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Acylation of N-Boc-N'-$COCF_3$ protected hydrazine

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Abstract:

Within the scope of the present thesis acylation of N-Boc-N'-$COCF_3$ protected hydrazine was investigated. Acetic anhydride, benzoyl and butanoyl chlorides, activated esters and benzyl chloroformate were tested as acylating agents. The implementation of highly reactive acyl chlorides led to very good yields of hydrazines monoacylated at the Boc-protected nitrogen. Application of an excess of acyl chlorides promoted the formation of diacylated Boc-protected hydrazines and an unexpected loss of the trifluoroacetic group. Acylation by activated esters or benzyl chloroformate resulted solely in monoacylated products isolated in fair to poor yields. Reactions utilizing acetic anhydride gave no product irrespective of reaction conditions.

Keywords:

Hydrazine, acylation, orthogonal protecting groups.

CERCS: P390 - Organic chemistry.

N-Boc-N'-$COCF_3$ kaitstud hüdrasiini atsüülimine

Lühikokkuvõte:

Käesoleva töö raames uuriti N-Boc-N'-$COCF_3$ atsüülimise võimalusi. Atsüülivate reagentidena kasutati etaanhappe anhüdriidi, bensoüül- ja butanoüülkloriidi, aktiivseid estreid ja bensüülkloroformaati. Atsüülimine väga reaktsioonivõimeliste atsüülkloriididega võimaldas modifitseerida Boc-kaitstud lämmastiku aatomit. Produktide saagised olid väga head. Atsüülkloriidide liia kasutamine soodustas diatsüülitud produkti N-Boc kaitstud produkti teket, millega kaasnes ootamatu trifluoroatsetüülse rühma eemaldumine. Atsüülimine aktiivsete estritega ja bensüülkloroformaadiga andis ainult monoatsüülitud produkte. Saagised olid keskpärased või madalad. Etaanhappe anhüdriidi kasutamine ei andnud atsüülitud produkti sõltumata reaktsiooni tingimustest.

Võtmesõnad:

Hüdrasiin, atsüülimine, ortogonaalsed kaitserühmad.

CERCS: P390 – Orgaaniline keemia.

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TERMS, ABBREVIATIONS AND NOTATIONS

ACN	acetonitrile
Ac	acetyl
Bn	benzyl
Boc	*tert*-butoxycarbonyl
Boc_2O	di-*tert*-butyl dicarbonate
BuLi	butyllithium
Cbz	benzyloxycarbonyl
DCC	N, N'-dicyclohexylcarbodiimide
DCM	dichloromethane
DIC	N, N'-diisopropylcarbodiimide
DiPEA	N, N-diisopropylethylamine
DMAP	N, N-dimethylaminopyridine
DO	1,4-dioxane
Et	ethyl
Et_2O	diethyl ether
EtOAc	ethyl acetate
EtOH	ethanol
Fmoc	fluorenylmethyloxycarbonyl
HBTU	benzotriazole tetramethyl uronium hexafluorophosphate
HOBt	hydroxybenzotriazole
HOMO	highest occupied molecular orbital
Me	methyl
MeOH	methanol
NHS	N-hydroxysuccinimide
PG	protecting group
PE	petroleum ether
Ph	phenyl
PTC	phase transfer catalysis
TBAHS	tetrabutylammonium hydrogensulfate
t-Bu	*tert*-butyl
TEA	triethylamine
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
Ts	*p*-toluenesulfonyl
TsOH	*p*-toluenesulfonic acid

INTRODUCTION

Hydrazine derivatives are ubiquitous in both rocket fuel and pharmaceutical industry. Hydrazine, violently igniting on heating or contact with metal oxides, decomposes into gaseous nitrogen and ammonia, a property attractive for use in rocket fuel. UDMH, unsymmetrical dimethyl hydrazine, fueled a liquid RD-119 engine for Kosmos-2 rockets that orbited 200 artificial Earth satellites in 1962-1967. Manifesting high biological activity, isonicotinic acid hydrazide is used as a tuberculosis and depression treatment [1].

Orthogonally protected hydrazine derivatives find their application as precursors for the synthesis of α-aza-amino acids, which when integrated in peptides in lieu of the native residues increase their proteolytic stability [2].

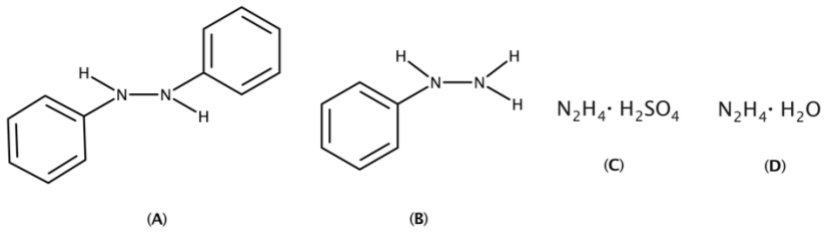
Regioselective modification of hydrazine is a longstanding problem in organic synthesis. There exists a plethora of strategies for the alkylation of hydrazine and its derivatives, such as monoprotection by alkoxycarbonyl groups, orthogonal protection, alkylation under PTC or Mitsunobu conditions, but most notably direct alkylation, reductive alkylation and polyanionic methods. Direct alkylation, though somewhat mediated by sterically hindered substituents or an excess of hydrazine, proceeds unselectively on account of the increased reactivity of the products as compared to that of the substrates [3]. Reductive alkylation is impeded by its multi-stage character and the instability of some starting carbonyl compounds [2]. Polyanionic methods exploit strongly nucleophilic bases, complicating handling and demanding of unfaltering maintenance of unconventional reaction conditions [4].

An ever more present interest in the acidity of hydrazine derivatives brought about reports on the drastically diverse pKa values of the two nucleophilic centres of several protected hydrazines, suggesting a possible manipulation of the substitution reaction through protecting groups [5]. As such, a method for benzylation of $BocNHNHCOCF_3$ was devised, basing on a selective deprotonation of the acidic trifluoroacetylated nitrogen, a convenient and fast pathway to monosubstituted hydrazines produced in excellent yields [6]. This work inspired us to investigate the possibility of acylation of the same substrate on the principles of acidity of the deprotonated species.

1 LITERATURE REVIEW

1.1 Historical considerations

Hydrazine is a compound of the molecular formula N_2H_4. By hydrazine derivatives we understand compounds containing the R($R^{'}$)-N-N-$R^{''}$($R^{'''}$) moiety, where R, $R^{'}$, $R^{''}$, $R^{'''}$ are either alkyl, acyl, aryl groups or H. The first mention of the formation of the N-N bond is attributed to N. Zinin's synthesis of hydrazobenzene ((**A**), *Scheme 1*) in 1845 [1], followed by E. Fischer's preparation of phenyl hydrazine ((**B**), *Scheme 1*) in 1875 [7], the generation of hydrazine sulphate ((**C**), *Scheme 1*) and hydrazine hydrate ((**D**), *Scheme 1*) by T. Curtius in 1887 [8], and, eventually, the isolation of anhydrous hydrazine in 1895 by Lobry De Bruyn [9].



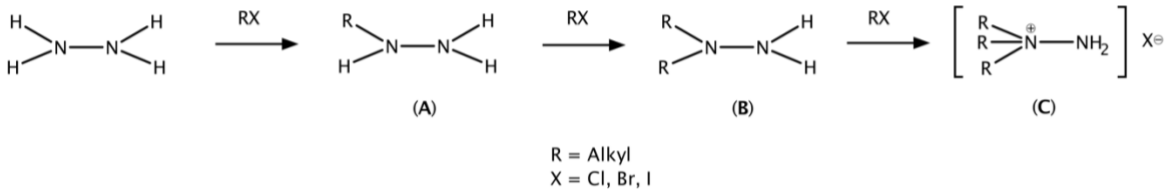
Scheme 1. Structures of the first synthesized hydrazines.

