ‘n etoksiegroep teweeggebring. Ons kon geen soortgelyke transformasie in die literatuur opspoor nie en glo dat dit ‘n unieke en nuwe reaksie is.

Ons het van ^{19}F KMR gebruik gemaak om die teenwoordigheid of afwesigheid van fluoriede en CF_3- moeïeteite in ons produkte aan te dui. Die groottes van die ^{13}C-^{19}F koppelingskonstantes in ons proton-ontkoppelde ^{13}C KMR spektra lei tot die akkurate toekenning van resonansies en struktuuropklaring.

Ons sal hierdie nuwe analoë van bikalutamied in kanker bio-assesering toets en as interne standaarde gebruik vir die kwantifisering van bikalutamied en analoë in liggaamsvloeistowwe.

Abbreviations

The following abbreviations were used to describe the solvent systems and reagents used in this study:

A	acetone
BuLi	butyllithium
$CDCl_3$	chloroform-d
DCM	dichloromethane
EtOAc	ethyl acetate
H	hexane
K_2CO_3	potassium carbonate
LAH	lithium aluminum hydride
NH_3	ammonia
TEA	triethylamine
THF	tetrahydrofuran
NaOH	sodium hydroxide
H_2SO_4	sulphuric acid

Chapter 1

Literature Review

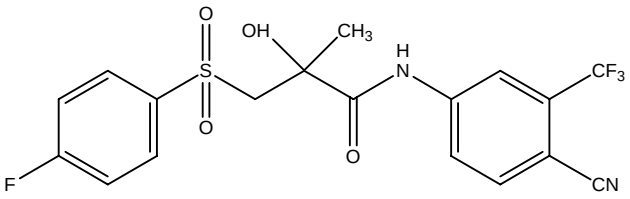
1.1 Introduction

Carcinoma of the prostate is the third leading cause of death for US men, after heart disease and stroke. It is estimated that one in six men will develop prostate cancer in the United States. The American Cancer Society estimated that in 2005 alone, 232 090 new cases were diagnosed with prostate cancer and from these 30 350 deaths occured.[1, 2]

(*R*,*S*)-Bicalutamide [N-(4-cyano-3-trifluoromethylphenyl)-α-hydroxy-α-methyl-β-(4-fluorophenylsulfonyl)propanamide] **(1)** sold as Casodex® is the leading antiandrogen currently used to treat prostate cancer.[3] Casodex® was first launched in 1995 as a combination treatment (with surgical or medical castration) and subsequently launched as monotherapy for the treatment of earlier stages of the disease.[4]

The growth of prostate cancer is stimulated by androgens, the male sex hormones. The pioneering work of Huggins and Hodges in 1941 showed the hormone dependence of this tumor. Androgen deprivation thus becomes the main treatment. This is achieved by castration, either alone or in combination with an antiandrogen such as bicalutamide.[5, 6]

The median survival of patients with metastatic androgen-independent prostate cancer is one year without the use of second-line hormonal therapy such as bicalutamide. Although most men with androgen-independent prostate cancer eventually develop symptoms related to metastases, often the first indication of disease progression is an asymptomatic increase in serum prostate-specific antigen (PSA) levels noted during routine surveillance.[7]



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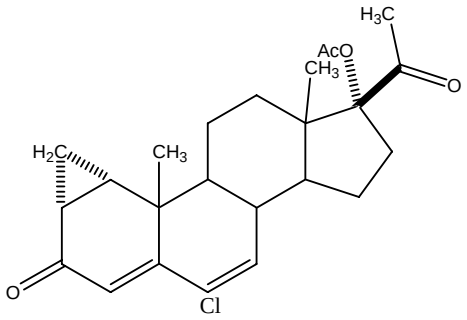
1.2 Pharmacology of bicalutamide

Most deaths from prostate cancer are caused by metastases. Although localized cancer can be treated with a good prognosis, metastatic prostate cancer resists conventional anti-cancer drugs and develops into incurable androgen refractory prostate cancer. This has been attributed to the genetic, biological, biochemical and immunologic diversity of prostate cancer cells. Research now concentrates on the mechanism of tumerogenesis and metastases in the hope of overcoming the heterogeneity of the disease.[2, 8]

Testosterone is the major endogenous ligand that complexes with the androgen receptor. It is synthesized in the testes (85%) and the adrenal cortex (15%). During the early stages of prostate cancer androgen activation of the androgen receptor exacerbates the disease and stimulates hyperplasia of the prostate. Removal of the testes is the simplest and most frequently used method to remove the stimulatory effect of testosterone on the growth of prostate cancer metastases. The adrenal gland however remains and only partial androgen deprivation is achieved.[2, 8]

Antiandrogens that bind to the androgen receptor and thereby block androgen action, from whatever source, have the potential to affect maximum androgen withdrawal. Early treatment of the disease during the so-called hormone receptive stage with a non-steroidal androgen receptor prostate-selective antagonist now benefits patients with a 99.8% 5-year survival rate.[2, 8]

Cyproterone acetate **2** was the first clinically used antiandrogen (early 1960s). It is effective in most patients and produces fewer side effects than estrogens. The steroidal nature of the drug is probably responsible for most of its remaining side effects including cardiovascular effects, adverse effects on serum lipoproteins, effects on carbohydrate metabolism (and diabetes) and severely depressed libido.



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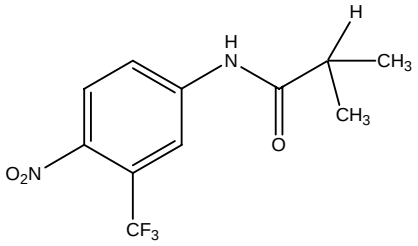
Flutamide **3** was the first non-steroidal antiandrogen. It is a pure antiandrogen that does not exhibit any of the side effects associated with steroidal drugs. It rarely causes loss of libido. It represents an important improvement but still causes side effects in some patients including gastrointestinal intolerance and gynecomastia. Irreversible liver function abnormalities can be serious. Flutamide antagonises the action of androgen at the hypothalamus and pituitary gland. This leads to an increase in luteinizing hormone (LH) that stimulates androgen secretion by the testes and progressively higher doses of flutamide is required if the testes had not been removed. The active metabolite of flutamide, hydroxyflutamide **(4)** has a biological half-life of 5.2 hours. This requires the drug to be administered three times daily.



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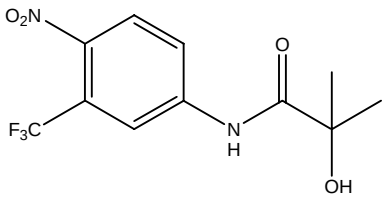

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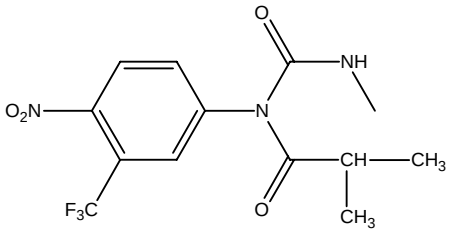
Nilutamide **5** is structurally related to flutamide and replaced it. It has an improved biological half-life (two days) that allows once daily administration. It causes side effects, including alcohol intolerance, interstitial pneumosis and problems with light-dark adaptation. It was eventually replaced by bicalutamide as the treatment of choice.



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Bicalutamide is a peripherally selective non-steroidal anti-androgen that was selected from 2000 compounds specifically designed for anti-androgen activity. It has a long half-life (once daily oral administration), does not stimulate LT production (does not require removal of the testes and can consequently be used as a monotherapy), does not interfere with libido and is generally better tolerated than flutamide or nilutamide. Its peripheral selectivity (little effect on serum LH and testosterone) is due to poor penetration of the blood-brain barrier.[8]

Bicalutamide has also been indicated as treatment for familial male-limited precocious puberty. It leads to decreased facial acne and pubic hair. It achieved a marked decrease in growth velocity and skeletal advancement in treated patients.[9]

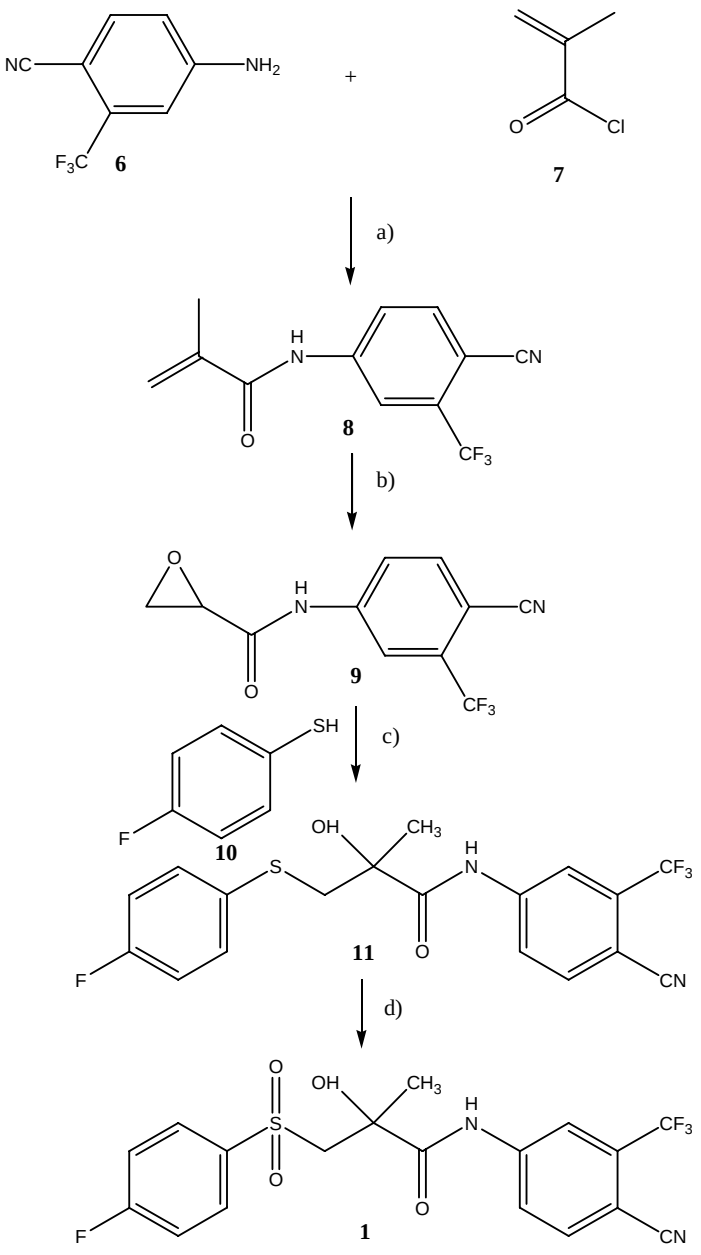
The (*S*)-isomer of bicalutamide is metabolized much faster than the (*R*)-isomer and is thus eliminated faster. The (*S*)-isomer consequently has a shorter half-life, put more stress on the liver and requires larger doses to be effective. It would be advantageous, particularly for patients with hepatic impairment, to administer the (*R*)-isomer only. Astrazeneca has recently patented the formulation of (*R*)-bicalutamide.[3, 10]

Analytical methods to quantify levels of bicalutamide in body fluids has been described in detail by Rao and co-workers.[10]

1.3 Synthesis of (*R*,*S*)-bicalutamide

Tucker and co-workers[6] reported the original synthesis of racemic bicalutamide **(1)** in 1988. It is comprised of four steps and has an overall yield of 50% **(Scheme 1)**.

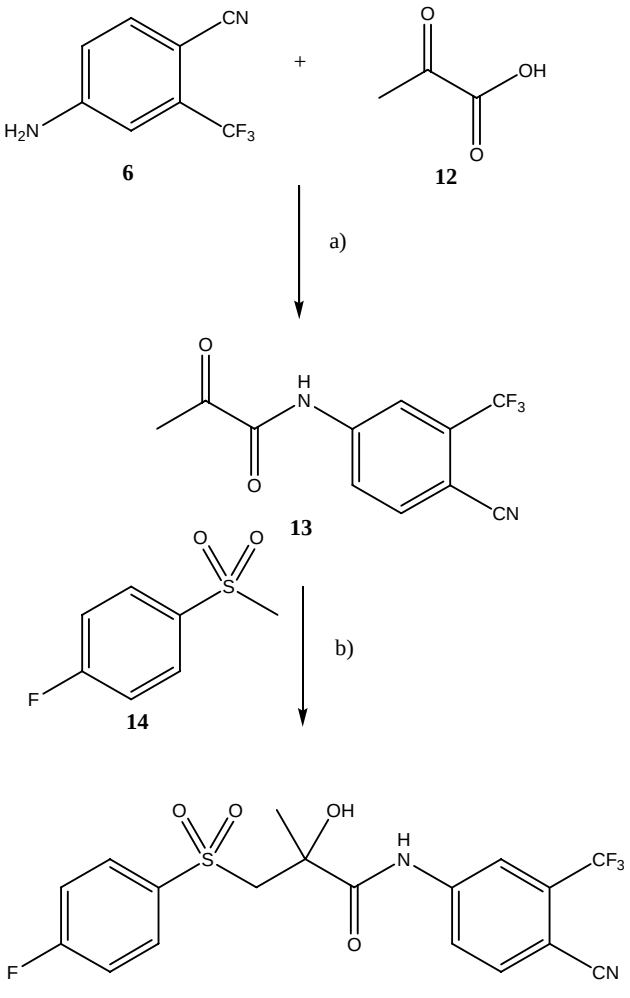
Scheme 1



Conditions: (a) CH_3CONMe_2, 2,6-Dimethylphenol; (b) CH_3CCl_3, mCPBA ; (c) NaH, THF; (d) CH_2Cl_2, mCPBA .

In 2002 James and Ekwuribe[11] described an improved synthesis of the enantiomeric mixture in two steps with an overall yield of 73%, **(Scheme 2)**. Thionyl chloride was found to catalyse the nucleophilic addition of the aniline **(6)** derivative (a poor nucleophile due to two electron withdrawing groups on the aromatic ring) to pyruvic acid to give the α-keto acid **13** in a good yield (80%). Sodium hydride was used to deprotonate the methyl sulfone to form the dimer. Use of a stronger base (pentylmagnesium bromide or *n*-butyllithium) improved the desired deprotonation.

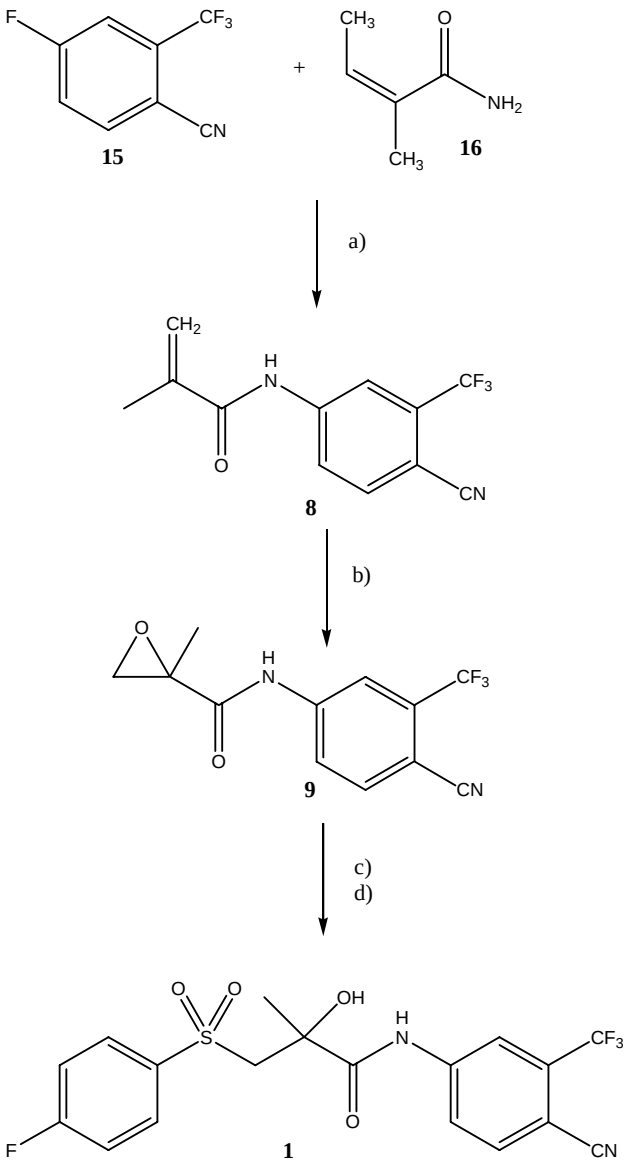
Scheme 2



Conditions: (a) DMA, $SOCl_2$; (b) BuLi, THF .

Chen and co-workers[12] synthesized (*R,S*)-bicalutamide from the less expensive 4-fluoro-2-trifluoromethylbenzonitrile **15**. They described the first use of the methylacrylamide anion in nuleophilic aromatic substitution. The three-step synthesis was reported to give an overall yield of more than 90% **(Scheme 3)**.

Scheme 3

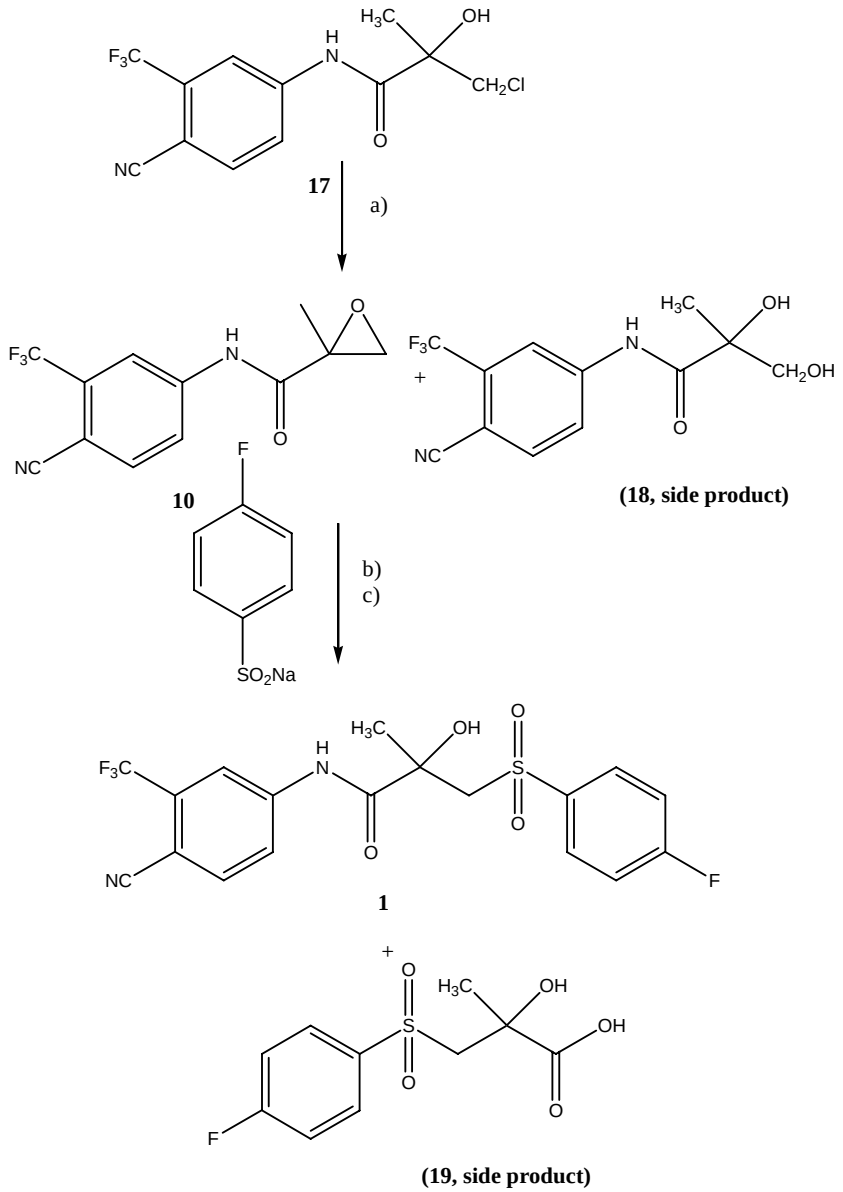


Conditions: (a)(i) H_2O/HCl, (ii) 2.8 eq. NaH/DMF, 97%; (b) H_2O_2/$(CF_3CO)_2O$, CH_2Cl_2, 98%; (c) 4-FC_6H_4SH/NaH/THF; (d) H_2O_2/$(CF_3CO)_2O$, CH_2Cl_2, 97%.

1.4 Production of enantiopure (*R*) - and (*S*)-bicalutamide

Torok and co-workers[13] developed a new route for the production of racemic (R,*S*)-bicalutamide **(Scheme 4)**. High-performance liquid chromatographic methods were developed for the enantioseparation of (*R*,*S*)-bicalutamide.

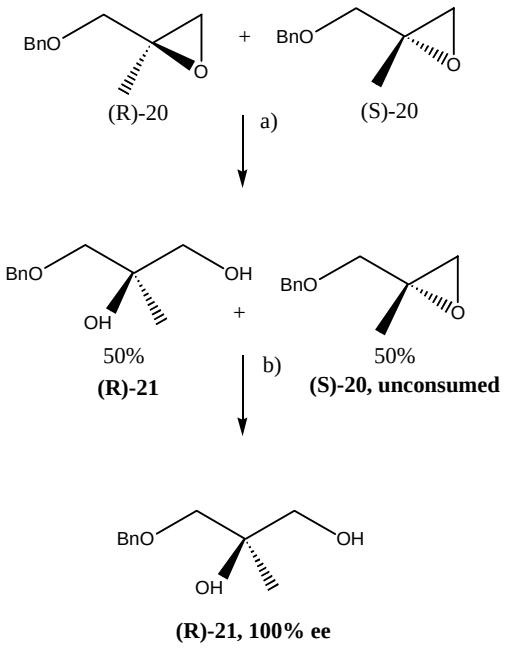
Scheme 4



Conditions: (a) NaOH in acetone, 68%; (b) AcOH in MeOH, 43%; (c) tetrabutylammonium bromide, MeOH, 62% **(18)** and **(19)** isolation by chromatography.

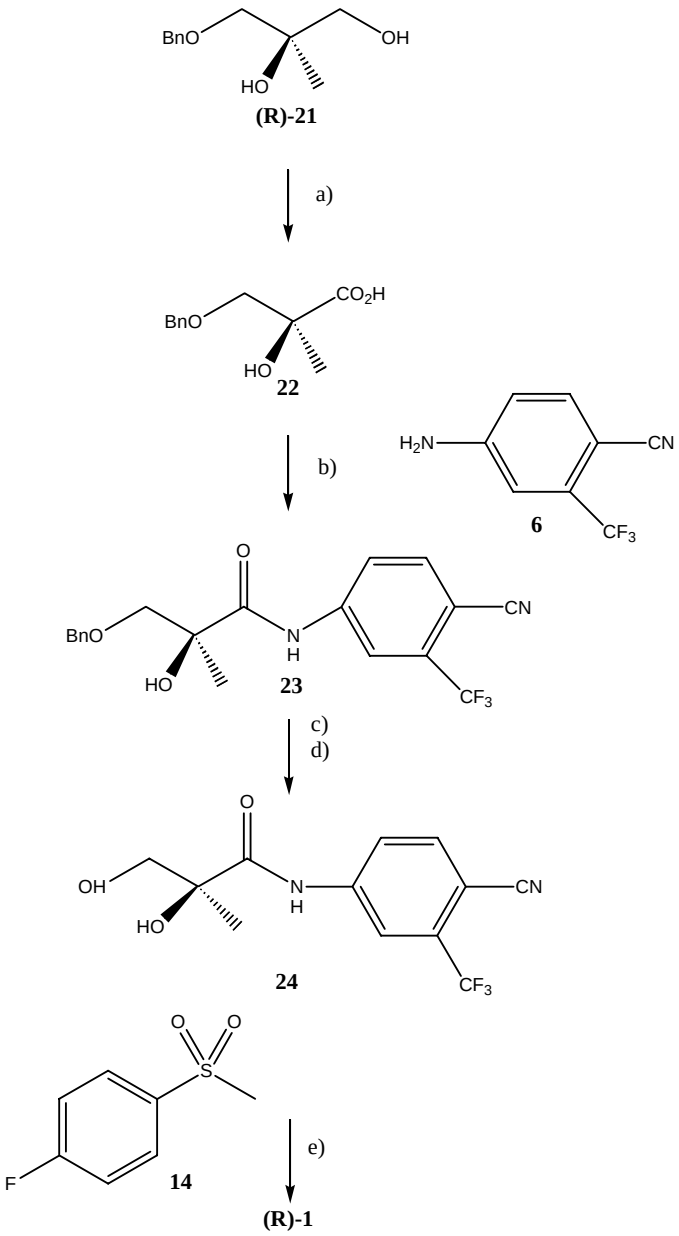
Fujino and co-workers[14] published a synthesis of enantiopure (*R*)-bicalutamide. They started with an epoxide mixture of (*R*)-**20** and (*S*)-**20**. Treatment with an engineered *Bacillus subtilis* transforms only the (*R*)-isomer and left the (*S*)-isomer unconsumed. Subsequent treatment of the resulting mixture of (*R*)-**21** and unconsumed (*S*)-**20** with dilute H_2SO_4 transforms unconsumed *R*-enantiomer to (*R*)-**21** with 100% ee conversion **(Scheme 5a)**. They also developed a method to synthesize (*R*)-bicalutamide from (*R*)-**21 (Scheme 5b)** and (*R*)-**20 (Scheme 5c)**.

Scheme 5a



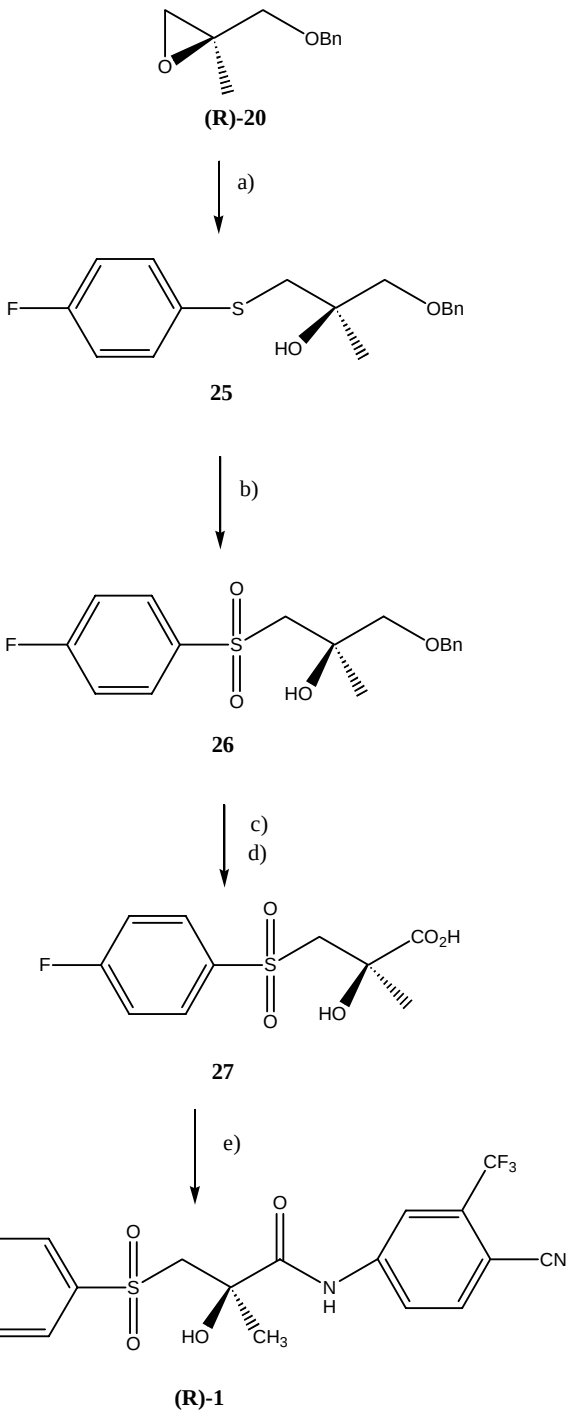
Reagents and conditions: (a) *B. subtilis* epoxide hydrolase, 30 ^{o}C, 7 days, conv 50%; (b) dilute H_2SO_4, room temperature.

Scheme 5b



Reagents and conditions: (a) TEMPO, NaClO, $NaClO_2$, MeCN-buffer, 35 ^{0}C, 24 h, 97%; (b) $SOCl_2$, THF, DMAP, room temperature, 5days; (c) Ac_2O, pyridine, 83% ; (d) DDQ, hv (352 nm, 15 W), MeCN, 85%; (e) K_2CO_3, MeOH, 85%;

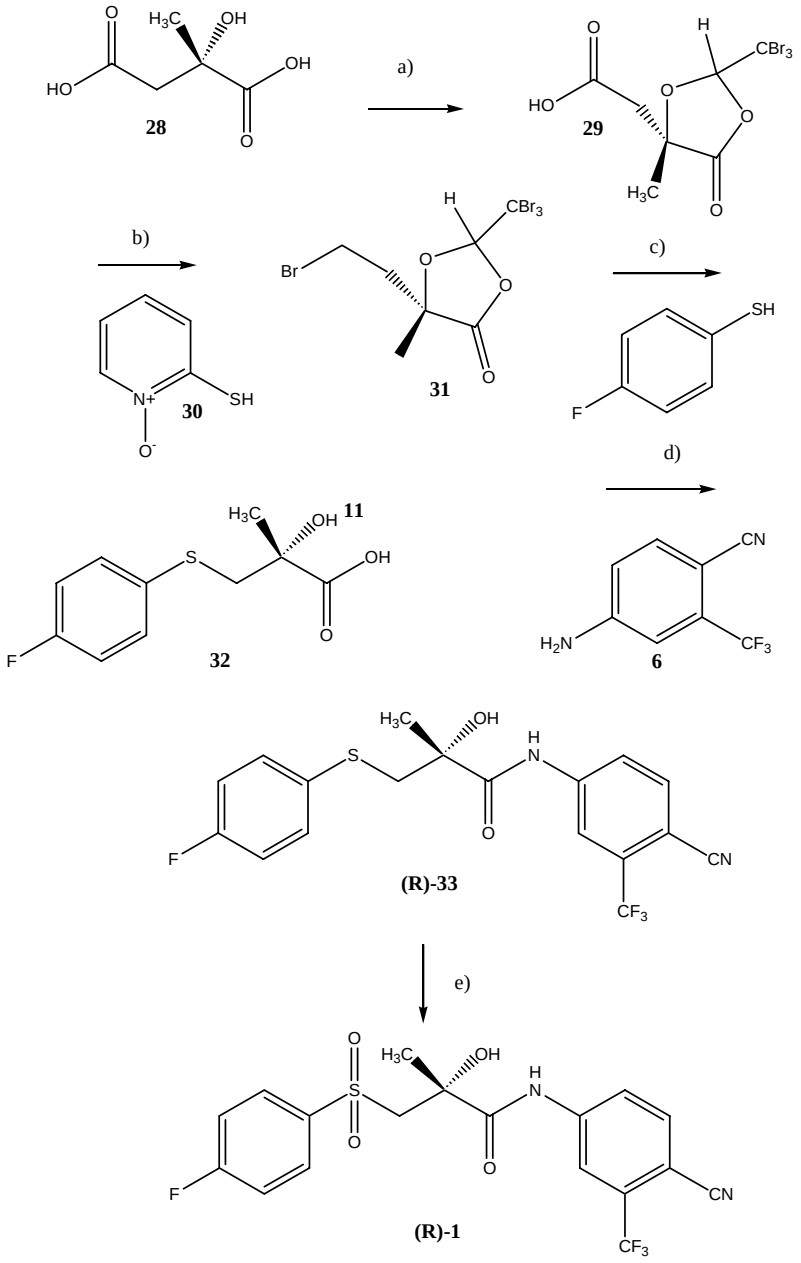
Scheme 5c



Reagents and conditions: (a) 4-fluorothiophenol , NaH, THF, room temperature, 90 min, 93%; (b) H_2O_2, AcOH, 60 ^oC, 24h; (c) H_2, Pd-C, EtOH, room temperature, 48h, 91% from **25**; (d) TEMPO, NaClO, $NaClO_2$, MeCN-buffer, 93%; (e) $SOCl_2$, THF, 4-cyano-3-trifluoromethyl-aniline, room temperature, 5 days, 91%.

In 2002, James and Ekwuribe[3] published an improved version of (*R*)-bicalutamide synthesis based on naturally occurring (*S*)-citramalic acid **(30)** as starting material **(Scheme 6)**. This route has the advantage of one less step and the use of natural (S)-citramalic acid in more cost effective synthesis.

Scheme 6



Reagents and conditions: (a) Tribromo acetalaldehyde, H_2SO_4; (b) DCC, $CBrCl_3$; (c) NaOH, i-PrOH ; (d) $SOCl_2$, DMA; (e) mCPBA.

1.5 Synthesis of derivatives of (*R,S*)-bicalutamide

Tucker and co-workers[6] successfully prepared analogs of bicalutamide **Table 1** using the synthetic route illustrated in **Scheme 1**. These compounds were also tested for anti-androgen activity.

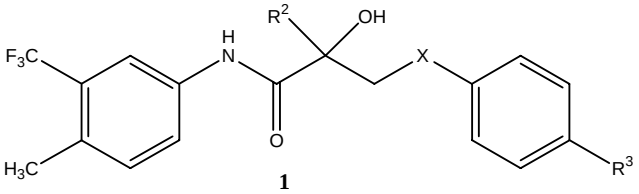


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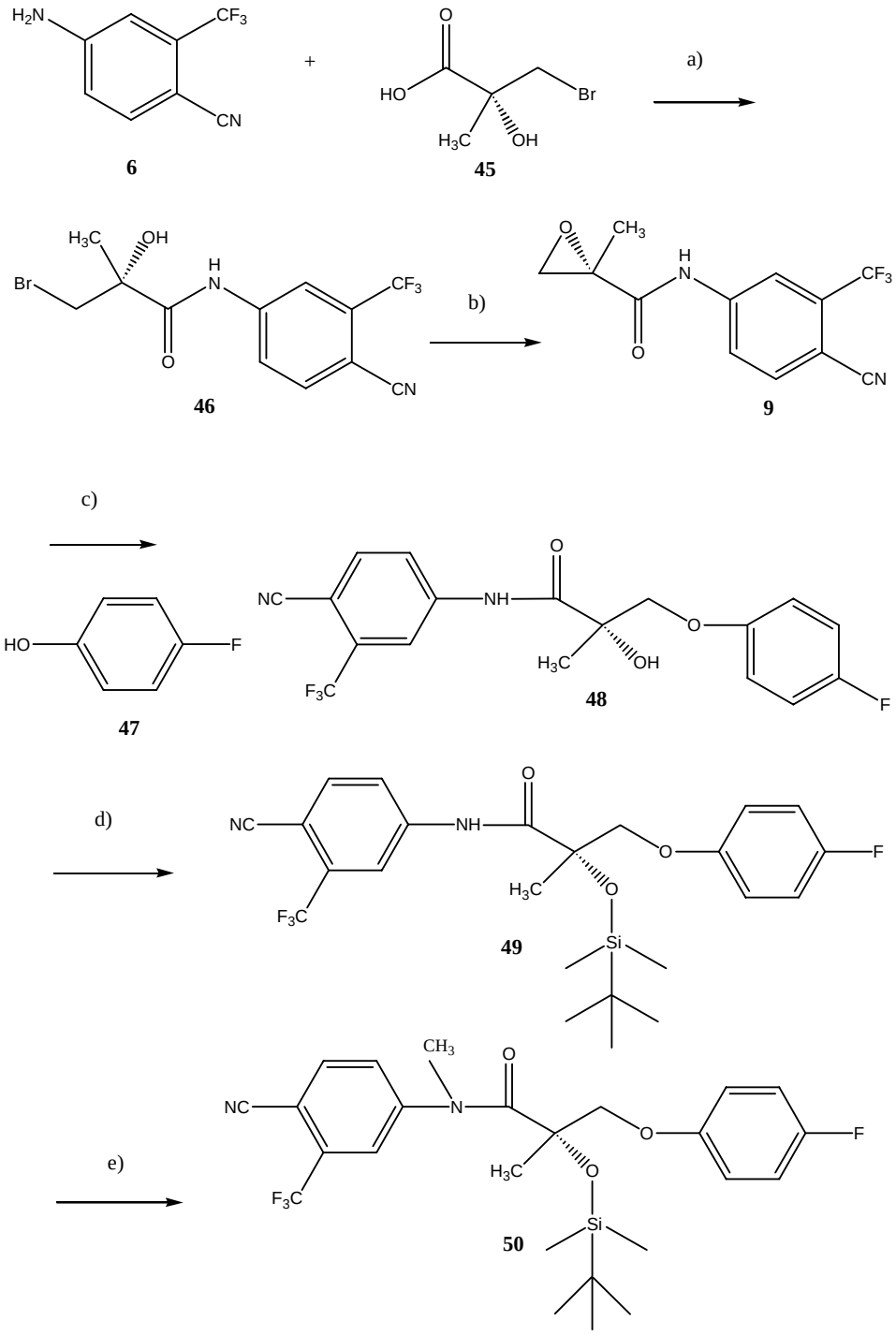
Table 1: Analogues of Bicalutamide

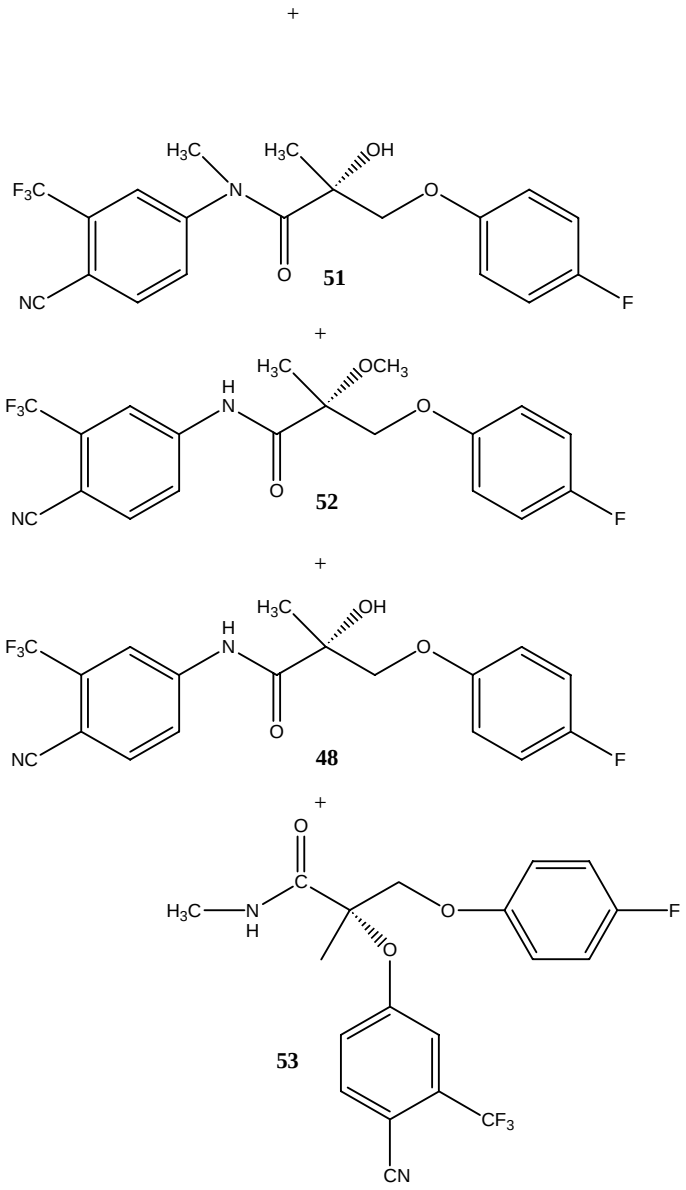
Compound	R^2	R^3	X	Formula
34	NO_2	3-Cl	S	$C_{17}H_{14}ClF_3N_2O_4S$
35	NO_2	2-Cl	S	$C_{17}H_{14}ClF_3N_2O_4S$
36	NO_2	4-F	S	$C_{17}H_{14}F_4N_2O_4S$
37	NO_2	4-F	SO_2	$C_{17}H_{14}F_3N_2O_6S$
38	NO_2	4-NO_2	S	$C_{17}H_{14}F_3N_3O_6S$
39	NO_2	4-CN	S	$C_{18}H_{14}F_3N_3O_4S$
40	NO_2	4-CH_3O	S	$C_{18}H_{17}F_3N_2O_5S$
41	CN	4-CH_3S	S	$C_{18}H_{17}F_3N_2O_4S_2$
42	CN	4-F	S	$C_{18}H_{14}F_4N_2O_2S$
1	CN	4-F	SO_2	$C_{18}H_{14}F_4N_2O_4S$
43	CN	4-Cl	S	$C_{17}H_{14}ClF_3N_2O_2S$
44	CN	4-CH_3S	S	$C_{19}H_{17}F_3N_2O_2S_2$

Patil and co-workers[15] synthesized a derivative of bicalutamide **(Scheme 7)** in which the sulfone group was replaced by oxygen **48.** The hydroxy group of compound **48** was protected using tert-butyldimethylsilyl trifluoromethane sulfonate with 2,6-lutidine in dichloromethane. *N*-methyl derivatives of compound **49** were prepared using a CsF-Celite/alkyl halide/acetonitrile combination. During

the *N*-methylation a 1, 4-*N*→*O* migration of the disubstituted phenyl ring was observed to yield compound **53**.