1.2 Reactivity

Hydrazine is known to be a strong nucleophile and a relatively weak base (pKa (NH_2NH_3(+) = 8.11 [10]) due to the presence of non-bonding lone pairs of electrons on both nitrogen atoms. Nucleophiles are termed a species hosting a pair of electrons in the highest occupied molecular orbital (the HOMO) [11]. The excess reactivity manifested in the nucleophiles that bear an unshared pair of electrons at the atom adjacent to the nucleophilic centre, *viz.*, the hydrazide anion, is termed the α-effect [12]. The electrostatic repulsion of the adjacent electronic lone pairs causes p_π orbital splitting, raising the HOMO and expediating the interaction with an electrophile. The pK_a of the conjugate acids of α-nucleophiles is diminished in light of the inductive electron-withdrawing effect of the immediately adjacent to the nucleophilic centre electronegative atom [13].

1.3 Direct alkylation of hydrazine and its derivatives

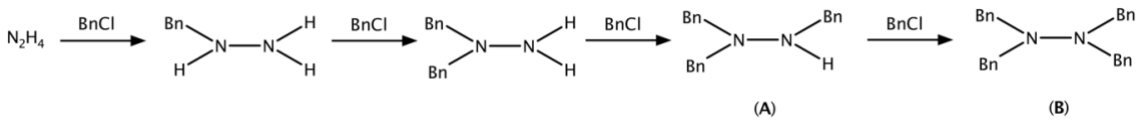
Direct alkylation of hydrazine with electrophiles such as alkyl halides leads ordinarily to asymmetrically substituted derivatives [3], [14]. The primary product, monoalkylhydrazine ((**A**), *Scheme 2*), undergoes further alkylation on the nitrogen bearing the alkyl substituent, due to the electron-donating effect of the latter imparting to it increased nucleophilicity and basicity. The proceeding alkylation reaction eventually yields dialkylhydrazine ((**B**), *Scheme 2*) and a quaternary hydrazinium salt ((**C**), *Scheme 2*), further alkylation attempts of the salt prompting the breaking of the N-N bond [2], [3].



Scheme 2. Direct alkylation of hydrazine by alkyl halides.

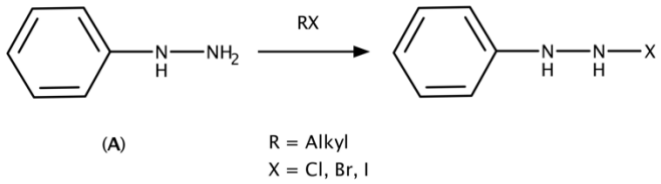
The selectivity of the alkylation is strongly dependent on the ratio of the reagents. Reacting hydrazine with an equimolar amount of the alkylating agent favours polyalkylation, whereas a 10-fold excess of the former produces satisfactory to good yields of the target monoalkylated product. The incorporation of sterically hindered groups, such as isopropyl, is conducive to higher yields of monosubstituted hydrazines even on a smaller (4-fold) excess of the substrate [3].

Steric hindrance of alkylating agents substantially altering the course of the reaction, alkylating hydrazine with benzyl chloride produced tri- ((**A**), *Scheme 3*) and tetrabenzylhydrazine ((**B**), *Scheme 3*) without quarternization [3].



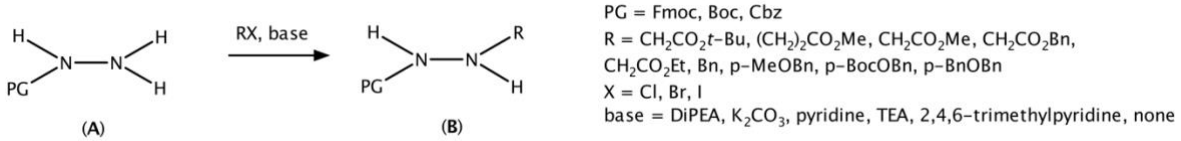
Scheme 3. Benzylation of hydrazine.

Regioselectivity is manifest on alkylating hydrazine carrying antecedently introduced substituents that incorporate the electronic lone pair on the adjacent nitrogen into resonance with phenyl group hitherto reducing the nucleophilicity of the atom, phenylhydrazine ((**A**), *Scheme 4*) serving an illustrative example [2], [3].



Scheme 4. Alkylation of phenylhydrazine.

Monoprotection of hydrazine with alkoxycarbonyl groups such as Boc or Fmoc greatly decreases its nucleophilicity, enhancing the selectivity of direct alkylation. To illustrate, alkoxycarbonyl protected hydrazines ((**A**), *Scheme 5*) were alkylated with α-halo esters, benzyl halides and their ether derivatives in the presence of organic and inorganic bases alike, or without any, under a multitude of temperature settings affording asymmetrically substituted products ((**B**), *Scheme 5*) [15], [16], [17], [18], [19]. The factors conducive to monoalkylation comprise a 3- or 4-fold excess of the protected hydrazine, the implementation of a base compatible with the protecting group, ACN as a solvent and bromides and iodides as alkylating agents [18]. Considering the general instability and the limited commercial availability of alkyl iodides, convenient *in situ* generation of iodides from less reactive halides in the presence of catalytic amounts of inorganic iodides was developed. This modification greatly increased the rate of hydrazine alkylation reaction [19]. It is to be noted that despite the improvements, direct alkylation of monoprotected hydrazines still suffers from polyalkylation and excellent yields of monoalkylated products are rarely encountered on this approach.



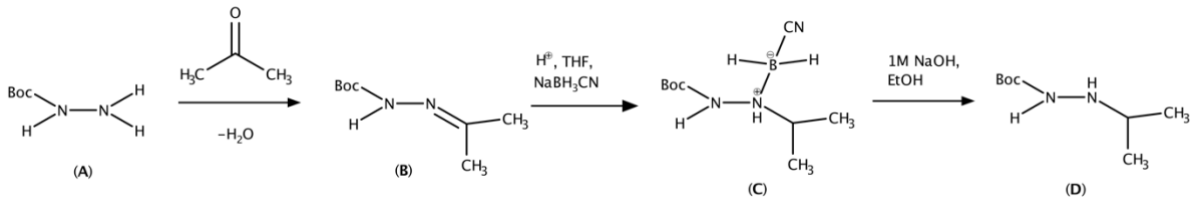
Scheme 5. Alkylation of monoprotected hydrazines.

Insofar as polyalkylation is not to be resolved via the excess in either hydrazine or its monoprotected derivatives on direct alkylation, the methods that do address the issue, namely reductive alkylation, those exploiting an ensemble of orthogonal protection with phase transfer catalysis, conversely polyanions, are to be considered in the sequel.

1.4 Reductive alkylation

Reductive alkylation proceeds via the generation of hydrazones, compounds of the form $R(R')\text{-}C{=}N\text{-}N\text{-}R''(R''')$, where R, R', R'' are hydrogens or alkyl groups and R''' is a protecting group requisite to prevent their spontaneous conversion into dihydrazones. This is effectuated by condensing monoprotected hydrazines with carbonyl compounds, followed by catalytic hydrogenation or reduction of C=N bond with complex hydrides [2], [20]. The maintenance of mild conditions on the condensation stage ensures selectivity [2].

For instance, condensation of *tert*-butyl carbazate ((**A**), *Scheme 7*) with acetone produced N-Boc-N'-isopropyl hydrazone ((**B**), *Scheme 7*). Reduction of the hydrazone with sodium cyanoborohydride in THF and hydrolysis of the resulting hydrazine-cyanoborane adducts ((**C**), *Scheme 7*) by 1M NaOH in ethanol afforded N-Boc-N'-isopropyl hydrazine ((**D**), *Scheme 7*) in 75% yield. It was found that catalytic reduction is best suited for aromatic hydrazones, whereas hydride addition for aliphatic ones [21], [22].

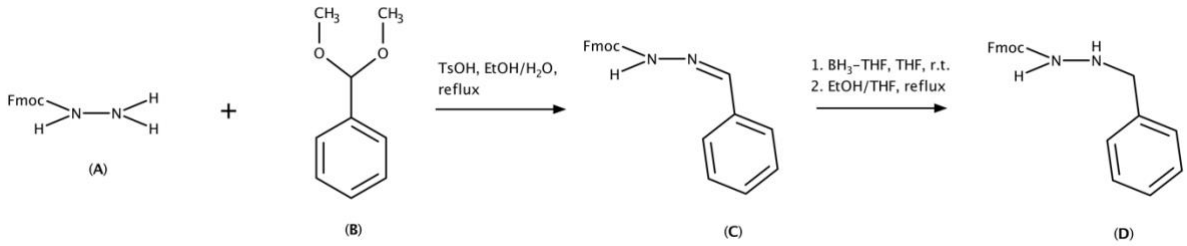


Scheme 7. Synthesis of N-Boc-N'-isopropyl hydrazine via reductive alkylation.

Temporal optimization of the multi-stage approach, demanding of frequent separation of the intermediates and prior deprotection of the carbonyl compounds, that in view of their instability are stored as acetals and ketals, sees itself in one-pot reductive alkylation [2].

One-pot reductive alkylation allowed for a 93% conversion of 9-fluorenylmethyl carbazate ((**A**), *Scheme 8*) into N-Fmoc-N'-benzyl hydrazine ((**D**), *Scheme 8*) by refluxing the former with benzaldehyde dimethyl acetal ((**B**), *Scheme 8*) in the $EtOH/H_2O$ solvent system in the

presence of a catalytic amount of *p*-toluenesulfonic acid. The obtained hydrazone ((**C**), *Scheme 8*) was reduced with borane-tetrahydrofurane having had exchanged the solvent for THF, and the reaction was quenched on addition of ethanol and reflux of the mixture [23].



Scheme 8. One-pot reductive alkylation of FmocNHNH₂ with benzaldehyde dimethyl acetal.

1.5 Methods based on NH acidity

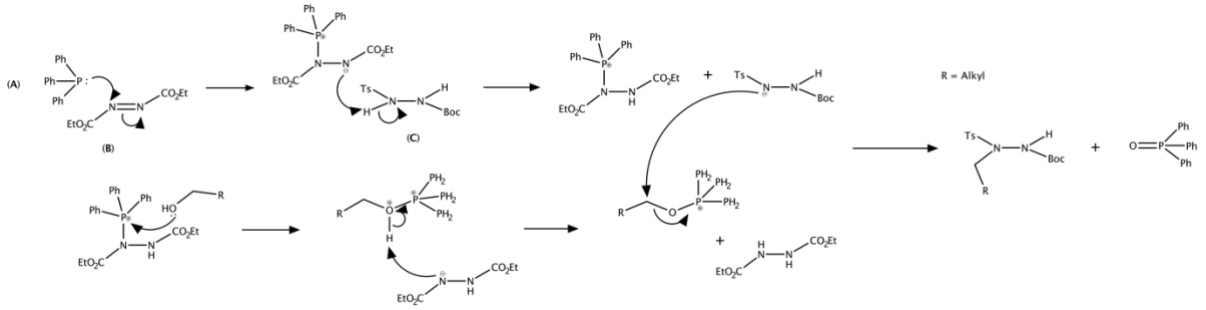
N-H acidity can be controlled via the substituents at the amine group. The pK_a of ammonia, acetamide, trifluoroacetamide and *p*-toluenesulfonamide in water gradually decrease along the series: 38.00, 15.10, 12.33, 10.21 [24], [25], [26]. On increasing the electron-withdrawing nature of the substituent, the electron density of the nitrogen is diminished, weakening the N-H bond [27]. The pK_a of hydrazine derivatives likewise distinctly reflects structural features. Increasing N-H acidity is required for regioselective deprotonation and subsequent alkylation under a variety of conditions [5].

Protecting groups are reversibly installed functional groups employed in order to temporarily block one of the reactive sites of hydrazine and carry out regioselective reactions [28]. The acidifying effect is determined by a combination of the field inductive effects of the protecting groups and by the resonance stabilization of the anionic species by the electron-accepting groups at the ionization center [5].

1.5.1 Mitsunobu alkylation

Mitsunobu conditions promote selective alkylation of hydrazines, *e.g.*, TsNHNHBoc ((**C**), *Scheme 9*) by alcohols in the presence of diethyl azodicarboxylate ((**B**), *Scheme 9*) and triphenylphosphine ((**A**), *Scheme 9*). The breaking of the N-H bond is a rate-determining step,

dictated by the pK_a of the nitrogen, which occurs preferentially within the TsNH moiety due to the electron-withdrawing nature of the protecting group [11], [29], [30].

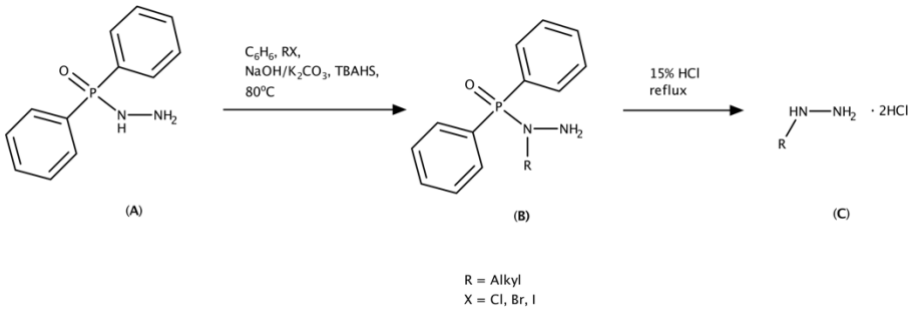


Scheme 9. Alkylation of diprotected hydrazines via the Mitsunobu protocol.

1.5.2 Phase transfer catalysis

The phenomenon of rate enhancement of a reaction between compounds in two immiscible phases via the use of a phase-transfer agent, a reactant capable of repeated transfer of either one across the interface into the opposite phase, bringing the starting compounds in close proximity so as to instigate the reaction, whilst itself not being consumed, is termed phase-transfer catalysis (PTC) [31]. The high performance of organic-soluble quaternary ammonium or phosphonium cations as phase-transfer agents is attributed to their anion-partitioning selectivity [31]. The weak anion-cation complex brings the transferred species into a reactive form and allows for efficient displacement, satisfying the additional requirement imposed on phase-transfer agents, apart from their fulfillment of the transfer function [31].

By virtue of solid-liquid PTC alkylation with alkyl halides, in a benzene-$NaOH_{(s)}/K_2CO_{3(s)}$ system with TBAHS as a phase-transfer agent and an eventual addition of aqueous hydrochloric acid to prompt the protecting group cleavage, diphenylphosphinic hydrazide ((**A**), *Scheme 10*) was selectively transformed into monoalkylhydrazine dihydrochloride ((**C**), *Scheme 10*). Curiously, similar regioselectivity had not been achieved on the implementation of a saturated NaOH solution under conventional PTC conditions [20], [32].



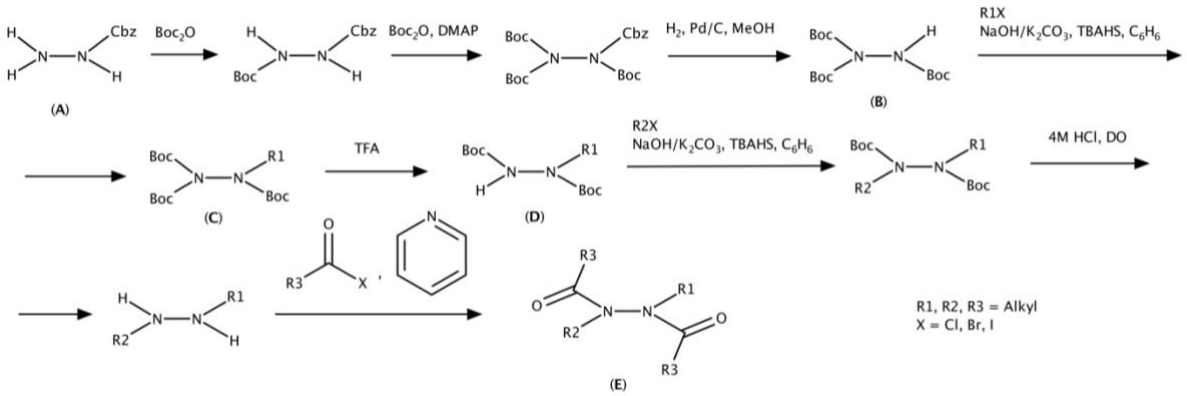
Scheme 10. Synthesis of monoalkylhydrazine via solid-liquid PTC.

Further, N, N'-dialkylhydrazine dihydrochloride was successfully prepared by the acetylation of N-alkyldiphenylphosphinic hydrazide ((**B**), *Scheme 10*), for the purposes of protection of the terminal nitrogen, followed by liquid-liquid PTC alkylation [33].