Scheme 7



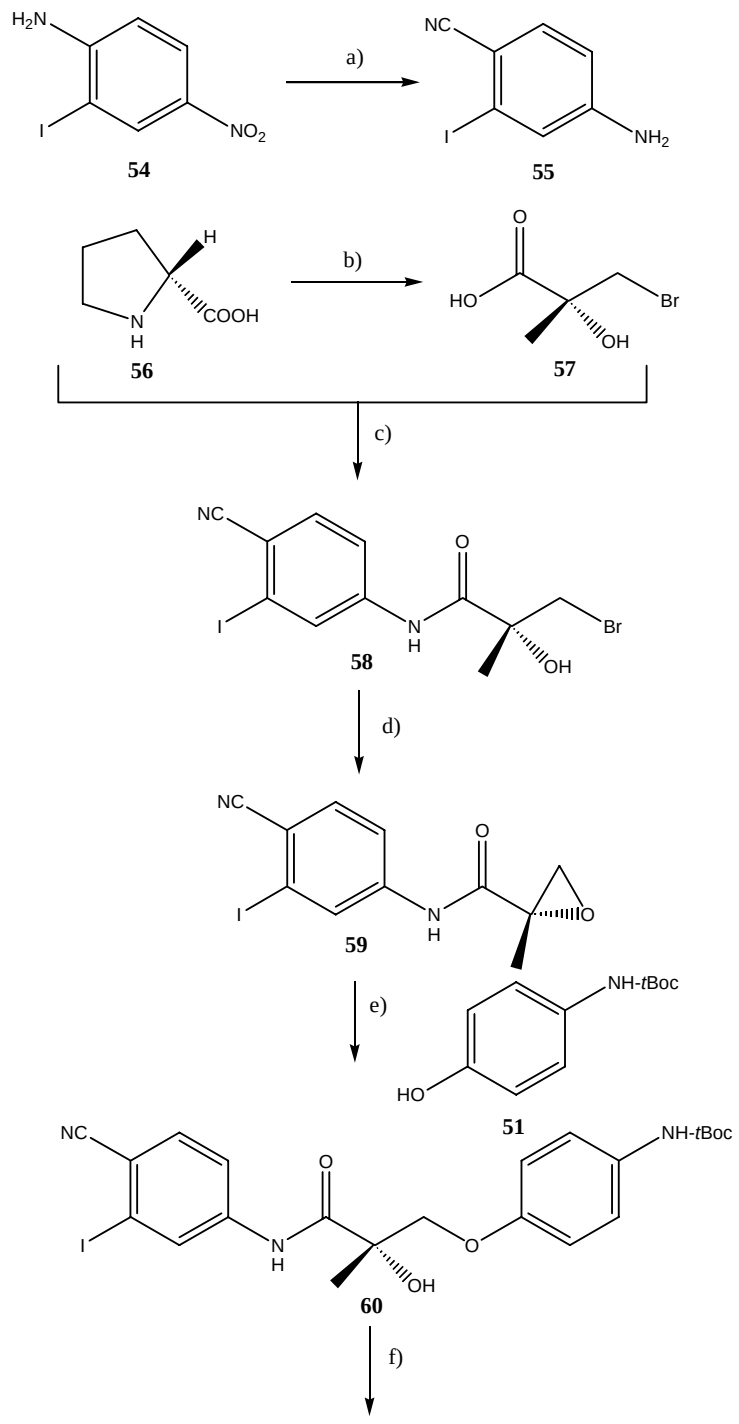


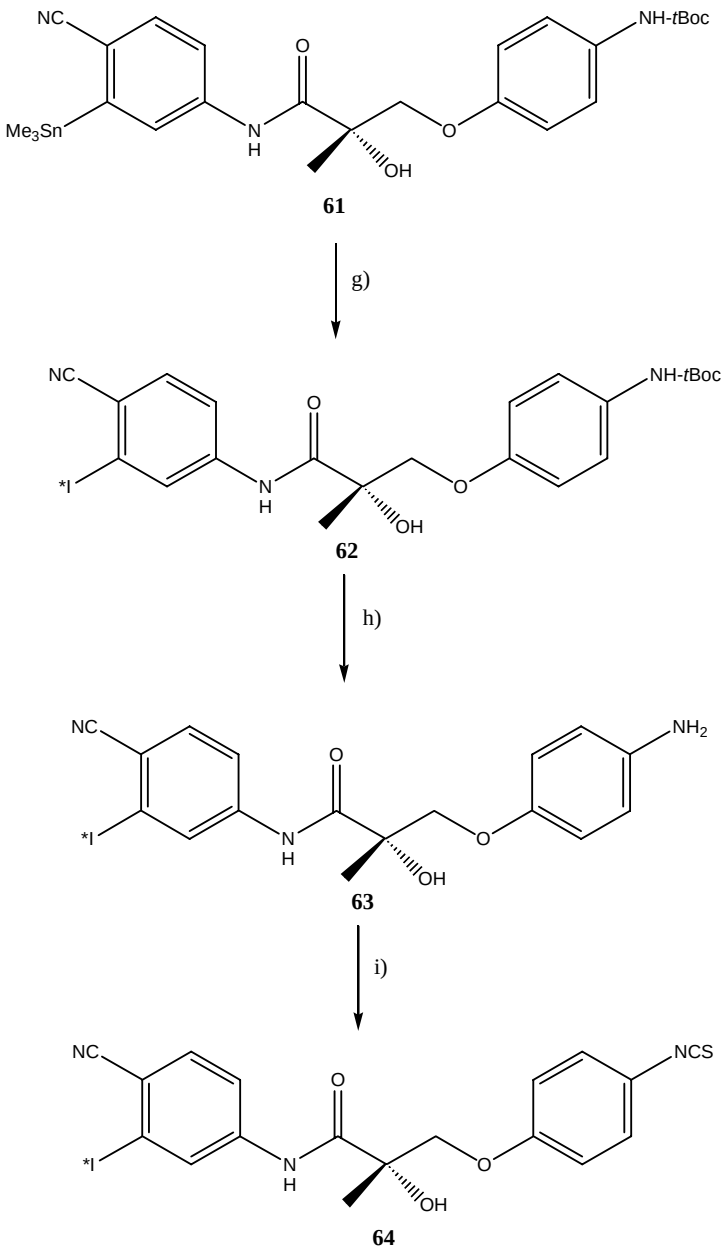
Reagents and conditions: (a) $SOCl_2$, TEA, THF; (b) anhyd. K_2CO_3, anhyd. acetone; (c) anhyd. K_2CO_3; (d) TBDMSOTf, 2,6-lutidine, CH_2Cl_2; (e) CH_3ICsF-Celite, Δ.

In 2005 Nair and co-workers[16] synthesized iodo analogs of bicalutamide **(Scheme 8)** that contained a radioactive isotopic label (^{125}I) to allow the radioimaging of prostate cancer. These compounds were obtained by replacing the trifluoromethyl and sulfonyl groups with iodine and oxygen respectively, as well as the introduction of chirality at the stereogenic centre. In 2006 Nair and co-workers[17]

also synthesized chiral oxazolidinedione derived bicalutamide analogs **(Scheme 9a and b).**

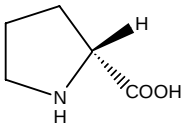
Scheme 8





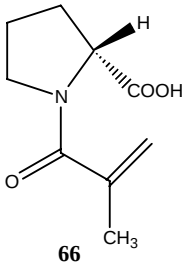
Reagents and conditions: (a) (1) $NaNO_2$, H_2SO_4, CuCN, NaCN, (2) HCl, $SnCl_2 \cdot 2H_2O$, EtOH; (b) (1) methacryloyl chloride, NaOH, acetone, (2) NBS, DMF, (3) HBr; (c) $SOCl_2$, THF; (d) K_2CO_3, acetone; (e) K_2CO_3, 2-propanol; (f) $Pd(PPh_3)_4$, hexamethyl ditin, toluene; (g) NaI ($Na^{125}I$), chloramines T, MeOH; (h) acetyl chloride, absolute EtOH; (i) chloroform, thiophosgene, $NaHCO_3$; *I = I or ^{125}I.

Scheme 9a



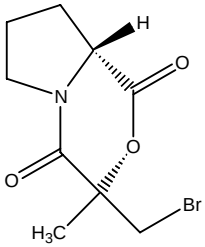
65

a)

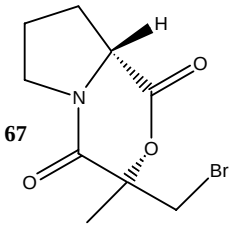


66

b)

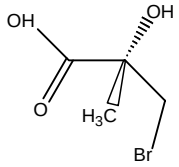


67



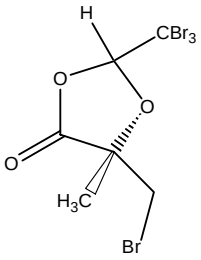
67

e)



68

f)



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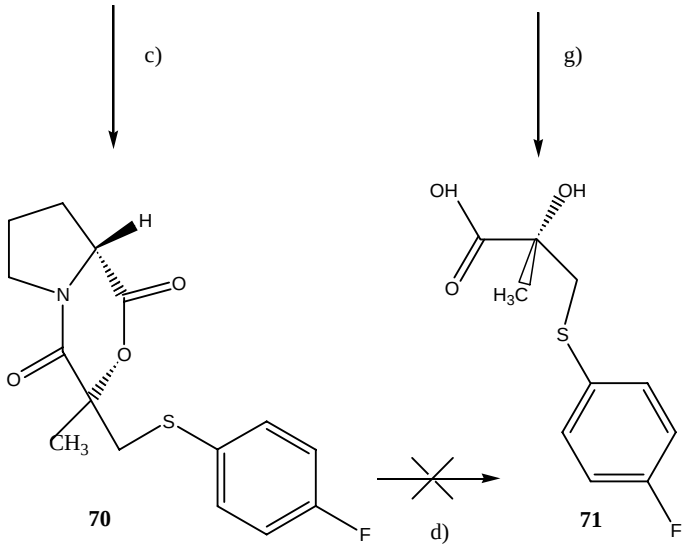
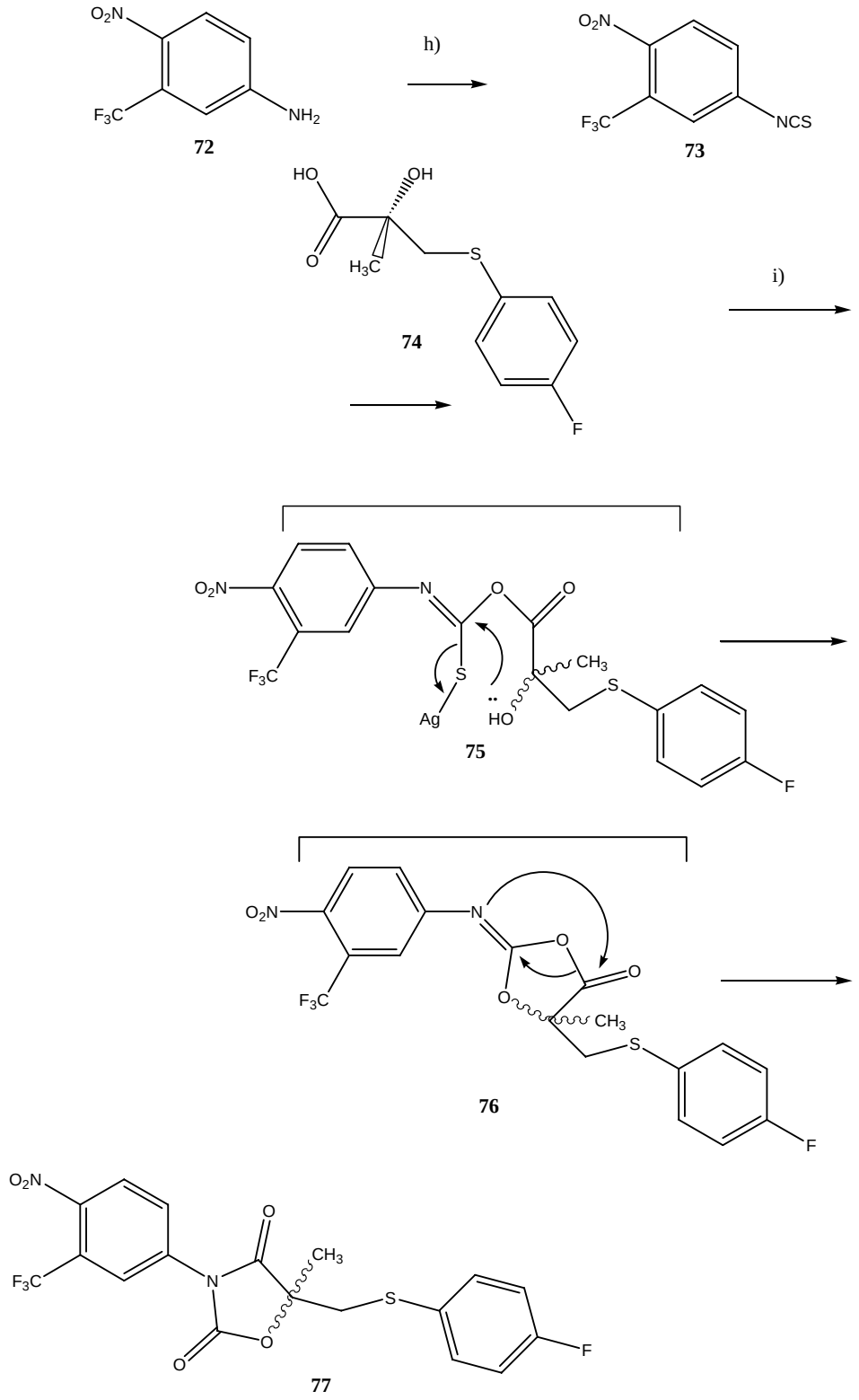


Reagents: (a) Methacryloyl chloride, NaOH, acetone; (b) NBS, DMF; (c) NaOH, 4'-fluorobenzenethiol, 2-propanol; (d) concd HCl; (e) 24% HBr; (f) tribromoacetalaldehyde, concd H_2SO_4; (g) (1) NaOH, 4-fluorobenzenethiol, 2-propanol, (2) conc. HCl.

Scheme 9b



Reagents: (h) $NaHCO_3$, thiophosgene, CH_2Cl_2; (i) acetonitrile, silver trifluoroacetate, triethylamine.

1.6 Chemical and biochemical transformations of bicalutamide

No references for the *in vivo* chemical degradation of bicalutamide to its metabolites or *in vitro* chemical transformations could be found.

1.7 References

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17. Nair, V. A.; Mustafa, S. M.; Mohler, M. L.; Dalton, J. T.; Miller, D. D. *Tetrahedron Lett.*, **2006**, *47*, 3953-3955.

Chapter 2

Results and Discussion

2.1 Introduction

The registration of a new medicine requires clinical trials. These are not complete without quantitative bio-analysis of body fluid samples. Regulatory authorities (e.g. the Food and Drug Administration or FDA in the United States) require extensive studies on the concentration of the new medicine (analyte) in human blood, it's half life (how long it stays in the human body) and products that it is metabolized to (metabolites) by enzymes in the blood, before they will allow it to be registered. To determine the concentration of medicine in human body fluids a unique internal standard is required for each new medicine.

PAREXEL is a leading global bio/pharmaceutical services organization that helps clients expedite time-to-market through their development and launch services. These include a broad range of clinical development capabilities, integrated advanced technologies, regulatory affairs consulting, and commercialization services.

Over the past 25 years, PAREXEL has developed significant expertise to assist clients in the worldwide pharmaceutical, biotechnology and medical device industries with the development and launch of their products in order to bring safe and effective treatments to the global marketplace for the patients who need them.

For drug and device developers, clinical research is a complex, uncertain, yet highly important and necessary endeavor. While innovative ideas are put to the test, substantial investments weigh in the balance and regulators oversee everything. These researchers need a proven partner that can assemble and deploy all the necessary capabilities required to fulfill the various activities of clinical research.

FARMOVS-PAREXEL has a bio-analytical service laboratory in Bloemfontein. This company analyses blood and other biological fluids for pharmaceuticals and pharmaceutical metabolites to assist local and international pharmaceutical companies to register new medicines in South Africa and overseas.

Reference materials of internal standards and other metabolites are required to validate their analytical methods. These are not always commercially available. The capacity to manufacture these internal standards and metabolites enables FARMOVS-PAREXEL not only to expand its services into trials from which it has been excluded but also to offer services not available internationally. The ability to custom synthesize internal standards provides FARMOVS-PAREXEL with a unique competitive advantage in this already sophisticated and competitive field.

This project aims to develop internal standards for the bio-analytical component of clinical trials that are required to register bicalutamide, an anticancer drug (see the literature review for more information on the pharmacology and chemistry of bicalutamide) and analogues.

2.2 Internal standards

An internal standard is used for calibration and validation in quantitative bio-analytical chemistry. The internal standard is added to the body fluid sample (mostly blood) at the beginning of the sample work up at about the same concentration of the analyte to be quantified. It controls variability during extraction, sample preparation and quantification during mass spectrometry analysis.

FARMOVS-PAREXEL uses mass spectrometry (total ion current of a specific mass fragment of the analyte under investigation). This represents a sophisticated and selective method of quantification. By choosing the right fragment to monitor,

interference from other metabolites and impurities in a complex body fluid sample can for all practical purposes be eliminated. The ideal internal standard should behave exactly like the metabolite under investigation during extraction from body fluids and sample preparation but should be distinguishable during the quantification process.

An ideal internal standard for quantification with mass spectrometry is an isotopically labeled form of the molecule with a difference of at least 3 mass units from the analyte that is to be quantified. An isotopically labeled internal standard will have a similar extraction recovery, ionization response in ESI mass spectrometry and a similar retention time in HPLC but differs in its recorded mass. Polarity and pKa plays an important role in these parameters.

If isotopically labeled internal standards are not available, structural analogues may be used. Of importance is that it has the same minimum of three mass unit difference that is required for isotopically labeled versions and that it co-elutes with the compound to be quantified. A chlorinated version of the parent molecule often has the same chromatographic retention time and differs sufficiently in mass. Hydroxylated (+16 amu) and demethylated (-14 amu) versions should be avoided as the human body often manufactures these analogues in unknown quantities from the parent compound as part of its normal metabolic processes. The human body normally metabolizes pharmaceuticals to more polar metabolites to allow secretion into urine. [1, 2]

2.3 Synthesis of internal standards (mass spectrometry quantification)

Three possible strategies were investigated to synthesize internal standards:

1) *De novo* synthesis of an isotopic isomer. The isomer is identical in all aspects except the mass difference due to the replacement of, for instance,

hydrogen with deuterium or ^{16}O with ^{18}O. To achieve the required mass unit difference, three hydrogens or two oxygens must be replaced.

2) *De novo* synthesis of a closely related analogue. Replacement of, for instance a methyl group with an ethyl or chlorine with a fluorine atom may yield the desired internal standard. The assumption is that there will be only a very small change in polarity and behaviour of the molecule during the extraction process.

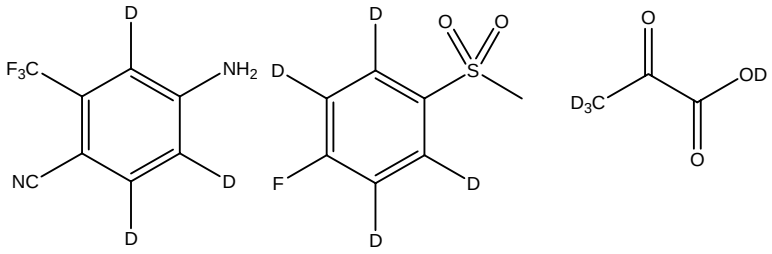
3) Modification of the analyte itself to a closely related analogue with a difference of at least three mass units.

2.4 Synthesis of internal standards for bicalutamide

2.4.1 Synthesis of deuterated bicalutamide

The synthesis of deuterated bicalutamide depends on the commercial availability of deuterium labeled starting materials. According to the review of synthetic procedures in Chapter 1**,** the following starting materials should be suitable.

Figure 1



78 **79** **80**

Two factors affected our decision not to proceed with this strategy:

1) The deuterated starting materials **(78, 79** and **80)** are not readily available commercially. Previous efforts in our laboratory to exchange H with D using D_2O obtained from published methods gave poor results.

2) A regulatory requirement is that isotopically labeled internal standards should contain less than 1% unlabeled product to ensure that only the analyte is recorded. Marginal isotopic purity of commercially available deuterated starting materials (99%) are often not high enough to achieve this. We have developed a strategy to overcome this problem by starting with two separately labeled building blocks. This, however, increases the cost and complexity of any potential synthetic method.

2.4.2 *De novo* synthesis of structural analogues of bicalutamide

Most of the published syntheses of bicalutamide uses the following starting materials (literature review, chapter 1):

1) 4-Amino-2-(trifluoromethyl)benzonitrile **(6**)

2) Phenylmethyl sulfone **(14)**

3) Pyruvic acid **(12)**

For the *De novo* synthesis of structural analogues many possibilities exist as any analogue of **6**, **14** or **12** could be used.

Two options were investigated:

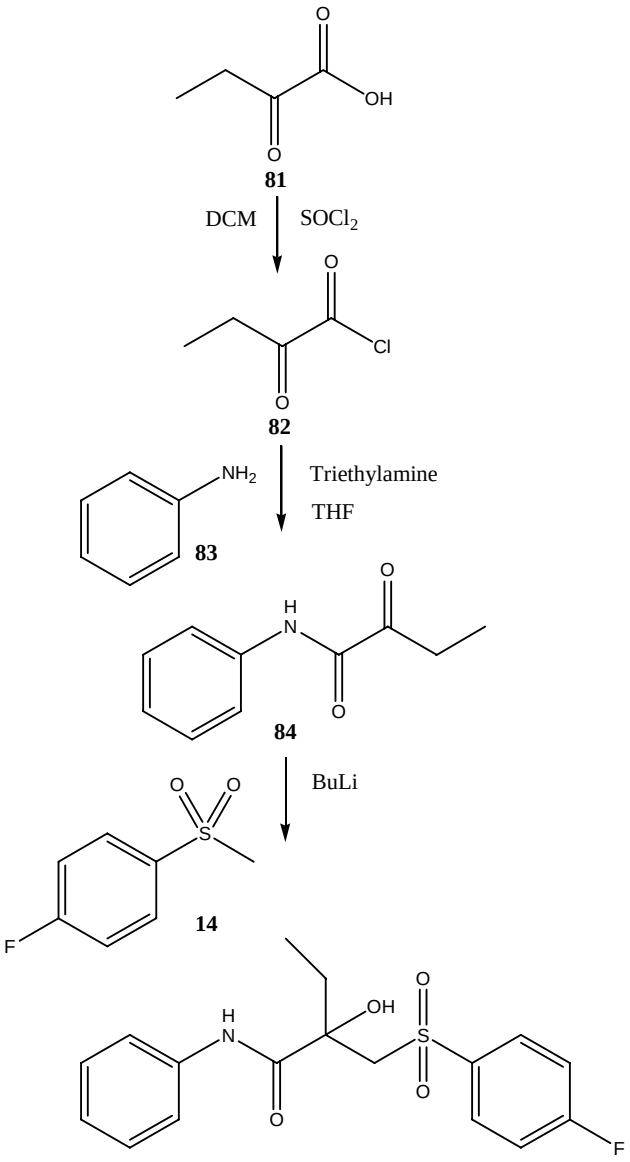
1) We decided to use 2-ketobutyric acid **81** and aniline **83** instead of pyruvic acid **12** and 4-amino-2-(trifluoromethyl)benzonitrile **6**. The use of aniline in a model reaction saved costs on the use of expensive material. The synthesis of the amide **84** was successful but the coupling between **14** and **84** failed to produce compound **85**. However the starting materials were recovered **(Scheme 13)**

2) The other option was to replace pyruvic acid **12** with 2-ketobutyric acid **81** in order to transform the 2-methyl group to a 2-ethyl group. This would not change the polarity significantly and would add 14 g/mol in the mass spectrometry. The rest of the starting materials remained the same. The synthesis of the amide **86** was successful. The coupling between the sulphone **14** and **86** did not succeed and compound **87** was not isolated **(Scheme 14).**

The coupling required deprotonation of the sulphone **14** and we suspect that the deprotonation of the amide interfered.

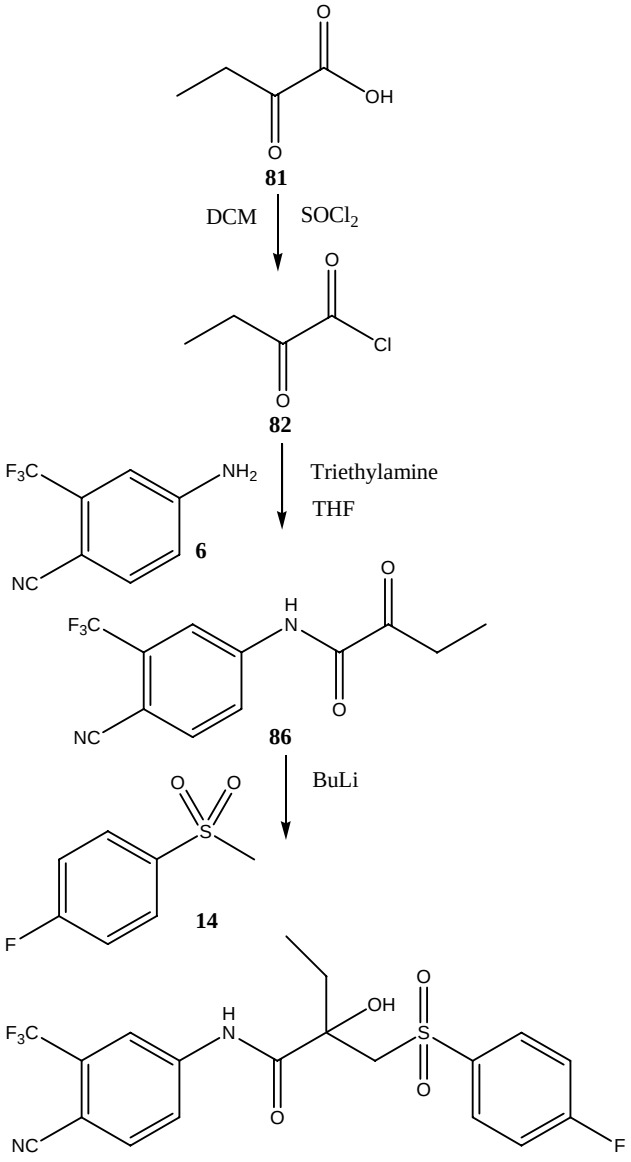
Gas chromatography was used to monitor the formation of **82** as this compound is volatile and TLC was unsuitable (Chapter 3). This enabled us to optimize our conditions in improving the yields of **85** and **87**. (GC Plate 13).

Scheme 13

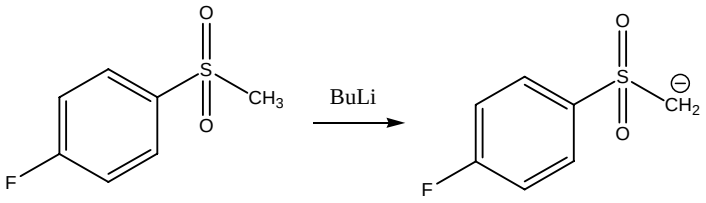


85, NOT ISOLATED

Scheme 14


O
OH
O
81
DCM
SOCl2
O
Cl
O
82
F3C
NH2
NC
6
Triethylamine
THF
H
N
F3C
NC
O
O
86
BuLi
O
O
S
F
14
OH
H
N
F3C
NC
O
O
S
O
F
87, NOT ISOLATED

Scheme 15



14 **14a**

In view of the fact that our other efforts to modify bicalutamide directly (described below) had succeeded and we had already produced three internal standard analogues, we decided not to pursue our *de novo* syntheses further. Our decision was also influenced by the realization that the published syntheses of bicalutamide were based on patents. It is a well known fact that inventors often do not give full disclosure of the finer details of their invention in order to frustrate attempts to improve on their invention. From previous experience we suspect that essential finer details were omitted.

2.4.3. Modifications of bicalutamide

Inspection of bicalutamide revealed the following functional groups that could be modified:

1) A fluorine atom which could be replaced by hydrogen or another halogen.

2) A C≡N group, which could be reduced or oxidized.

3) An SO_2 group, which could be reduced.

4) An OH group, which could be derivatized or eliminated.

5) An amide group that could be alkylated.

We decided to explore the following avenues:

A. Reduction of the C≡N group

B. Elimination of the OH group.

C. Methylation of the OH group.

A. Reduction of the C≡N group.

Reduction of a nitrile group to an amine is a well-known reaction.[3] Selectivity and efficiency in the reduction of nitriles are important for the preparation of amino derivatives in organic synthesis. This reduction is important in industry. Numerous reagents and methods have been developed to reduce aromatic nitrile groups, such as:

1) Metal/acid reduction

2) Catalytic hydrogenation

3) Electrolytic reduction

4) Homogeneous catalytic transfer hydrogenation

5) Heterogeneous catalytic transfer hydrogenation

All these methods, however, have limitations, such as:

1) The metal/acid system lacks selectivity and requires a strong acidic medium.

2) Catalytic hydrogenation employs highly diffusible, low molecular weight, flammable hydrogen gas.

3) In electrolytic reduction yields are low and there is a lack of practical utility in academic institutions.

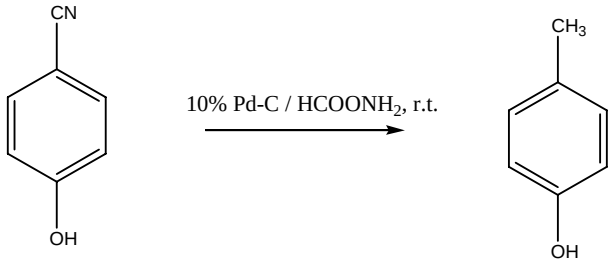
4) Homogenous catalytic transfer hydrogenation requires expensive reagents as catalysts. Work up and isolation of the products are not trivial.

5) Heterogeneous catalytic transfer hydrogenation employs metals like palladium, platinum and ruthenium. These catalysts require stringent precautions, because of their flammable nature in the presence of air and hydrogen. Because of the small quantities of the internal standard required for bio-analysis, cost of the catalyst is not important for this application.

Raney nickel is routinely used as a catalyst in the field of catalytic hydrogenation as well as in the field of heterogeneous catalytic transfer hydrogenation.[4]

Reduction of the cyano group to an alkylamine is the general transformation. However, further reduction to a methyl group has only rarely been reported. Brown and co-workers[5] reported this rare reduction using ammonium formate as the hydrogen source in the presence of a 10 % palladium on carbon catalyst **(Scheme 16)**. This hydrogenation occurred at room temperature over a 24 hour period. The reaction rate is influenced by the amount of catalyst employed and temperature. Equal weights of catalyst to nitrile have to be used to ensure a smooth conversion to the corresponding methyl derivative.

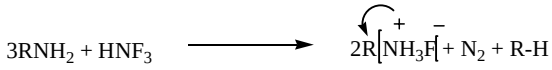
Scheme 16



88 **89**

Bumgardner and co-workers[6] reported the reduction of primary aliphatic amines to alkanes **(90)** with HNF_2 **(91)**. It was postulated that R−N=N−H is an intermediate, so that the reaction proceeds through the carbocation **(92) (Scheme 17)**.

Scheme 17



90 **91** **92** **93**

An indirect means of achieving the same result is conversion of the primary amine to sulfonamide $RNHSO_2R'$ and treatment of this with hydroxylamine-*O*-sulfonic acid. The same intermediate, R−N=N−H is postulated in this case.

Brieger and Nestrick[7] investigated C≡N reduction to CH_3 and reported various reaction conditions such as nature of the donors, solvent effects, and the effects of temperature on reduction.

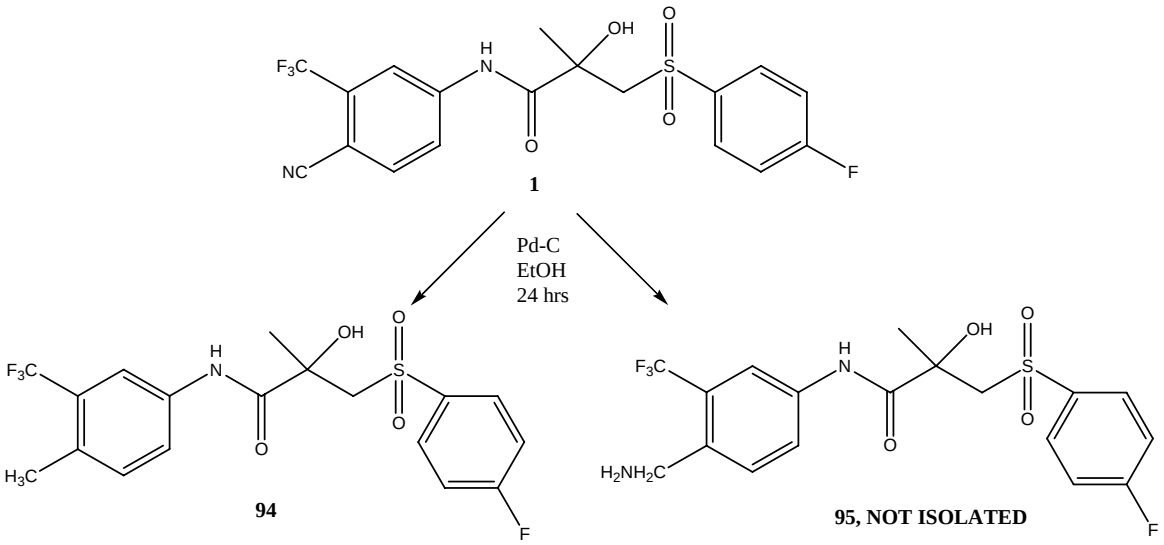
We decided to investigate heterogeneous catalytic transfer hydrogenation because pressure equipment and the supported metals such as palladium and nickel were readily available to us as only a small quantity of the internal standard were required.

Sulfones are resistant to reducing agents and were not expected to interfere in the reduction of the nitrile group. It seems that α-anion formation makes the sulfone inert with reducing agents such as $LiAlH_4$.[8]

Our first step was to reduce the cyano group with Raney nickel using an autoclave at high temperature and pressure. This method however was unsuccessful probably due to the poor quality of the Raney nickel (W-11) used.

We then decided to use palladium on activated charcoal and the result was unexpected as the C≡N group was smoothly reduced to a CH_3 group to afford compound **94 (Scheme 18**). This reaction is reminiscent to the palladium-catalyzed hydrogenolysis of benzyl alcohols, yielding toluene.[9] Amine **95** was not isolated but served as an intermediate to the formation of **94**.

Scheme 18



A full structure elucidation on **94** is given in section 2.8, but the following salient features of the unexpected compound are important:

1) The mass spectrum is in full agreement with this assignment; $(M^+)^-$ ($C_{18}H_{17}NO_4F_4S$) in negative electron ion mode: m/z = 419.0895 compared to M^+ ($C_{18}H_{18}N_2O_4F_4S$): m/z = 435 for the amine **95.**

2) In the ^1H NMR spectrum a new doublet was observed at δ 2.45 (J = 1.4 Hz), which integrated for three protons. This peak was assigned to the benzylic CH_3 because the COSY shows a coupling between CH_3 and H-5". Benzylic four bond coupling splits the CH_3 resonance into a doublet (J = 1.4 Hz). A CH_2 group attached to an amine **95** would integrate for two protons only.

3) In the ^{13}C NMR spectrum the C≡N group at δ 114.98 was replaced by a new resonance at δ 18.81 which was assigned to the CH_3 group. (*CH_2NH_2* would be expected at about δ 46.0).[8]

4) The HMBC experiment shows the three-bond coupling (correlation) between C-3" and CH_3 as well as a three-bond coupling between C-5" and CH_3.

These features confirm the postulated structure. This compound is an ideal internal standard because there is a difference in mass of about 11 mass units and the polarity difference is acceptable.

B. <u>The elimination of the OH group</u>.

Bicalutamide has a tertiary hydroxy group, which is normally difficult to eliminate. The following options to eliminate the OH group were investigated to overcome this problem:

a) Acid catalysis

b) Base catalysis

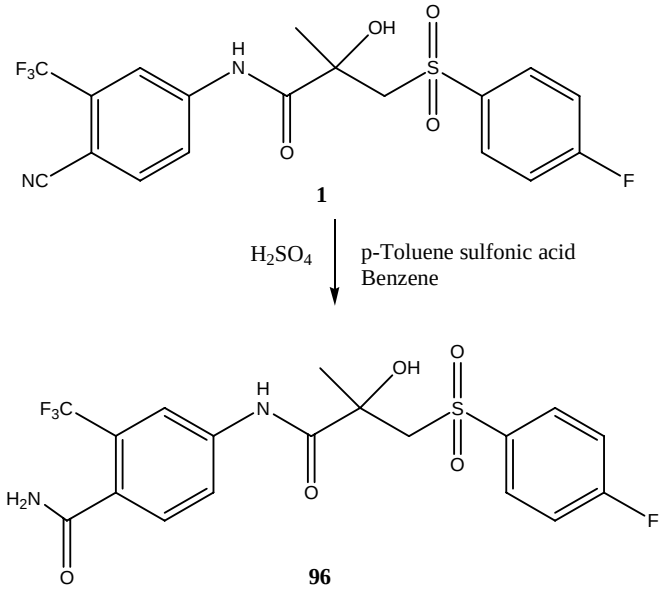
c) Thermal elimination

d) Photochemistry

a) <u>Acid Catalysis</u>

Acid catalysis was attempted (employing 1M HCl in ethanol). The reaction was refluxed for a few days, however, no reaction took place. Refluxing bicalutamide in benzene (bp 110 ºC) with *p*-toluenesulfonic acid also produced no results. The addition of H_2SO_4 to the benzene reaction mixture resulted in isolation of **96 (Scheme 19**), i.e. hydrolysis of the nitrile group to an amide.

<u>Scheme 19</u>



A full structure elucidation on compound **96** is given in section 2.8 but the following salient features of the amide are important:

1) The amide is characterized by an $(M-H)^-$ mass of 447.0640 in electron ion negative mode.

2) The amide is further characterized by an additional carbonyl group at δ 168.03 in the ^{13}C NMR spectrum.

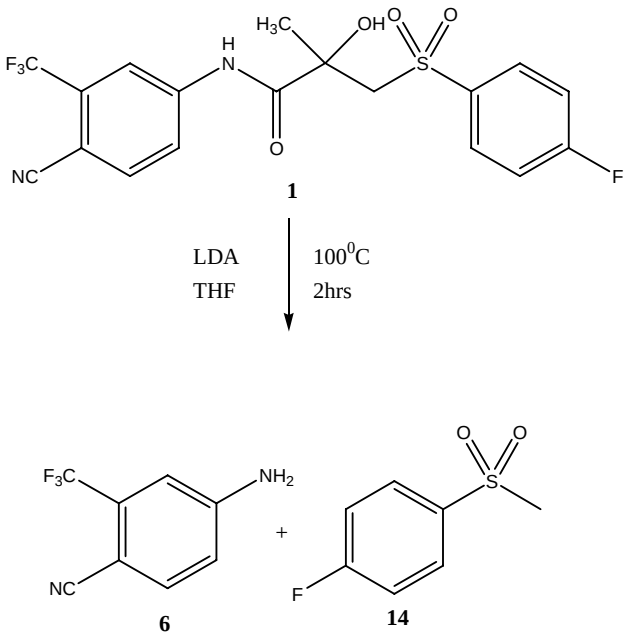
3) The IR spectrum shows the characteristic amide stretching frequency which consists of two stretching frequencies at 3448 cm^{-1}(plate 6h).

The product **96** was inert to methylation with diazomethane. This proves that hydrolyses to a carboxylic acid did not take place. The acid would require a mass of $(M^+)^-$ 448 in the negative mode and (M^+) 449 $[M + H]^+$, these peaks, however, were not observed in the mass spectrum.

b) <u>Base Catalysis</u>

To eliminate the OH group we tried a strong hindered base, lithium diisopropylamide (LDA). This resulted in cleavage of bicalutamide and the isolation of **6** and **14 (Scheme 20)**. This is probably due to the presence of trace amounts of water. Water reacts with LDA to give lithuim hydroxide which hydrolyses the amide bond of bicalutamide. A retro-aldol reaction results in the isolation of **14**.

Scheme 20



A weaker base such as K_2CO_3 was used but it could not deprotonate the OH group, and no reaction took place.

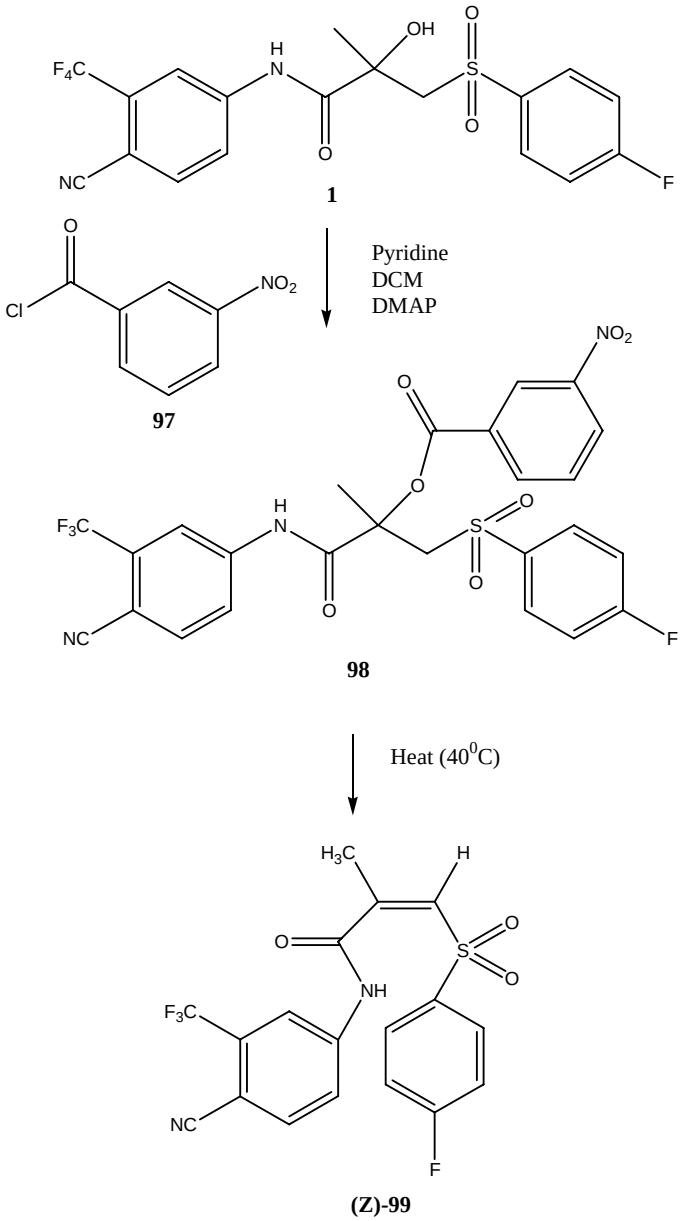
c) <u>Thermal elimination</u>

Heating of bicalutamide up to 200 ºC under argon did not produce any transformations.

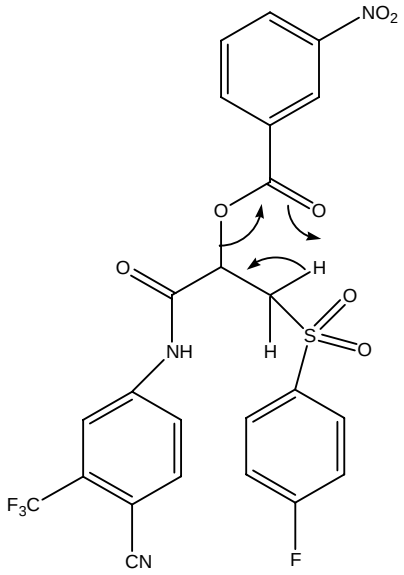
To transform the OH group into a better leaving group we derivatized it with 3-nitrobenzoylchloride **97**. Stirring bicalutamide at room temperature with nitrobenzoylchloride[10] in DCM with catalytic amounts of pyridine gave the 3-nitro-benzoyl ester **98**.

Heating of the ester **98** to 40 ºC yielded the target alkene **99** via elimination of 3-nitrobenzoic acid **(Scheme 21)**. The same result was achieved if **98** were not isolated and the reaction mixture was heated directly.

Scheme 21

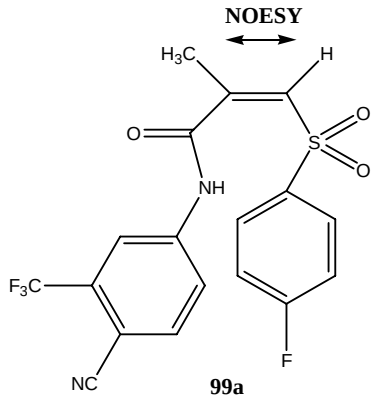


The thermal elimination of the nitrobenzoyl group is expected to take place via a syn-periplanar cyclic transition state **98a**.