1.5.3 Orthogonal protection

An orthogonal system is defined as a set of independent classes of protecting groups, wherein each class can be removed in any sequence in the presence of all the other classes [34]. The concept lays foundation for the orthogonal protecting group synthetic methodology, which employs hydrazine derivatives bearing a combination of orthogonal protecting groups, which are sequentially derivatized and deprotected, allowing for regioselective synthesis [20].

The efficiency of this approach was demonstrated using N, N, N'-tris-Boc-hydrazine ((**B**), *Scheme 11*) as the key intermediate compound. It was generated via a dual acylation of Cbz-hydrazine ((**A**), *Scheme 11*) with Boc_2O in the presence of DMAP and a subsequent removal of the Cbz group through hydrogenation over a Pd/C catalyst. The obtained precursor was treated with primary alkyl halides under PTC conditions to yield N, N-di-Boc-N'-alkyl-Boc-hydrazine ((**C**), *Scheme 11*). Geminal Boc groups are significantly more labile than a single such functionality, granting orthogonal protection. Thus, one Boc group from the $N(Boc)_2$ moiety was cleaved by TFA, the resulting product ((**D**), *Scheme 11*) alkylated in like manner and successfully converted into N, N'-di-acyl-di-alkyl-hydrazine ((**E**), *Scheme 11*) via a multi-step synthesis including deprotection and acylation reactions [35].

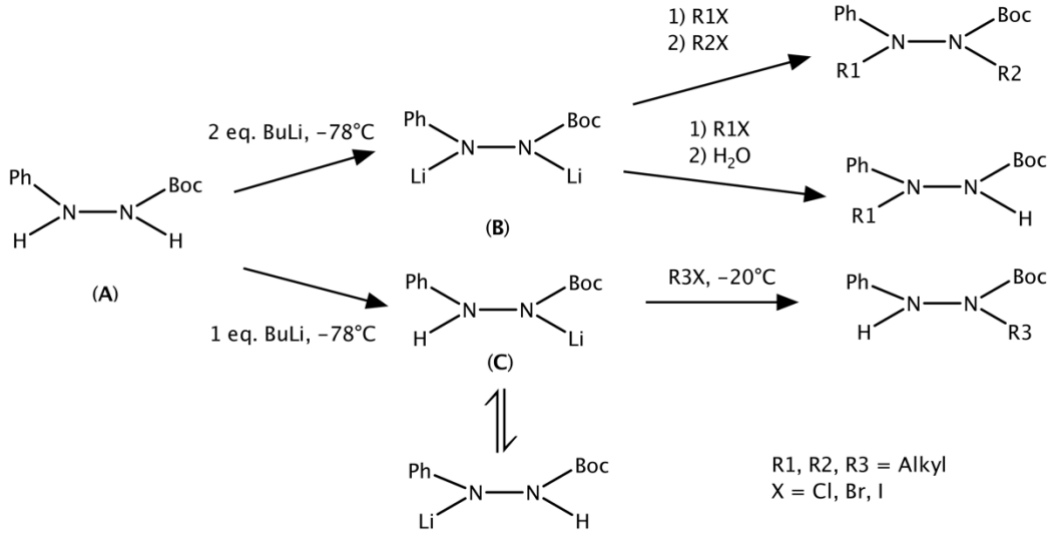


Scheme 11. Synthesis of N, N'-di-acyl-di-alkyl-hydrazine from a triprotected precursor.

1.5.4 Polyanions

The interplay of the electronic effects of hydrazine derivatives on their metalation and the subsequent selective alkylation stands at the heart of the polyanionic method. On treating substituted hydrazine derivatives with butyllithium, a very strong and nucleophilic base, in a dry and inert environment at exceedingly low temperatures, a stable polyanion is formed. Its multiplicity is controlled by the number of the equivalents of the base and the acidity of the nitrogen atom, and the species is alkylated fastest at the most nucleophilic and basic nitrogen [20].

To illustrate, PhNHNHBoc ((**A**), *Scheme 12*) was transformed into a dianion ((**B**), *Scheme 12*) on reacting it with 2 eq. of BuLi at -78°C and alkylated at -60°C either symmetrically with 2 eq. of an alkyl halide, or consecutively with two distinct electrophiles in one-pot fashion. The latter reaction is possible due to the difference in nucleophilicity of the nitrogen atoms connected to different substituents. Evidently, selective alkylation at the more nucleophilic nitrogen pertaining to the PhNH moiety would prove readily attainable by adding 1 eq. of an electrophile. Moreover, the more acidic and less nucleophilic BocNH moiety of the model compound was selectively alkylated via its monoanion ((**C**), *Scheme 12*), generated from PhNHNHBoc and 1 eq. of BuLi [4].



Scheme 12. Alkylation of mono- and dianions.

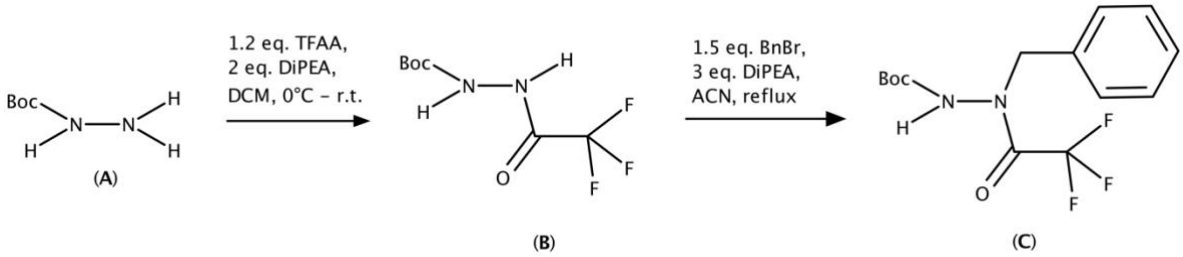
Despite its remarkable selectivity, the meticuluous temperature control, maintenance of inertness and dryness of the system, application of very strong, nucleophilic, moisture-sensitive and difficult-to-handle bases render this method ill-adapted for large-scale preparative procedures. These complications necessitate a much more reliable and robust method for regioselective alkylation of hydrazine derivatives.

1.5.5 Experiment design

Recently our group reported a method for regioselective benzylation of BocNHNHCOCF$_3$. Requisite of no exceptional conditions, it builds on the contrasting reactivity of the amide moieties and promises an exciting and convenient pathway to monoalkylated hydrazines. Trifluoroacetic moiety being one of the most electron-withdrawing groups [5], it attracts the electron density of the adjacent nitrogen onto itself. As a result, the nucleophilicity and basicity of the site are diminished, marking it a principal target for deprotonation by a much milder base, *viz.*, DiPEA than that discussed in the preceding section [36], [37] and a suitable negatively charged agent to perform the nucleophilic attack thereafter.

The synthesis starts from trifluoroacetylation of *tert*-butyl carbazate ((**A**), *Scheme 13*) by treating it with TFAA in the presence of DiPEA, the obtained N-Boc-N'-COCF$_3$-hydrazine ((**B**), *Scheme 13*) is alkylated with benzyl bromide on selective deprotonation by DiPEA, furnishing N-Boc-N'-COCF$_3$, N'-Bn-hydrazine ((**C**), *Scheme 13*) in 94% yield. DiPEA

preferentially deprotonates the trifluoroacetylated nitrogen due to pK_a compatibility and quarternization-preventing steric hindrance, unlike stronger bases such as Cs_2CO_3, K_2CO_3, NaH or *t*-BuOK, which produce disubstituted products by deprotonating both NH moieties. Pyridine was ascertained as too weak a base to prompt the reaction altogether [6].



Scheme 13. Regioselective benzylation of N-Boc-N'-$COCF_3$-hydrazine.

The uniqueness of the substrate is upheld by the scarcity of research examining its reactivity, much as that of the method, the breadth of the applicability of which remains obscure. The primary undertaking of the thesis lies in addressing the issues by investigating the reactivity of $BocNHNHCOCF_3$ with respect to acylating agents, ranging from acyl halides to activated esters, and exploring the scope of the method.

2 THE AIMS OF THE THESIS

The thesis explores the acylation of $BocNHNHCOCF_3$ and aims to investigate:

- The regioselectivity of the reaction;
- Acylation with carboxylic acid anhydrides, acyl halides, activated esters, benzyl chloroformate;
- The reaction pathway on the variation of certain parameters such as reagent proportions, base, solvent system and temperature.

3 EXPERIMENTAL PART

3.1 Instruments and techniques

TLC analyses were performed on a Sigma Aldrich silica gel plate 60 F254 UV 254 with a pore size of 60 Å and a UV lamp was used for vizualisation.

NMR ^{1}H and ^{13}C spectra measurements were performed on 700 MHz Bruker Avance-III instrument with $CDCl_3$ as a solvent and solvent residual signals as an internal reference.

HPLC analyses were conducted using a Shimadzu Prominence system fitted with a Phenomenex 2.6 μm (C18, 100 Å, 150 x 4.6 mm) column. Mobile phase composition comprised 0.1% TFA in water for phase A and ACN for phase B. 5-99% B (*Method 1*) or 10-99% B (*Method 2*) gradient elution programmes were employed, with the initial 5 minutes being held at constant composition and a 32-minute gradient to follow. The detection signal was monitored at 220 nm.

Column chromatography was performed using Merck Silica gel 60, mesh 70-230 (0.063-0.2 mm).

Solvent evaporation was performed on a BUCHI Rotavapor R-210 rotary evaporator and a Martin Christ Alpha 1-2 LD plus lyophilizer.

3.2 Physical properties of reagents and solvents

Compound	Molar mass, g/mol	Boiling point, °C	Melting point, °C	Density, g/ml	Refractive index
$BocNHNH_2$	132.16	63-65	39-42	1.020	1.4496
DCM	84.933	39.8	-95	1.327	1.4242
DiPEA	129.244	114	-	0.742	1.4138
TFAA	210.031	39.5	-65	1.490	1.2690
$KHSO_4$	136.169	-	200	2.320	-
NaCl	58.443	1465	802	2.170	-
Na_2SO_4	142.043	-	884	2.700	-

$BocNHNHCOCF_3$	212.17	-	130-132	-	-
Benzoyl chloride	140.567	201	-0.5	1.2120	1.5537
Cs_2CO_3	325.82	-	793	4.240	-
2,4,6-collidine	121.180	170	-44.3	0.9166	1.4959
NaH	23.998	-	638	1.390	-
EtOAc	88.106	77.1	-83.8	0.9003	1.3723
PE	-	90-100	-	0.77	1.428
Butanoyl chloride	106.551	101	-89.0	1.0277	1.4121
HBTU	379.25	-	200	-	-
Butanoic acid	88.106	163.7	-5.12	0.9528	1.3980
DCC	206.327	123	34.5	-	-
DIC	126.199	147	-	0.806	1.4320
HOBt x H_2O	153.14	-	155-158	-	-
NHS	115.088	-	96.3	-	-
p-nitrophenol	139.109	-	113.8	1.479	-
Pentachlorophenol	266.336	310	189.5	1.978	-
$CHCl_3$	119.378	61.2	-63.47	1.4788	1.4459
CbzCl	170.594	103	-	1.195	1.5190
Et_2O	74.121	34.4	-116.22	0.7138	1.3526

Table 1. Physical properties of reagents and solvents [5], [26], [38].

3.3 General synthetic procedures and characterization of synthesized substances

Samples for reaction monitoring were prepared by mixing a few drops of the reaction mixture with 1 ml of 0.1M $KHSO_4$ in an Eppendorf tube, extracting with 0.1 ml of EtOAc to analyze the organic layer via TLC.

HPLC samples were prepared by taking a few drops of the organic layer, evaporating EtOAc in an N_2 stream and dissolving the residue in 0.5 ml of ACN.

NMR samples were prepared by dissolving 30-50 mg of the analyte in 500 µl of $CDCl_3$.

3.3.1 Synthesis of N-Boc-N'-$COCF_3$-hydrazine

$BocNHNH_2$ (1 eq, 3.05 g) was weighed in a 250 ml round bottom flask equipped with a magnetic stirring bar, dissolved in 60 ml of DCM, the reaction flask was flushed with N_2, closed, placed in an ice and water bath over a stir plate, stirring started. DiPEA (2 eq, 8.2 ml) was added. TFAA (1.2 eq, 5.82 g) was separately dissolved in 30 ml of DCM, the solution transferred into a dropping funnel previously flushed with N_2 and closed with a rubber septum with an N_2 filled balloon. The funnel was installed over the flask and a dropwise addition of the contents was initiated, continuing for the next hour and a half. On the termination of the addition, the cold bath was removed, and the reaction mixture was allowed to warm up to room temperature for half an hour, all the while being stirred. The reaction mixture was analyzed by TLC, the plate (R_f(EtOAc/PE (1/2)) = 0.64) containing only one spot, that of the target compound. The mixture was then transferred into a 500 ml separatory funnel and diluted with 150 ml of DCM. The obtained solution was washed 5 times with 40 ml of 0.1 M $KHSO_4$, 30 ml of water, 20 ml of saturated NaCl solution. The collected water phase was extracted twice with 30 ml of DCM, the extracts washed with 20 ml of NaCl and combined with the main organic phase, dried over anhydrous Na_2SO_4, rotary evaporated and put into the refrigerator. The crystallized substance was transferred into a mortar, crushed into fine grains, transferred into a 100 ml round bottom flask and dried in high vacuum for 1.5 h.

(1) N-Boc-N'-$COCF_3$-hydrazine. 86% as a white solid, mp 125 °C (lit.[5] mp 130-132 °C), R_f(EtOAc/PE (1/2)) = 0.64. IR (cm^{-1}): 3291, 3191, 2988, 2911, 1737, 1687, 1503, 1367, 1292, 1241, 1205, 1154, 1133, 856, 730, 674. NMR ($CDCl_3$): ^1H (700 MHz) δ = 1.476 (s, 9H, (3xCH_3)), 6.774 (s, 1H, N-H(Boc)), 8.775 (s, 1H, N-H($COCF_3$)); ^{13}C (176 MHz) δ = 28.00, 83.38, 113.10, 114.73, 116.37, 118.00, 154.49, 156.42, 156.63, 156.86.

3.3.2 Syntheses with acyl chlorides

$BocNHNHCOCF_3$ (1 eq, 0.15 g (reactions 3A, 3B, 3C, 3D, 3E, 3G, 3H, 3I in *Table 3* and all reactions in *Table 4*), 0.075 g (reaction 3F)) was weighed in a 10 ml round bottom flask equipped with a magnetic stirring bar and dissolved in the solvent, specified for each experiment in *Table 3* and *Table 4*. The flask was put over a stir plate and stirring was started. Base (listed in *Table 3* and *Table 4*) and acyl chloride were sequentially added. The flask (a

cold water filled condenser installed on top in reaction 3F in *Table 3*) was closed with a rubber septum and the reaction was allowed to proceed at a chosen temperature (*Table 3* and *Table 4*). The reactions were monitored via TLC with EtOAc/Hex (1/2) as the eluent system and HPLC (*Method 1*). On termination, the reaction mixtures 3A in *Table 3* and 4A, 4C, 4E in *Table 4* were rotary evaporated at 40°C and partitioned between EtOAc and 0.1 M $KHSO_4$, 40 ml of each. The organic phase was washed with 25 ml of saturated NaCl solution, the water phase was extracted 3 times with 30 ml of EtOAc, the organic extracts were washed with 30 ml of saturated NaCl, combined with the main organic phase, dried over anhydrous Na_2SO_4 and rotary evaporated at 45°C. The crude mixture was purified by means of column chromatography using EtOAc/PE (1/2) system as eluent in reaction 3A (*Table 3*) or EtOAc/PE (1/4) system in reactions 4A, 4C, 4E (*Table 4*). The eluate was collected in 5 ml fractions and analyzed by TLC with EtOAc/PE (1/2) system as eluent in reaction 3A (*Table 3*) or EtOAc/PE (1/4) system in reactions 4A, 4C, 4E (*Table 4*). Subsequent rotary evaporation and lyophilization of the target fractions afforded two different compounds.