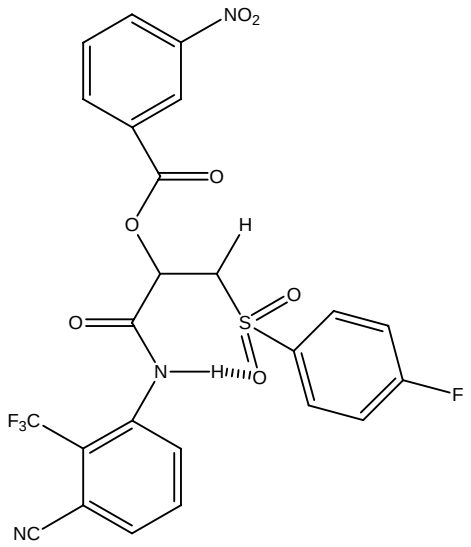
98a

The two protons on the CH_2 group adjacent to the sulfonyl group are diastereotopic. Elimination of the pro-*R* hydrogen will give a *Z* alkene and of the pro-*S* hydrogen will give an E alkene.[11] The presence of a NOESY between the CH_3, H (plate 7d) proves that the pro-R hydrogen was eliminated to give an alkene with a Z-configuration **(99a)**.



99a

A probable explanation for the observed stereoselectivity and the absence of any E-alkene is that a hydrogen bond between the SO_2 group and the N-H of the amide stereoselectively favours the transition state that the selective removal of the pro-R hydrogen to form the Z-configurated alkene **(99b)**.



99b

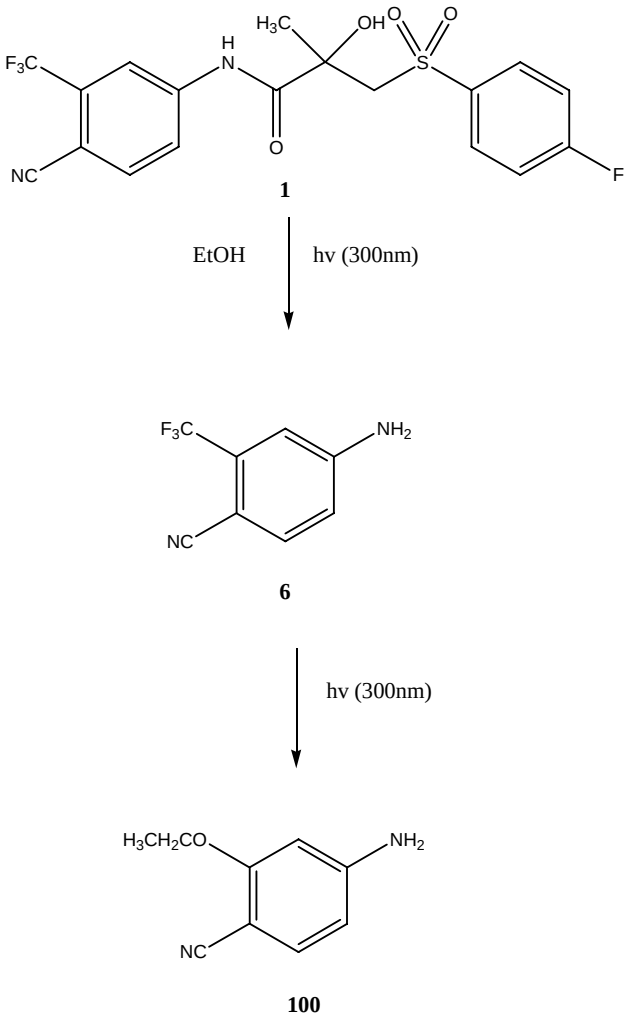
C. Photochemistry

Examples of elimination of OH using photochemistry have been reported.[12] Irradiation of bicalutamide at 300 nm, however, unexpectedly yielded compound **100**. This product requires hydrolysis of the amide bond to yield the aniline derivative **6**, followed by replacement of the CF_3 group by an ethoxy group in a typical photocatalyzed S_N reaction. The trifluoromethyl group is normally chemically inert. It is also resistant to many metabolic transformations.[13] The high carbon-fluorine bond energy makes fluorine a poor leaving group in nucleophilic substitution reactions. This indicates that the CF_3 group was directly substituted **(Scheme 22)**. The nature of the substituent (OCH_2CH_3) indicates an ionic mechanism via a π,π*-excited singlet. Radical substitution would have yielded a $CH(OH)CH_3$ derivative.[12] During photochemical reactions excitation of π, π*-singlets are normally associated with ionic reactions and π,π*-triplets with radical mechanisms

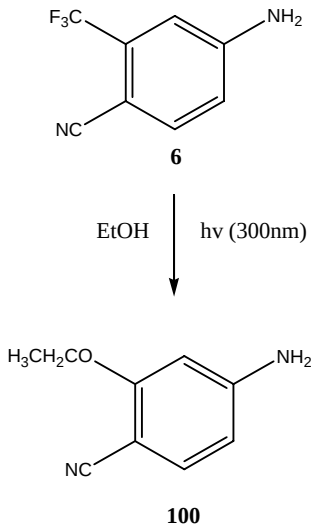
We repeated the photolytic reaction starting with 4-amino-3-(trifluoromethyl)benzonitrile **(Scheme 23)** and obtained the same product **100** under the same photolytic conditions in 50 % yield. We could not find any

reference to this transformation in the literature and believe it to be a new reaction. We will investigate the synthetic potential of this further.

Scheme 22



Scheme 23



A full structure elucidation is given in section 2.8. The following features however are the most important:

1) The absence of carbon-fluorine couplings in the ^{13}C NMR spectrum and fluorine resonances in the ^{19}F NMR spectrum indicate the absence of fluorine present.

2) The NOESY experiment demonstrates a coupling between H-2 and the ethyl CH_2 as well as coupling between H-2 and the ethyl CH_3. (Plate 8c).

3) The NOESY experiment also shows the coupling of the NH_2 group to H-3 and H-5 and proves that the NH_2 group is flanked by these protons. (Plate 8e)

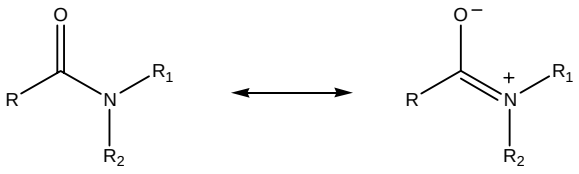
4) Mass spectrometry agrees with the replacement of CF_3 (M^+ ($C_8H_5N_2F_3$): m/z = 186.1) with OCH_2CH_3 (M^+ ($C_9H_{10}N_2O$): m/z = 163.0874).

D. Methylation

Our first attempt to methylate the aliphatic hydroxy group of bicalutamide involved the use of K_2CO_3 as base and the addition of MeI in DMA. This reaction gave no results as the base used could not deprotonate the OH in bicalutamide. We then used sodium as a base and then afterwards MeI was added. Purification by chromatography was difficult but from cursory NMR it was observed that cleavage of bicalutamide occurred. This is attributed to a typical retro-aldol reaction.

Methylation with LDA and MeI gave an intractable mixture. We assumed that methylation of the amide gives rise to rotational isomers which gives complicated NMR spectra. The amide N-CO bond has a partial double-bond character arising from the contribution of resonance structure **102 (Scheme 24)** to the ground state of the amide. A large barrier to rotation around the amide N-CO bond is provided. This leads to geometrical and magnetical nonequivalence of the N-substituents.[14]

Scheme 24



101 **102**

2.5 ^{19}F NMR Spectroscopy

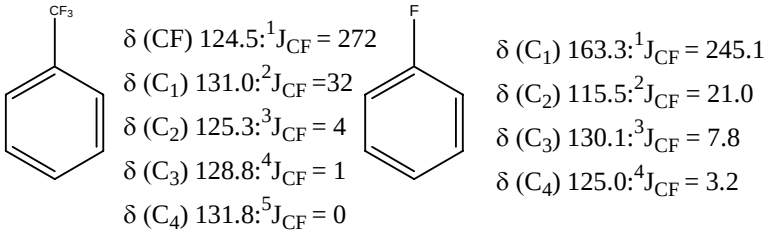
The ^{19}F nucleus (I = ½, natural abundance 100%) has 83% of the sensitivity to NMR spectroscopy detection compared to that of a ^{1}H nucleus. As such it is a useful tool to detect the presence of fluorine in a molecule. Most modern probe-heads can be tuned to the ^{19}F frequency because of the proximity of the resonance frequencies of ^{1}H and ^{19}F. No special equipment other than a ^{19}F preamplifier is needed for a standard experiment.

Introduction of fluorine into a potential drug can produce a wide range of biological effects, ranging from complete metabolic inertness to high specificity of binding to a particular protein receptor site. Because fluorine rarely occurs in natural compounds, it has become a very useful tool to investigate and monitor biological processes (eg. enzyme product identification and monitoring a complex biological mixture). Because of fluorine's high sensitivity, it has also become a tool to determine the concentration of fluorine in solutions (quantitative NMR). The concentration of fluorinated molecules in a range of 10 μM can be conveniently determined in about 15 minutes. [15]

Due to the large range in fluorine chemical shifts (from -131 to + 129 relative to CF_3COOH in ppm) no single fluorine-containing compound can be used experimentally as a universal reference compound (c.f. tetramethylsilane in ^{1}H and ^{13}C NMR spectroscopy). Any fluorine containing compound that resonates in the region of the internal standard can be used as reference. We used trifluoroacetic acid which resonates at about δ -78.7 relative to TMS. An NMR tube insert was used to keep the reference and the fluorine containing compound under investigation apart and to avoid the effect of dilution on the chemical shift of the reference compound (This enabled us to use 99% trifluoroacetic acid as a reference). Fluorine chemical shifts are significantly more sensitive than proton chemical shifts to concentration and temperature of the sample and these should be specified.

C-F couplings are visible in proton decoupled (CPD) ^{13}C NMR spectra because only ^{1}H is decoupled and not ^{19}F. The size of the couplings between fluorine and carbon is very useful in determining the position of fluorine in a molecule and is given in **(Scheme 25).** It gives an indication of the size of the coupling (J_{CF}) as a function of the number of bonds (x) between carbon and fluorine.[16]

Figure 2



We observed the aromatic CF_3 group in our products and starting materials at about -63 ppm and the aromatic F substituent at about -106 ppm (in acetone-d_6) **(Table 2)**.

Table 2. Chemical shift of ^{19}F in bicalutamide, derivatives of bicalutamide and fragments of bicalutamide

Compound	CF_3 (δ)	4'-F (δ)
1	-62.60	-106.31
6	-63.00	
14		-106.7
86	-62.7	
94	-61.74	-106.61
96	-59.57	-106.68
98	-62.82	-105.63
99	-62.74	-105.28

2.6 Our investigation achieved the following:

1. Four new derivatives of bicalutamide have been synthesized.

2. Three of these derivatives are suitable internal standards for quantitative bio-analysis of body fluid samples.

3. These derivatives are novel and will be submitted for testing in anti-cancer bioassays.

4. a) A simple method for C≡N reduction to CH_3 in bicalutamide has been developed.
   b) A method for hydrolysis of C≡N to $CONH_2$ in bicalutamide has been established.
   c) A reaction for the facile elimination of OH to form an alkene under mild conditions has been developed.

5. A new photochemical method for the substitution of CF_3 by an ethoxy group in aromatic amines has been developed.

6. We used the ^{13}C-^{19}F coupling as a useful tool to identify and elucidate the structures of all the derivatives of bicalutamide.

7. We used fluorine NMR to validate the presence of a CF_3 and F group in a reaction product.

2.7 Future Work

We plan the following:

1) Methylation of bicalutamide with diazomethane indicates that a very slow reaction takes place. Heating is not permissible as diazomethane will evaporate and an autoclave cannot be used because of the explosion danger associated with diazomethane. We will reinvestigate this reaction.

2) We will investigate the synthetic potential of the photolytic replacement of the aromatic CF_3 with OCH_2CH_3 starting with model compounds. This reaction appears novel as we could not find a literature reference.

3) The synthesis of bicalutamide and bicalutamide derivatives from the deprotonation of 4-fluorophenyl methyl sulfone and amide addition will be attempted again with different bases and under anhydrous conditions.

4) We will test our novel derivatives in anti-cancer screening and as anti-androgens.

2.8 Structure Elucidation

Comprehensive interpretations of the ^{1}H, ^{13}C, APT, COSY, HSQC, HMBC, IR and NOESY spectra of bicalutamide and its reaction products are given below:

2.8.1 2-Oxo-*N*-phenylbutanamide (84)

A. Mass spectrometry confirmed assignment of structure **84,** found (ES) $[M+H]^{+}$, 178.0870 ($C_{10}H_{11}NO_2 + H^{+}$) required m/z 178.0868.

B. The salient features in the ^{1}H NMR spectrum are (Plate 1a):

1) According to the integrals in the ^{1}H NMR spectrum (H-2'/ H-6') and (H-3'/ H-5') in the aniline ring resonates at δ 7.65 (d, J = 8.0 Hz), δ 7.37 (t, J = 8.0 Hz), and integrated for two protons each.

2) H-4' at δ 7.18 (t, J = 8.0 Hz) integrated for one proton.

3) The C-2 (CH_2) resonance is observed as a quartet at δ 3.05 (J = 7.3 Hz) and integrated for two protons.

4) The C-3 (CH_3) resonated as a triplet at δ 1.16 and integrated for three protons.

C. The salient features in the ^{13}C NMR spectrum are (Plate 1b):

1) (C-2'/C-6') and (C-3'/ C-5') resonates at δ 119.8 and δ 129.2, respectively. These resonances were confirmed by correlation with (H-2'/ H-6') and (H-3'/ H-5') at δ 7.65 and δ 7.37 in the HSQC (Plate 1d).

2) C-4' resonates at δ 125.2. This was confirmed by correlation with H-4' at δ 7.18 in the HSQC (Plate 1d).

D. The salient features in the COSY experiment (Plate 1c):

Cross peaks in the COSY confirmed the expected aliphatic coupling between CH_2 δ 3.05 (J = 7.3 Hz) and CH_3 at δ 1.16 (J = 7.3 Hz).

E. The HMBC and APT data did not add any additional information that could not be obtained from the other methods.

2.8.2 *N*-[4-(cyano-3-(trifluoromethyl)phenyl]-2-oxobutanamide (86)

A. Mass spectrometry confirmed assignment of structure **(86),** found (ES) $[M-H^+]^-$ 269.0541 ($C_{12}H_9F_3N_2O_2$ - H^+) required m/z 269.0538.

B. The salient features in the ^1H NMR spectrum are (Plate 2a):

1) According to the integrals in ^1H NMR spectrum, H-2, H-6, H-5 in the AMX system resonated at δ 8.55 (J = 1.9 Hz), δ 8.37 (J = 1.9 Hz,) and δ 8.09 (J = 8.5 Hz) respectively and integrated for one proton each.

2) The C-2 (CH_2) two doublets at δ 3.02 (J = 7.2 Hz) was clearly visible and integrated for two protons.

3) The C-3(CH_3) resonated as a triplet at δ 1.10 and integrated for three protons.

C. The salient features in the ^{13}C NMR spectrum are (Plate 2b):

1) Assignment of C-2, C-5, C-6 at δ 117.82, δ 123.04 and δ 136.25 respectively was confirmed by HSQC (plate 2d).

D. The salient features in the COSY spectrum are (Plate 2e):

Cross peaks in the COSY confirmed the expected aliphatic coupling between CH_2 at δ 3.02 (J = 7.2 Hz) and CH_3 at δ 1.10 (J = 7.2 Hz).

E. The HMBC and APT did not add any additional information that could not be obtained from the other methods.

2.8.3 Starting Material (bicalutamide) (1)

A. Mass spectrometry confirmed assignment of structure **(1),** found (ES) MW (M^+) 430.38 ($C_{18}H_{14}F_4N_2O_4+H^+$) requires m/z 430.

B. The salient features in the ^1H NMR spectra are (plate 3a):

1) The aliphatic CH_3 hydrogen resonance was observed as a singlet at δ 1.42.

2) The aliphatic CH_2 group resonance was observed as two geminal doublets (J = 14.8 Hz) at δ 3.57 and δ 4.00.

3) H–2", H–6" and H–5" of the tri-substituted aniline moiety were easily recognized as an AMX system at δ 8.28 (J = 2.0 Hz), δ 8.08 (J = 2.0 Hz, J = 8.5 Hz), δ 8.7.89 (J = 8.5 Hz), respectively. No fluorine couplings were observed.

4) The observed hydrogen resonances on the *p*-fluorophenyl sulphone ring showed clear hydrogen fluorine couplings. The AA'BB' system at δ 7.86 and δ 7.16 is split by ($^3J_{FH}$ = 9.0 Hz, ortho coupling), (H-3', H–5', closest to fluorine) and $^4J_{FH}$ = 5.0 Hz (H-2', H–6', next to the sulfone group). Because $^3J_{FC}$ and $^3J_{FC}$ are both 9.0 Hz, H-3'and H–6' appeared as a triplet.

C. The salient features in the ^{13}C NMR spectrum (Plate 3b) are:

a) The aliphatic region

1) The amide carbonyl resonance was observed at δ 172.60.

2) The CH_3 resonance was observed at δ 26.52 (negative APT, plate 3d and it correlated with CH_3 at δ 1.42 in HSQC, plate 3e).

3) The CH_2 at C–3 was deshielded by the sulfone group and the resonance was observed at δ 62.74. This was confirmed by correlation with CH_2 at δ 4.1 and δ 3.7 in the HSQC (plate 3e).

4) The quaternary C–2 had a small intensity. It was deshielded by the adjacent hydroxy carbonyl groups and the resonance was observed at δ 73.25 (positive APT, plate 3d).

b) The aniline ring

1) The CF_3 carbon resonance was observed as a quartet at δ 122.25 (negative APT, plate 3d) with the characteristic large $^1J_{FC}$ = 273.0 Hz coupling.[1]

2) The quartenary 3"- carbon, next to the CF_3 group resonance was observed at δ 132.02 with ($^2J_{FC}$ = 32.0Hz).

3) The C≡N group resonates at δ 114.98 which was characteristic of this functional group.[15]

4) C–2", C–5" and C–6" correlated with the proton AMX system in the HSQC (plate 3e) and the resonance was observed at δ 117.01 ($^3J_{FC}$ = 5.1), δ 135.51 and δ 122.29, respectively (negative APT, plate 3d).

5) C–1" and C–4" resonances were observed at δ 142.70 and δ 102.87 (J = 2.2 Hz), respectively.

c) Phenyl sulfone ring

1) The C–4' resonance at δ 165.03 was characterized by $^{1}J_{FC}$ = 253.0 Hz.

2) C–3' and C–5' resonance at δ 115.47 were symmetrical and correlated with H–3' and H–5' at δ 7.16 in the HSQC spectra (plate 3e). They were also identified by $^{2}J_{FC}$ = 22.9 Hz.

3) C–2' and C–6' resonated at δ 131.10 which correlated with H–2' and H–6' at δ 7.86 in the HSQC spectra (plate 3e). They were also identified by $^{4}J_{FC}$ = 9.8 Hz.

4) The resonance observed at δ 136.91 ($^{1}J_{FC}$ = 2.0 Hz) was assigned to C–1'. The proximity to the sulfone group explained the large deshielding. It appeared as a positive resonance in the APT (plate 3d) which confirmed the quartenary nature.

2.8.4. 3-(4-Fluorophenylsulfonyl)-2-hydroxy-2-methyl-*N*-[4-methyl-3-(trifluoromethyl)phenyl]propanamide (94)

A. Mass spectrometry confirmed assignment of structure **94,** found (ES) $[M-H]^{+}$ ($C_{18}H_{17}NO_4F_4S+H^{+}$) required m/z 419.0893, in negative electron ion mode.

B. The salient features in the ^{1}H NMR spectrum (Plate 4a)**:**

The NMR spectrum was similar to that of bicalutamide, the starting material, with the following exceptions:

1) The AMX system of the aniline ring has lost deshielding of about 0.8ppm. This illustrated the reduction of the C≡N group to CH_3. (Plate 4a).

2) The new doublet at δ 2.45 (J = 1.4 Hz) that appeared in the spectrum was assigned to the aromatic CH_3.

C. The salient features in the ^{13}C NMR spectrum (Plate 4b):

1) The new peak at δ 18.78 was assigned to the CH_3 group.

2) The C≡N group at δ 114.98 ppm had disappeared.

3) C-4" attached to the C≡N group in bicalutamide had moved to δ 135.20 and the resonance was observed as a doublet with ($^3J_{CF}$ = 3.1 Hz).

D. The salient features in the COSY spectrum are (Plates 4d and 4e):

1) The COSY data demonstrated a strong cross coupling between H-5" at δ 7.24 and CH_3 of C-4" at δ 2.45 (J = 1.4 Hz), benzylic coupling.

2) The CH_3 of C-4" at δ 2.45 also showed weaker cross couplings with H-6" at δ 7.46 and H-2" at δ 7.68 on the aniline ring.

E. The salient features in the HSQC spectrum (plate 4f) are:

The HSQC data confirmed the direct correlation between the CH_3 carbon δ 135.20 and the CH_3 hydrogen at δ 2.45.

F. The salient features in the HMBC spectrum(Plate 4g) are:

HMBC data illustrated the three bond coupling between C-3" at δ 129.37 and CH_3 at δ 2.45 as well as a three bond coupling between C-5" and CH_3. This confirmed the attachment of CH_3 to C-4" of the aniline moiety in place of the CN group.

H. The salient features in the IR (Plate 4h and Plate 5h) are:

The disappearance of the characteristic C≡N group at 2229.3 was illustrated in the IR comparison with bicalutamide **(1)**.[15]

2.8.5 4-[3-(4-Fluorophenylsulfonyl)-2-hydroxy-2-methylpropanamido]-2-(trifluoromethyl)benzamide (96)

A. Mass spectrometry confirmed assignment of structure **(96),** found (ES) $[M-H]^+$ 447.0640 ($C_{18}H_{17}NO_4F_4S$-H^+) required m/z 447.0638, in the negative electron ion mode.

B. The salient features in the ^1H NMR spectrum (Plate 5a) are:

The ^1H NMR spectrum was similar to that of bicalutamide, the starting material.

C. The salient features in the ^{13}C NMR spectrum (Plate 5b) are:

The amide was further characterized by an additional carbonyl group at δ 168.03 in the ^{13}C spectrum.

D. The salient features in the IR spectrum (Plate 6h) include:

The IR data showed the characteristic amide stretching frequency which consisted of two stretching frequencies at about 3448 cm^{-1} .[15]

2.8.6 1-(4-Fluoro-3-(trifluoromethyl)phenylamino)-3-(4-fluorophenylsulfonyl)-2-methyl-1-oxopropan-2-yl 3-nitrobenzoate (98)

A. The mass spectrum confirmed structure **98**, found (ES) $[M-H]^-$, 579.0650 ($C_{25}H_{17}F_4N_3O_2S$ - H^+) required m/z 579.0645.

B. The salient features in the ^{1}H NMR spectrum (Plate 6a) are:

In addition to the observed proton resonances from the two aromatic moieties, the AMX system and the AB system, four additional protons were observed in the aromatic region. These protons were assigned as H-2''' at δ 8.58 (J = 0.4 Hz, 1.6 Hz, 2.3 Hz), H-4''' at δ 8.42 (J = 1.1 Hz, 2.3 Hz, 8.2 Hz), H-6''' at δ 8.25 (J = 1.1 Hz, 1.6 Hz, 7.4 Hz) and H-5''' at δ 7.74 (J = 0.4 Hz, 7.5 Hz, 8.2 Hz), J = 0.4 Hz corresponds with *p*-coupling.

C. The salient features in the ^{13}C NMR spectrum (Plate 6b) are:

1) There were two C=O resonances at δ 168.87 and δ 165.90. The benzoyl ester C=O group was assigned to δ 165.90.

2) C-4' directly attached to fluorine also resonated in the same area as the C=O at δ 165.90 as a doublet with coupling (^{1}J = 254.2 Hz).

3) The C-1'' resonance was observed as a doublet at δ 142.27 ($^{1}J_{CF}$ = 3.4 Hz).

4) The CF_3 resonance was observed as a quartet at δ 122.10 ($^{1}J_{CF}$ = 273.0 Hz).

5) C-NO_2 resonated at δ 129.75 which was characteristic of this functional group.[15]

6) The aliphatic carbon resonances were observed at δ 22.40 (CH_3), δ 57.07 (CH_2) and δ 80.24 (C-2). These resonances were similar to bicalutamide.

7) The C-1''' resonance was observed at δ 147.77 and C-1'' resonated at δ 142.26.

8) C-2'' resonated at δ 117.40 ($^{1}J_{CF}$ = 4.6 Hz).

9) The C-3'/ C-5' resonance was observed as a doublet at δ 116.54 ($^2J_{CF}$ = 22.8 Hz). This assignment was confirmed by the coupling between H-3'/ H-5' at δ 7.3 and C-3'/ C-5' at δ 116.54 in the HSQC (plate 6e).

10) C-2'/ C-6' resonated as a doublet at δ 131.30 ($^3J_{CF}$ = 9.8 Hz). This assignment was confirmed by the coupling between H-2'/ H-6' at δ 8.01 and.C-2'/ C-6' at δ 131.30 in the HSQC (plate 6e).

11) The remaining six hydrogen attached aromatic carbons resonated at:

δ 117.46 (C-6'')
δ 124.43 (C-2'')
δ 127.53 (C-4''')
δ 129.81 (C-5''')
δ 135.34 (C-6''')
δ 135.61 (C-5'').

These assignments were confirmed with HSQC (plate 6e).

D. The salient features in the COSY spectrum (Plate 6d)

1) The COSY data demonstrated the correlation between the four nitrobenzoyl protons at δ 8.58 (H-2'''), δ 8.42 (H-4'''), δ 8.25 (H-6''') δ 7.74 (H-5''').

2.8.7 (Z)-N-[4-Cyano-3-(trifluoromethyl)phenyl]-3-(4-fluorophenylsulfonyl)-2-methylacrylamide (99)

A. The mass spectrum confirmed structure **99**, found (ES) $[M+H]^+$, 413.0587 ($C_{18}H_{12}F_4N_2O_3S+H^+$) required m/z 413.0583.

B. The salient features in the ^1H NMR spectrum (Plate 7a):

The NMR spectrum was similar to that of bicalutamide, the starting material, with the following exceptions:

1) The expected resonances of the protons in the AMX H–2", H–6" and H–5" of the tri-substituted aniline ring was easily recognized at δ 8.3 (J = 1.9Hz), δ 8.2 (J = 1.9Hz, J = 8.6Hz), δ 8.0 (J = 8.6Hz), respectively.

2) The expected resonances of the protons in the AB system was observed i.e. H-2' and H-6' resonated at δ 8.1 (J = 5.1Hz, 8.9Hz) and H-3' and H-5' resonated at δ 7.5 (J = 8.9Hz).

3) The CH_3 group resonated as a doublet at δ 2.41 (J = 1.5Hz) due to the cross-coupling with the vinylic proton.

4) The vinylic proton resonance was observed at δ 7.2 (J = 1.4Hz, 2.9Hz).

C. The salient features in the NOESY spectrum (Plate 7d):

1) The NOESY data demonstrated the expected resonances correlation between the vinylic proton and the methyl group.

2.8.8 4-Amino-2-ethoxybenzonitrile (100)

A. The mass spectrum confirmed structure **100**, found (ES) $[M+H]^+$, 163.0874 ($C_9H_{10}N_2O + H^+$) required m/z 163.0872.

B. The salient features in ^1H NMR spectrum (Plate 8a):

The characteristic features of the NMR spectra are the following:

1) H-5, H-2 and H-6 in AMX system resonated at δ 7.52 (J = 8.5 Hz), δ 7.36 (J = 2.5 Hz) and δ 6.94 (J = 2.5 Hz, 8.5 Hz), respectively.

2) The characteristic broad peak of NH_2 resonated at δ 5.86.

3) The CH_2 group resonates as a quartet at δ 4.37 (J = 7.1Hz) and CH_3 resonates as a triplet at δ 1.38 (J = 7.1Hz)

C. The salient features in the ^{13}C NMR spectrum (Plate 8b):

It is clear from this spectrum that there was no flourine present in the molecule.

D. The salient features in the NOESY spectrum (Plate 8c, d, e):

1) The NOESY demonstrated a weak coupling between H-2 at δ 7.36 and CH_2 at δ 4.37 (Plate 8c) as well as coupling between H-3 and CH_3 at 1.38 (Plate 8d).

2) The coupling of the NH_2 group to H-3 at δ 7.36 and H-5 at δ 6.94 proved that -NH_2 lies between these two protons (Plate 8e).

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Chapter 3

Experimental

3. Standard Experimental Procedures

The following general experimental techniques were used in this study.

3.1 Chromatographic Methods

3.1.1. Thin-Layer Chromatography

Qualitative thin-layer chromatography was performed on Merck aluminium sheets (silica gel 60 F_{254}, 0.25 mm). Preparative thin-layer chromatography was performed on glass plates (20x20 cm), covered with a thin layer (1.0 mm) Kieselgel PF254 (100 g Kieselgel in 230 mℓ distilled water per 5 plates). The plates were dried at room temperature and used unactivated. The plates were loaded to a maximum of 25 mg material per plate. After development the plates were dried at room temperature in a fast stream of air and the different bands were distinguished under UV light (254 nm), scraped off and eluted with acetone.

3.1.2. Centrifugal Chromatography

Centrifugal Chromatography was performed on a thin layer of silica gel coated with a circular glass plate called a rotor. A motor drove the rotor at constant speed by a shaft passing through a hole in the centre. The compound to be separated was applied as a solution at the centre of the pre-cast rotor by way of a solvent pump or hand held syringe. The chosen solvent mixture was then pumped onto the centre. The solvent is forced by centrifugal forces through the adsorbent layer effectively separating the individual components as a result of their different affinities for the silica layer and solvent mixture. A solvent channel collected the eluent and brought it to the output tube where the fractions were collected.

Centrifugal chromatography was performed with an Analtech Cyclograph™ with commercially available Analtech rotors (4 mm).

3.1.3 Column Chromatography

Separations on Sephadex LH-20 from Pharmacia and Kieselgel from Merck (Art 773, 170-230 mesh) were performed with various column sizes and at differing flow rates. Fractions were collected in test tubes.

3.1.4 Spraying Reagents

All TLC plates were sprayed with a 2% (v/v) solution of formaldehyde (40%) in concentrated sulfuric acid and subsequently heated to 110 ºC for maximum colour development.

3.2 Gas Chromatography

A VARIAN 3300 gas chromatograph was used to record and follow the rate of reactions.

Column Dimensions:

Column material	Silan glass	**Packing material**	Chromosorb
Mass	80/100	**Liquid phase**	OV-101
Column length	2 m	**Internal diameter**	3 mm
Rate	30 cm^3/min	**Detector:**	Flame ionization
Injection temperature	200 ºC	**Column temperature**	60-200 ºC
Detector temperature	250 ºC		

Conditions:

1) Initial column temperature 60 ºC, Initial retention time 0.5 min,
   Program 1: Final temperature: 200 ºC, Column 10.0 ºC/min
   Column retention time: 0.5 min.

2) Initial column temperature 35 ºC, Initial retention time 0.5 min,
   Program 1: Final temperature: 200 ºC, Column 10.0 ºC/min
   Column retention time: 0.5 min.

3.3 Spectroscopic Methods

3.3.1 Nuclear Magnetic Resonance Spectroscopy (NMR)

A 600 MHz Bruker spectrometer was used to record the ^{1}H NMR, COSY, HMQC, HMBC, ^{13}C and APT (150 MHz) experiments in either chloroform-d ($CDCl_3$) or acetone-d_6 with TMS (tetramethylsilane) as internal standard. Chemical shifts were expressed as parts per million (ppm) (δ scale) and coupling constants were measured in Hz. The following abbreviations of **s, d, dd, t, q, m** and **br** are used to denote **singlet, doublet, doublet of doublets, triplet, quartet, multiplet** and **broad**, respectively.

3.3.2 Mass Spectrometry (MS)

The samples were injected via a Rheodyne valve into a carrier solvent pump at a flow rate of 50 µl/minute by a Perkin Elmer series 200 micro pump. The sample was thus delivered into an electrospray ionization source of a Waters Quattro Ultima triple quadrupole mass spectrometer, operated in the positive or negative mode. The capillary voltage was 3.5 kV, the source temperature 80 ºC and the desolvation temperature was 150 ºC. The cone voltage was at 40 V and all other settings were adjusted for maximum detection of the ions. Data was acquired by scanning the mass range from mz = 100 to m/z = 1000 at a scan rate of 3 seconds/scan. A representative mass spectrum of the sample was produced by addition of the spectra across the injection peak and subtraction of the background.

3.3.3 Infrared Spectroscopy (IR)

Solid state FR-IR spectra were recorded as KBr pellets on a Bruker Tensor 27 spectrometer in the range of 3000-600 cm^{-1}.

3.4 Physical Properties Measurement

3.4.1. Melting Point

Melting points were recorded and are uncorrected on a REICHERT, AUSTRIA, No: 351375.

3.5 Photochemical Reactions

All photochemical reactions were carried out inside the photochemical reactor RAYON manufactured by SOUTHERN N. E. ULTRAVIOLET Co. Middletown, Connecticut, USA, equipped with RAYONET PHOTOCHEMICAL REACTOR lamps CAT. NO. RPR - 3000Å, 3500Å, 2537Å respectively.

3.6 Chemical Methods

3.6.1. Methylation with Diazomethane

Dried material (100 mg) was dissolved in methanol (20 mℓ) and cooled to -10 ºC. Diazomethane generated by the reaction of NaOH (16 mℓ, 30% in a 95 5 v/v ethanol solution), with *N*-methyl-*N*-nitroso-*p*-toluenesulphonamide (Diazald, 5 g) in ether (100 mℓ) and glycol (12.5 mℓ) as co-solvent was distilled slowly into the sample. The reaction mixture was stored at -10 ºC for 48 hours and the excess diazomethane was removed at room temperature in a fast moving air current.

3.7 Synthesis

3.7.1. a) Synthesis of 2-oxo-*N*-phenylbutanamide (84)

Thionyl chloride (0.5 mℓ, 6.4 mmol) was added slowly at 0 ºC under a N_2 atmosphere to a solution of 2-ketobutyric acid **(81)** (500 mg, 4.9 mmol) in anhydrous dichloromethane (10 mℓ). The reaction mixture was left to stir at 0 ºC for 2 hours after which aniline **(82)** (0.4 mℓ, 4.4 mmol) was added followed by TEA (1.4 mℓ, 10.2 mmol) at 0 ºC. The reaction mixture was allowed to attain room temperature and after 10 minutes the reaction mixture was extracted with ethyl acetate (200 mℓ) and washed with water. The solvent was then dried over anhydrous sodium sulfate and concentrated by rotary evaporation. Column chromatography (silica gel, H:EtOAc:DCM; 9:0.5:0.5 and 1 drop TEA) gave one fraction, R_F 0.10

The fraction R_F 0.10 was identified as 2-oxo-*N*-phenylbutanamide **84** as yellow crystals, mp 120 ºC, (80 mg, 27%). ^{1}H NMR: δ (600 MHz, Acetone-d_6, Me_4Si) 8.76 (1H, NH), 7.65 (2H, d, J = 8.0 Hz, H-2'/ H-6'), 7.37 (2H, t, J = 8.0 Hz, H-3'/ H-5'), 7.18 (1H, t, J = 8.0 Hz, H-4'), 3.05 (2H, q, J = 7.3 Hz, CH_2), 1.16 (3H, t, J = 7.3 Hz, CH_3). (^{1}H NMR Plate 1a).

^{13}C NMR: δ (150 MHz, Acetone-d_6, Me_4Si) δ 199.9 (C=O), 157.6 (C=O), 136.3 (C-1'), 129.2 (C-3'/ C-5'), 125.2 (C-4'),119.8 (C-2'/ C-6'), 30.0 (-CH_2), 7.2 (-CH_3) (^{13}C NMR Plate 1b).

IR (KBr): ν_{max} = 3318.4, 2360.2, 1742.2, 1670.4, 1536.1, 1444.3, 1399.9, 1105.8, 753.0 cm^{-1}; m/z (ES) 178.08 (100 %, $[M+H]^+$); Found (ES) $[M+H]^+$, 178.0870 ($C_{10}H_{11}NO_2 + H^+$) requires m/z 178.0868.

3.7.1. b) Attempted synthesis of 2-[(4-fluorophenylsulfonyl)methyl]-2-hydroxy-*N*-phenylbutanamide (85)

4-Fluorophenyl methyl sulphone **14** (164.0 mg, 0.9 mmol) was dissolved in anhydrous THF (10 mℓ) and the temperature was dropped to -75 ºC at which time BuLi (0.1 ml, 0.9 mmol) was added, in a N_2 atmosphere.The reaction mixture was allowed to reach room temperature and stirred for 1 hour at room temperature. After cooling to -75 ºC again, 2-oxo-*N*-phenylbutanamide **84** (83.9 mg, 0.47 mmol) was dissolved in anhydrous THF (2 mℓ) and added to the reaction mixture. The mixture was left overnight to attain room temperature and stirred at this temperature. NH_4Cl was added to the mixture and dried under N_2. Column chromatography (silica gel, H:EtOAc:DCM; 4:3:3 and 1 drop TEA) gave two fractions, R_F 0.60 and R_F 0.40.

The fraction R_F 0.60 yielded unreacted 2-oxo-*N*-phenylbutanamide **84** as yellow crystals, mp 120 ºC, (80 mg). NMR identical to plate 1.

The fraction R_F 0.40 yielded unreacted 4-fluorophenyl methyl sulfone **14** as a white amorphous solid (150 mg).

^{1}H NMR: δ (600 MHz, Acetone-d_6, Me_4Si) 7.9 (dd, J = ,5.2 Hz, 8.9 Hz, H-2/ H-6), 7.3 (t, J = 8.9 Hz, H-3/ H-5), 3.0 (1H, s,CH_3). (^{1}H NMR plate 10a).

^{13}C NMR: δ (150 MHz, Acetone-d_6, Me_4Si) 166.4 (d, J = ,C-4), 137.8 (d, J = 3Hz , C-1), 130.4 (d, J = 9.7Hz, C-2/ 6), 116.3 (d, J = 22.8Hz, C-3/ 5), 43.6 (-CH_3). (^{13}C NMR plate 10b).

^{19}F NMR: δ (564.2MHz, Acetone-d_6, Trifluoroacetic acid) -106.7 (F). (^{19}F NMR plate 10c).

3.7.2. a) *N*-[4-Cyano-3-(trifluoromethyl)phenyl]-2-oxobutanamide (86)

Thionyl chloride (1 mℓ, 13.7 mmol) was added slowly at 0 ºC under a N_2 atmosphere to a solution of ketobutyric acid **81** (100 mg, 0.98 mmol) in anhydrous dichloromethane (10 mℓ). The reaction mixture was left to stir at 0 ºC for 2 hours after which 4-amino-3-(trifluoromethyl)benzonitrile **6** (150 mg, 0.8 mmol) was added followed by TEA (2.7 mℓ, 19.6 mmol) at 0 ºC. The reaction mixture was allowed to attain room temperature and after 10 minutes the reaction mixture was extracted with ethyl acetate (200 mℓ) and washed with water. The solvent was dried over anhydrous sodium sulfate and concentrated by rotary evaporation. Column chromatography (silica gel, H:EtOAc:DCM; 4:3:3 and 1 drop TEA) gave one fraction, R_F 0.60.

The fraction R_F 0.60 was identified as *N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-oxobutanamide **86**, orange crystals, mp 120 ºC, (80 mg, 36 %). ^{1}H NMR: δ (600 MHz, Acetone-d_6, Me_4Si) 10.30 (1H, s, NH), 8.55 (1H, d, J = 2.0 Hz, H-2'), 8.37 (1H, dd, J = 1.9 Hz, 8.6 Hz, H-6'), 8.09 (1H, d, J = 8.6 Hz, H-5'), 3.02 (2H, q, J = 7.18 Hz, CH_2), 1.10 (3H, t, J = 7.16 Hz, CH_3). (^{1}H NMR plate 2a).

^{13}C NMR (150 MHz, Acetone-d_6, Me_4Si): δ 159.5 (C=O), 142.4 (C-1'), 136.3 (C-5'), 132.8 (q, $^2J_{FC}$ = 34.6 Hz, C-3'), 123.0 (C-6'),122.6 (q, $^1J_{FC}$ = 273.5 Hz, CF_3), 117.8 (q, $^3J_{FC}$ = 5.4 Hz, C-2'), 115.3 (C≡N), 104.1 (d, $^3J_{FC}$ = 2.26 Hz, C-4), 29.6 (CH_2), 6.4 (CH_3). (^{13}C NMR Plate 2b).

^{19}F NMR δ: (564.2 MHz, Acetone-d_6, Trifluoroacetic acid) -62.63 (CF_3). (^{19}F NMR plate 2c).

IR (KBr): ν_{max} = 3333.6, 2232.4, 1703.1, 1585.8, 1434.7, 1324.2, 1181.3, 1097.4 cm^{-1}; m/z (ES) 269 (100 %), $[M\text{-}H^+]^-$); Found (ES) $[M\text{-}H]^-$, 269.0541 ($C_{12}H_9F_3N_2O_2$ - H^+) requires m/z 269.0538.

3.7.2. b) Attempted synthesis of N-(4-cyano-3-(trifluoromethyl)phenyl)-2-[(4-fluorophenylsulfonyl)methyl]-2-hydroxybutanamide (85)

4-Fluorophenyl methyl sulphone **14** (164.0 mg, 0.9 mmol) was dissolved in anhydrous THF (10 mℓ) and cooled to -75 ºC at which time BuLi (0.1 mℓ, 0.9 mmol) was added (under N_2 atmosphere). The reaction mixture was allowed to reach room temperature and stirred for 1 hour at room temperature. N-(4-cyano-3-(trifluoromethyl)phenyl)-2-oxobutanamide **86,** (83.9 mg, 0.5 mmol) was dissolved in anhydrous THF (2 mℓ) and added to the reaction mixture at -75 ºC. The reaction mixture was left overnight to attain room temperature and stirred at this temperature. The reaction mixture was divided and NH_4Cl was added to one half and the other half was dried under N_2. Column chromatography (silica gel, H:EtOAc:DCM; 4:3:3 and 1 drop TEA) gave two fractions, R_F 0.60 and R_F 0.40.

The fraction R_F 0.60 yielded amide **86** as orange crystals, mp 120 ºC, (80 mg). NMR identical to Plate 2.

The fraction R_F 0.40 yielded 4-fluorophenyl methyl sulfone **(14)** as a white amorphous solid, (160 mg). NMR identical to Plate 10.

3.7.3. Extraction of bicalutamide; *N*-[4-cyano-3-(trifluoromethyl)phenyl]-3-[(4-fluorophenyl)sulfonyl]-2-hydroxy-2-methylpropanamide (1) from Casodex®

A Casodex® tablet (50 mg) was crushed, extracted with acetone and concentration by rotary evaporation gave 48.9 mg of bicalutamide **(1)**.

^{1}H NMR: δ (600 MHz, Acetone-d_6) 9.84 (1H, NH), 8.43 (1H, d, J = 2.0 Hz, H-2''), 8.08 (1H, dd, J = 8.56 Hz, 2.0 Hz, H-6''), 7.89 (1H, d, J = 8.56 Hz, H-5''), 7.86 (2H, dd, J = 9.0 Hz, 5.0 Hz, H-2'/ 6'), 7.16 (2H, t, J = 9.0 Hz, H-3'/ 5'), 4.0 (1H, d, J = 14.7, H-3), 3.57 (1H, d, J = 14.7, H-3), 1.42 (3H, s, CH_3).(^{1}H NMR Plate 3a).