(2) N-Boc, N-Bz-N'-$COCF_3$-hydrazine. 83% as a white solid, mp 115-117 °C, R_f(EtOAc/PE (1/4)) = 0.47. IR (cm^{-1}): 3214, 3056, 2982, 1757, 1704, 1547, 1367, 1230, 1201, 1178, 1140, 969, 917, 835, 737, 698, 670. NMR ($CDCl_3$): ^1H (700 MHz) δ = 1.232 (s, 9H, (3xCH_3)), 7.433 (t, 2H, J = 7.7 Hz, Ar(H)), 7.554 (t, 1H, J = 7 Hz, Ar(H)), 7.686 (d, 2H, J = 7.7 Hz, Ar(H)), 8.968 (s, 1H, N-H($COCF_3$)); ^{13}C (700 MHz) δ = 27.41, 86.17, 113.10, 114.74, 116.38, 118.01, 128.40, 128.55, 132.68, 134.66, 150.16, 155.93, 156.15, 156.36, 156.58, 170.26.

(3) N-Boc, N-Bz-N'-Bz-hydrazine. 10% as a white solid, mp 119-127 °C (decomposed), R_f(EtOAc/PE (1/2)) = 0.5. IR (cm^{-1}): 3269, 2981, 1751, 1695, 1660, 1525, 1490, 1367, 1291, 1233, 1150, 974, 905, 853, 835, 807, 762, 718, 698, 670. NMR ($CDCl_3$): ^1H (700 MHz) δ = 1.226 (s, 9H, (3xCH_3)), 7.406-7.440 (m, 4H, Ar(H)), 7.522 (t, 2H, J = 7.7 Hz, Ar(H)), 7.779 (d, 2H, J = 7.7 Hz, Ar(H)), 7.849 (d, 2H, J = 7.7 Hz, Ar(H)), 8.507 (s, 1H, N-H(Bz)); ^{13}C (700 MHz) δ = 27.55, 84.93, 127.68, 128.28, 128.48, 128.86, 131.71, 132.05, 132.64, 135.87, 151.48, 166.68, 171.47.

(4) N-Boc, N-butanoyl-N'-$COCF_3$-hydrazine. 78% as a white solid, mp 68-72 °C, R_f(EtOAc/PE (1/4)) = 0.53. IR (cm^{-1}): 3255, 2975, 2941, 2882, 1788, 1750, 1698, 1538, 1460, 1373, 1285, 1247, 1202, 1130, 1034, 995, 916, 860, 765. NMR ($CDCl_3$): ^1H (700

MHz) δ = 0.966 (t, 3H, J = 7.7 Hz, CH_3), 1.498 (s, 9H, (3xCH_3)), 1.681 (sex., 6H, J = 7.7 Hz, CH_2), 2.908 (t, 2H, J = 7.7 Hz, CH_2), 8.530 (s, 1H, N-H($COCF_3$)); ^{13}C (176 MHz) δ = 13.63, 18.12, 27.76, 39.07, 85.91, 113.12, 114.75, 116.39, 118.02, 149.87, 155.29, 155.50, 155.72, 155.94, 172.81.

(5) N-Boc, N-butanoyl-N'-butanoyl-hydrazine. 35% as a white solid, mp 60-63 °C, R_f(EtOAc/PE (1/2)) = 0.54. IR (cm^{-1}): 3206, 3032, 2965, 2934, 2875, 1740, 1727, 1664, 1545, 1458, 1370, 1297, 1240, 1198, 1149, 1091, 1027, 984, 859, 842, 764. NMR ($CDCl_3$): ^1H (700 MHz) δ = 0.951 (t, 3H, J = 7.7 Hz, CH_3), 0.982 (t, 3H, J = 7.7 Hz, CH_3), 1.492 (s, 9H, 3xCH_3), 1.675 (sext, 2H, J = 7.42 Hz, CH_2), 1.706 (sext, 2H, J = 7.42 Hz, CH_2), 2.244 (t, 2H, J = 7.7 Hz, CH_2), 2.865 (t, 2H, J = 7.7 Hz, CH_2), 7.504 (s, 1H, N-H($CH_3CH_2CH_2C(O)$)); ^{13}C (176 MHz) δ = 13.75, 13.84, 18.26, 18.87, 27.96, 36.04, 39.15, 84.45, 151.39, 171.63, 173.67.

3.3.3 **Syntheses with activated esters**

A 10 ml round bottom flask was flame-dried and cooled in an N_2 stream in reactions 5B, 5C, 5D, 5E, 5F, 5G, 5H, 5J, 5K, 5L (*Table 5)*, for reactions 5A and 5I (*Table 5)*, a non-dried flask was used. The flask was equipped with a magnetic stirring bar, in reactions 5C and 5G (*Table 5)* a cold water filled condenser was installed on top, the system closed with a rubber septum, with an N_2-filled balloon installed on top, and put over a stir plate (and an oil bath equipped with a thermometer in reaction 5G (*Table 5*). Then, 4 ml of ACN were added to the flask and stirring was started. Carbodiimide or HBTU and butanoic acid were sequentially added, the mixture was allowed to stir, meanwhile in reactions 5D-5L (*Table 5)* the nucleophilic additive was weighed, added, pre-activation continued whilst $BocNHNHCOCF_3$ and DiPEA were dissolved in 2 ml of ACN in a separate vial. The obtained solution was added to the reaction flask, the reaction was allowed to proceed at a chosen temperature (r.t.-Δ). The process was monitored via TLC with EtOAc/PE (1/2), EtOAc/PE (1/4) or $CHCl_3$/EtOAc (7/1) as eluents and HPLC (*Method 1*). On termination, the reaction mixtures 5E, 5F, 5I in *Table 5* were centrifuged to discard the precipitate. The reaction mixtures or the supernatants collected were rotary evaporated at 40°C and partitioned between EtOAc and 0.1 M $KHSO_4$, 40 ml each. The organic phase was washed with 25 ml of saturated NaCl solution, the water phase with 3 times 30 ml of EtOAc, the organic extracts rinsed with 30 ml of saturated NaCl, combined with the main organic phase, dried over anhydrous Na_2SO_4 and rotary evaporated at 45°C. The crude

mixture was purified by the means of column chromatography using EtOAc/PE (1/4) system as eluent. The eluate was collected in 5 ml fractions, analyzed by TLC with the same eluent as employed in column chromatography and HPLC (*Method 1*). Subsequent rotary evaporation and lyophilization of the target fractions afforded N-Boc, N-butanoyl-N'-$COCF_3$-hydrazine. In experiments 5A 5D, 5E, 5F in *Table 5* the product was additionally purified via a similar protocol using $CHCl_3$/EtOAc (10/1) mixture as eluent.

(4) N-Boc, N-butanoyl-N'-$COCF_3$-hydrazine. 49% as a white solid, mp 68-72 °C, R_f(EtOAc/PE (1/4)) = 0.53. IR (cm^{-1}): 3255, 2975, 2941, 2882, 1787, 1751, 1698, 1537, 1460, 1372, 1285, 1246, 1201, 1136, 1034, 995, 916, 860, 764. NMR ($CDCl_3$): ^1H (700 MHz) δ = 0.959 (t, 3H, J = 7.7 Hz, CH_3), 1.491 (s, 9H, (3xCH_3)), 1.671 (sex., 6H, J = 7.7 Hz, CH_2), 2.899 (t, 2H, J = 7.7 Hz, CH_2), 8.675 (s, 1H, N-H($COCF_3$)); ^{13}C (176 MHz) δ = 13.61, 18.11, 27.74, 39.05, 85.89, 113.13, 114.76, 116.40, 118.03, 149.88, 155.33, 155.55, 155.76, 155.98, 172.89.

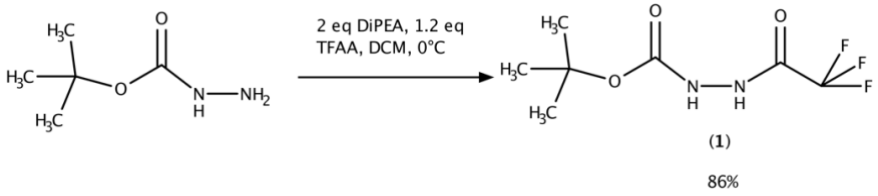
3.3.4 Synthesis of N-Boc, N-Cbz-N'-$COCF_3$-hydrazine

BocNHNHCOCF$_3$ (1 eq, 0.15 g) was weighed in a 10 ml round bottom flask equipped with a magnetic stirring bar and dissolved in 6 ml of ACN. The flask was put over a stir plate and stirring started. DiPEA (3 eq, 344 μl) and benzyl chloroformate (1.1 eq, 103 μl) were sequentially added. The flask was closed with a rubber septum and the reaction was allowed to proceed at room temperature for 9-10 hours. The process was monitored via TLC with $CHCl_3$/EtOAc (7/1) as eluent and HPLC (*Methods 1 and 2*). On termination, the reaction mixture was rotary evaporated at 40°C and partitioned between EtOAc and 0.1 M $KHSO_4$, 40 ml of each. The organic phase was washed with 25 ml of saturated NaCl solution, the water phase was extracted 3 times with 30 ml of EtOAc, the organic extracts were washed with 30 ml of saturated NaCl, combined with the main organic phase, dried over anhydrous Na_2SO_4 and rotary evaporated at 45°C. Column chromatography was performed three times using $CHCl_3$/EtOAc (7/1), $CHCl_3$/EtOAc (13/1), distilled Et_2O/PE (1/1) systems as eluents. Eluate was collected in 5 ml fractions, analyzed by TLC with the same eluent as employed in column chromatography and HPLC (*Methods 1 and 2*). Subsequent rotary evaporation and lyophilization of the target fractions afforded N-Boc, N-Cbz-N'-$COCF_3$-hydrazine in 51% yield.

(6) N-Boc, N-Cbz-N'-$COCF_3$-hydrazine. 51% as a white solid, mp 58-61°C, R_f(EtOAc/PE (1/2)) = 0.64. IR (cm^{-1}): 3224, 3039, 2983, 1799, 1780, 1750, 1533, 1457, 1372, 1281, 1250, 1206, 1154, 1141, 1095, 917, 845, 768, 695, 669. NMR ($CDCl_3$): ^1H (700 MHz) δ = 1.451 (s, 9H, (3xCH_3)), 5.199 (s, 2H, CH_2), 7.325 (m, 5H, Ar(H)), 8.915 (s, 1H, N-H($COCF_3$)); ^{13}C (176 MHz) δ = 27.67, 69.74, 85.87, 113.07, 114.70, 116.34, 117.97, 128.32, 128.72, 128.77, 134.55, 148.64, 150.97, 155.48, 155.69, 155.91, 156.13.

4 RESULTS AND DISCUSSION

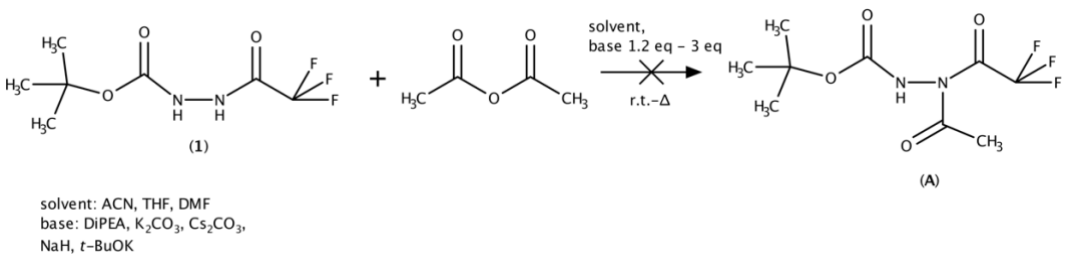
The study started with the preparation of BocNHNHCOCF$_3$ (**1**) ((**B**), *Scheme 14*) from *tert*-butyl carbazate according to the protocol described by Mastitski *et al.*, [6]. The synthesis was carried out at 0°C with a gradual addition of TFAA to prevent polysubstitution, and an excess of DiPEA and TFAA to reach completion.



Scheme 14. Synthesis of BocNHNHCOCF$_3$.

The series of acylations was commenced with the assumption of the introduced substituent eventually replacing the hydrogen at the nitrogen adjacent to the trifluoroacetyl group due to the increased acidity of the site, an extension of the results of the benzylation of BocNHNHCOCF$_3$ [6].

The synthesized substrate was first reacted with 1.5 eq of acetic anhydride at various temperatures, in the presence of different bases (DiPEA, K_2CO_3, Cs_2CO_3, NaH, t-BuOK) and in diverse solvents (ACN, DMF, THF) as is encapsulated in *Table 2*, and the reaction was monitored via TLC. The formation of the expected N-Boc-N'-COCF$_3$, N'-Ac-hydrazine ((**A**), *Scheme 15*) or its N-Ac isomer did not take place in all cases, a possible explanation being the weak reactivity of the acylating agent.



Scheme 15. Acetylation of BocNHNHCOCF$_3$.

Reaction	Base	Solvent	Temperature	Reaction time, h
2A	DiPEA, 3 eq	ACN, 6 ml	reflux	18.5
2B	K_2CO_3, 3 eq			24
2C	NaH, 1.2 eq (+1.2 eq 3 h in)	dry DMF, 2 ml	r.t. - 90°C	6.5
2D	t-BuOK, 1.2 eq (+1.2 eq 4.5 h in)	DMF, 2 ml	r.t. - 70°C	23
2E	t-BuOK, 1.2 eq	THF, 2 ml		4.5
2F	Cs_2CO_3, 1.5 eq (+1.5 eq 5.5 h in)	ACN, 6 ml	reflux	168
2G		DMF, 2 ml	85°C	

Table 2. Acetylation of BocNHNHCOCF$_3$.

Benzoylation of the substrate under the conditions specified in *Table 3* had surprisingly produced N-Boc, N-Bz-N'-COCF$_3$-hydrazine ((**2**), Scheme 16) instead of the expected N-Boc-N'-COCF$_3$, N'-Bz-hydrazine and N-Boc, N-Bz-N'-Bz-hydrazine ((**3**), Scheme 16) as a product of overacylation. Application of a greater excess of benzoyl chloride results in an increase of N-Boc, N-Bz-N'-Bz-hydrazine (**3**) content in the reaction mixture, as was demonstrated most prominently in reactions 3C and 3F in *Table 3*. DiPEA was found to be the most suitable base and, drawing on the results of the benzylation experiments [6], it may be concluded that its compatibility with N-Boc-N'-COCF$_3$-hydrazine is the only requisite parameter as per the choice of the deprotonating agent that determines the outcome of the reaction. It would take as little as 1.5 h to generate the target compound (**2**) in 83% yield in reaction 3A, since the acylating agent is extremely reactive.

Reaction	Acylating agent eq	Base	Solvent	Conditions	Reaction time, h	Product by HPLC, 5-99% B	Yield of isolated products
3A	1.1		ACN, 6 ml		1.5	-	ω(**2**) = 83%,

		DiPEA, 3 eq		Room temperature			ω(**3**) = 10%
3B					5	70.0% (**2**), 12.3% (**3**)	*
3C	1.5				2	47.7% (**2**), 43.2% (**3**)	*
3D	1.05		$CHCl_3$, 6 ml		2.5	84.1% (**2**), 3.4% (**3**)	*
3E					1	79.2% (**2**), 5.1% (**3**)	*
3F	2	DiPEA, 4 eq	ACN, 3 ml		1	31.0% (**2**), 48.1% (**3**)	*
3G	1.1	Cs_2CO_3, 1.5 eq	ACN, 6 ml		5 h	62.9% (**2**), 7.4% (**3**)	*
3H	1.1	2,4,6-collidine, 3 eq	ACN, 6 ml		5 h	59.5% (**2**), 16.5% (**3**)	*
3I	1.05 (+ 0.5 eq 3.5 h since the start)	NaH, 1.2 eq	dry DMF, 6 ml		4.5 h	9.0% (**2**), 1.2% (**3**)	*

Table 3. Benzoylation of $BocNHNHCOCF_3$ with benzoyl chloride.