^{13}C NMR: δ (150MHz, Acetone-d_6) 172.6 (amide C=O), 165.0 (d, $^{1}J_{FC}$ = 253.3, C-4'),142.7 (C–1"), 136.9 (d, $^{1}J_{FC}$ = 2.0 Hz, C–1'), 135.5 (C-5"), 132.0 (q, $^{2}J_{FC}$ = 29.41, C-3"), 131.1 (d, $^{4}J_{FC}$ = 9.67 Hz, C-2'/ 6'), 122.3 (q, $^{1}J_{FC}$ = 273.77, -CF_3), 122.29 (C-6"), 117.0 (q, $^{3}J_{FC}$ = 5.42 Hz, C-2"), 115.5 (d, $^{2}J_{FC}$ = 22.9 Hz, C-3'/ 5'), 114.9 (C≡N), 102.9 (d, J = 2.2 Hz, C-4"), 73.3 (C-2), 62.7 (CH_2), 26.5 (CH_3).(^{13}C NMR Plate 3b).

^{19}F NMR: δ (564.2MHz, Acetone-d_6, Trifluoroacetic acid) -62.60 (CF_3), -106.50 (F). (^{19}F NMR Plate 3c).

IR (KBr): ν_{max} = 3338.2, 2230.2, 1689.2, 1516.78, 1328.1, 1328.1, 1290.4, 1179.5, 1140.8 cm^{-1}; M^+ 430.38.

3.7.4. 3-(4-Fluorophenylsulfonyl)-2-hydroxy-2-methyl-*N*-[4-methyl-3-(trifluoromethyl)phenyl]propanamide (94)

Bicalutamide **(1)** (50 mg, 0.2 mmol) was dissolved in ethanol (20 mℓ) and equal equivalents palladium on activated charcoal was added. The reaction mixture was stirred at room temperature under H_2 gas for 24 hours. The catalyst was filtered off and the solvent was evaporated under reduced pressure. Column chromatography (silica gel, H:EtOAc and 1 drop TEA 5:5) yielded two fractions, R_F 0.36 and R_F 0.18.

The fraction R_F 0.18 yielded unreacted bicalutamide **(1)** as white crystals from ethyl acetate and petroleum ether from acetone, mp 191-193 ºC, (20 mg). NMR identical to plate 3.

The fraction R_F 0.36 yielded 3-(4-fluorophenylsulfonyl)-2-hydroxy-2-methyl-*N*-[4-methyl-3-(trifluoromethyl)phenyl]propanamide **(94)** as a white amorphous solid, (15 mg, 50 %).^{1}H NMR: δ (600 MHz, $CDCl_3$, Me_4Si) 8.74 (1H, NH) 7.87 (2H, dd, J = 4.97 Hz, 8.88 Hz, H-2'/ 6'), 7.68 (1H, d, J = 2.30 Hz, H-2"), 7.46 (1H, dd, J = 2.30 Hz, 8.37 Hz, H-6"), 7.24 (1H, d, J = 8.37 Hz, H-5"), 7.11 (2H, t, J = 8.88 Hz, H-3'/ 5'), 4.02 (1H, d, J = 14.2 Hz, H-3), 3.48 (1H, d, J = 14.2 Hz, H-3), 2.45 (3H, d, J = 1.38 Hz, CH_3), 1.59 (3H, s, -CH_3). (^{1}H NMR Plate 4a).

^{13} C NMR: δ (150 MHz, $CDCl_3$,Me_4Si) 170.4 (amide C=O), 166.2 (d, $^2J_{FC}$ = 258.3 Hz, C-4'), 135 (d, $^1J_{FC}$ = 3.1 Hz, C-1'), 134.8 (C-1''), 132.9 (C-5''), 130.9 (d, $^4J_{FC}$ = 9.8 Hz, C-2''/ 6'), 129.4 (q, $^1J_{FC}$ = 30.1 Hz, C-3''), 125.0 (q, $^1J_{FC}$ = 273.02 Hz, CF_3), 122.3 (C-6''), 117,0 (q, $^3J_{FC}$ = 5.8 Hz, C-2''), 116.7 (d, $^2J_{FC}$ = 22.8 Hz, C-3''/ 5'), 74.2 (C-2), 61.6 (CH_2), 27.8 (CH_3), 18.8 (CH_3). (^{13}C NMR Plate 4b).

^{19}F NMR: δ (564.2 MHz, Acetone-d_6, Trifluoroacetic acid) -61.40 (CF_3), -106.60 (F). (^{19}F NMR Plate 4c).

IR (KBr): ν_{max} = 3362.2, 1530.2, 1320.4, 1281.4, 1137.3, 835.0 cm^{-1}; m/z (ES) 420 (100 %, $[M+H]^+$); Found (ES) $[M+H]^+$, 420.0895 ($C_{18}H_{17}NO_4F_4S + H^+$) requires m/z 420.0893.

3.7.5. Synthesis of (3-(4-fluorophenylsulfonyl)-2-hydroxy-2-methylpropanamido)-2-(trifluoromethyl)benzamide (96)

Bicalutamide **(1)** (50 mg, 0.2 mmol) was refluxed in benzene (5 mℓ) with equal equivalents of ρ-toluenesulfonic acid and a few drops of H_2SO_4 for 5 hours. Column chromatography (H:EtOAc and 1 drop TEA; 5:5) gave two fractions R_F 0.60 and R_F 0.30.

The fraction R_F 0.6 yielded unreacted bicalutamide **(1)** as white crystals from ethyl acetate and petroleum ether from acetone, mp 191-193 ^oC, (5 mg). NMR identical to plate 3.

The fraction R_F 0.3 yielded 4-[3-(4-fluorophenylsulfonyl)-2-hydroxy-2-methylpropanamido]-2-(trifluoromethyl)benzamide **(96)** as a white amorphous solid, (20 mg, 44 %). ^{1}H NMR: δ (600 MHz, Acetone-d_6, Me_4Si) 9.66 (1H, OH) 8,21 (2H, d, J = 2.1 Hz, H-3''), 8.0 (3H, m, H-2''/ H-6''/ H-5''), 7.58 (1H, d, J = 8.3 Hz, H-6''), 7.30 (2H, t, J = 8.7 Hz, H-3''/ 5'), 4.06 (1H, d, J = 14.8 Hz, H-3), 3.68 (1H, d, J = 14.8 Hz, H-3), 1.55 (3H, s, CH_3).(^{1}H NMR Plate 5a).

^{13}C NMR: δ (150 MHz, Acetone-d_6, Me_4Si) 171.7 (C=O), 168.0 (C=O), 164.9 (d, $^2J_{FC}$ = 253.1 Hz, C-4'), 138.9 (C-4''), 137.6 (d, $^1J_{FC}$ = 3.0 Hz, C-1'), 132.9 (C-6''),

130.8 (d, $^{4}J_{FC}$ = 9.60 Hz, C-2'/ 6'), 127.5 (C-1''), 126.6 (q, $^{1}J_{FC}$ = 34.6 Hz, C-2''), 124.1 (q, $^{1}J_{FC}$ = 273.7 Hz, CF_3), 122.5 (C-5''), 116,7 (q, $^{3}J_{FC}$ = 5.5 Hz, C-3''), 115.2 (d, $^{2}J_{FC}$ = 22.9 Hz, C-3'/ 5'), 73.0 (C-2), 62.5 (CH_2), 26.3 (CH_3). (^{13}C NMR Plate 5b)

^{19}F NMR: δ (564.2 MHz, Acetone-d_6, Trifluoroacetic acid) -59.63 (CF_3), -106.80 (F) (^{19}F NMR Plate 5c).

IR (KBr): ν_{max} = 3447.7, 2964.0, 2925.8, 1673.38, 1494.9, 1142.36 cm^{-1}; m/z (ES) 447 (100 %, $[M\text{-}H^{+}]^{-}$; Found (ES) $[M\text{-}H^{+}]^{-}$, 447.0640 ($C_{18}H_{17}NO_4F_4S$ - H^{+}) requires m/z 447.0638.

3.7.6. <u>Attempted synthesis of (Z)-N-[4-cyano-3-(trifluoromethyl)phenyl]-3-(4-fluorophenylsulfonyl)-2-methylacrylamide (99)</u>

Bicalutamide (50 mg, 0.1 mmol) was dissolved in THF (5 mℓ). LDA (0.1 mℓ) was added and the reaction mixture was then heated at 100 ºC in an autoclave under N_2 (1 bar) for 2 hours. The reaction mixture was allowed to cool overnight. Column chromatography (T:A; 8:2) gave three fractions R_F 0.70, R_F 0.60 and R_F 0.30.

The fraction R_F 0.30 yielded <u>unreacted bicalutamide</u> **(1)** as white crystals from ethyl acetate and petroleum ether from acetone, mp 191-193 ºC (20 mg). NMR identical to plate 3.

The fraction R_F 0.60 yielded <u>4-fluorophenyl methyl sulfone</u> **(14)** as a white amorphous solid, (10 mg).

^{1}H NMR: δ (600 MHz, $CDCl_3$, Me_4Si) 7.98 (dd, 2H, J = 5.2 Hz, 8.9 Hz, H-3/ H-5), 7.26 (t, 2H, J = 8.9 Hz, H-3/ H-5), 3.07 (CH_3). NMR identical to plate 10.

The fraction R_F 0.70 yielded <u>4-amino-3-(trifluoromethyl)benzonitrile</u> **(6)** as an orange amorphous solid, (5 mg). NMR identical to plate 9.

3.7.7. Synthesis of 1-(4-Fluoro-3-(trifluoromethyl)phenylamino)-3-(4-fluorophenylsulfonyl)-2-methyl-1-oxopropan-2-yl 3-nitrobenzoate (98) and Synthesis of (Z)-N-[4-cyano-3-(trifluoromethyl)phenyl]-3-(4-fluorophenylsulfonyl)-2-methylacrylamide (99)

Bicalutamide **(1)** (100 mg, 0.23 mmol) was dissolved in dichloromethane (10 mℓ) and pyridine (0.5 mℓ) and a solution of 3-nitrobenzoyl chloride **(97)** (100 mg, 0.4 mmol) in dichloromethane (0.5 mℓ) was added, the mixture was cooled to 0 ºC, followed by the addition of 4-dimethylaminopyridine (50 mg, 0.4 mmol). The reaction mixture was allowed to attain room temperature after which it was heated for 2 hours at 40 ºC and subsequently stirred for five days at room temperature. The reaction mixture was dried under N_2 and column chromatography (silica gel, H:EtOAc; 5:5 and 1 drop aq. NH_3) gave three fractions, R_F 0.60, R_F 0.40 and R_F 0.27.

The fraction R_F 0.27 yielded unreacted bicalutamide **(1)** as white crystals from ethyl acetate and petroleum ether from acetone, mp 191-193 ºC, (10 mg,). NMR identical to plate 3.

The fraction R_F 0.40 yielded 1-(4-Fluoro-3-(trifluoromethyl)phenylamino)-3-(4-fluorophenylsulfonyl)-2-methyl-1-oxopropan-2-yl 3-nitrobenzoate **(98)** as a yellow-white amorphous solid, (20 mg, 22 %).

^{1}H NMR: δ (600 MHz, acetone-d_6) 9.93 (s, NH), 8.58 (ddd, J = 0.4, 1.6, 2.3 Hz, H-2'''), 8.42 (ddd, 1H, J = 1.1, 2.3, 8.2 Hz, H-4'''), 8.25 (ddd, 1H, J = 1.1 Hz, 1.6 Hz, 7.5 Hz, H-6'''), 8.11 (d, J = 2.0 Hz, H-2''), 7.96 (dd, J = 2.0 Hz, 8.8 Hz, H-6''), 7.9-7.8 (m, H-5''/ H-3'/ H-5'), 7.74 (ddd, J = 0.4 Hz, 7.5 Hz, 8.2 Hz, H-5'''), 7.29 (t, J = 8.8 Hz, H-2'/ H-6') , 4.36 (d, J = 15.3 Hz, H-3), 4.12 (d, J = 15.3 Hz, H-3), 2.0 (s, CH_3). (^{1}H NMR Plate 6a).

^{13}C NMR: δ (150 MHz, acetone-d_6) 168.9 (C=O), 165.3 (d, $^1J_{FC}$ = 254.2 Hz, C-4'), 162.9 (C=O), 147.8 (C-1'''), 142.26 (C–1''), 136.5 (d, $^1J_{FC}$ = 3.4 Hz, C-1'), 135.3 (C-5''), 135.6 (C-6'''), 135.3 (q, $^2J_{FC}$ = 32.0 Hz, C-3''), 130.8 (d, $^4J_{CF}$ = 9.8 Hz , C-2'/ C-6'), 129.8 (C-5'''), 127.5 (C-4'''), 24.0 (C-2'''), 123.2 (C-6''), 122.6 (q, $^2J_{FC}$ =

272.2 Hz, C-3''), 122.1 (q, $^{1}J_{FC}$ = 273.8, CF_3) 117.9 (q, $^{1}J_{FC}$ = 4.6 Hz, C-2''), 116.5 (d, $^{1}J_{FC}$ = 22.8 Hz, C-3'/ 5'), 115.2 (C≡N), 103.5 (C-4''), 80.24 (C-2), 57.0 (CH_2), 22.4 (CH_3). (^{13}C NMR Plate 6b).

^{19}F NMR: δ (564.2 MHz, Acetone-d_6, Trifluoroacetic acid) -62.70(CF_3), -106.0 (F).(^{13}C NMR Plate 6c)

IR (KBr): ν_{max} = 3430.0, 3105.7, 2933.3, 2360.3, 2231,9, 1735.9, 1592.1, 1535.1, 1327.02, 1144.29, 842.2 cm^{-1}; m/z (ES) 579 (100 %, $[M-H]^-$); Found (ES) $[M-H]^-$, 579.0650 ($C_{25}H_{17}F_4N_3O_2S$ - H^+) requires m/z 579.0645.

The fraction R_F 0.60 yielded (Z)-N-(4-cyano-3-(trifluoromethyl)phenyl)-3-(4-Fluorophenylsulfonyl)-2-methylacylamide **(99)** as a white amorphous solid, (31.8 mg, 35.3 %). ^{1}H NMR: δ (600 MHz, Acetone-d_6) 10.14 (1H, OH), 8.30 (d, 1H, J = 1.9 Hz, H-2''), 8.14 (dd, 1H, J = 1.9 Hz, 8.6 Hz, H-6''), 8.08 (dd, 2H, J = 5.3 Hz, 8.9 Hz, H-2'/ 6'), 8.02 (d, J = 8.6 Hz, H-5''), 7.47 (t, 2H, J = 8.9 Hz, H-3'/ 5'), 7.22 (q, 1H, J = 1.5 Hz, C-3), 2.41 (d, 3H, J = 1.5 Hz, CH_3). (^{1}H NMR Plate 7a).

^{13}C NMR: δ (150 MHz, Acetone-d_6) 167.7 (C=O), 166.5 (d, $^{1}J_{FC}$ = 163.2 Hz, C-4'), 147.1 (C-2), 143.9 (C-1''), 138.5 (d, $^{1}J_{FC}$ = 3.2 Hz, C-1'), 137.0 (C-5''), 134.2 (C-3), 133.5 (q, $^{2}J_{FC}$ = 32.0 Hz, C-3''), 131.7 (d, $^{4}J_{FC}$ = 10.0 Hz, C-2'/ 6'), 123.9 (C-6''), 123.5 (q, $^{1}J_{FC}$ = 273.2 Hz, CF_3), 118.7 (q, $^{3}J_{FC}$ = 5.2 Hz, C-2''),117.7 (d, $^{2}J_{FC}$ = 2.3 Hz, H-3'/ 5'), 116.2 (CN), 104.8 (d, $^{1}J_{FC}$ = 2.4 Hz, C-4''), 14.2 (CH_3). (^{13}C NMR Plate 7b).

^{19}F NMR: δ (564.2 MHz, Acetone-d_6, Trifluoroacetic acid) -62.30 (CF_3), -105.50 (F). (^{19}F NMR Plate 7c).

IR (KBr): ν_{max} = 3320.2, 2231.5, 1697.03, 1592.4, 1533.2, 1328.4, 1146.0, 840.0 cm^{-1}; m/z (ES) 413 (100 %, $[M+H]^+$); Found (ES) $[M+H]^+$, 413.0587 ($C_{18}H_{12}F_4N_2O_3S$+ H^+) requires m/z 413.0583.

3.7.8 Synthesis of 4-amino-2-ethoxybenzonitrile (100) (I)

Bicalutamide **(1)** (100 mg, 0.16 mmol) was dissolved in ethanol (100 mℓ) and irradiated for 24 hours at 300 nm under a N_2 atmosphere. The solvent was concentrated by rotary evaporation. Column chromatography (silica gel, H:EtOAc:DCM and 1 drop TEA, 4:3:3) yielded three fractions R_F 0.50, R_F 0.38 and R_F 0.17.

The fraction R_F 0.17 yielded unreacted bicalutamide **(1)** as white crystals from ethyl acetate and petroleum ether from acetone, mp 191-193 ºC, (34.7 mg). NMR identical to plate 3.

The fraction R_F 0.38 yielded 4-amino-2-ethoxybenzonitrile **(100)** as a yellow amorphous solid, (20 mg, 30.6 %). ^{1}H NMR: δ (600 MHz, acetone-d_6) 7.52 (1H, d, J = 8.5 Hz, H-5), 7.36 (1H, d, J = 2.5 Hz, H-2), 6.94 (1H, dd, J = 2.5 Hz, , H-6), 5.86, (2H, NH_2) 4.37 (2H, dd, J = 7.1 Hz, 14.3 Hz, CH_2), 1.38 (3H, t, J = 7.1Hz, CH_3). (^{1}H NMR Plate 8a).

^{13}C NMR: δ (150 MHz, acetone-d_6) 164.3 (C-2), 152.3 (C-3), 136.0 (C–5), 134.0 (C–4), 118.4 (CN), 116.6 (C-6), 115.5 (C-6), 98.1 (C-NH_2), 61.3 (CH_2), 13.5 (CH_3). (^{13}C NMR plate 8b).

IR (KBr): υ 3458.1, 3372.6, 3234.2, 2207.7, 1705.4, 1601.7, 1267.8, 1247.5 cm^{-1}; m/z (ES) 163 (100 %, $[M+H]^+$); Found (ES) $[M+H]^+$, 163.0874 ($C_9H_{10}N_2O + H^+$) requires m/z 163.0872.

The fraction R_F 0.5 yielded 4-amino-3-(trifluoromethyl)benzonitrile **(6)** as an orange amorphous solid, (20 mg).

^{1}H NMR: δ (600 MHz, Acetone-d_6, Me_4Si) 7.6 (1H, d, J = 8.6 Hz, H-5), 8.4 (1H, d, J = 2.3Hz, 8.6Hz, H-2), 7.0 (1H, dd, J = 2.3Hz, 8.5Hz, H-5), 6.1 (2H, s, NH_2). (^{1}H NMR plate 9a).

^{13}C NMR: δ (150 MHz, Acetone-d_6, Me_4Si) 152.8 (C-4), 136.3 (C-6), 133.2 (q, $^2J_{FC}$ = 31.7 Hz, C-3), 122.2 (q, $^1J_{FC}$ = 272.9 Hz, CF_3), 116.7 (q, $^3J_{FC}$ = 4.9 Hz, C-

3), 111.3 (d, $^{3}J_{FC}$ = 2.1 Hz, C-5), 111.24 (CN), 94.1 (C-4 and C-NH_2). (^{13}C NMR plate 9b).

^{19}F NMR: δ (564.2 MHz, Acetone-d_6, Trifluoroacetic acid) -63.0 (CF_3). (^{19}F NMR plate 9c).

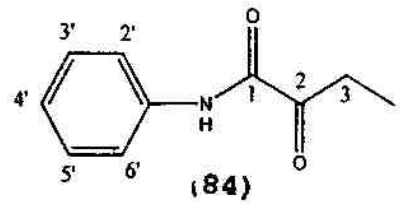
3.7.9. Synthesis of 4-amino-2-ethoxybenzonitrile (100) (II)

4-Amino-2-(trifluoromethyl)benzonitrile **(6)** (200 mg, 1.1 mmol) was dissolved in ethanol (100 mℓ) and irradiated for 24 hours at 300 nm under a N_2 atmosphere. The solvent was concentrated by rotary evaporation. Column chromatography (silica gel, H:EtOAc and 1 drop TEA, 5:5) yielded two fractions R_F 0.50 and R_F 0.38.

The fraction R_F 0.38 yielded 4-amino-2-ethoxybenzonitrile **(100)** as a yellow amorphous solid, (100 mg, 83 %). NMR identical to Plate 8.

The fraction R_F 0.5 yielded unreacted 4-amino-2-(trifluoromethyl)benzonitrile **(6)** as a orange amorphous solid, (80 mg). NMR identical to Plate 9.

Plate 1b($CDCl_3$,298K)



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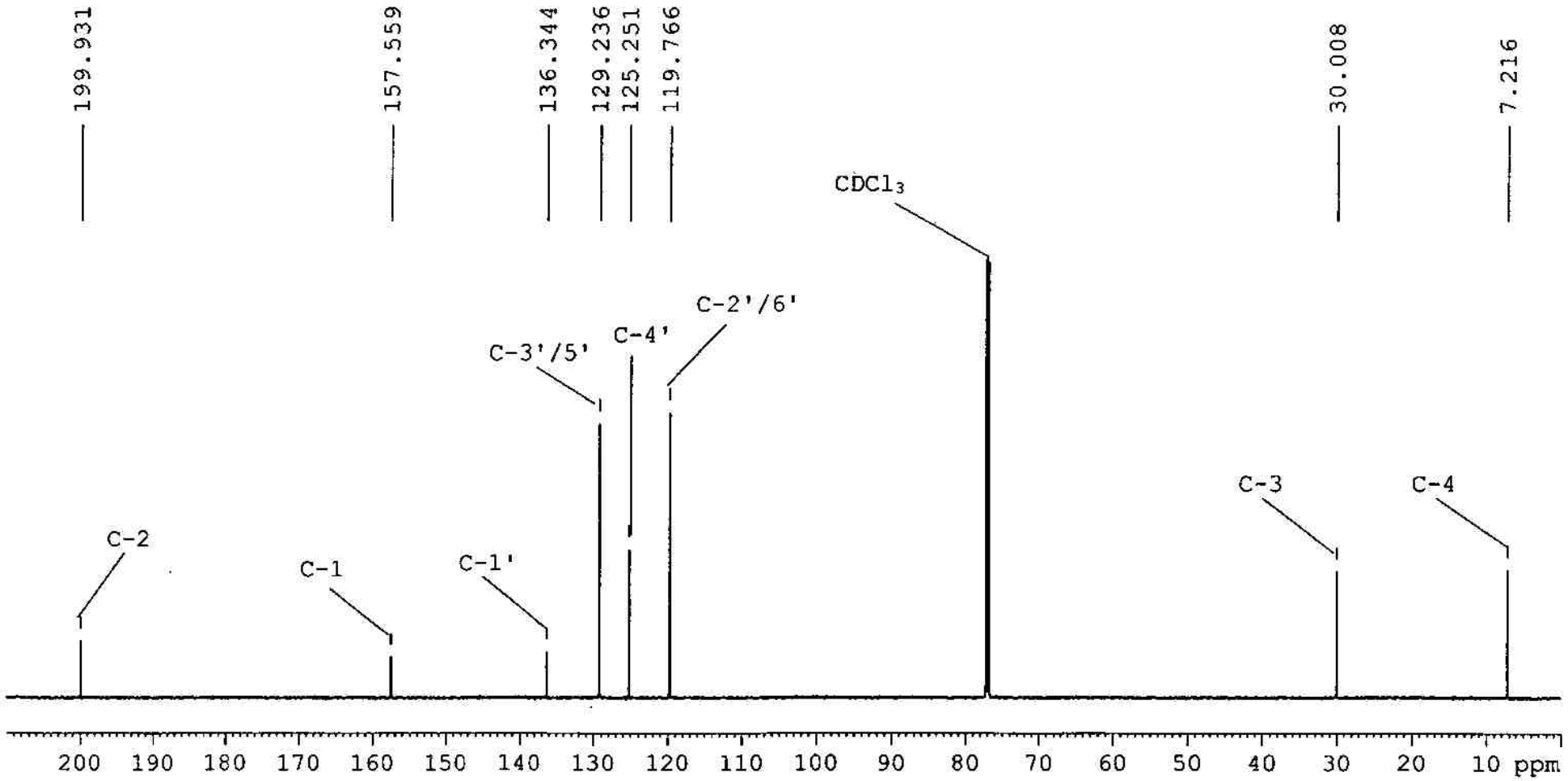
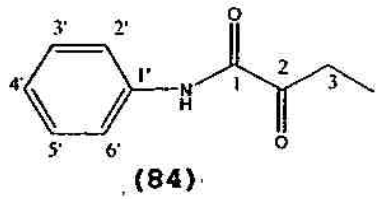


Plate 1c ($CDCl_3$, 298K)



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COSY

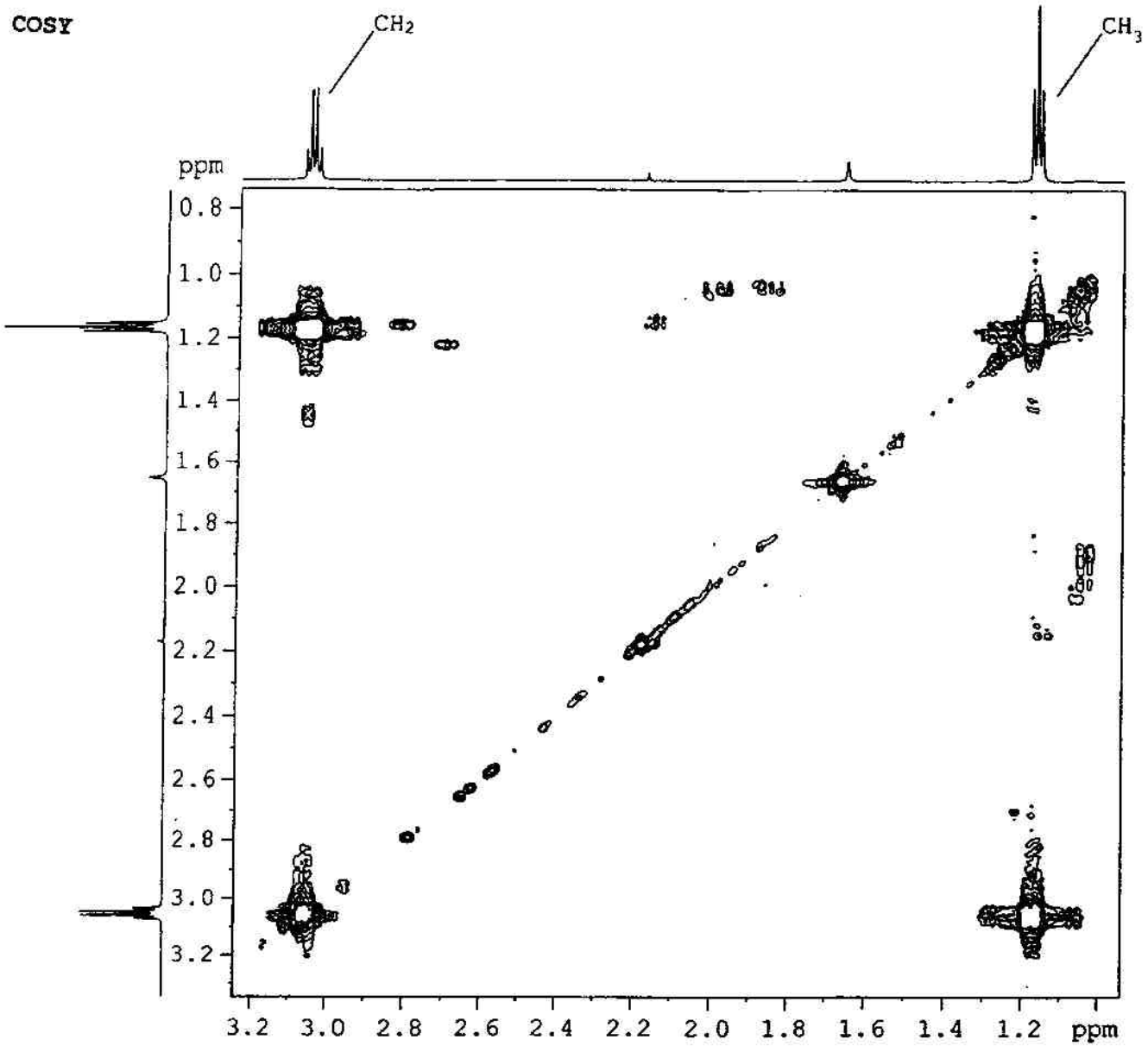
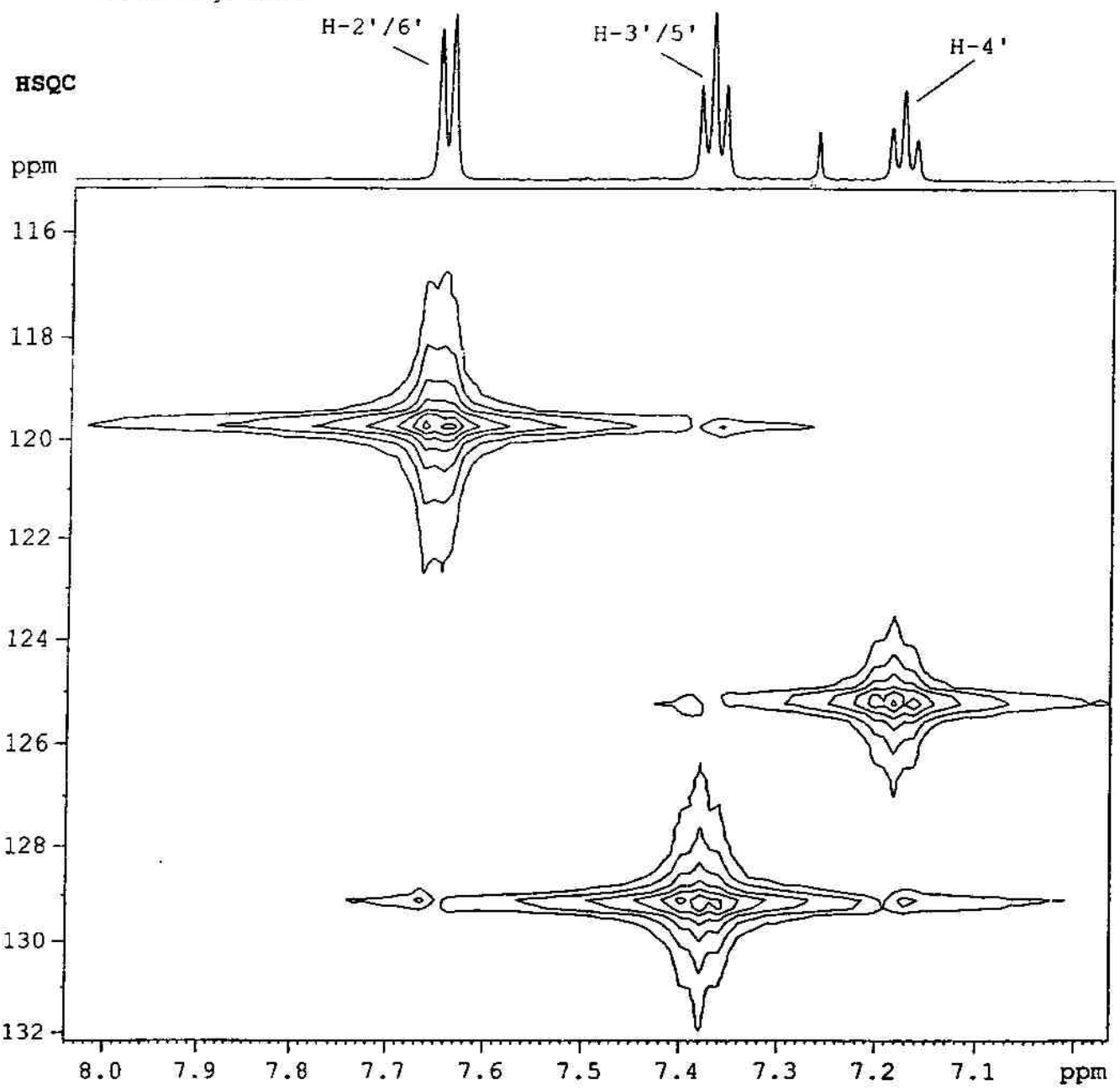


Plate 1d ($CDCl_3$, 298K)



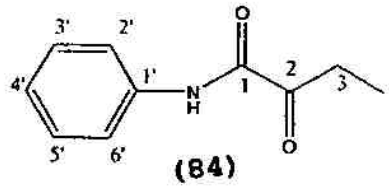


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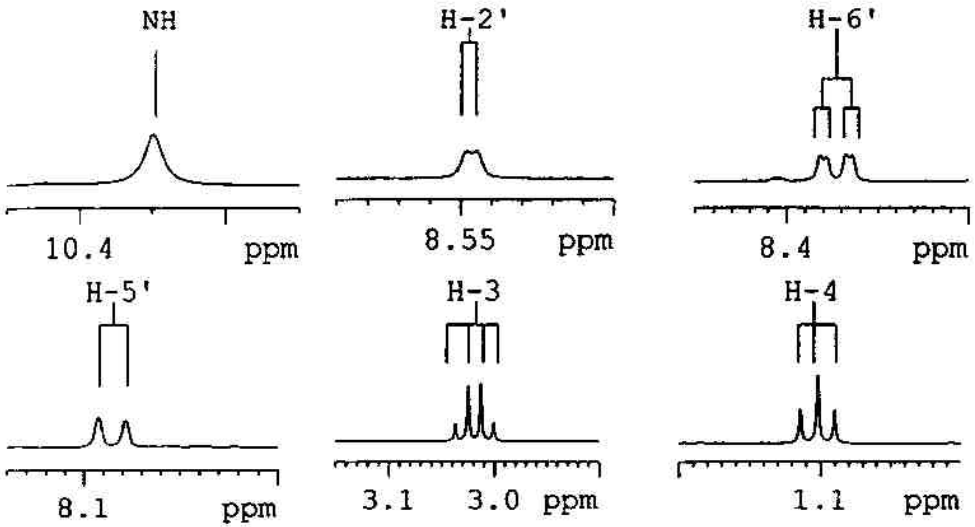
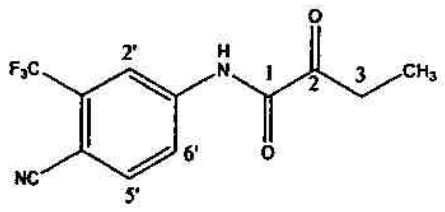
Plate 2a(Acetone-d $_6$,298K)





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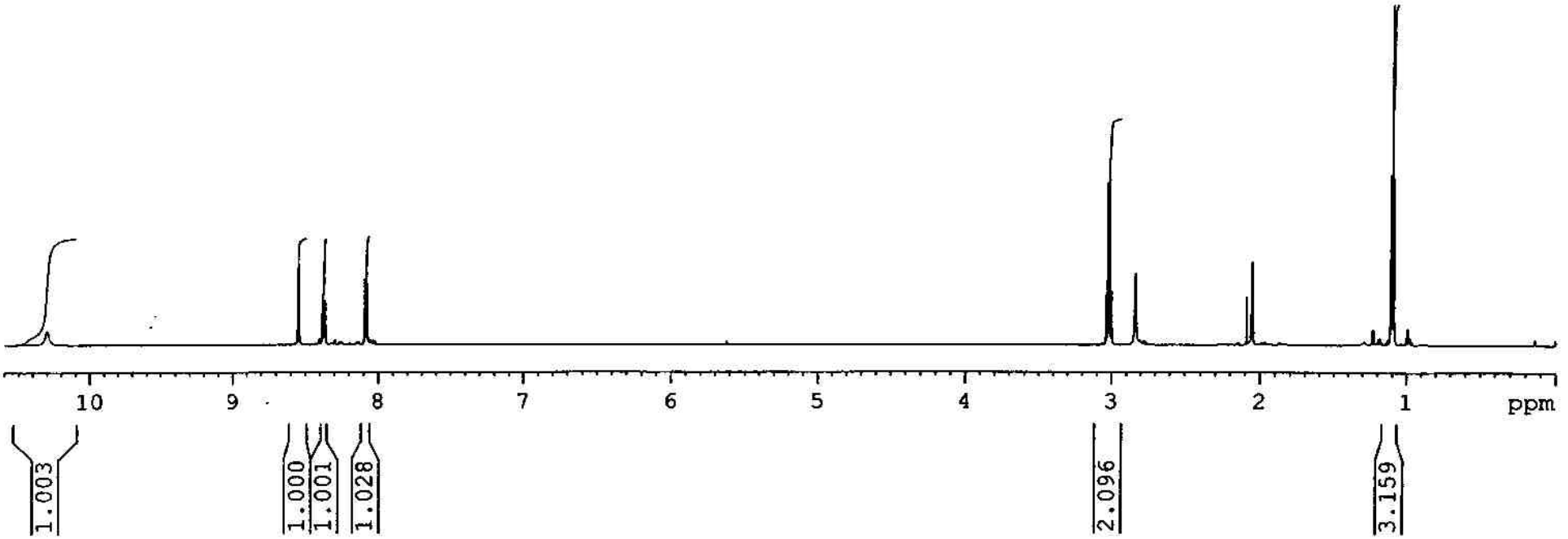
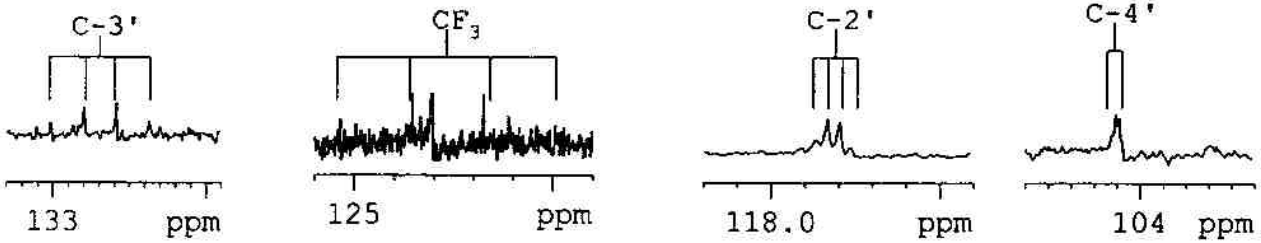
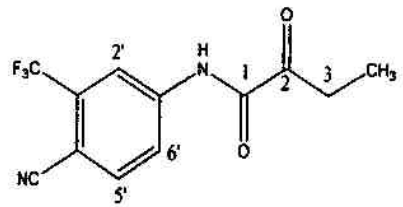


Plate 2b(Acetone-d $_6$, 298K)





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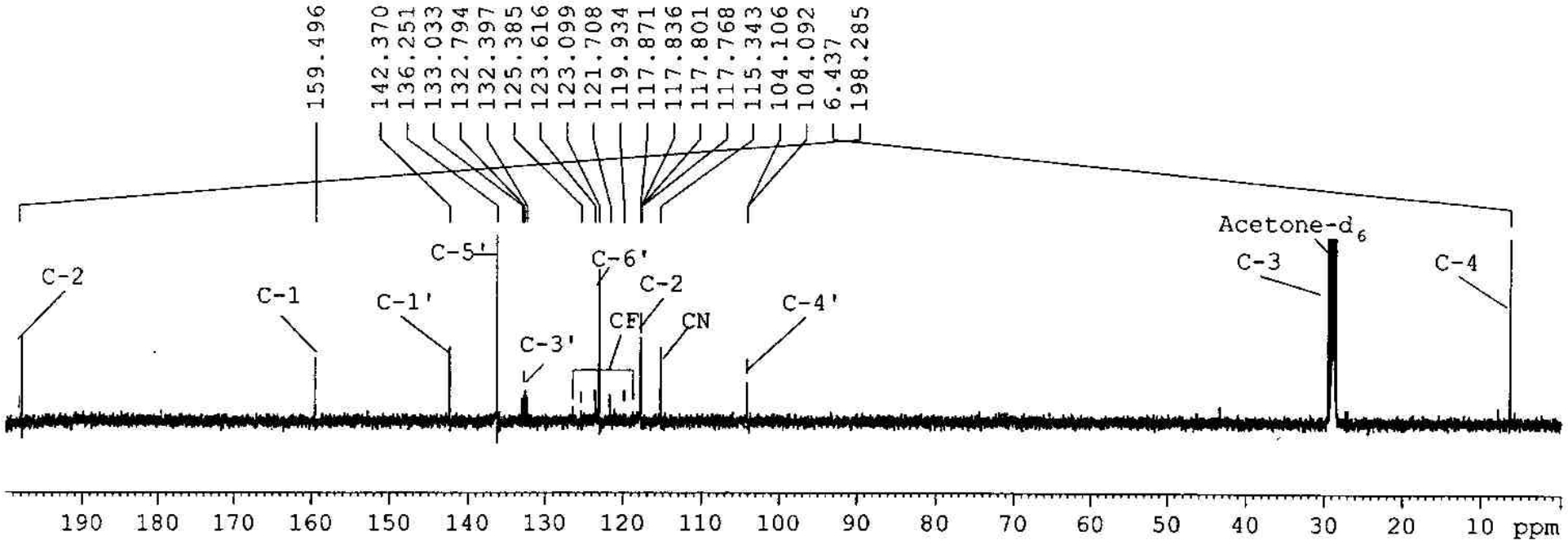
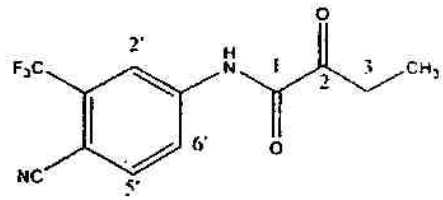


Plate 2c(Trfluoroacetic acid,298K)



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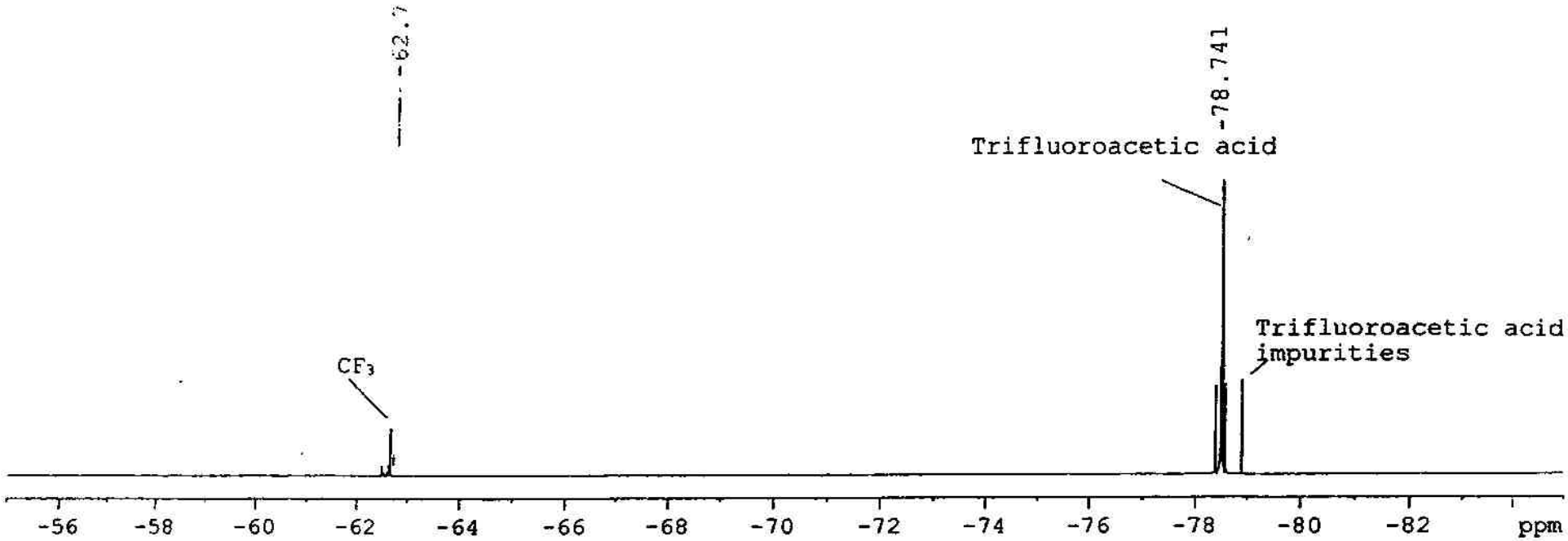
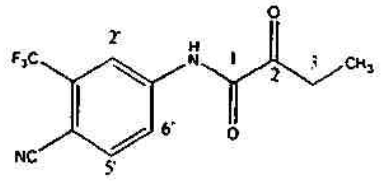


Plate 2d (Acetone-d $_6$, 298K)

HSQC



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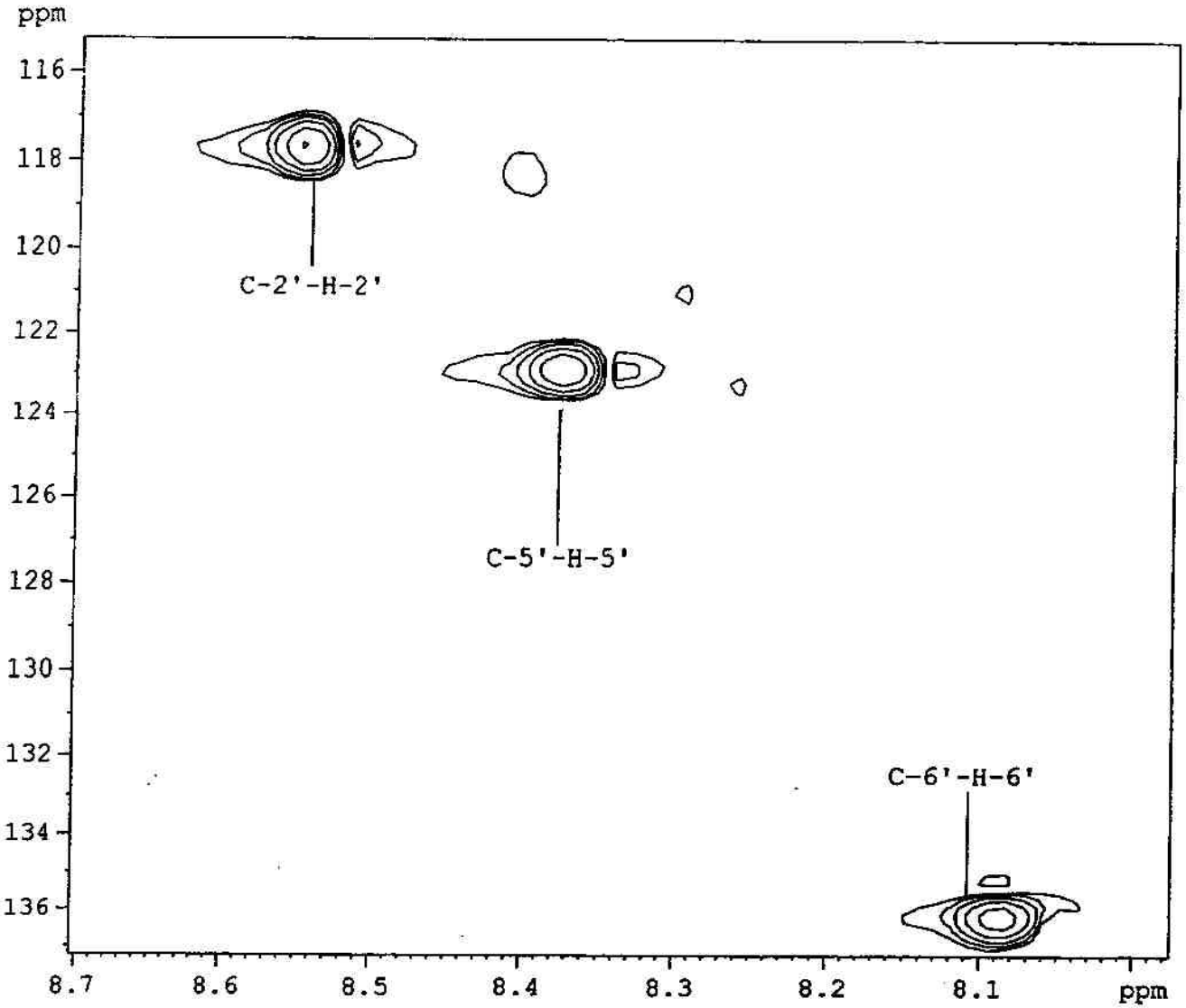
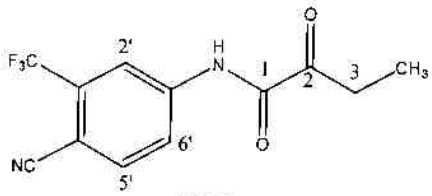


Plate 2e(Acetone-d_6, 298K)

COSY



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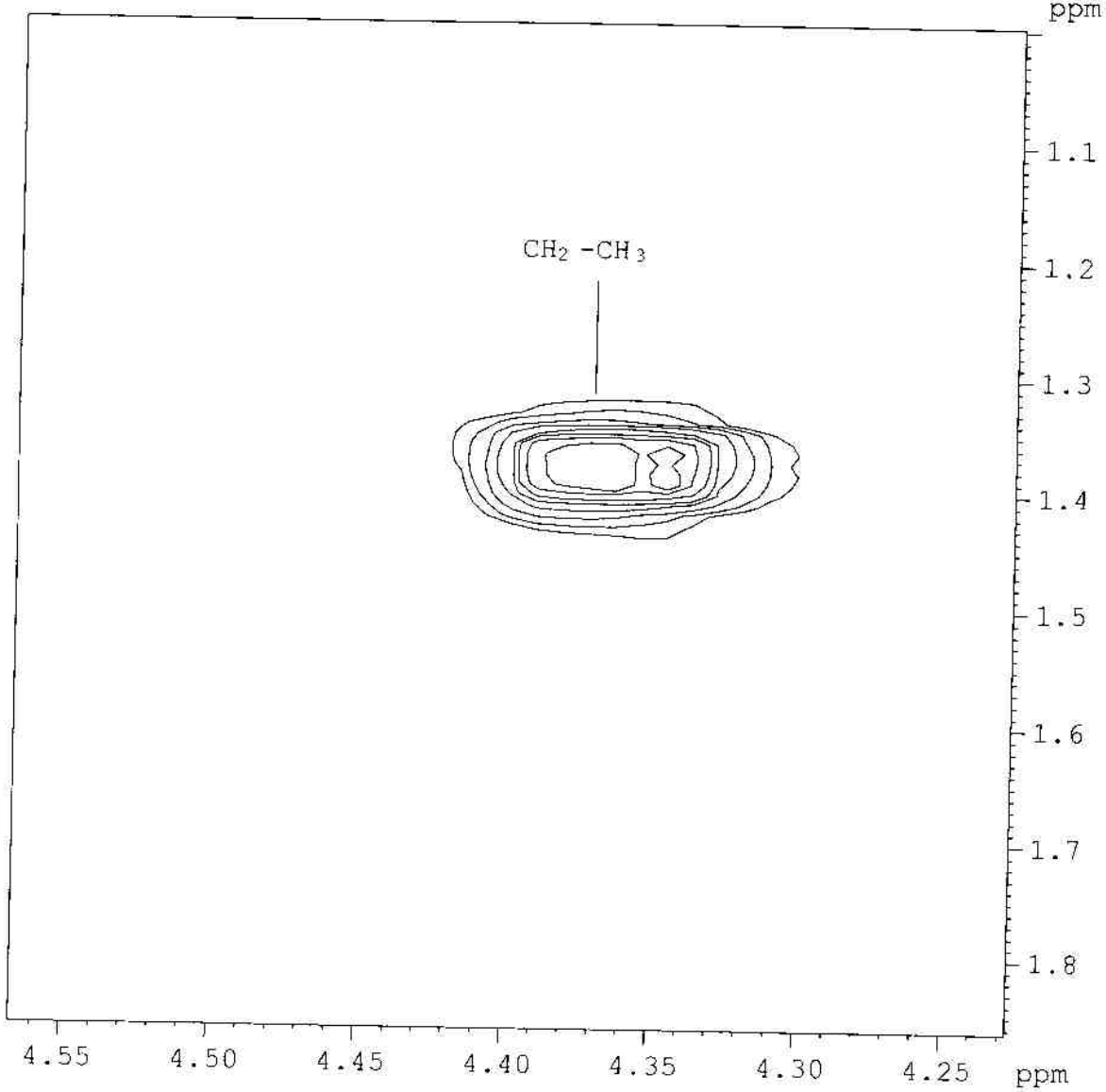


Plate 3a (acetone -d_6 298K)

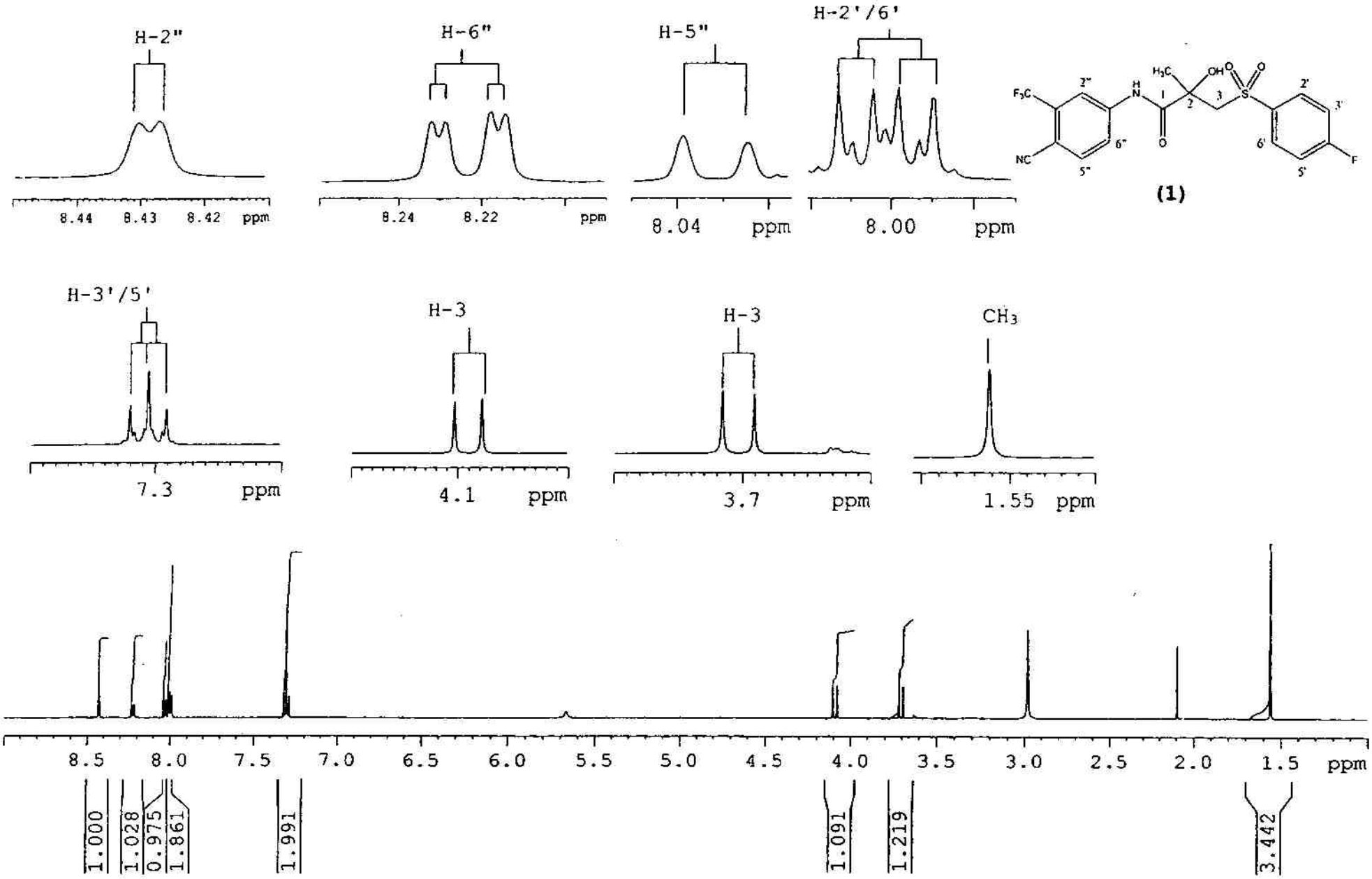
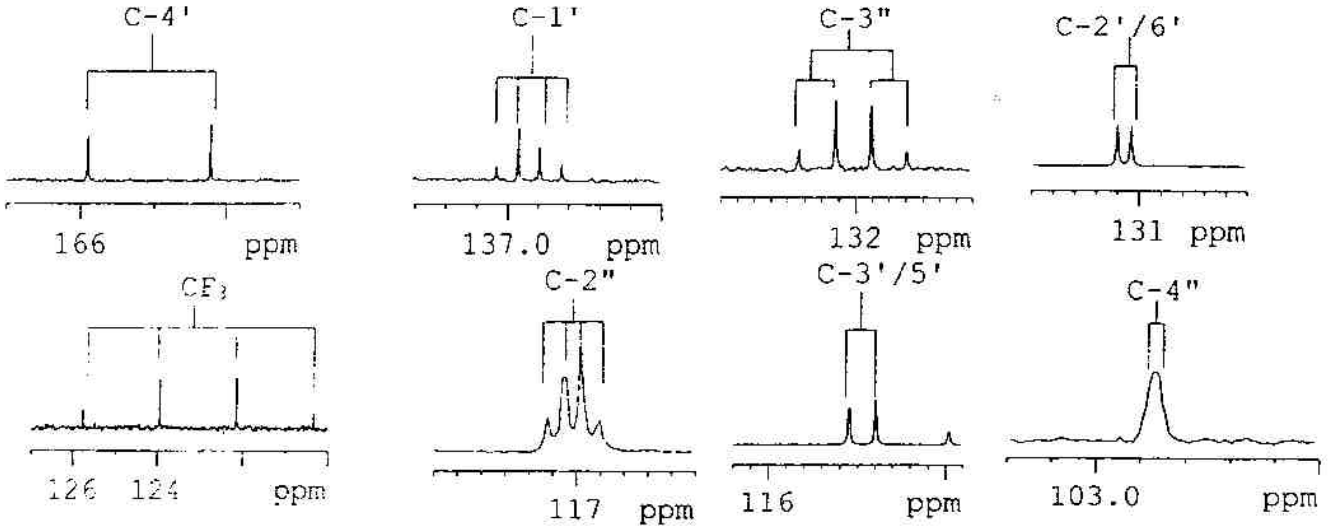
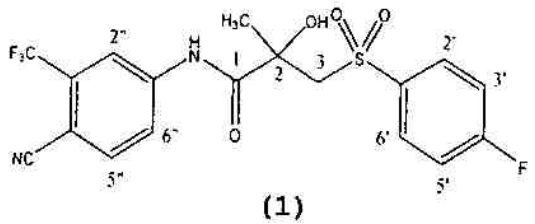


Plate 3b(Acetone-d $_6$,298K)





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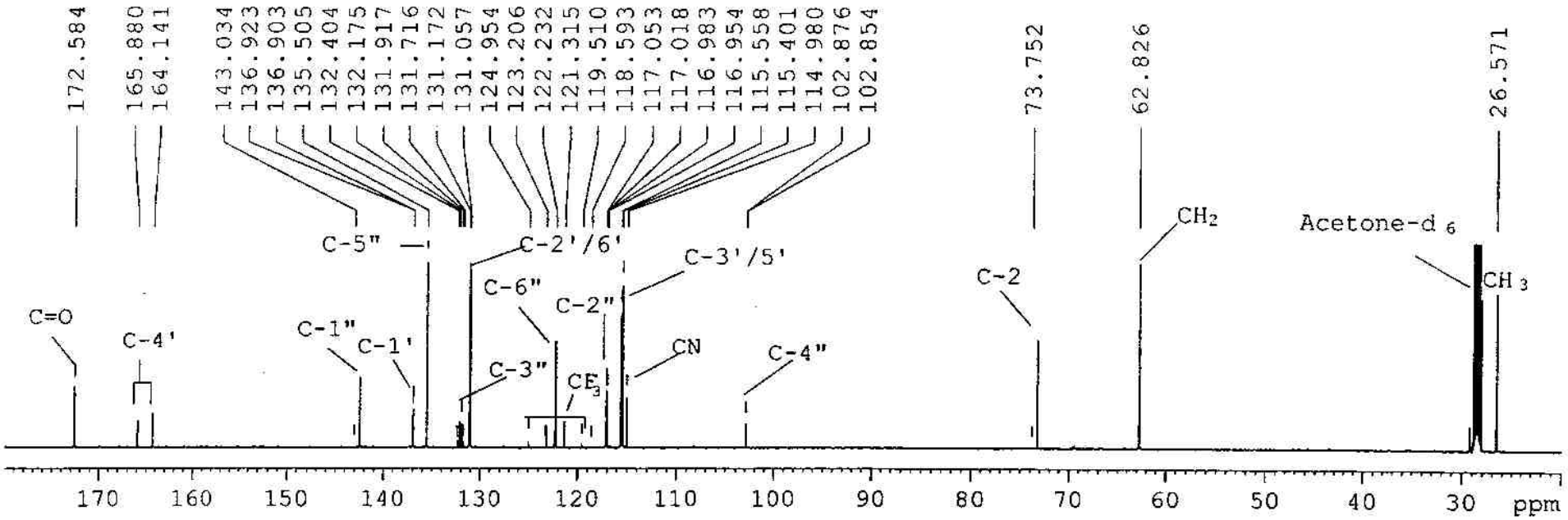
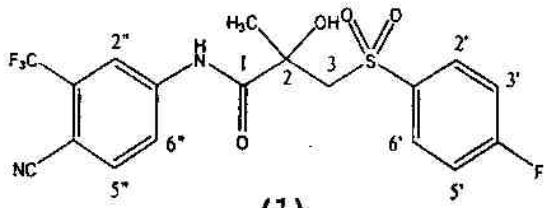


Plate 3c(Trfluoroacetic acid,298K)



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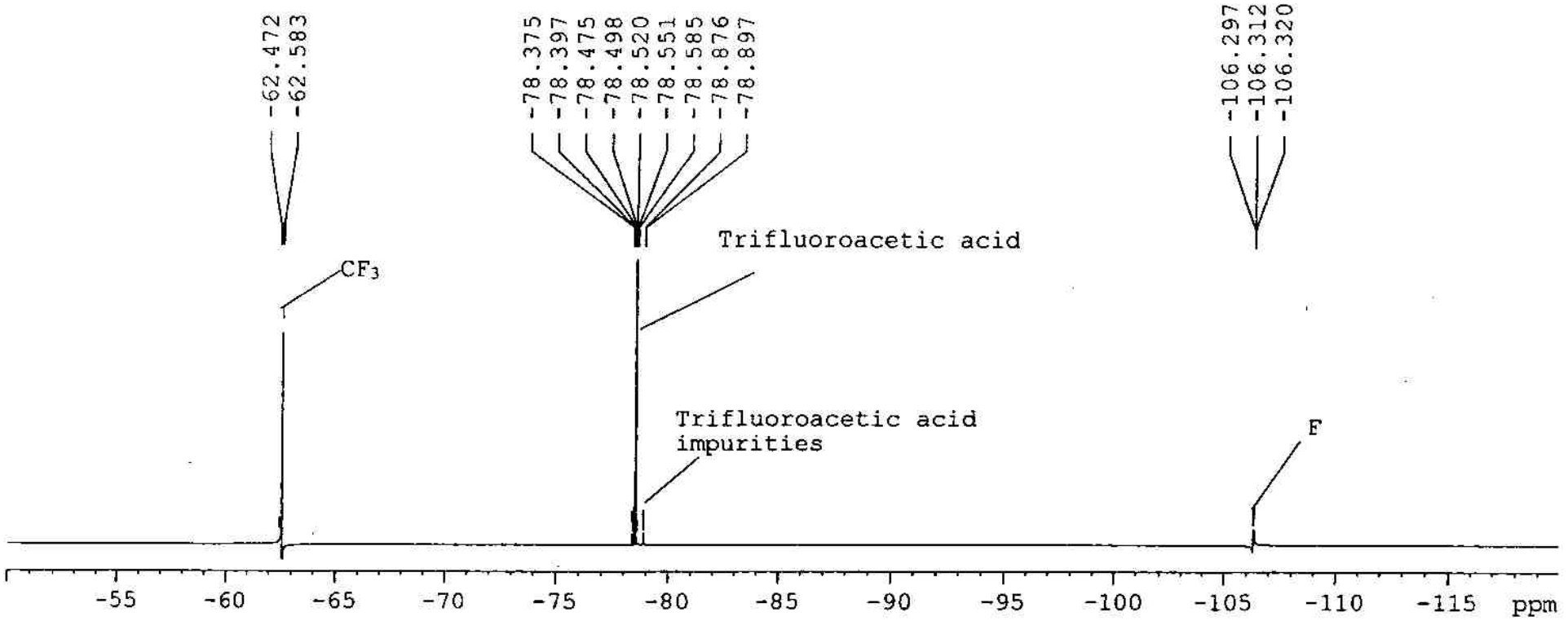
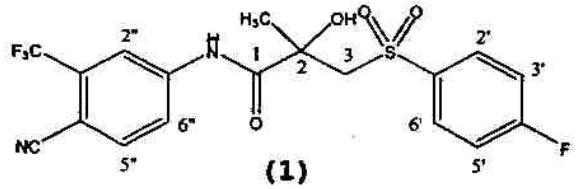


Plate 3d(Acetone-d $_6$,298K)

APT



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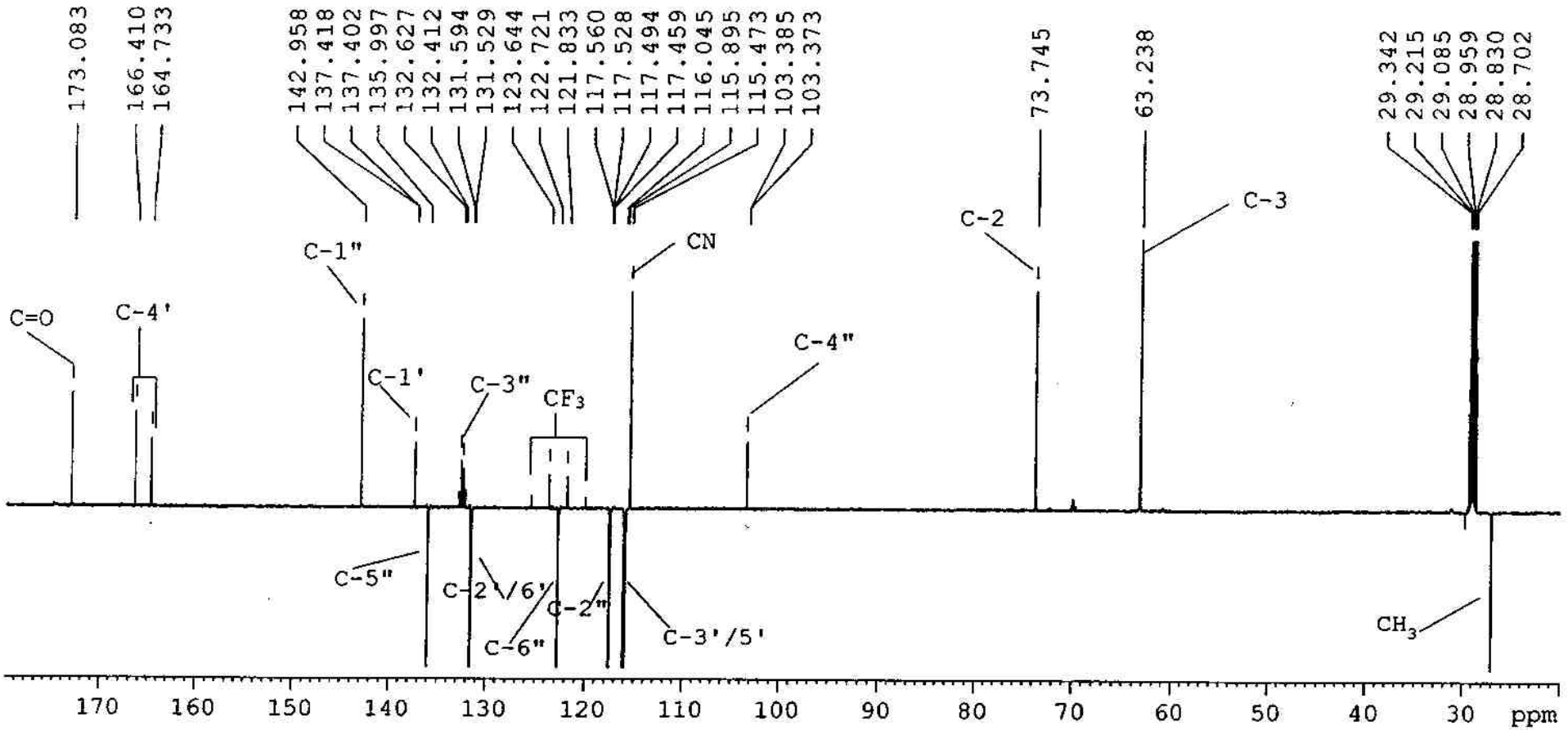
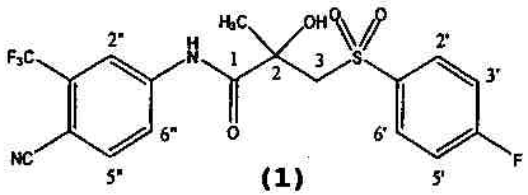


Plate 3e (Acetone-d $_6$, 298K)

HSQC



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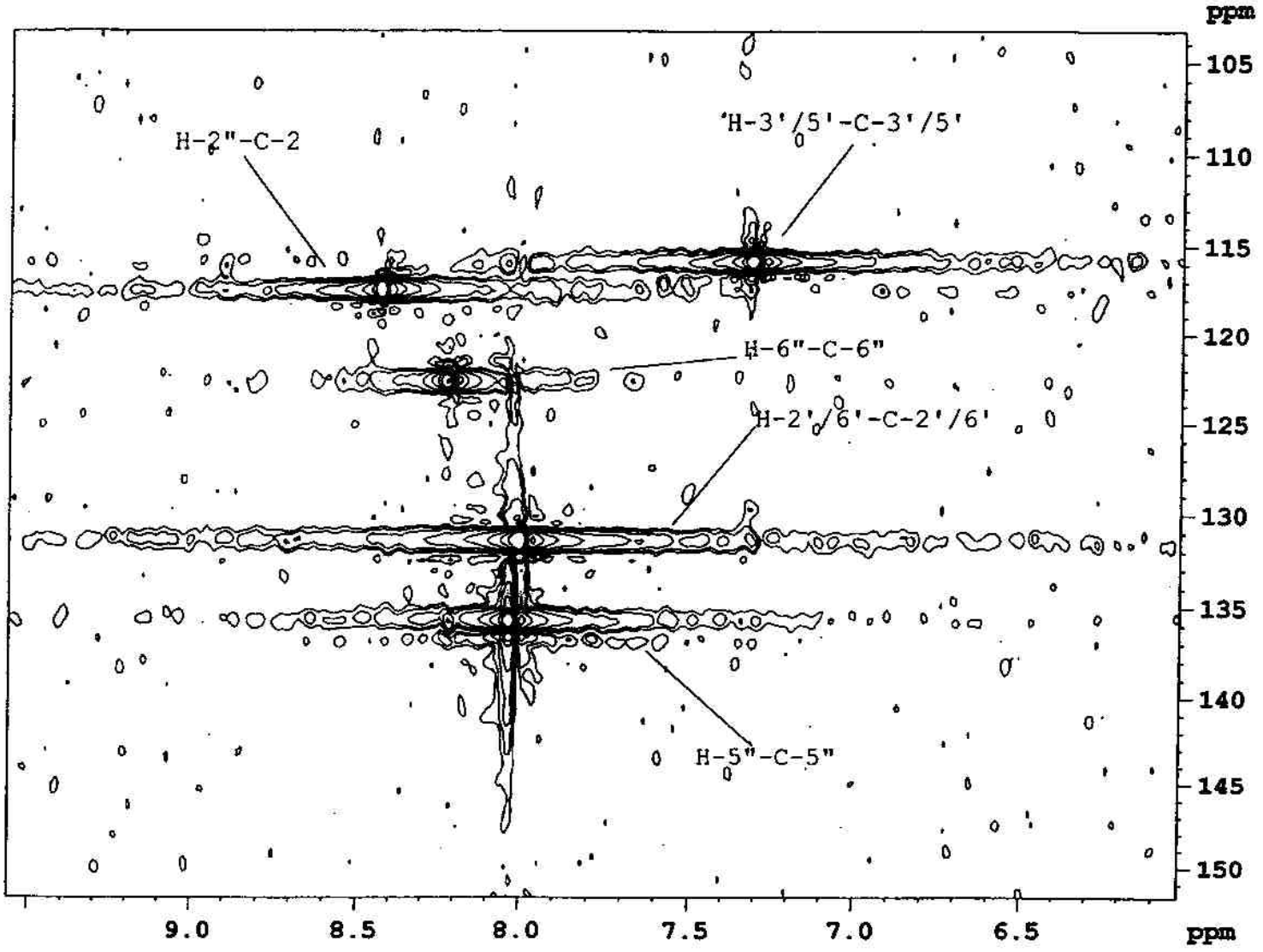
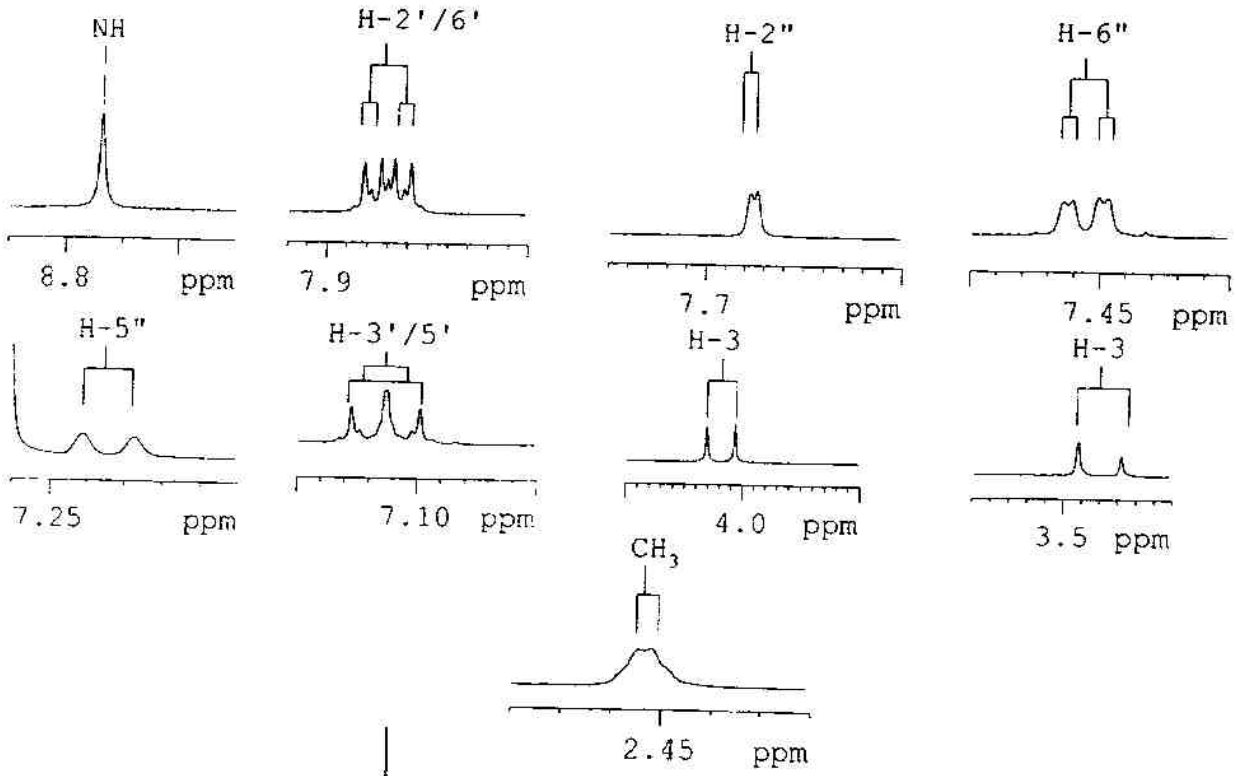
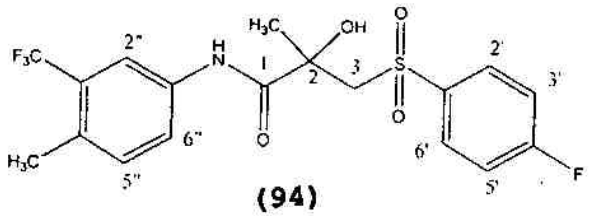


Plate 4a(CDCl ₃,298K)





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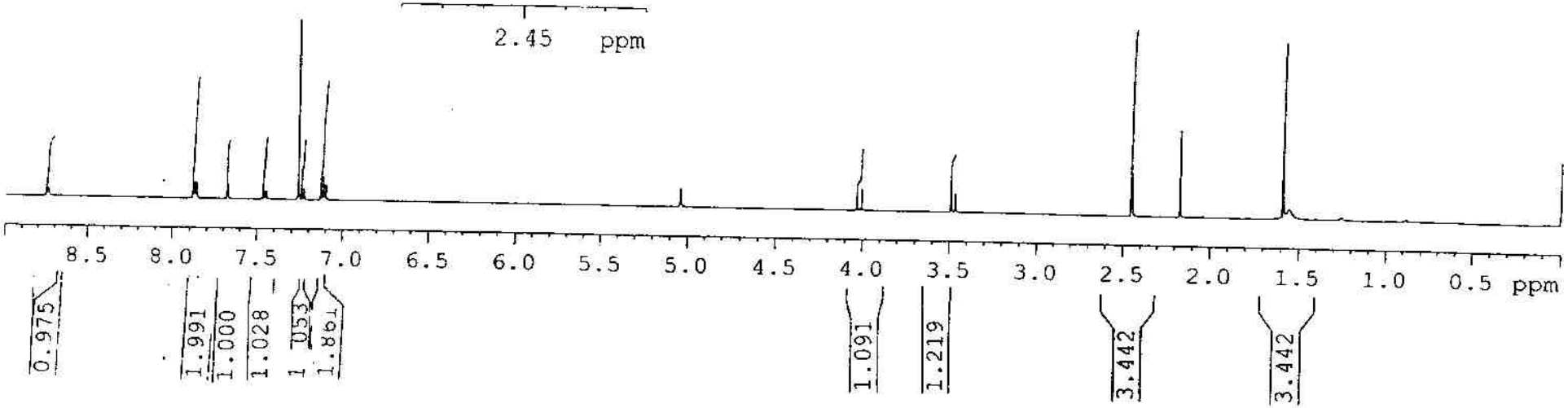
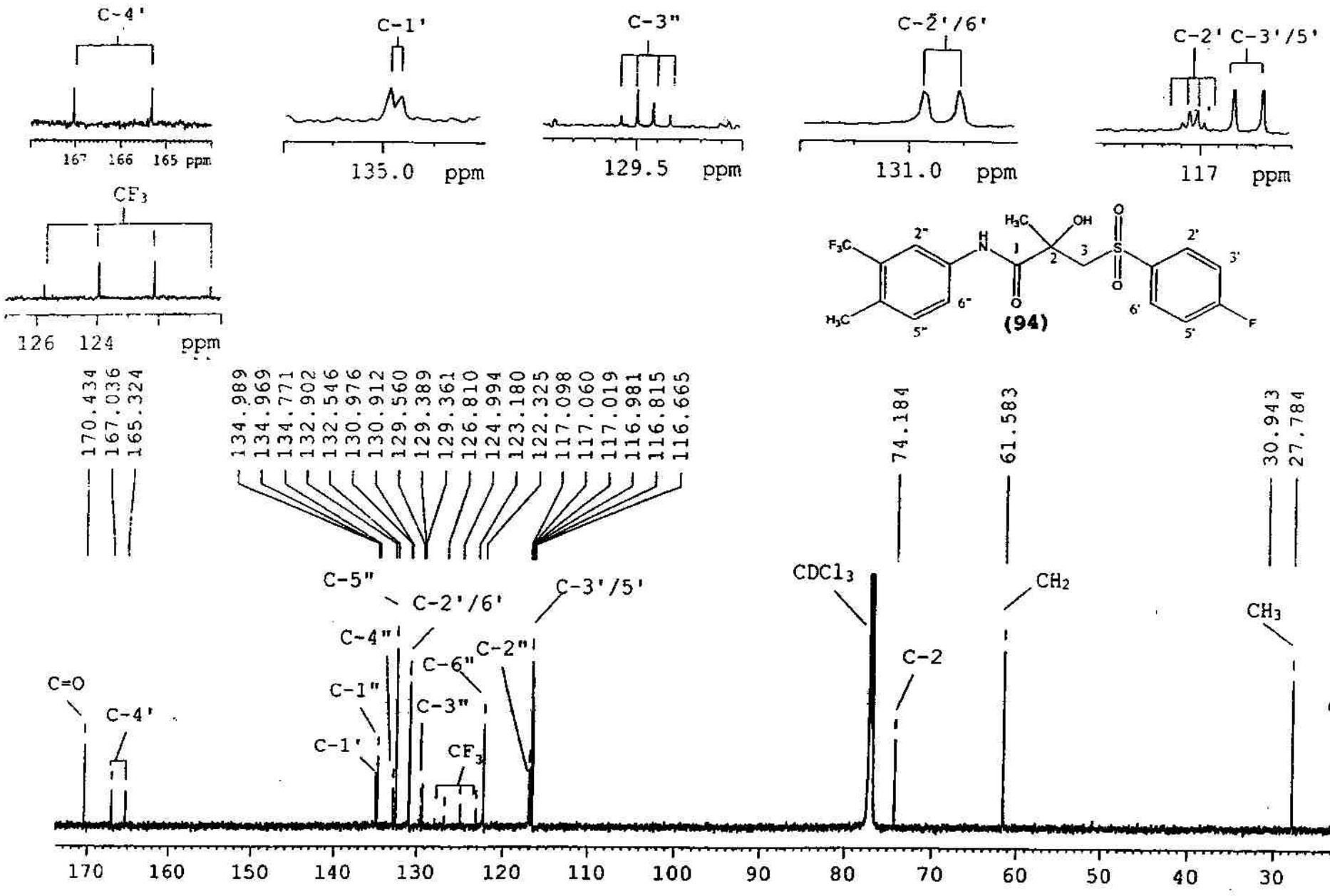
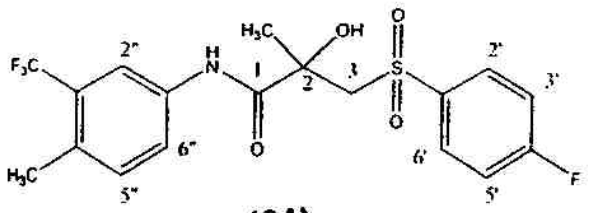


Plate 4b(CDCl $_3$,298K)



ate 4r(Trifluoroacetic acid),298K)



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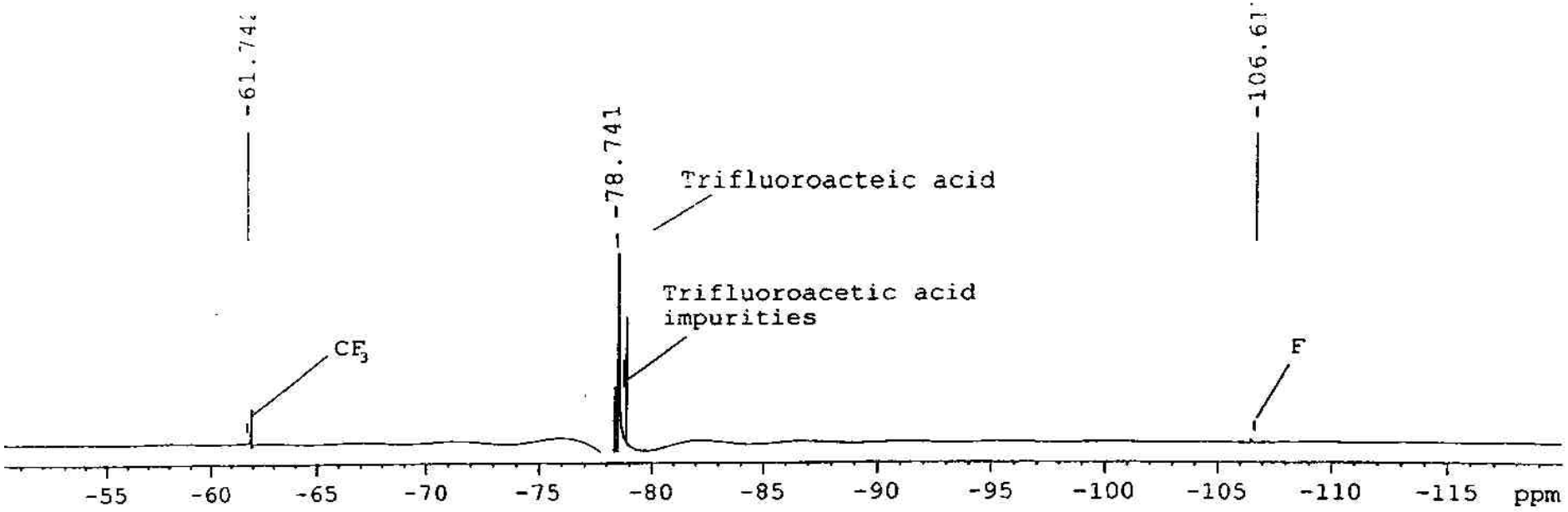
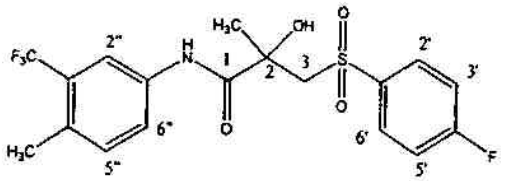


Plate 4d ($CDCl_3$, 298K)

COSY



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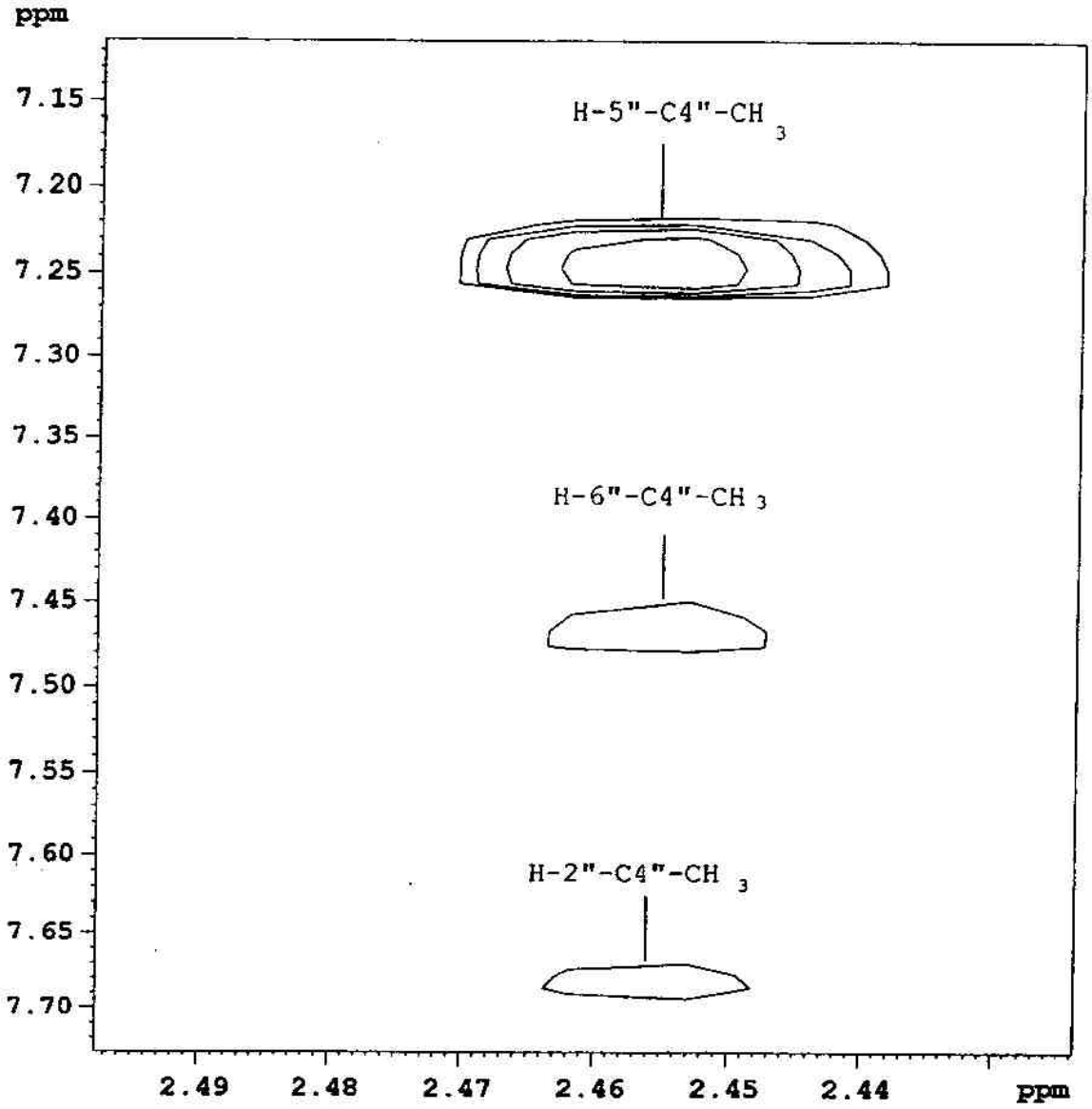
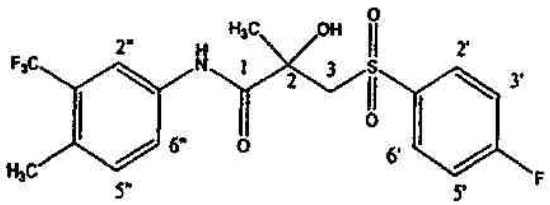


Plate 4e($CDCl_3$, 298K)

COSY



(94)

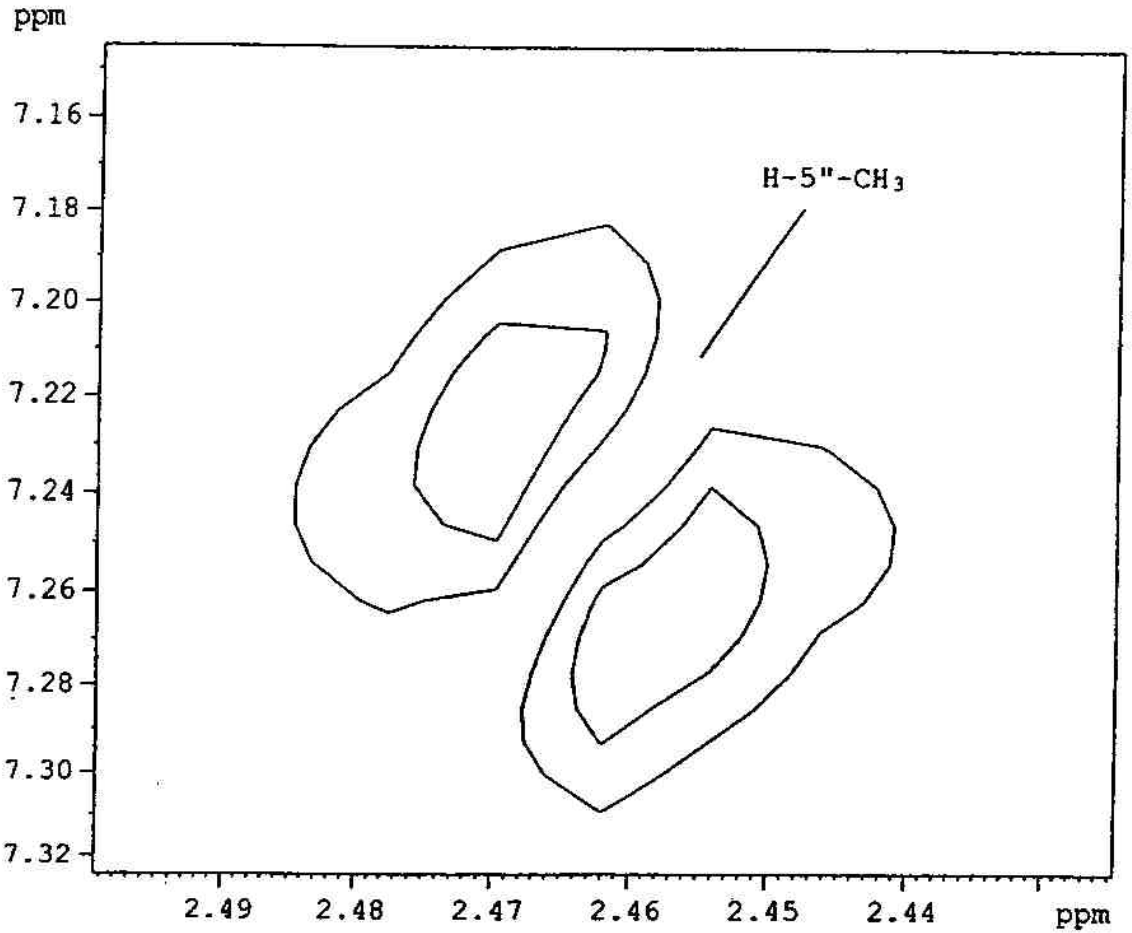
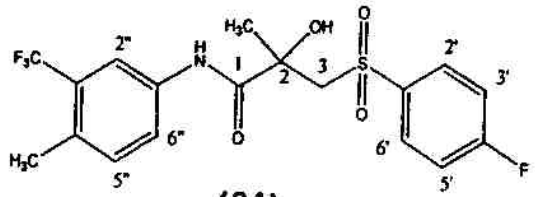


Plate 4f($CDCl_3$,298K)

HSQC



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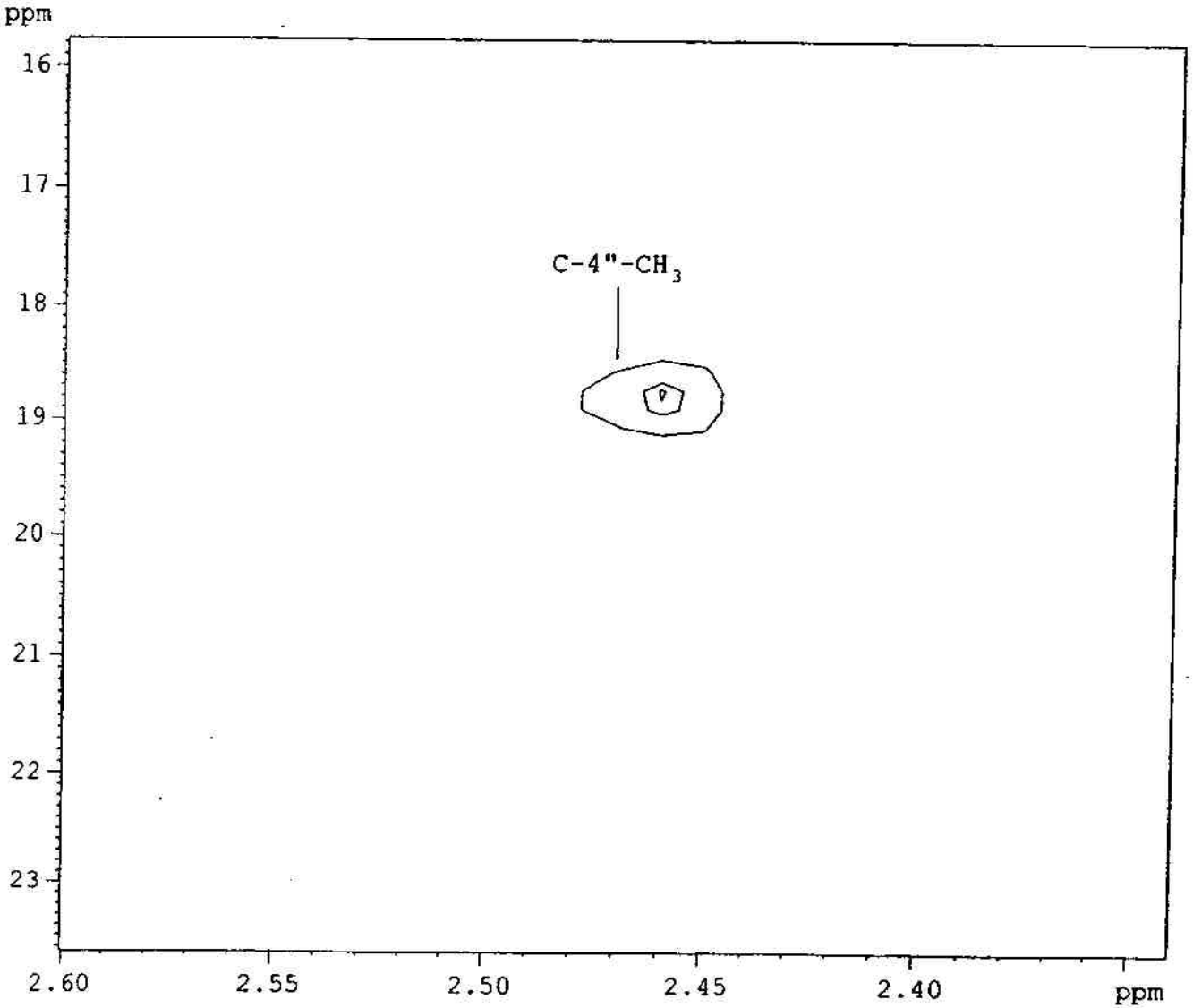
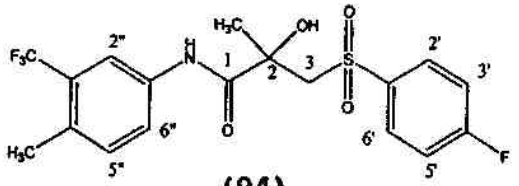
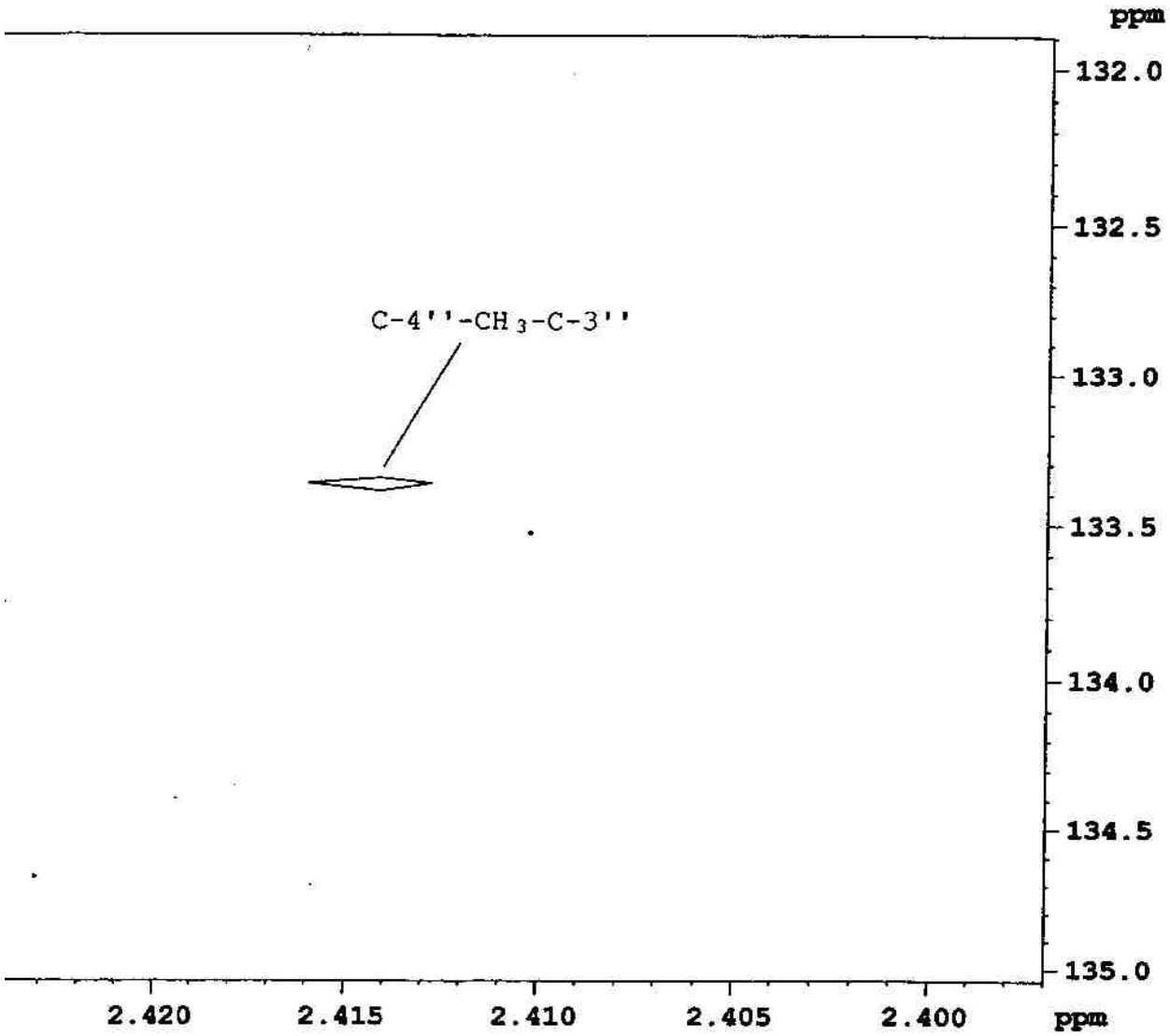


late 4g(CDCl 3,298K)

MBC



(94)



.ate 5a (Acetone-d 6 ,298K)

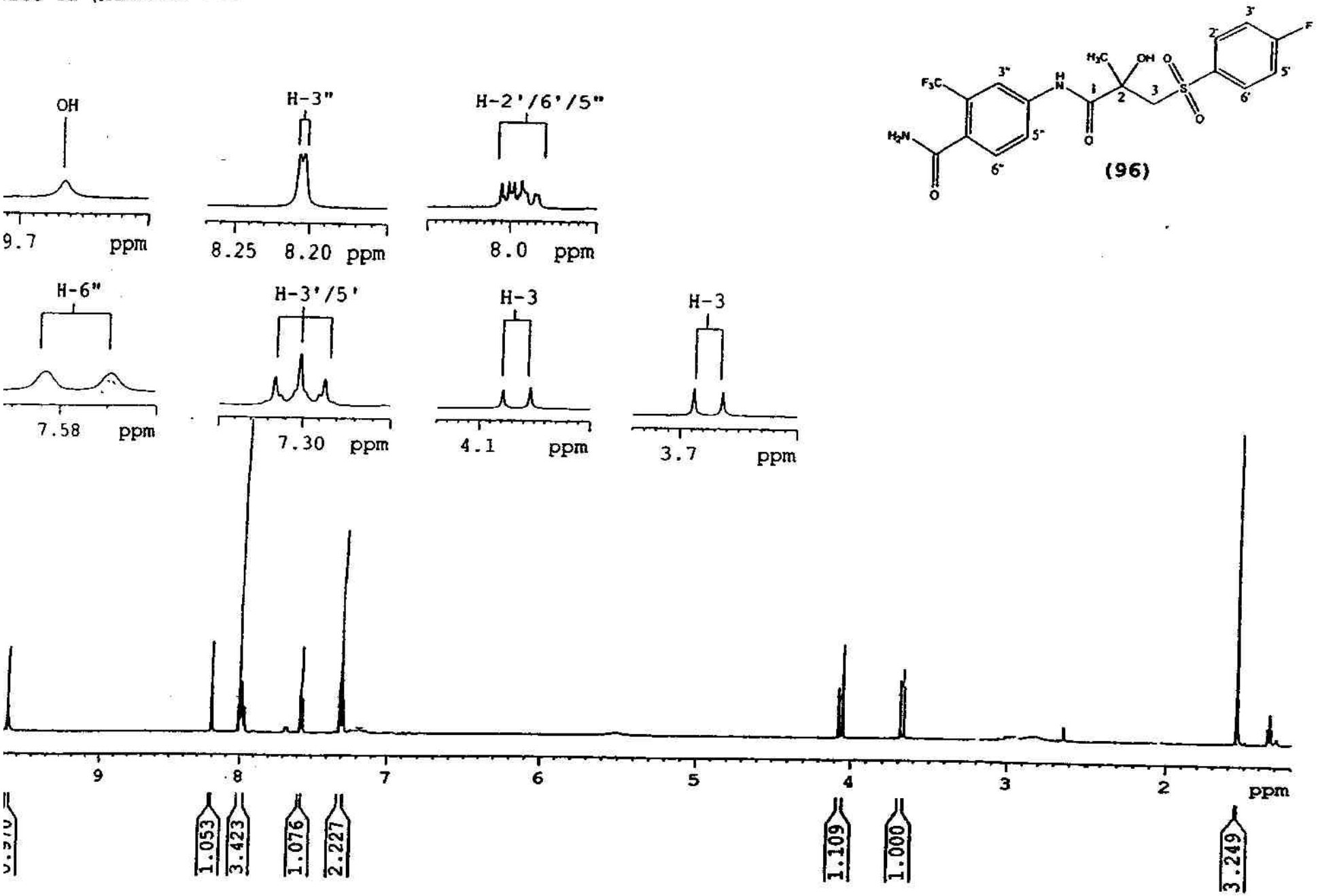
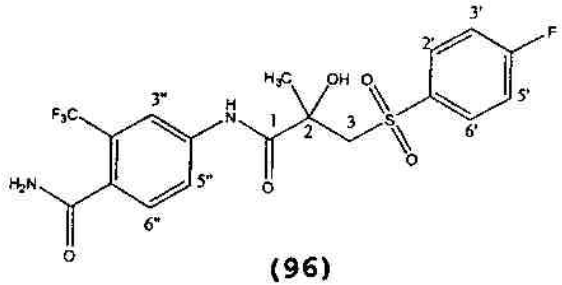
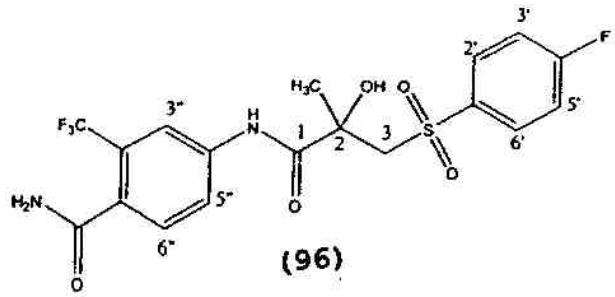


Plate 5b(Acetone-d $_6$, 298K)



(96)

Plate 5c (Trfluoroacetic acid, 298K)



(96)

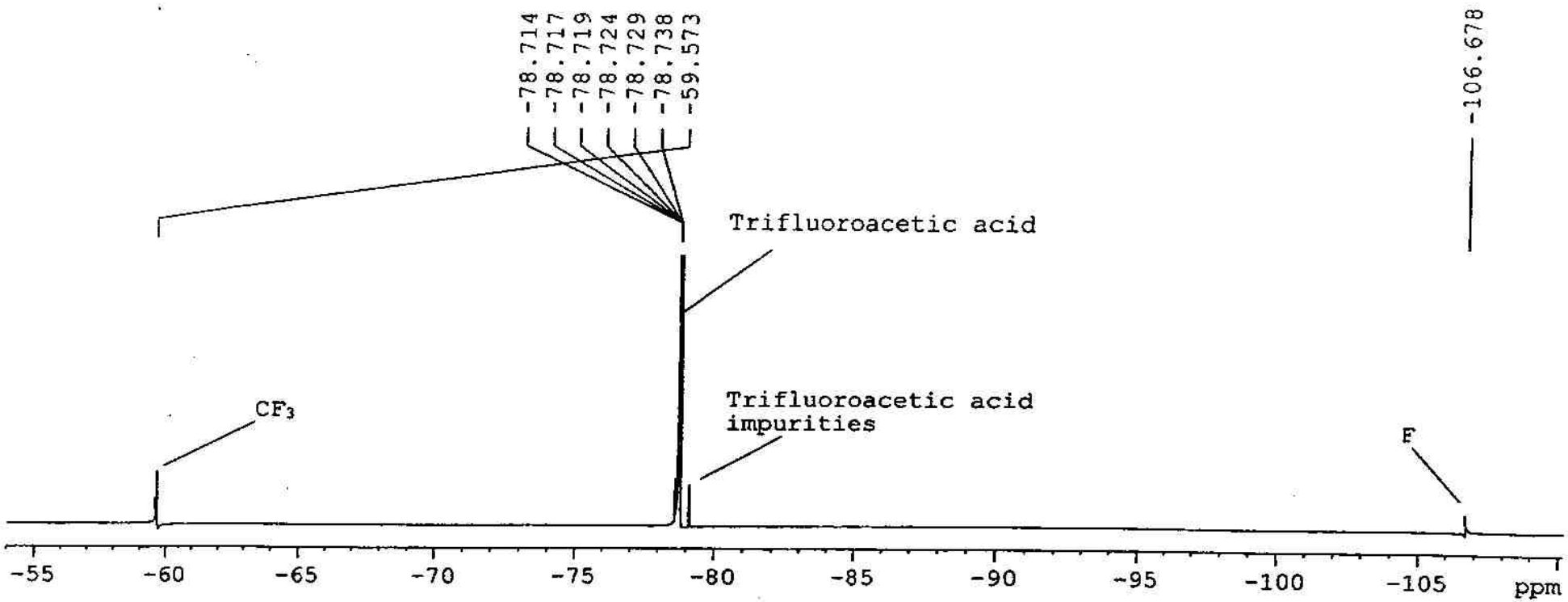
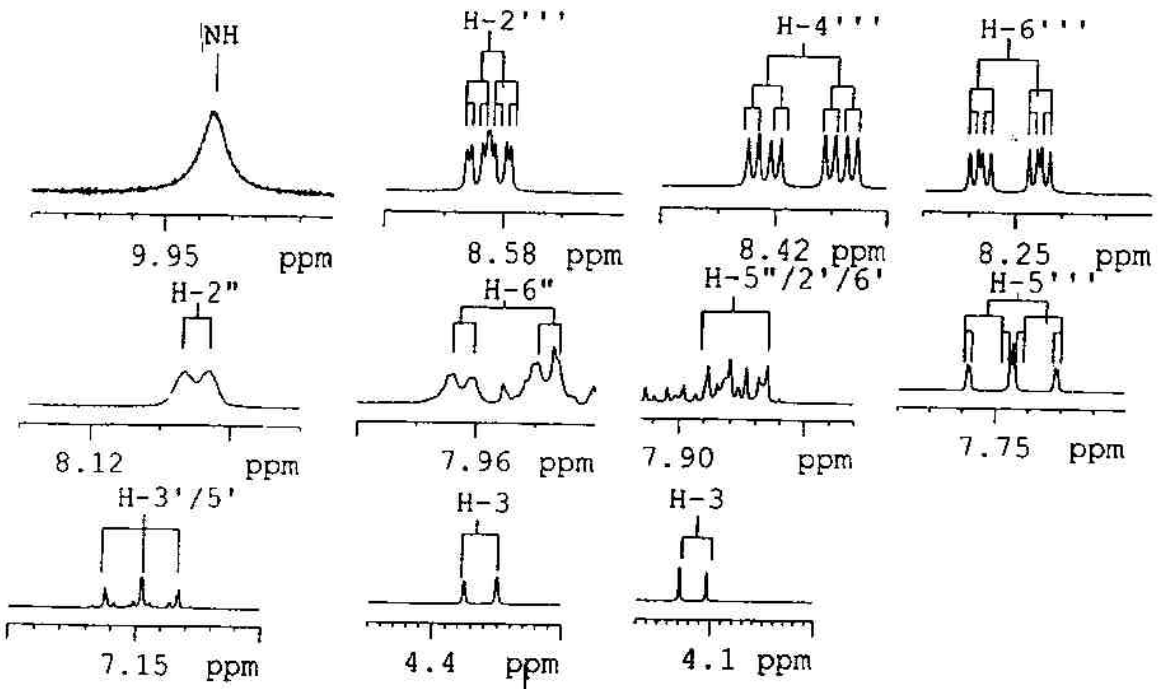
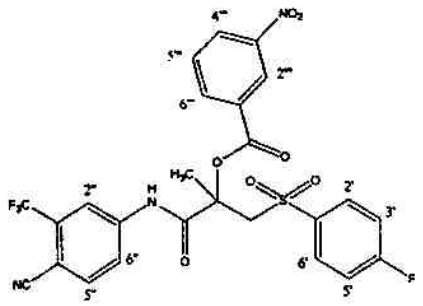


Plate 6a (Acetone-d $_6$ 298K)





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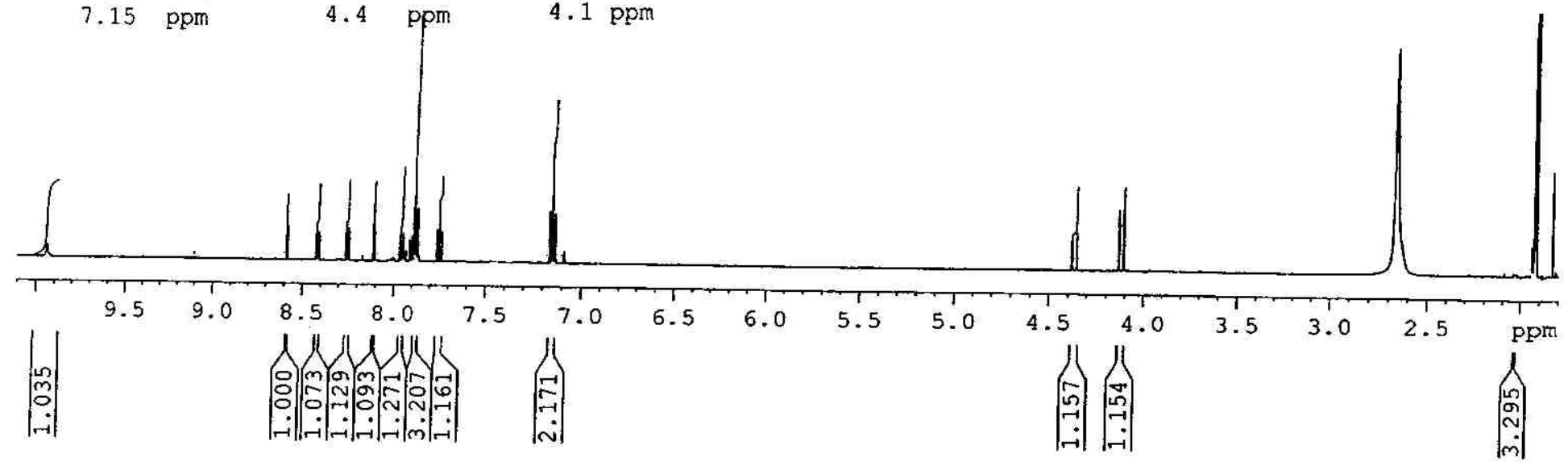


Plate 6b(Acetone-d $_6$, 298K)

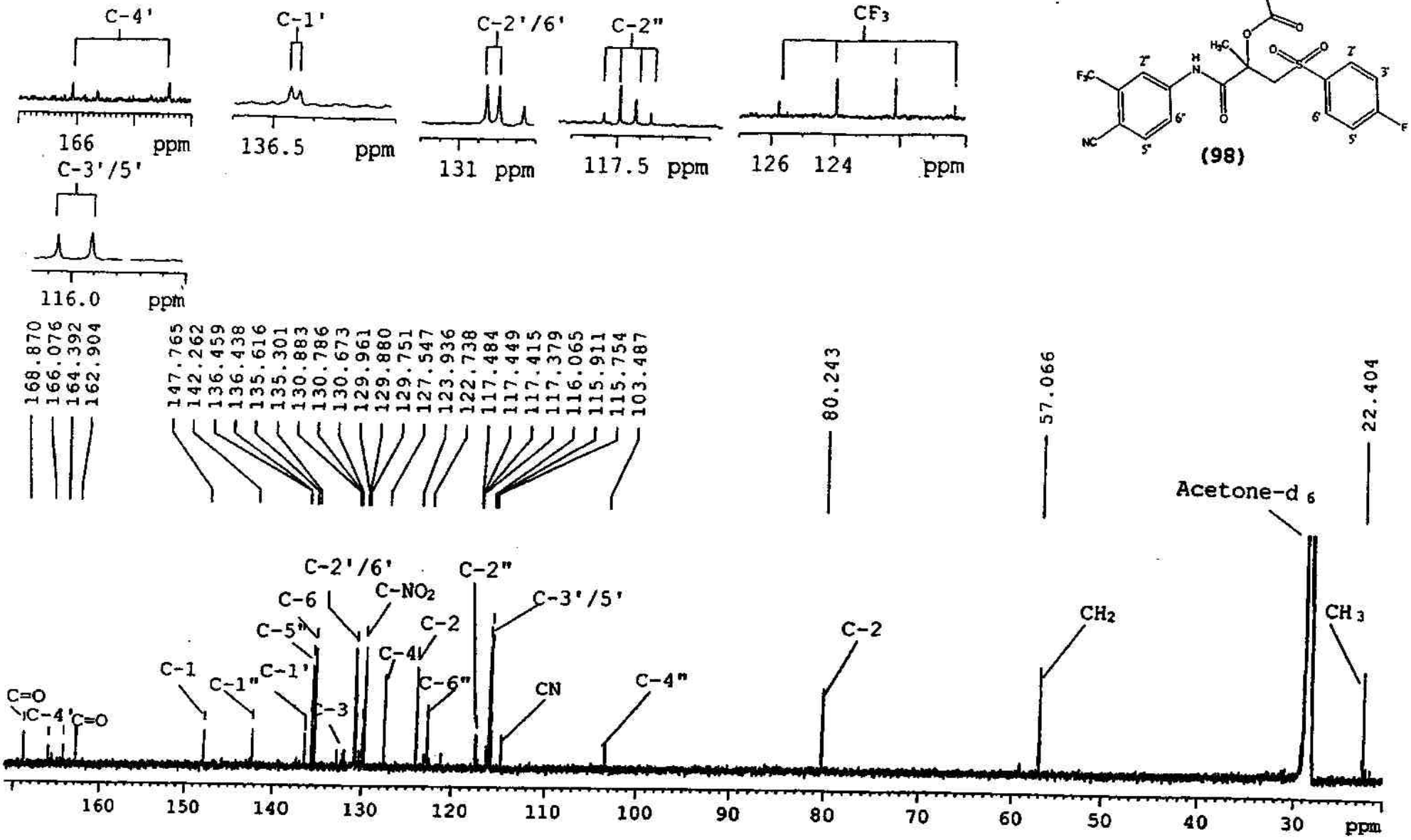
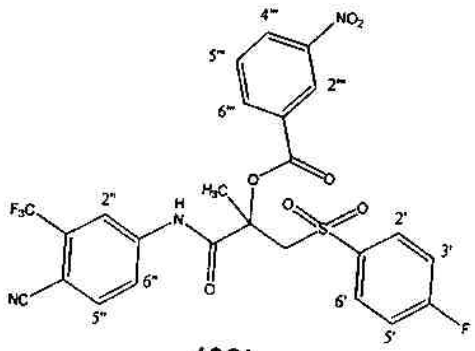


Plate 6c(Trifluoroacetic acid,298K)



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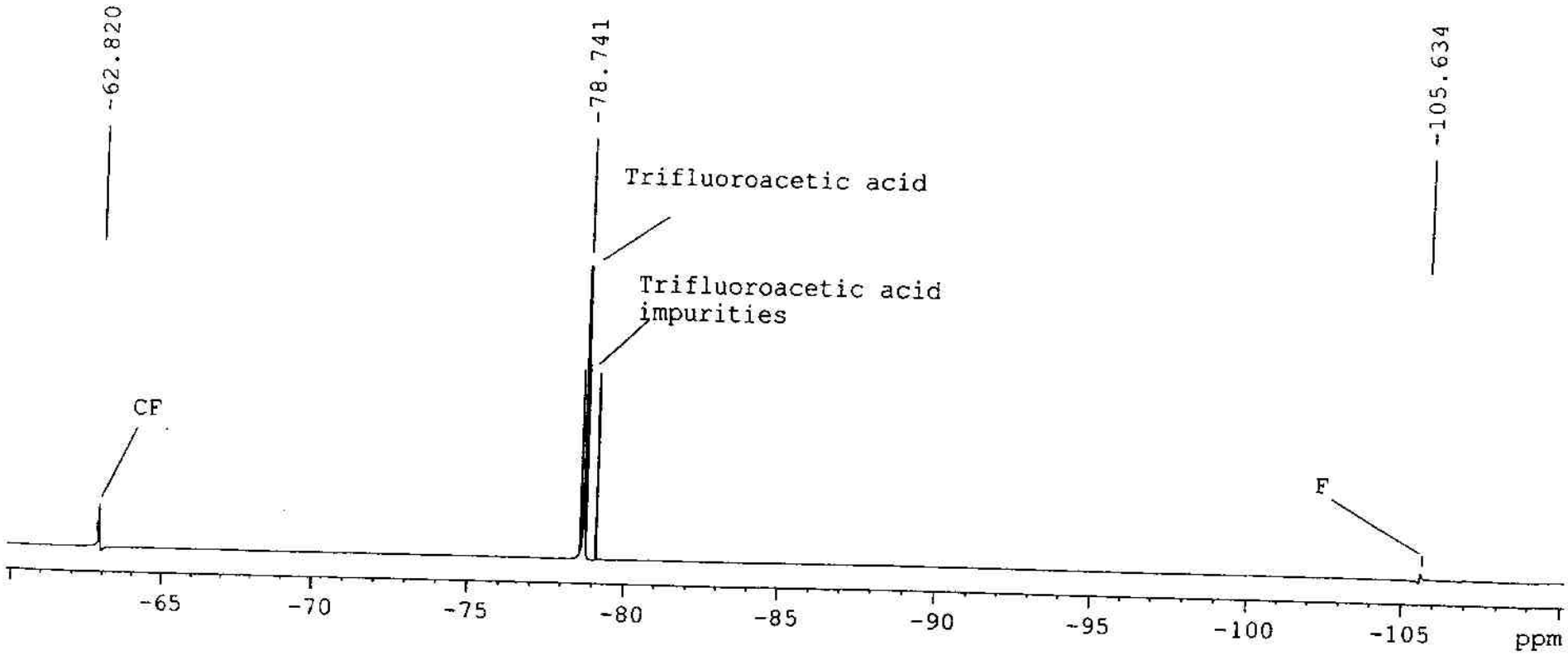
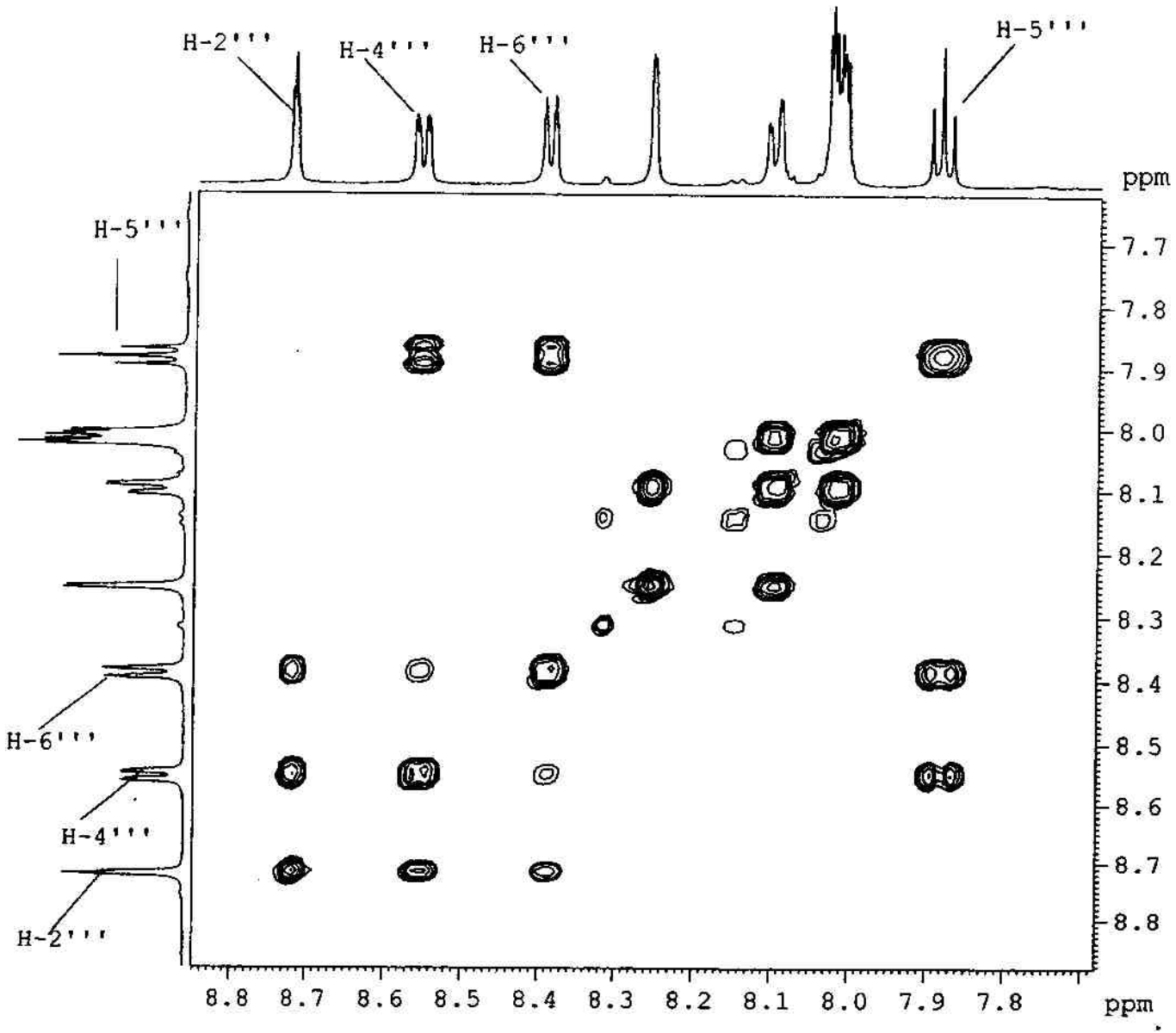
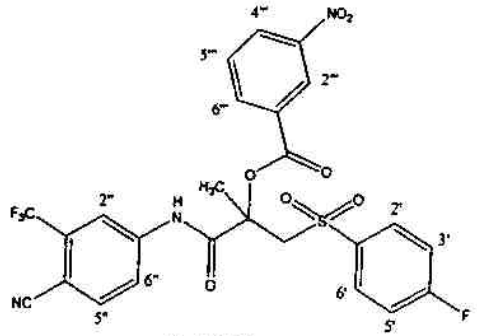


late 6d(Acetone-d_6, 298K)

COSY

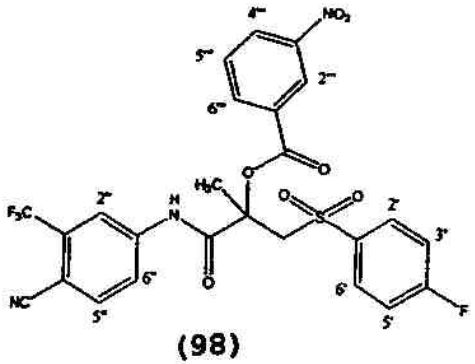




(98)

Plate 6e (Acetone-d $_6$,298K)

HSQC



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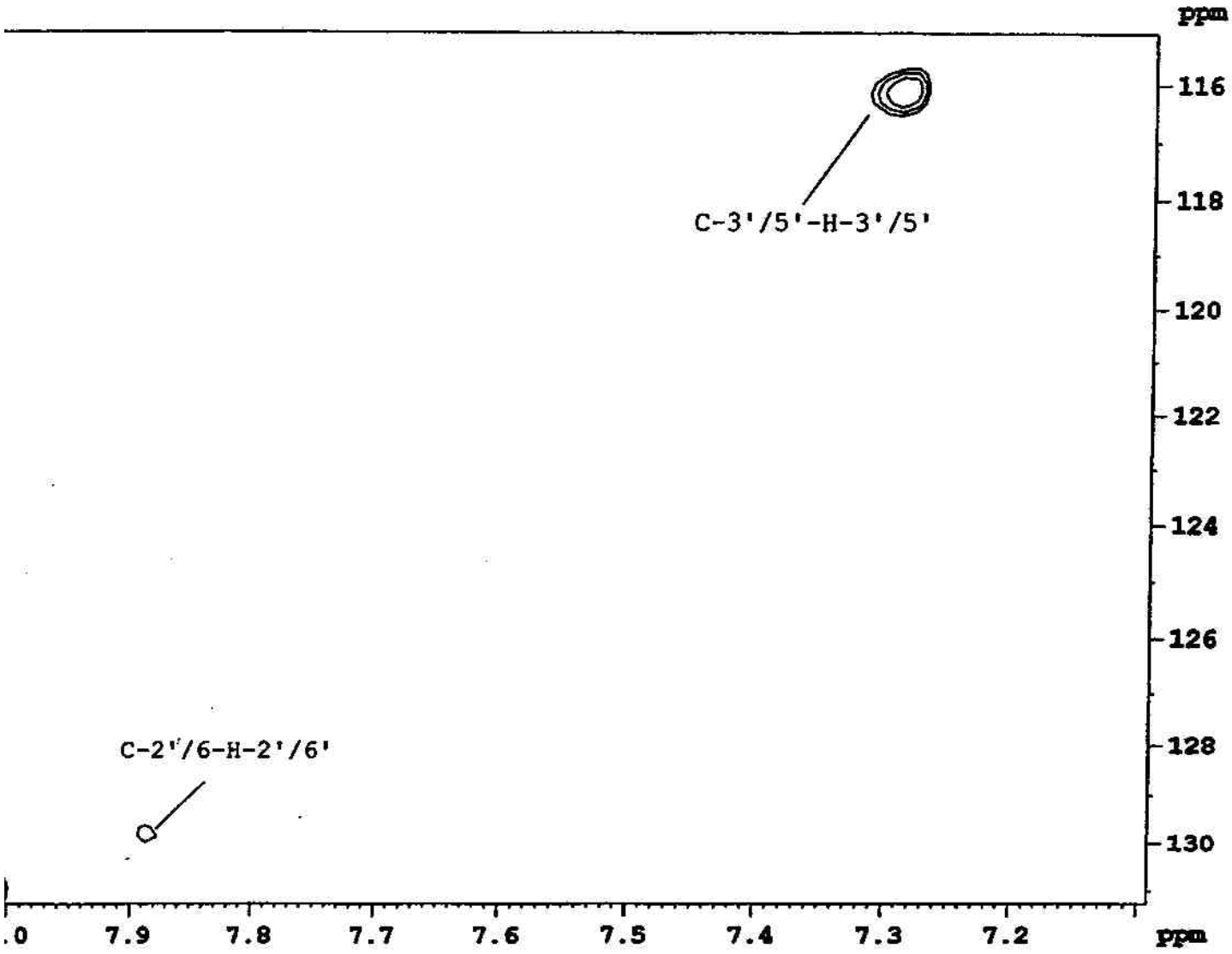
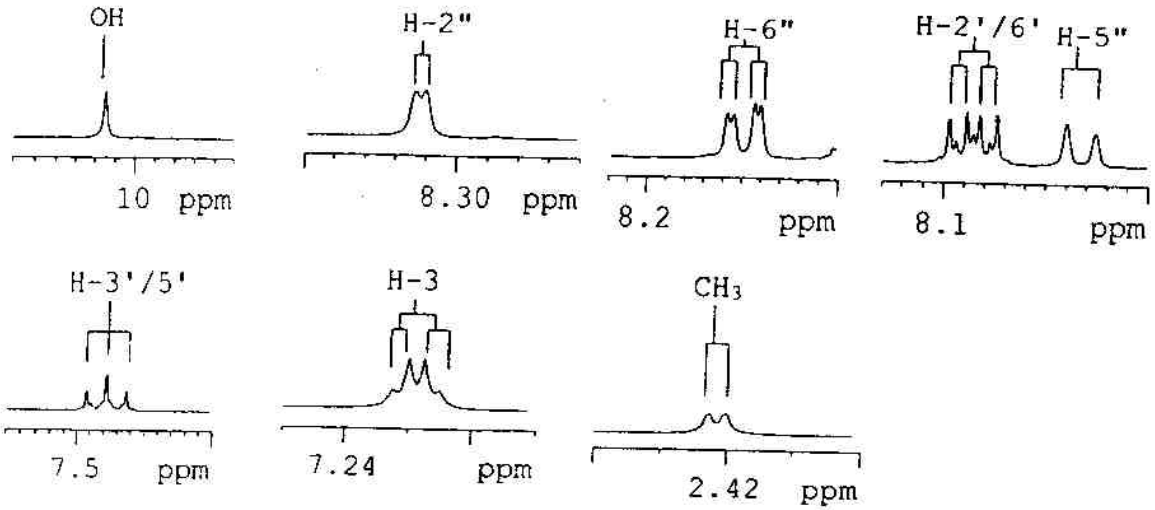
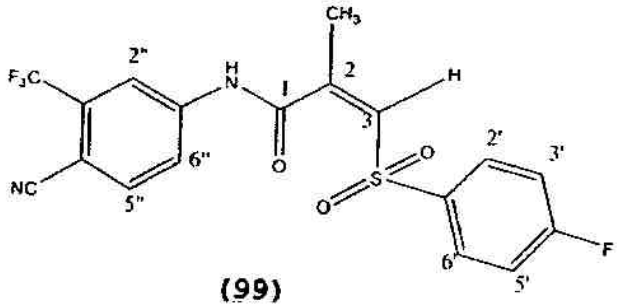


Plate 7a (Acetone-d $_6$, 298K)





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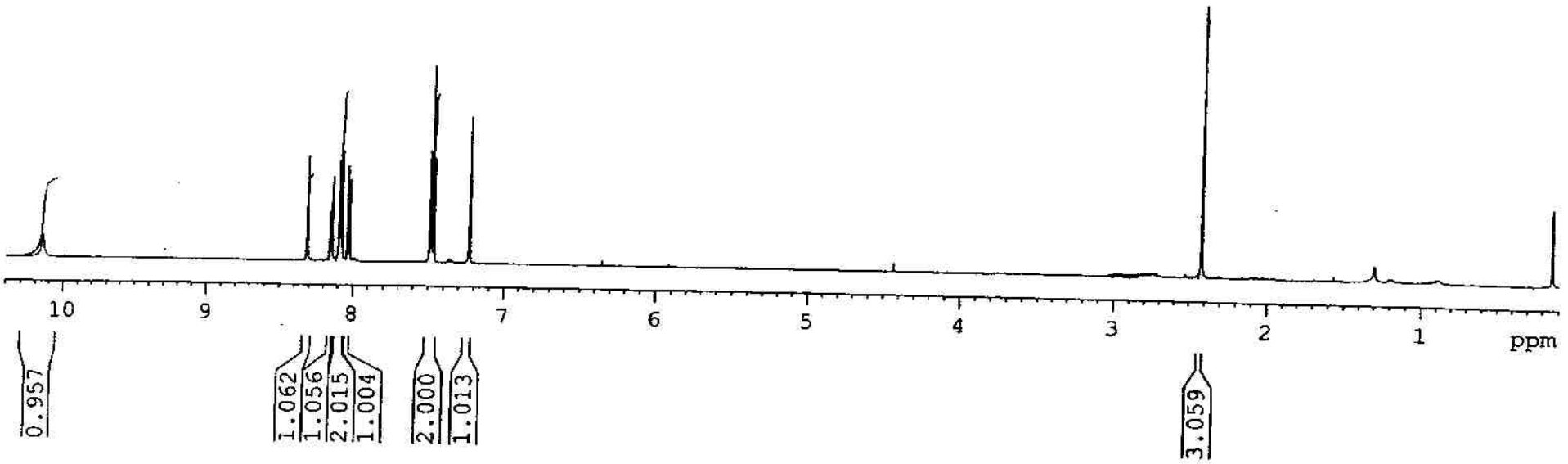
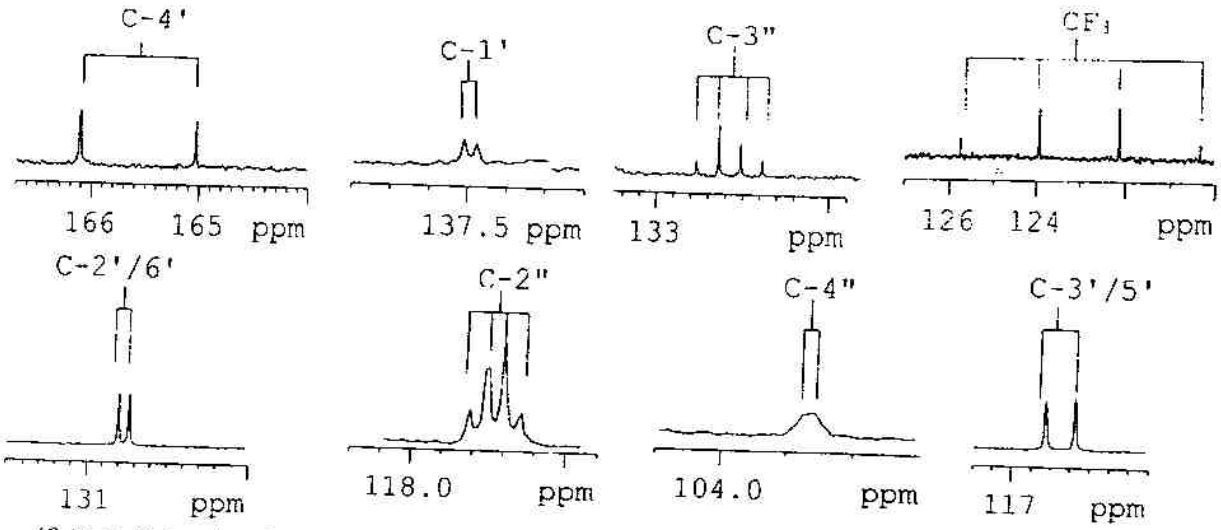
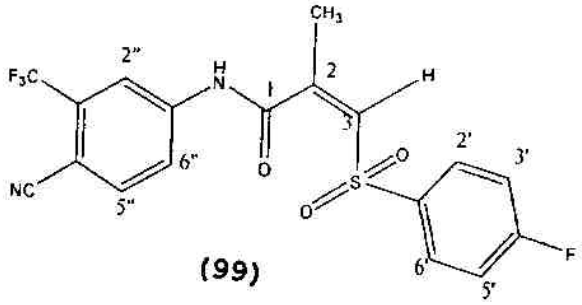


Plate 7b (Acetone-d $_6$, 298K)





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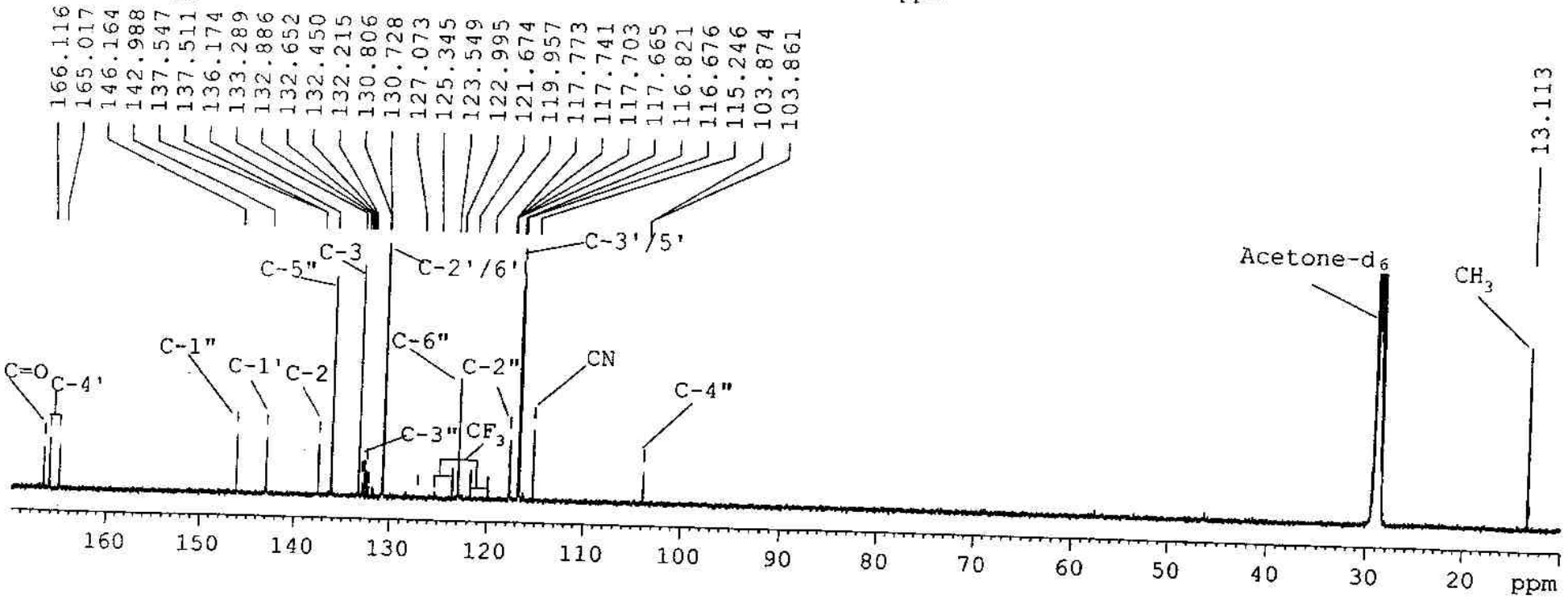
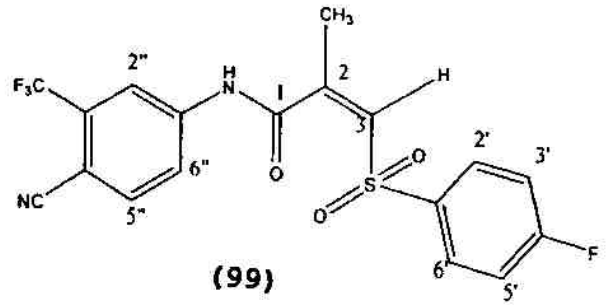


Plate 7c(Trifluoroacetic acid,298K)



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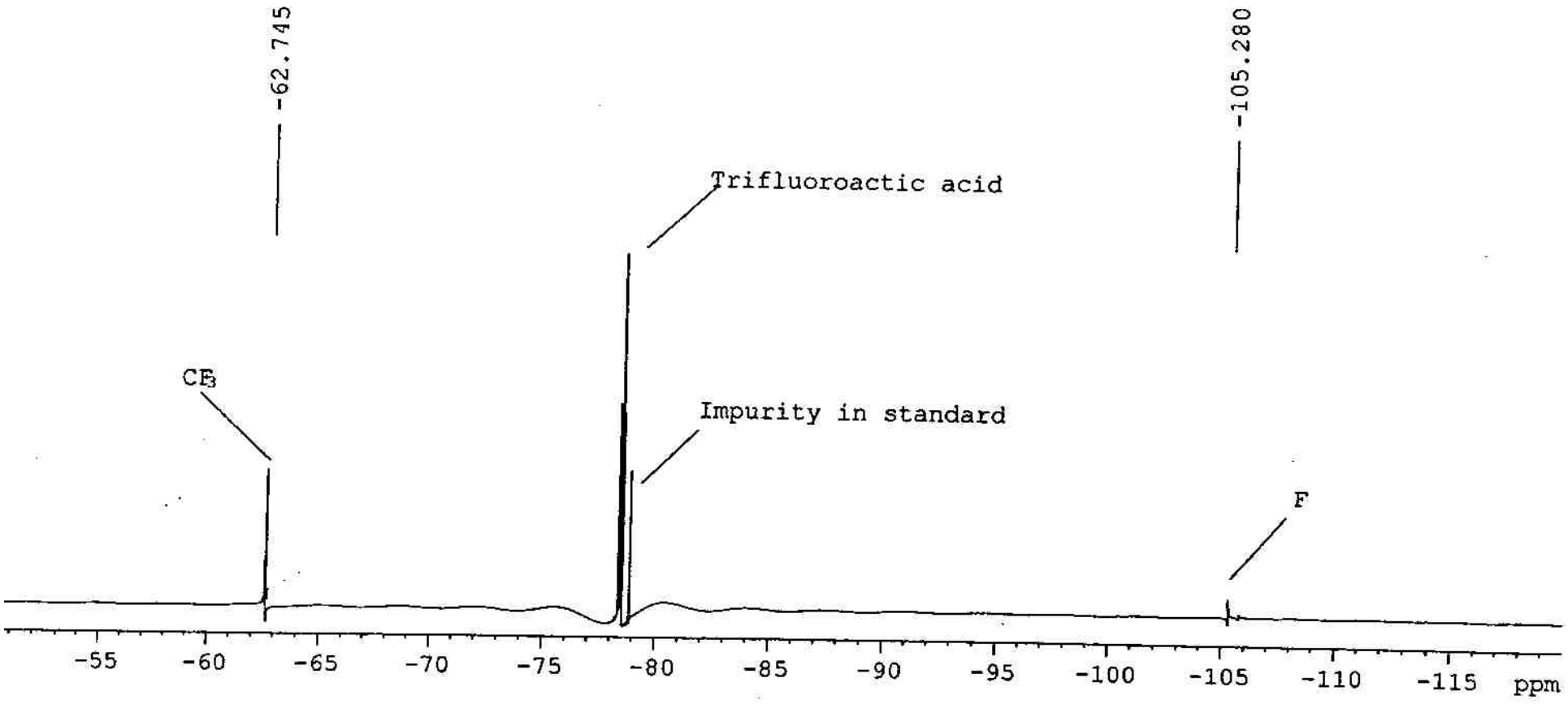
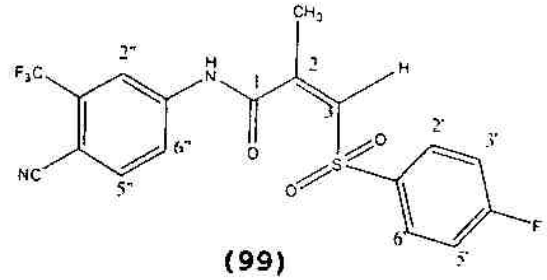


Plate 7d (Acetone-d_6, 298K)

NOESY



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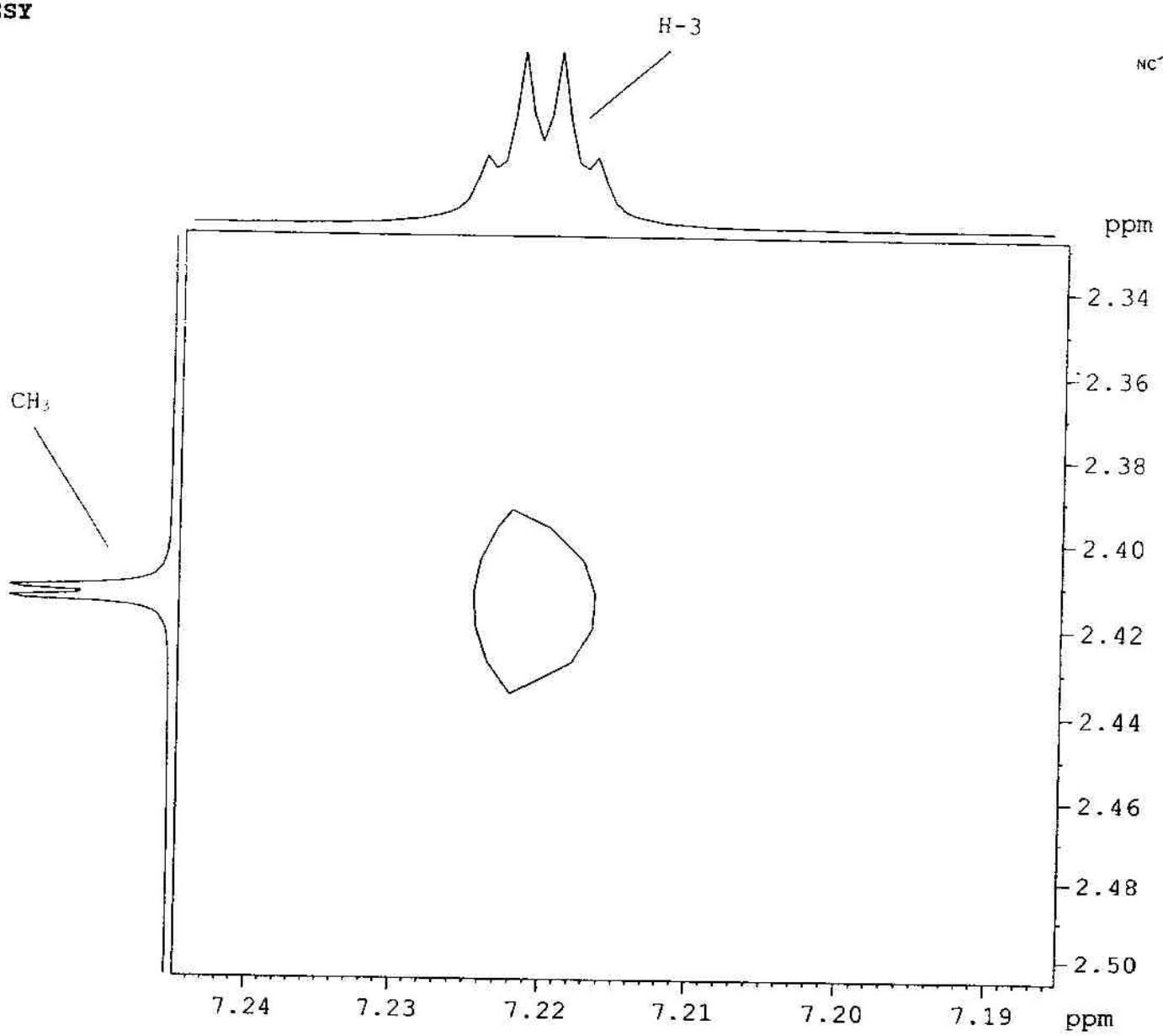
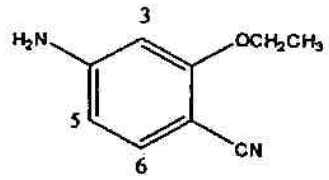


Plate 8a (Acetone-d $_6$, 298K)



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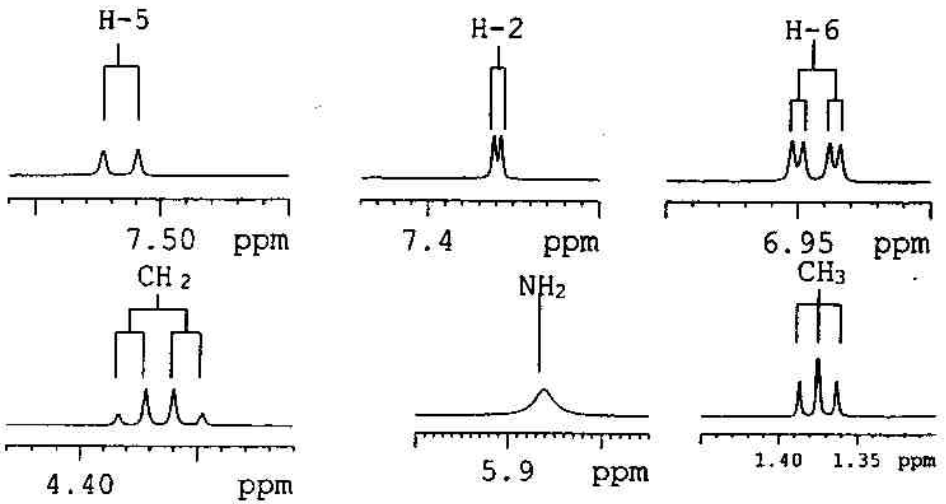


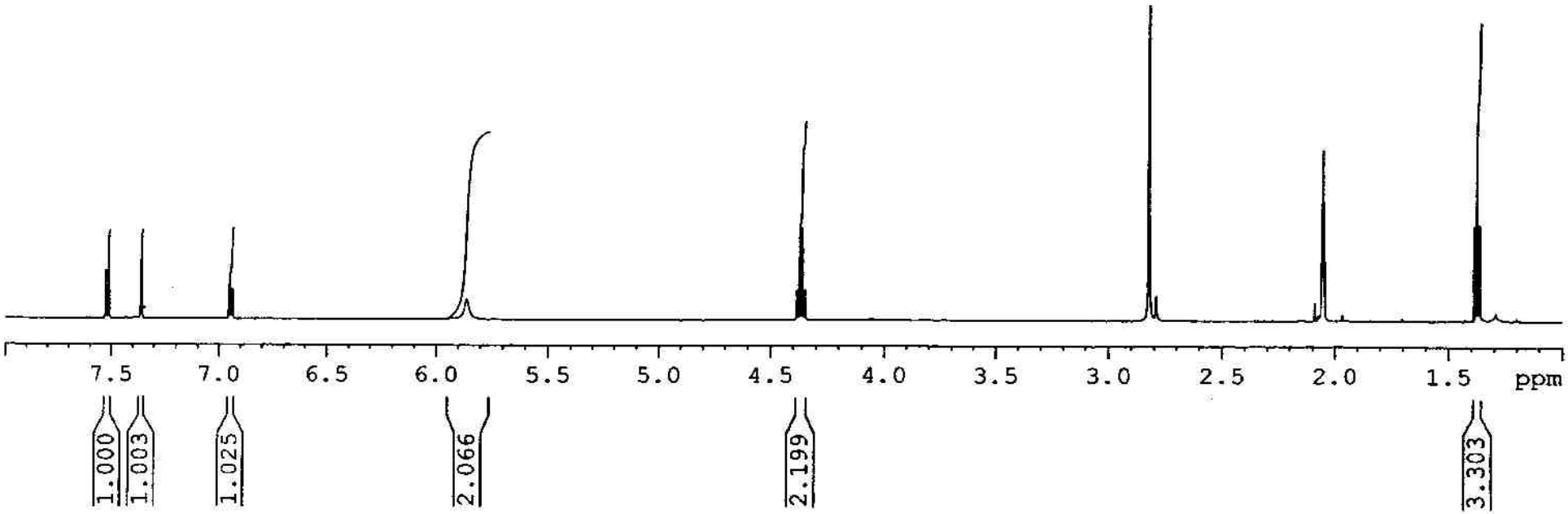
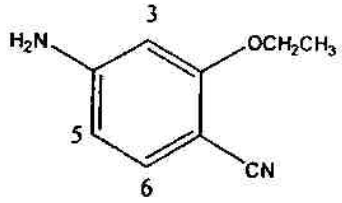


Plate 8b (Acetone-d $_6$, 298K)



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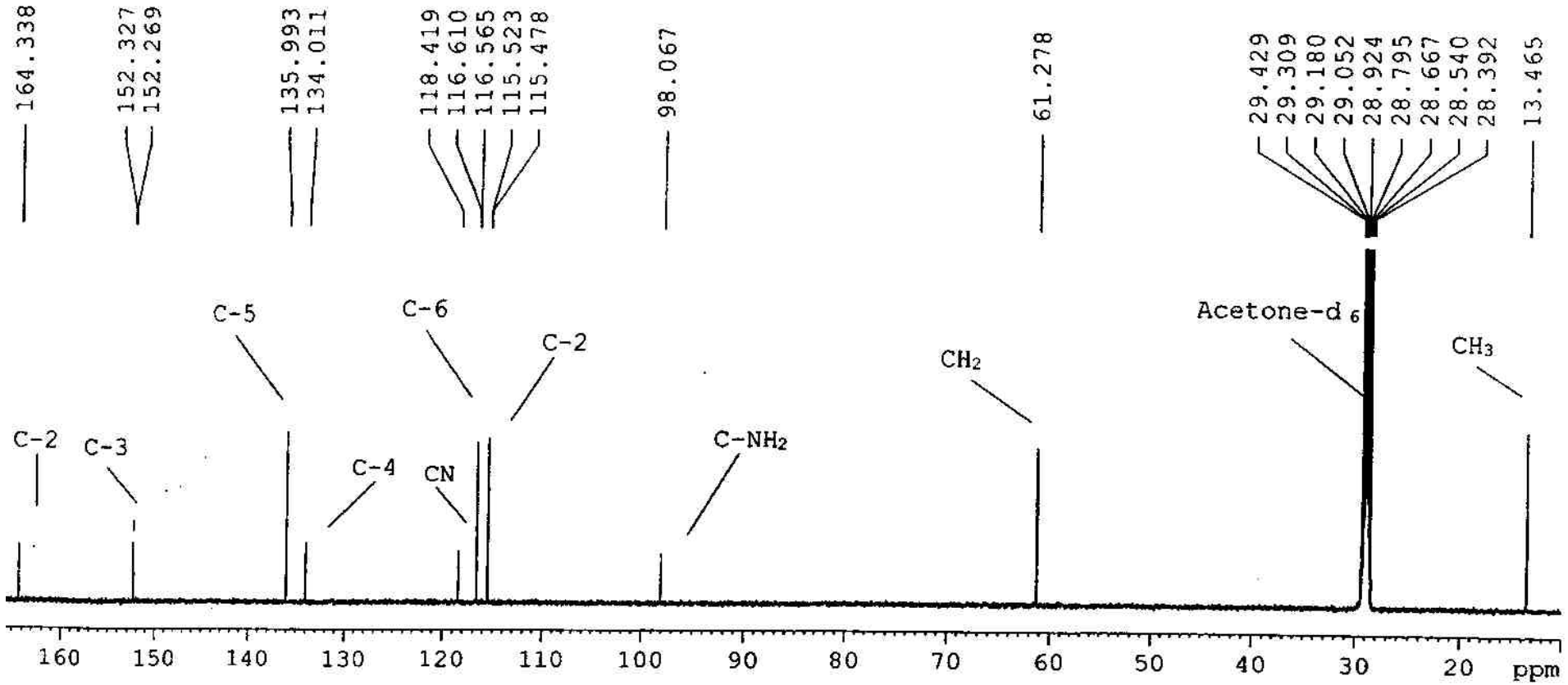
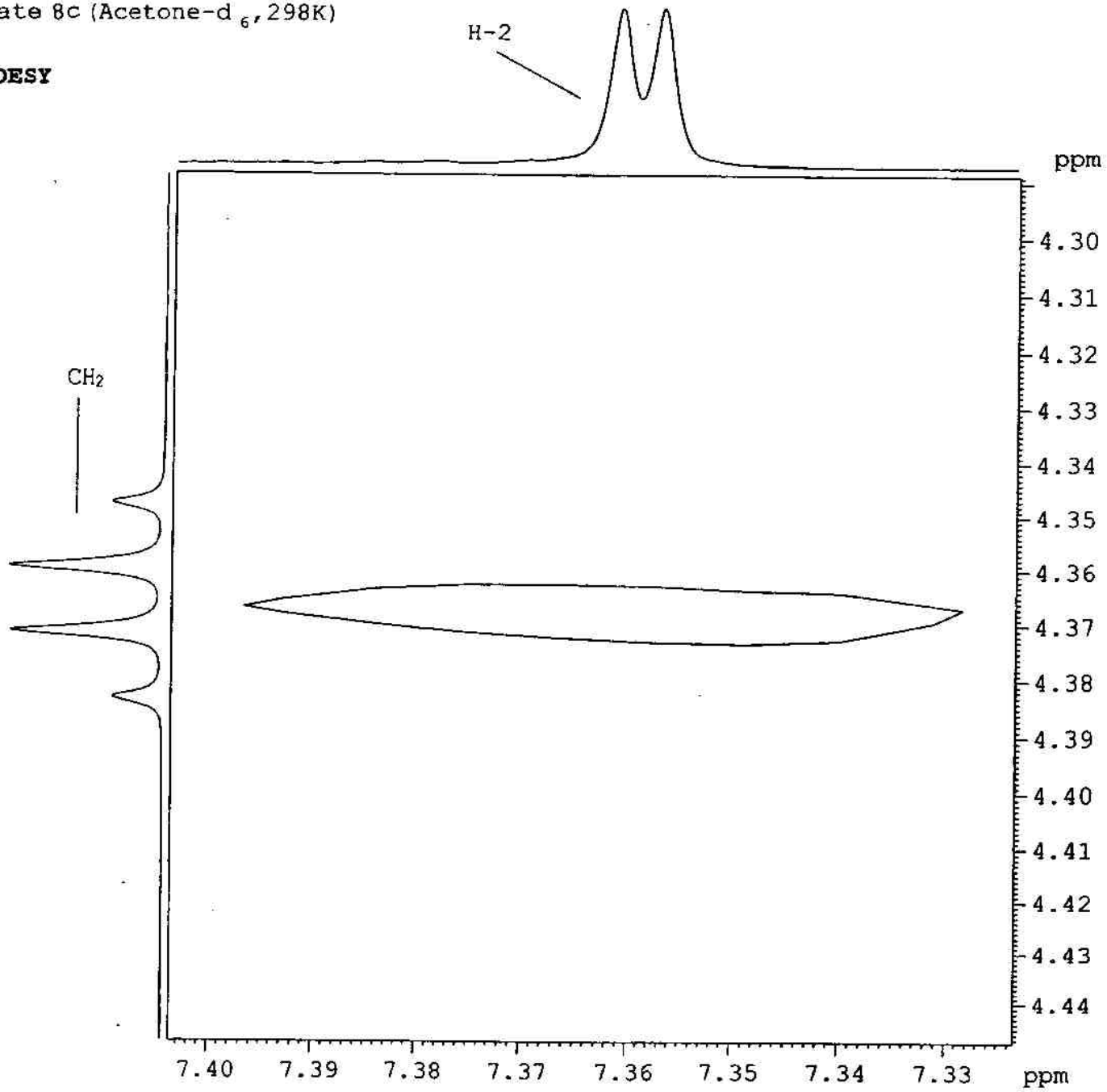


Plate 8c (Acetone-d_6, 298K)

NOESY



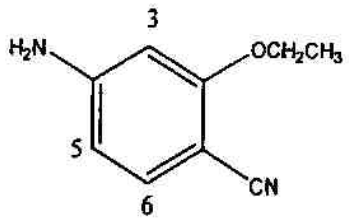


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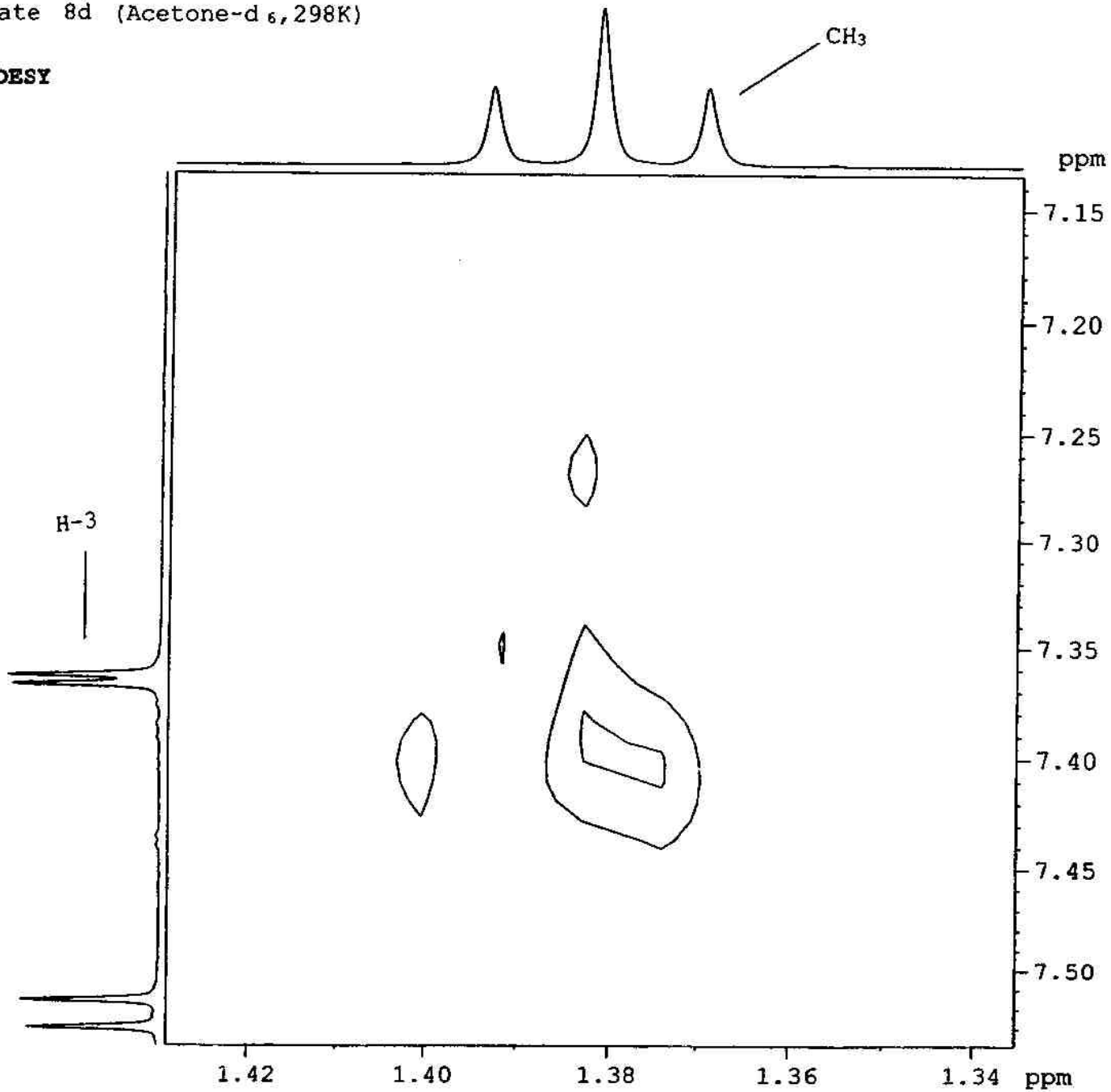
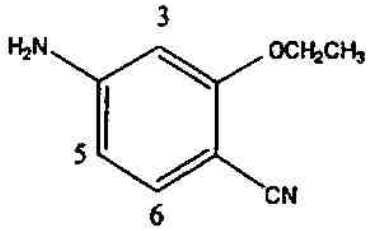
Plate 8d (Acetone-d $_6$, 298K)

NOESY





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late 8e (Acetone-d $_6$,298K)

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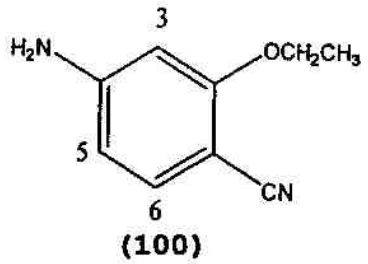

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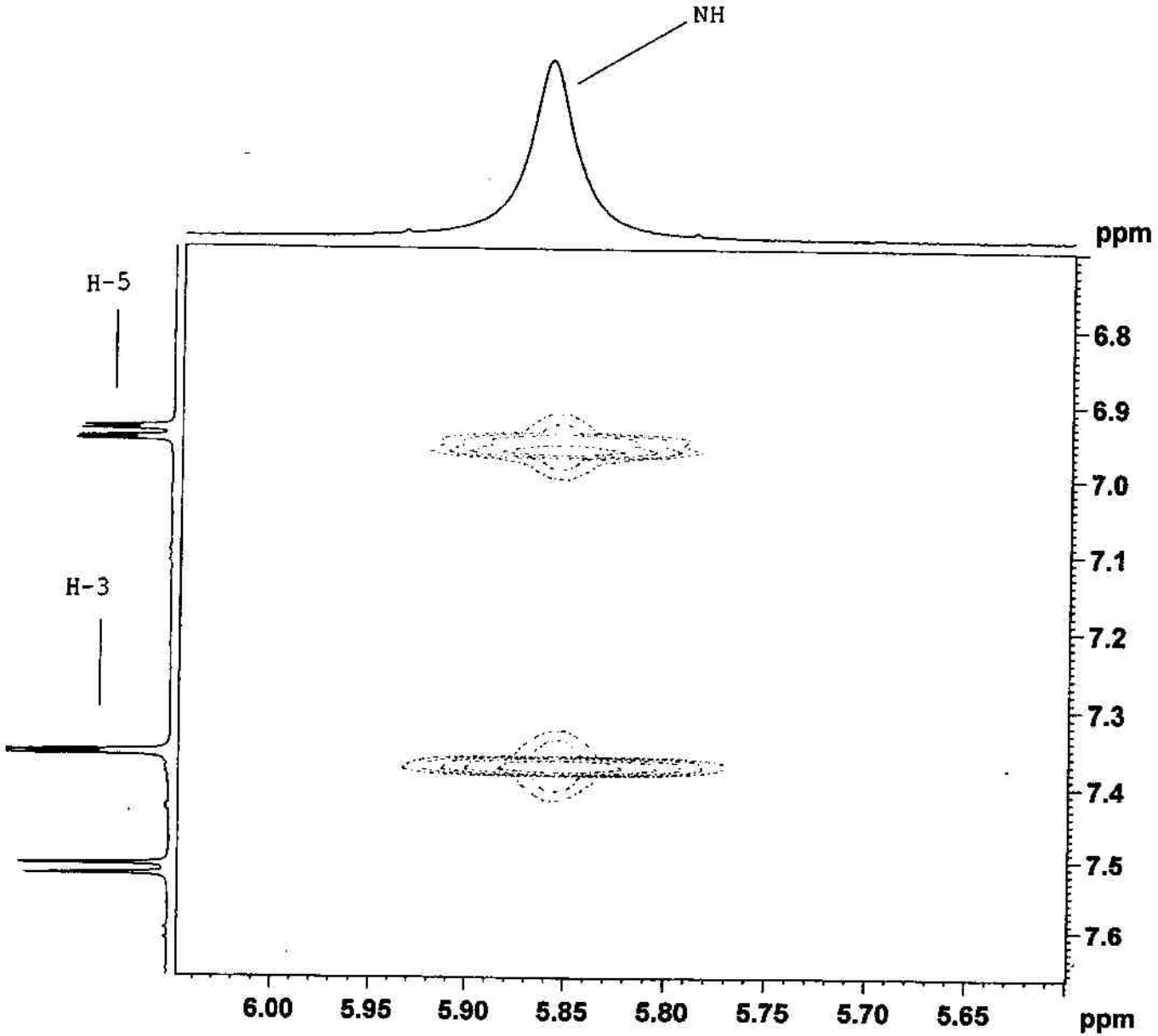
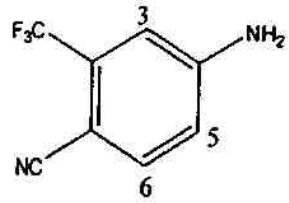


Plate 9a (Acetone-d $_6$,298K)



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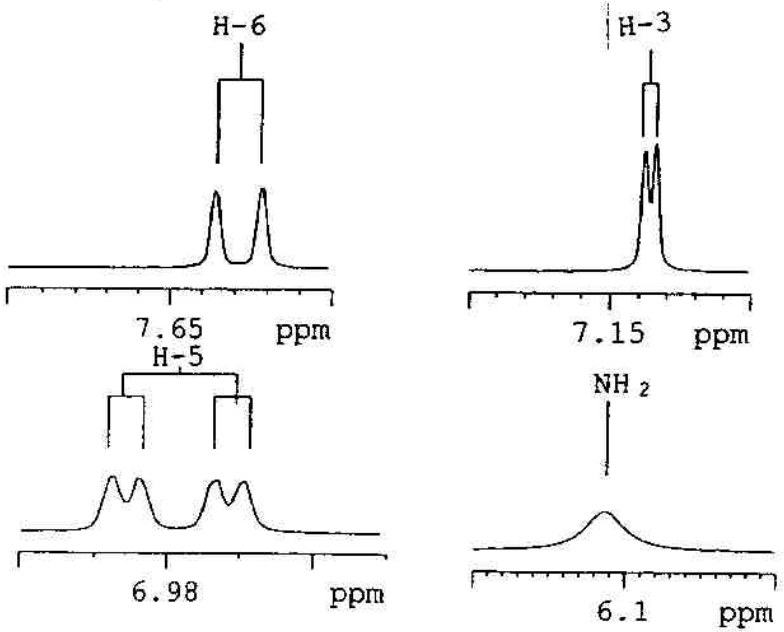


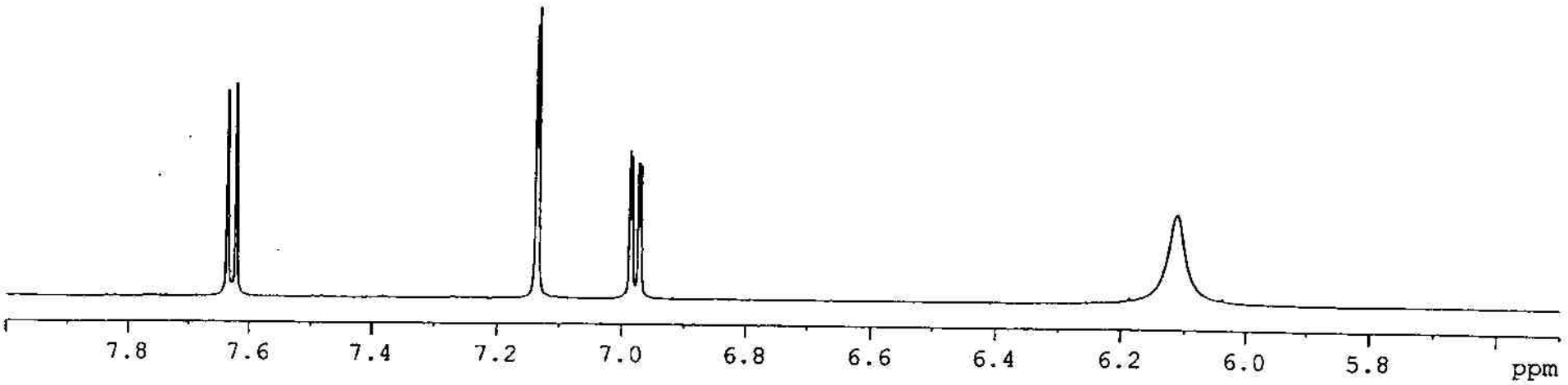
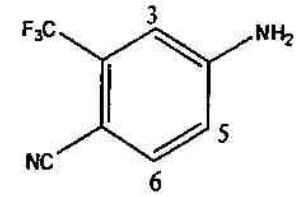


Plate 9b (Acetone-d $_6$, 298K)



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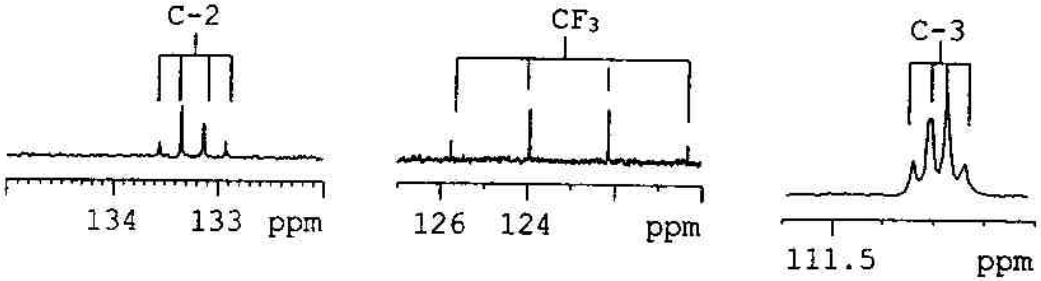


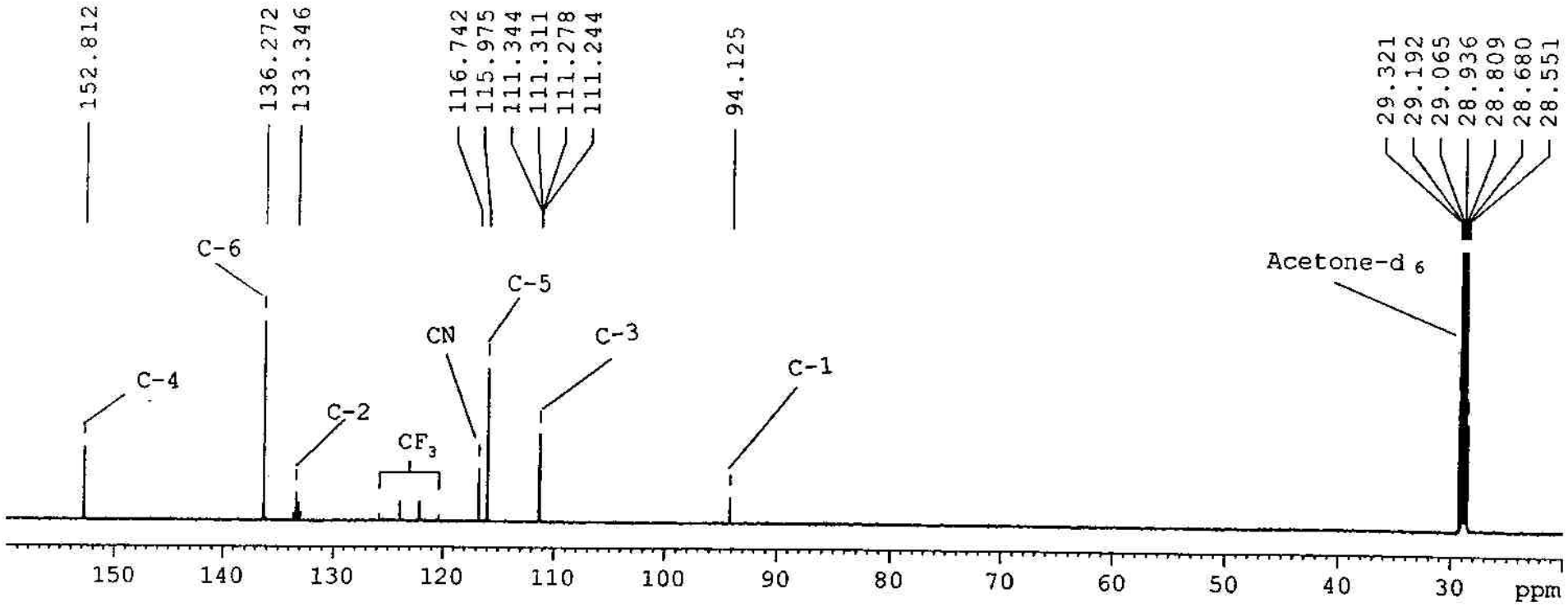
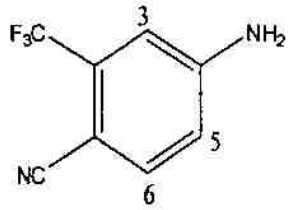


Plate 9c (Trifluoroacetic acid, 298K)



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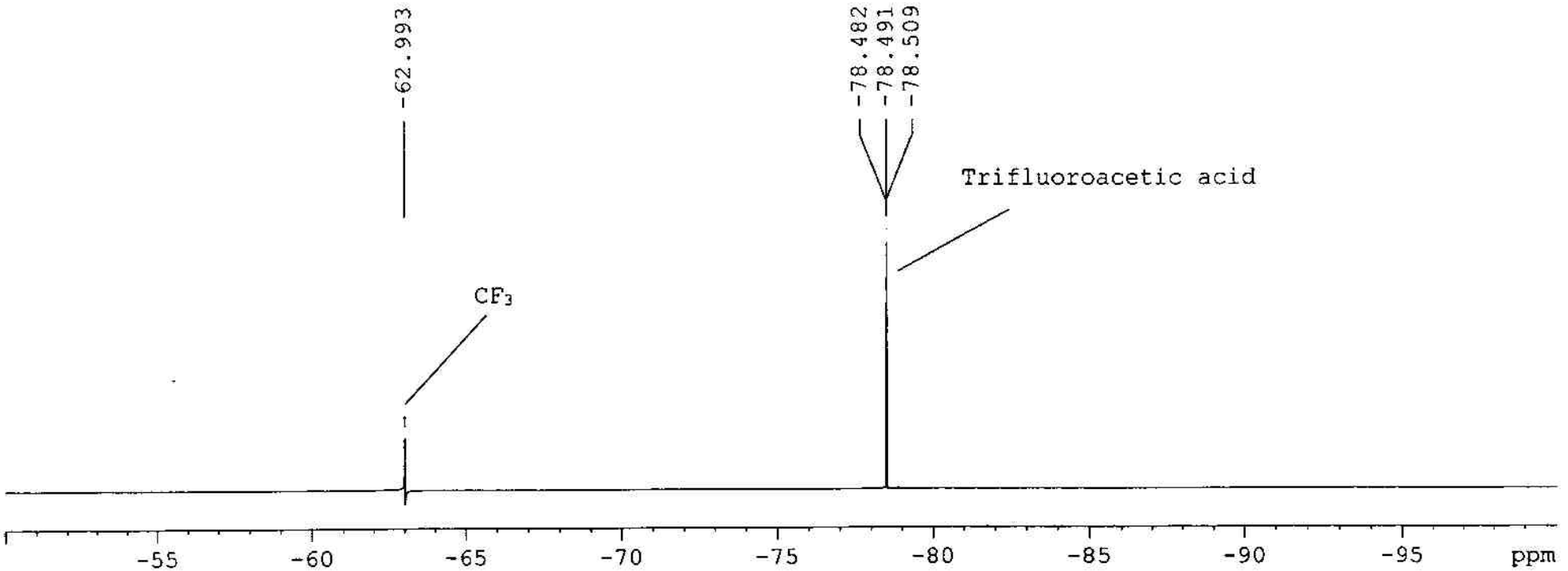


Plate 10a (Acetone-d_6, 298K)

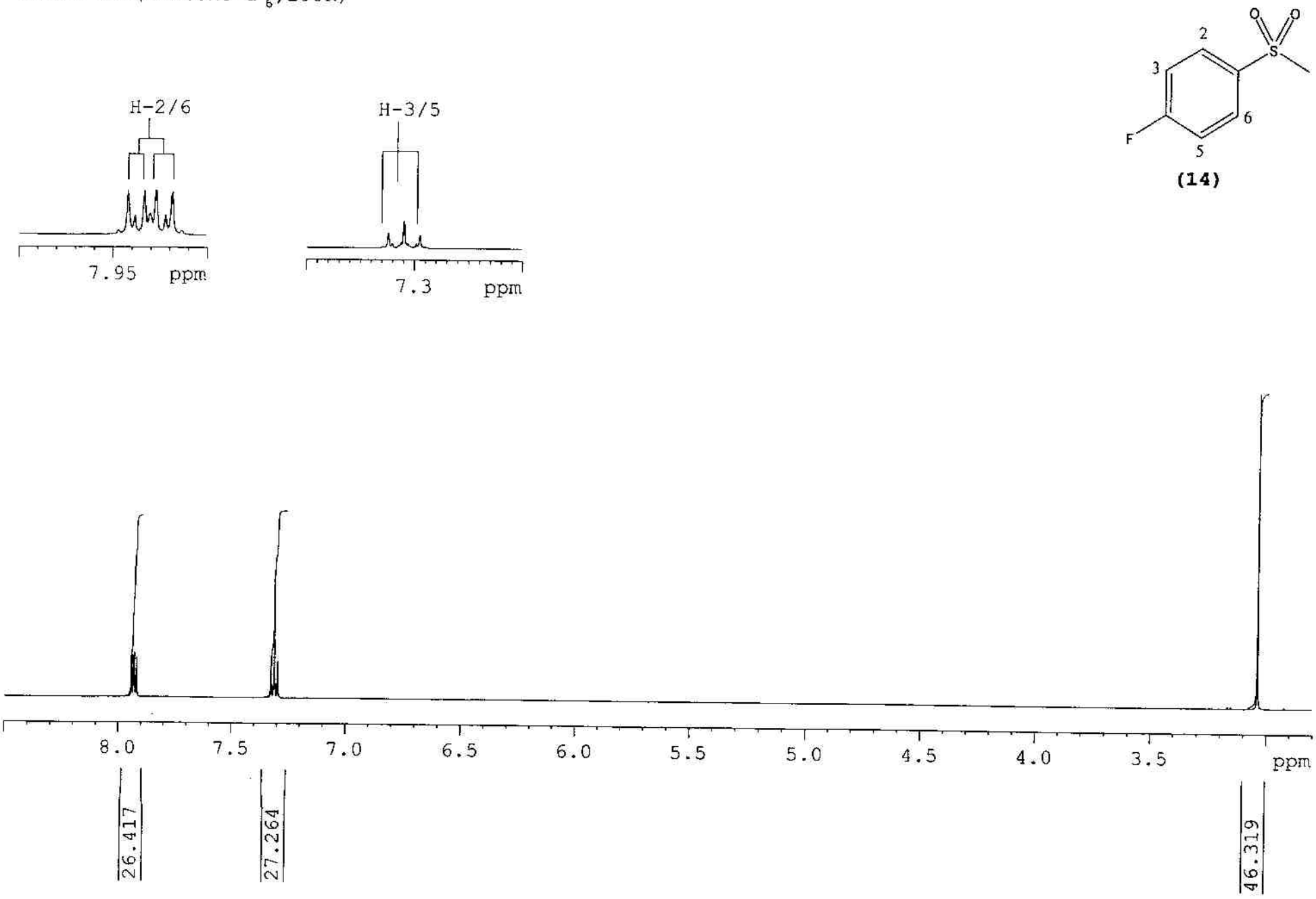
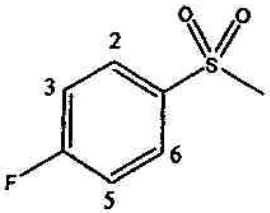


Plate 10b (Acetone-d $_6$, 298K)



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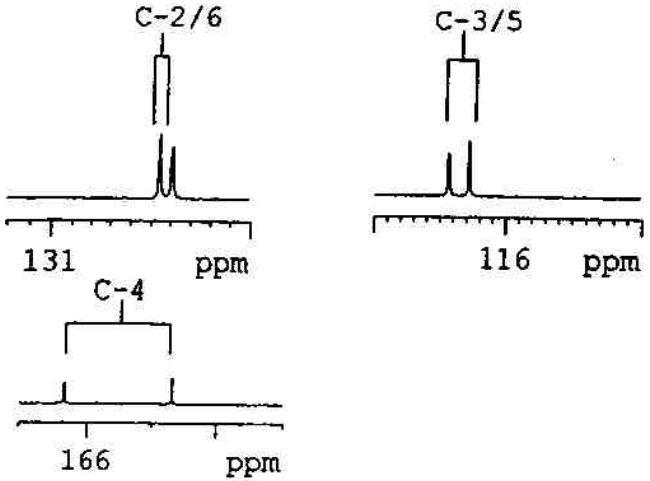


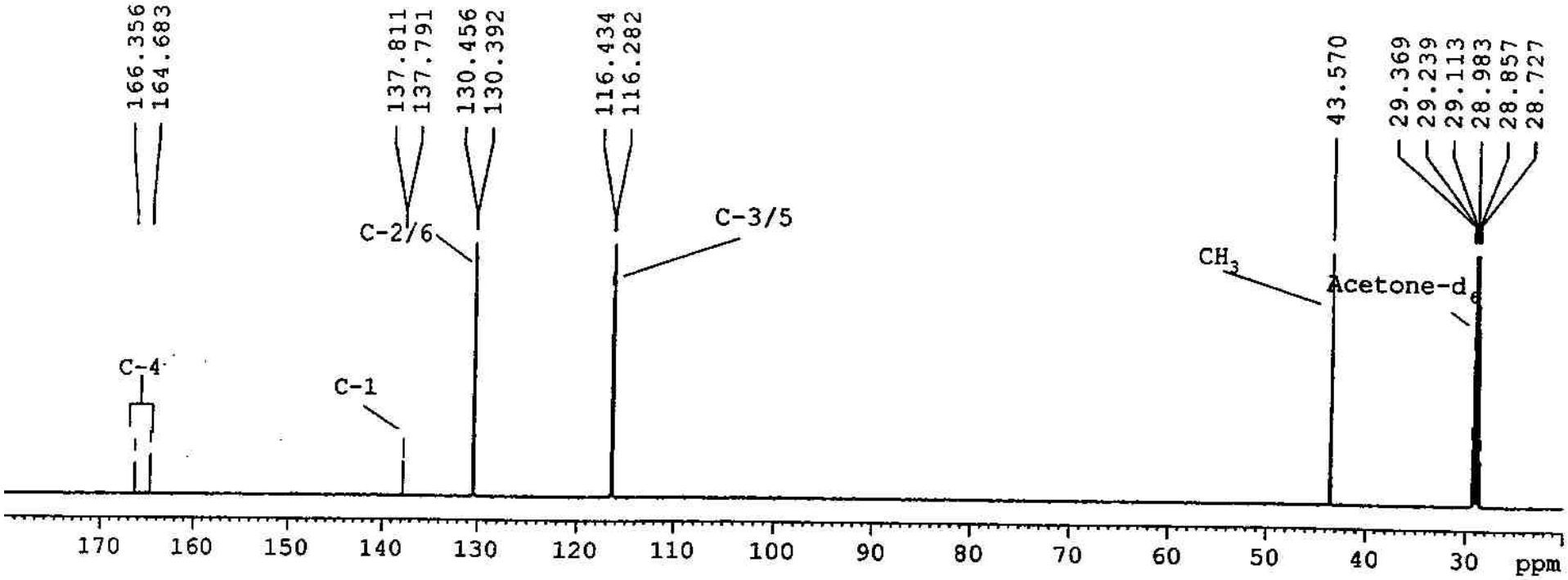
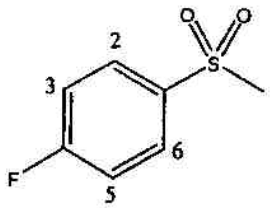


Plate 10c (Trifluoroacetic acid, 298K)



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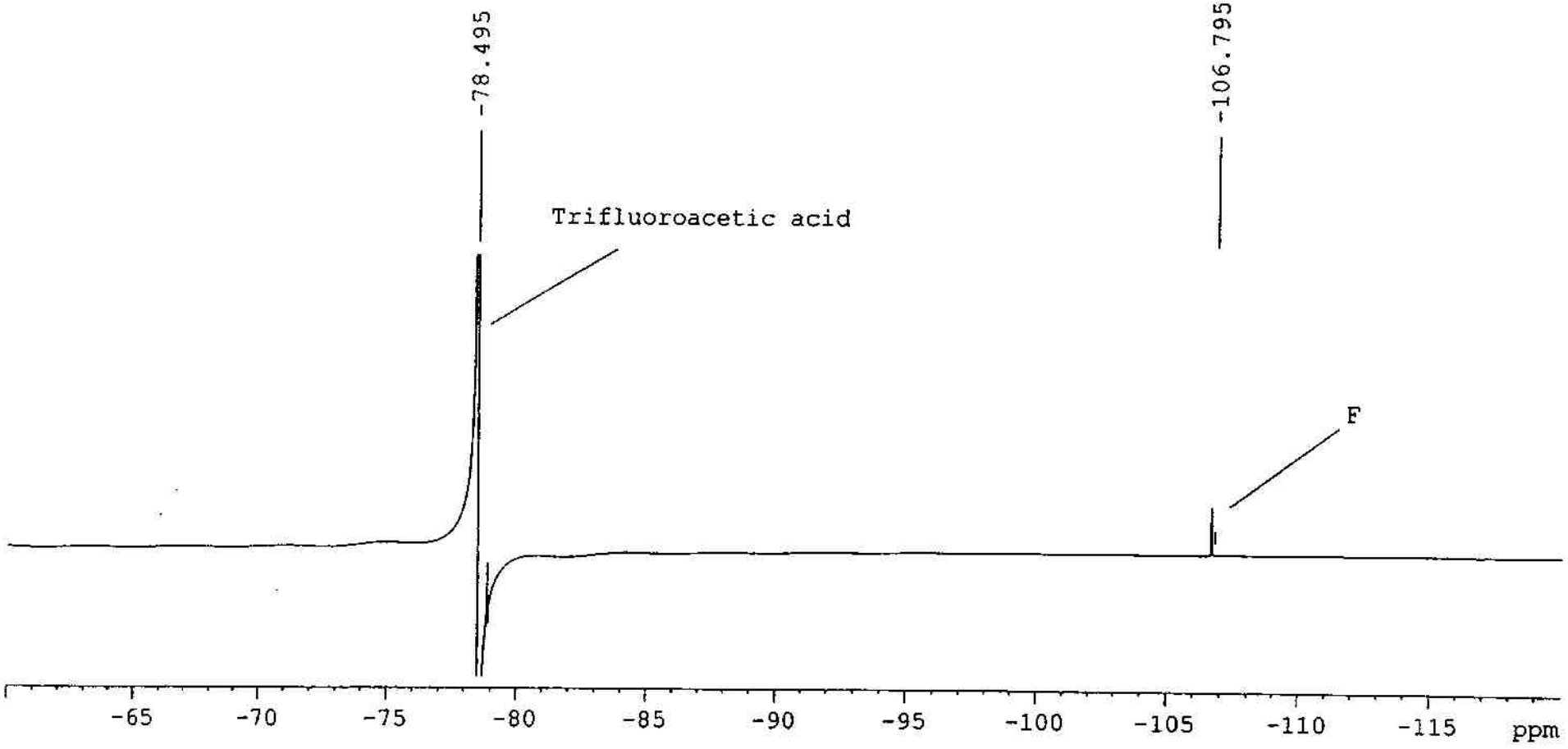

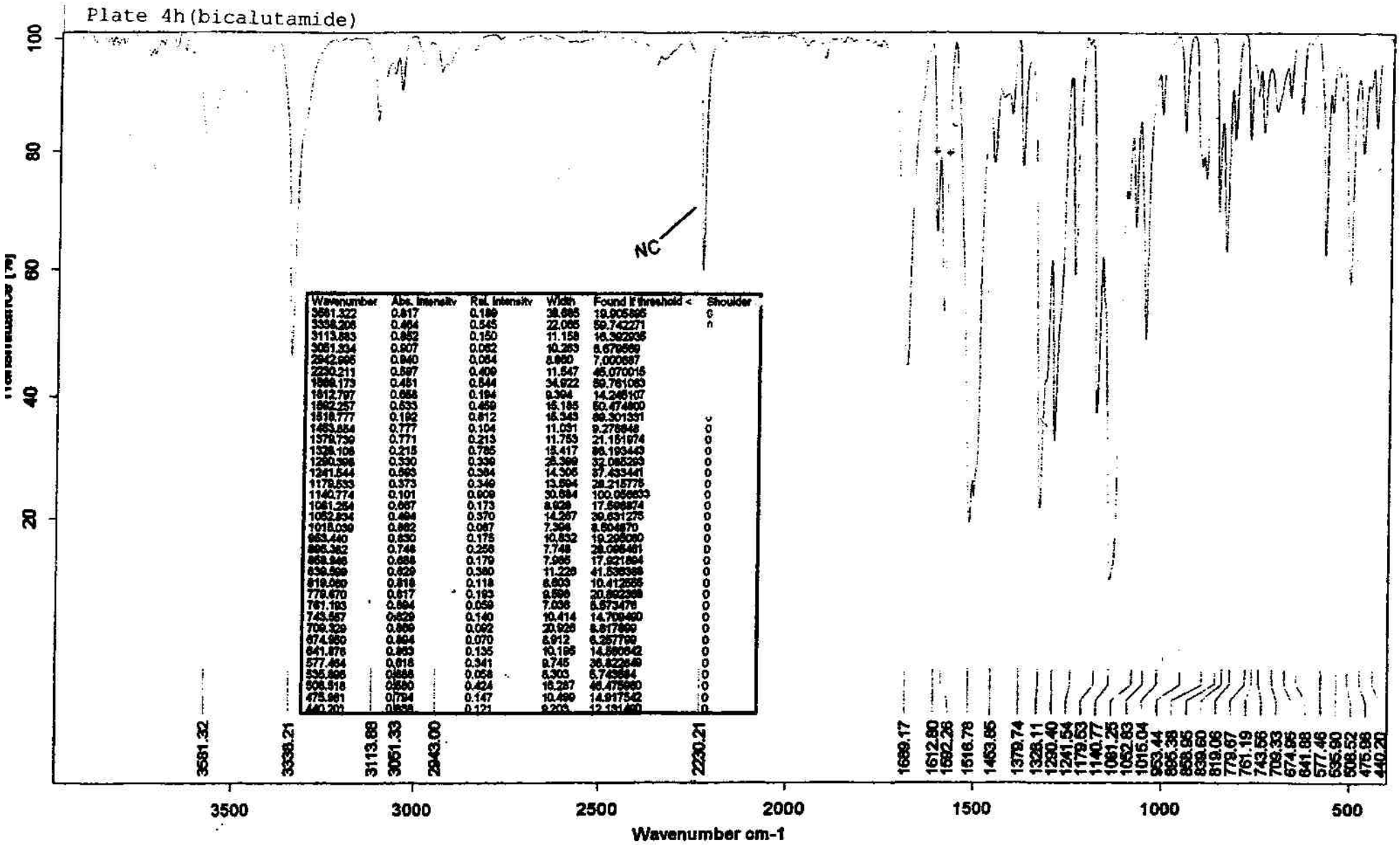

Wavenumber	Abs. Intensity	Rel. Intensity	Width	Found if threshold <	Shoulder
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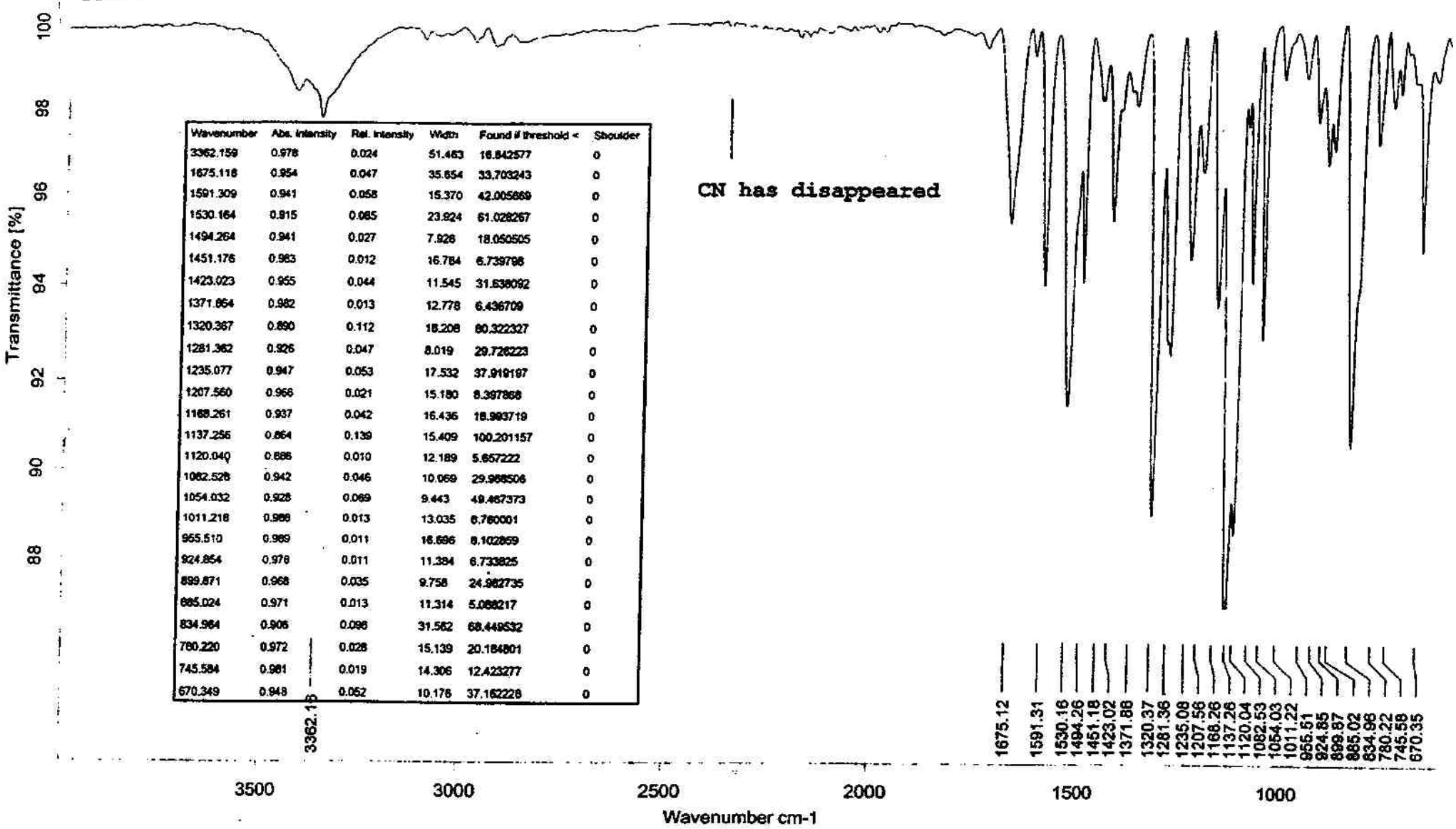
3338.206	0.464	0.545	22.065	59.742271	c
3113.883	0.852	0.150	11.158	16.392936	
3051.334	0.807	0.082	10.263	8.679569	
2942.995	0.840	0.064	8.950	7.000887	
2230.211	0.597	0.409	11.547	45.070015	
1689.173	0.451	0.844	34.922	89.761063	
1612.797	0.858	0.194	9.394	14.246107	
1592.257	0.833	0.459	15.185	50.474800	
1516.777	0.192	0.812	16.343	89.301331	
1453.854	0.777	0.104	11.051	9.278648	c
1379.739	0.771	0.213	11.753	21.161974	c
1328.106	0.215	0.785	15.417	86.193443	c
1290.398	0.330	0.339	25.399	32.085293	c
1241.544	0.593	0.394	14.305	37.433441	c
1179.533	0.373	0.349	13.594	28.215776	c
1140.774	0.101	0.909	30.594	100.000000	c
1081.254	0.667	0.173	8.928	17.866874	c
1052.834	0.494	0.370	14.267	39.631275	c
1015.039	0.862	0.087	7.396	8.604870	c
953.440	0.830	0.175	10.832	19.295090	c
895.382	0.748	0.256	7.748	28.096461	c
858.946	0.838	0.179	7.995	17.921894	c
839.599	0.629	0.380	11.228	41.536388	c
819.060	0.818	0.118	8.803	10.412555	c
779.670	0.817	0.193	9.599	20.892369	c
761.193	0.894	0.059	7.036	5.573478	c
743.557	0.829	0.140	10.414	14.709460	c
709.329	0.869	0.092	20.926	8.817899	c
674.950	0.864	0.070	8.912	6.357700	c
641.876	0.863	0.135	10.195	14.560642	c
577.464	0.618	0.341	9.745	36.822649	c
535.896	0.858	0.058	8.303	5.749594	c
508.518	0.550	0.424	16.287	46.475980	c
475.981	0.794	0.147	10.499	14.917542	c
440.203	0.858	0.121	9.203	12.131460	c

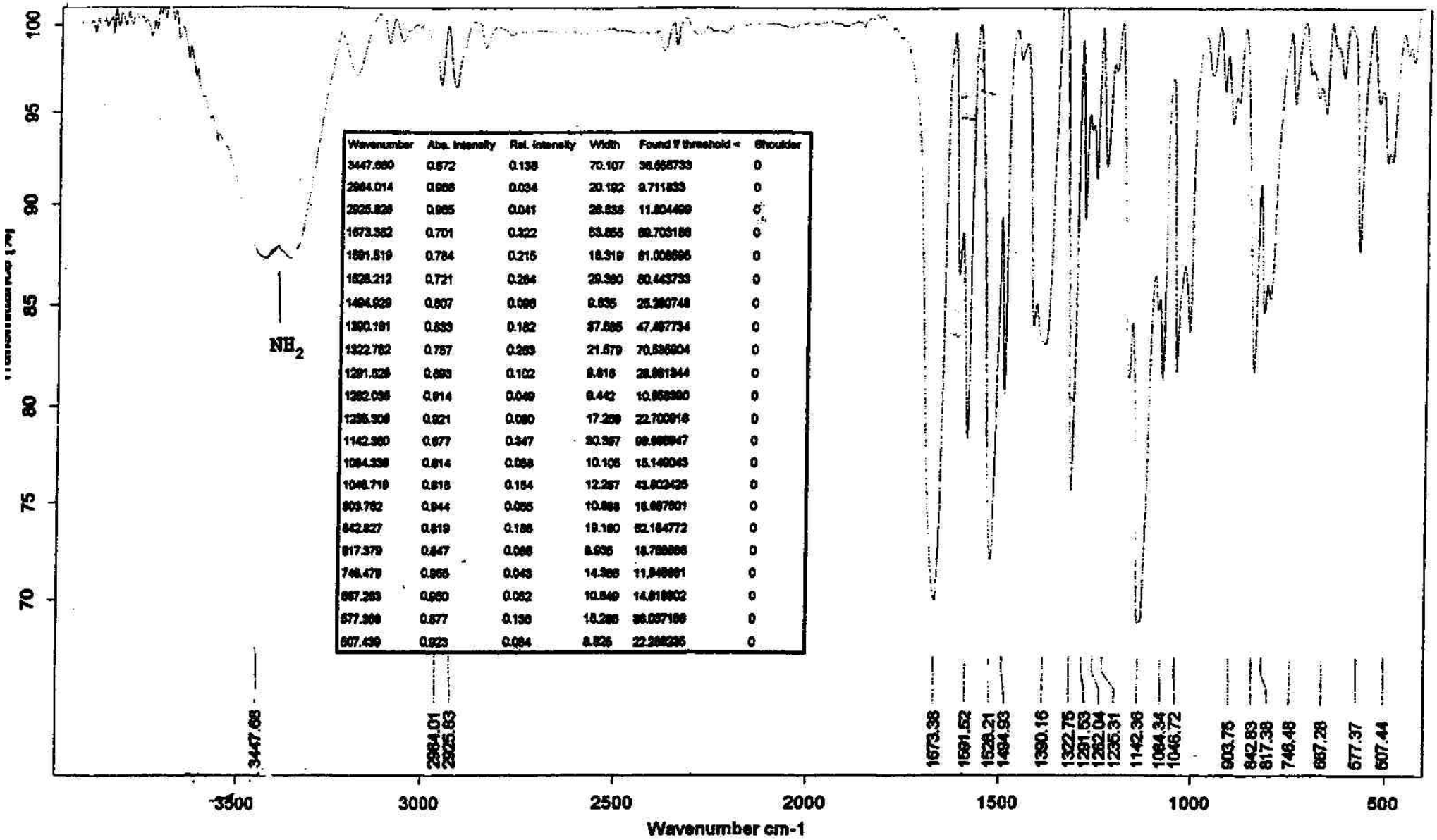
Plate 5h



CN has disappeared

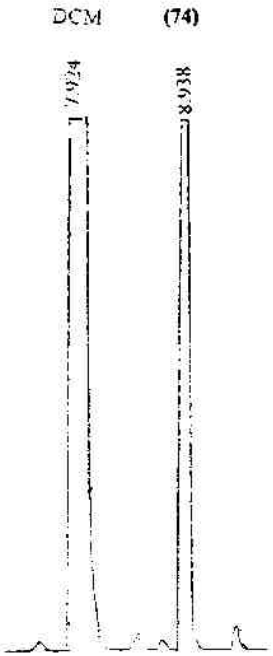
Wavenumber	Abs. intensity	Rel. intensity	Width	Found if threshold <	Shoulder
3362.159	0.978	0.024	51.463	16.842577	0
1675.118	0.954	0.047	35.654	33.703243	0
1591.309	0.941	0.058	15.370	42.005869	0
1530.164	0.915	0.085	23.924	61.028267	0
1494.264	0.941	0.027	7.926	18.050505	0
1451.176	0.983	0.012	16.784	6.739796	0
1423.023	0.955	0.044	11.545	31.638092	0
1371.864	0.982	0.013	12.778	6.436709	0
1320.367	0.890	0.112	18.208	80.322327	0
1281.362	0.926	0.047	8.019	29.726223	0
1235.077	0.947	0.053	17.532	37.919197	0
1207.560	0.966	0.021	15.180	8.397868	0
1168.261	0.937	0.042	16.436	18.993719	0
1137.256	0.864	0.139	15.409	100.201157	0
1120.040	0.988	0.010	12.189	5.657222	0
1082.528	0.942	0.046	10.069	29.988506	0
1054.032	0.928	0.069	9.443	49.467373	0
1011.218	0.986	0.013	13.035	6.760001	0
955.510	0.989	0.011	16.696	6.102859	0
924.854	0.976	0.011	11.384	6.733825	0
899.871	0.968	0.035	9.758	24.982735	0
885.024	0.971	0.013	11.314	5.088217	0
834.964	0.906	0.096	31.562	68.449532	0
780.220	0.972	0.028	15.139	20.184801	0
745.584	0.981	0.019	14.306	12.423277	0
670.349	0.948	0.052	10.176	37.162228	0

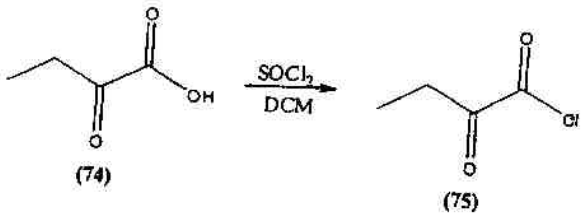
Plate 6h



Wavenumber	Abs. Intensity	Rel. Intensity	Width	Found If threshold <	Shoulder
3447.880	0.872	0.138	70.107	38.655733	0
2964.014	0.966	0.034	20.192	9.711833	0
2925.828	0.955	0.041	28.535	11.804469	0
1673.382	0.701	0.322	53.855	89.709186	0
1591.519	0.784	0.215	18.319	81.008696	0
1528.212	0.721	0.264	29.390	80.443733	0
1494.929	0.807	0.096	9.035	26.280748	0
1390.161	0.839	0.162	37.685	47.497734	0
1322.762	0.757	0.269	21.579	70.536804	0
1291.528	0.869	0.102	8.816	28.881344	0
1262.036	0.914	0.049	9.442	10.858990	0
1235.308	0.921	0.080	17.269	22.700916	0
1142.360	0.677	0.347	30.397	99.999947	0
1084.339	0.814	0.058	10.105	15.149043	0
1046.719	0.816	0.154	12.287	43.823426	0
903.762	0.944	0.055	10.888	15.697501	0
842.827	0.819	0.186	19.180	52.184772	0
817.379	0.847	0.066	8.935	18.788686	0
746.479	0.866	0.043	14.366	11.845691	0
687.283	0.950	0.052	10.849	14.818902	0
577.369	0.877	0.136	16.286	38.037186	0
507.439	0.923	0.084	8.525	22.286236	0

ATE 13

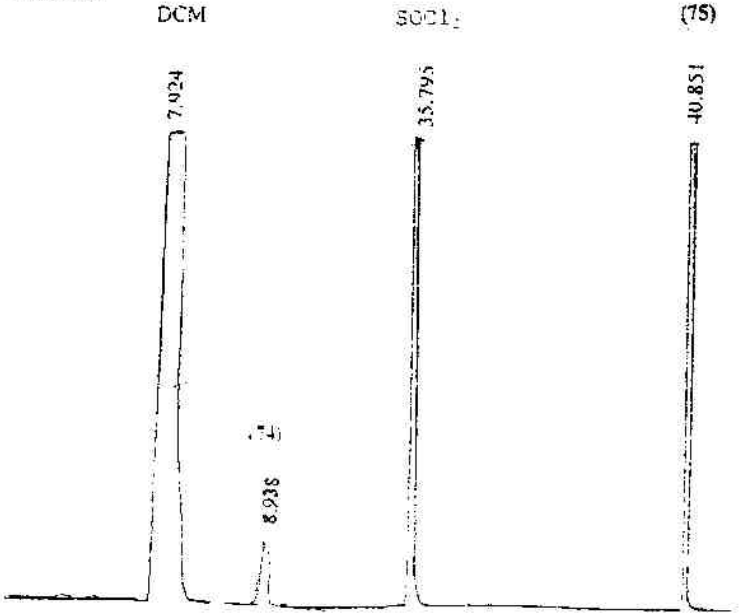




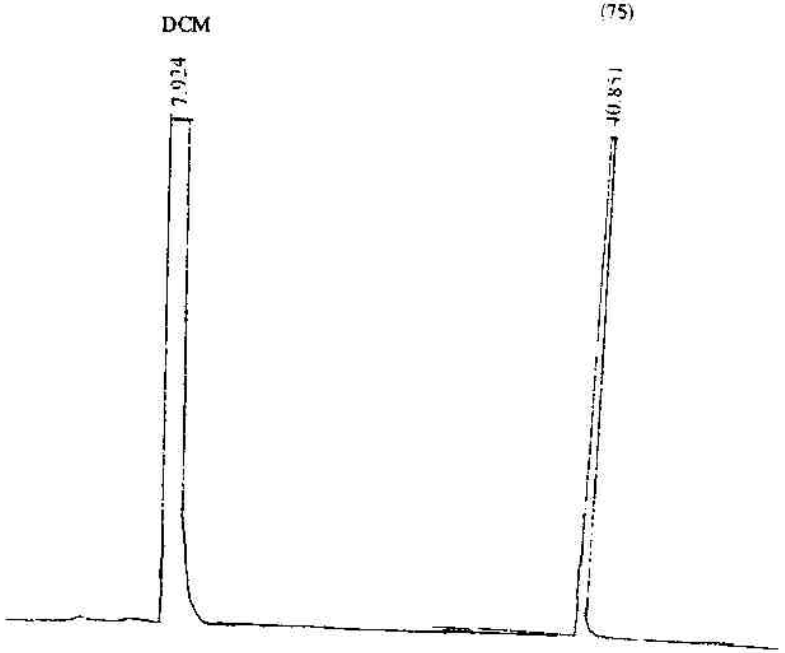
(74)

(75)

0 HOURS

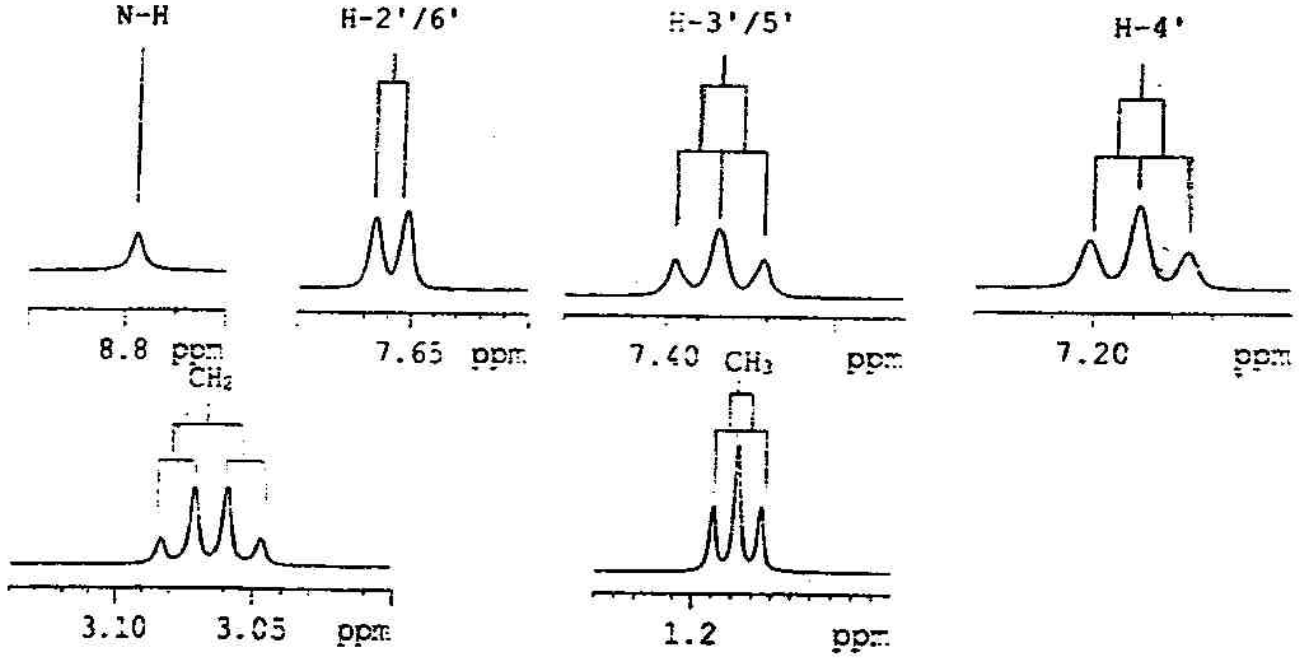


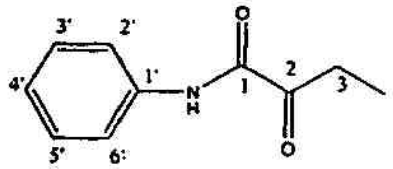
15 MINUTES



2 HOURS

Plate 1a($CDCl_3$, 298K)





(84)