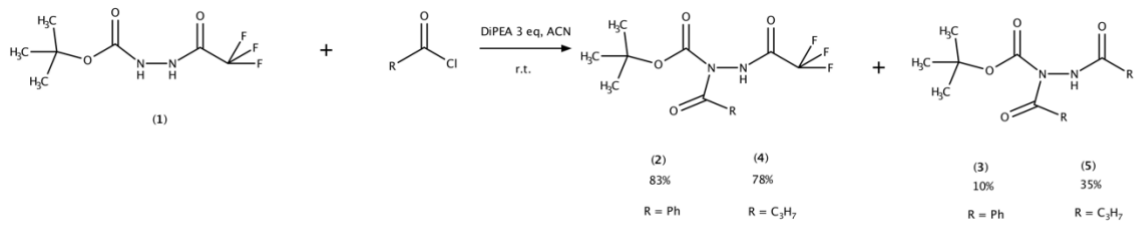
Reactions marked with "" were only monitored by HPLC.*

Butanoylation was carried out via the use of butanoyl chloride, the summary of the experiments presented in *Table 4*. Similarly to benzoylation, butanoyl chloride would furnish N-Boc, N-butanoyl-N'-$COCF_3$-hydrazine ((**4**), *Scheme 16*) and N-Boc, N-butanoyl-N'-butanoyl-hydrazine ((**5**), *Scheme 16*) over a span of at most 2 hours on account of its great reactivity, with (**4**) being isolated in good yields, and (**5**) present in a higher percentage on an increase of the quantity of the acylating agent employed.

Reaction	Acylating agent eq	Base	Conditions	Reaction time, h	Product by HPLC, 5-99% B	Yield of isolated products
4A	1.1	DiPEA, 3 eq	Room temperature	2	85.3% (**4**), 8.2% (**5**)	ω(**4**) = 74%
4B				27	84.6% (**4**), 10.5% (**5**)	*
4C			Reflux	0.7	77% (**4**), 10.5% (**5**)	ω(**4**) = 74%, ω(**5**) = 8%
4D		2,4,6-collidine, 3 eq	Room temperature	23	78.4% (**4**), 10% (**5**)	*
4E	1.5	DiPEA, 3 eq	Room temperature	1	68.6% (**4**), 29.9% (**5**)	ω(**4**) = 78%, ω(**5**) = 35%

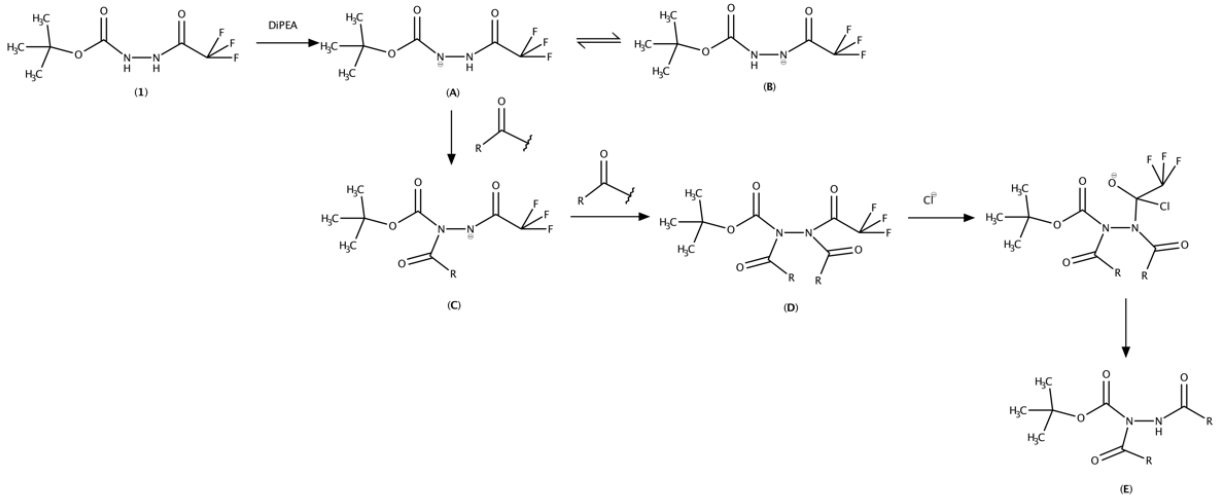
Table 4. Butanoylation of BocNHNHCOCF$_3$ with butanoyl chloride.

Reactions marked with "" were only monitored by HPLC.*



Scheme 16. Acylation of BocNHNHCOCF$_3$ via benzoyl and butanoyl chloride.

The tendency of the attachment of an acyl substituent at the nitrogen adjacent to the Boc group would carry on into the rest of the experiments. The electronic effects at play on acylation of the substrate being rather diverse from those occurring on alkylation, it can be hypothesized that the nucleophilicity of the anions (**A**) and (**B**) in *Scheme 17* comprising an equilibrium upon deprotonation is the deciding, rate-determining factor in our case, a situation reminiscent of that of polyanions [20]. Despite the facilitated deprotonation of the trifluoroacetylated nitrogen, its electronic lone pair is drawn onto the protecting group and is rather strongly incorporated into its electronic cloud on account of the electron-withdrawing nature of the fluorine atoms. Conversely, Boc group renders the electronic lone pair at the adjacent nitrogen more spatially and energetically available by not binding it onto itself quite as tightly, thereby increasing the efficacy of the nucleophilic attack. Thus, the more nucleophilic anion ((**A**), *Scheme 17*) is the first one to react with the acylating agent. Whenever a significant excess of the acylating agent is present, it reacts with the monoacylated anion ((**C**), *Scheme 17*), displacing the hydrogen at the trifluoroacetylated nitrogen, a step majorly impacted by the steric hindrance of the tetrahedral intermediate and the reactivity of the acylating agent. Once the species ((**D**), *Scheme 17*) adjoins some nucleophile at the carbonyl carbon, N-Boc, N-C(O)R-N'-C(O)R-hydrazine ((**E**), *Scheme 17*) acts as a leaving group.

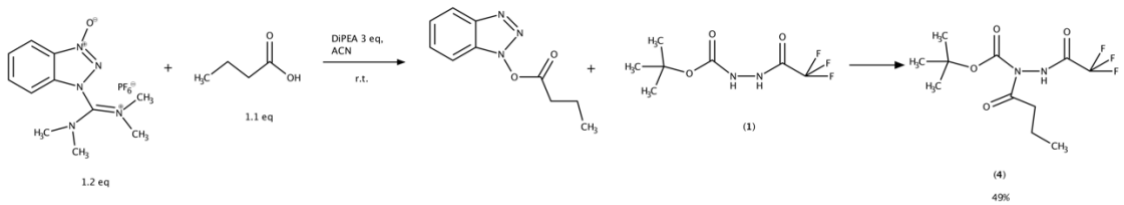


Scheme 17. Proposed acylation pathway.

As it will be best elucidated in the discussion of the results to follow, the geometries of the substrate and the acylating agent must be compatible and the reactivity of the acylating agent is directly correlated with product yields.

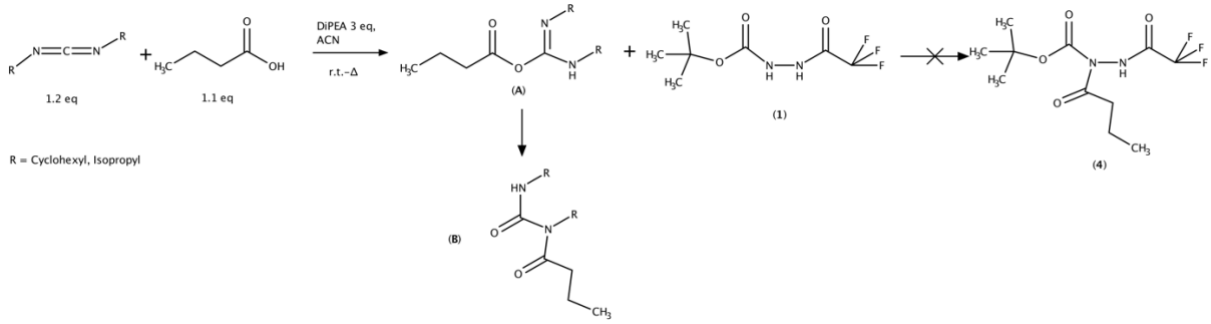
Butanoylation was additionally attempted via activated esters. Activated esters are those highly susceptible to nucleophilic attack due to the presence of good RO^- leaving groups, typically generated from carboxylic acids and coupling reagents such as carbodiimides or uronium salts, *viz.*, HBTU, often in the presence of a nucleophilic additive that catalyzes the activation. The use of activated esters is ubiquitous in peptide synthesis, the reason being their weaker reactivity as compared to that of acyl halides [11], [27], [39].

Acylation via a HBTU-activated ester, alternatively, would result in (**4**) obtained in 49% yield, identical to the product of acylation of $BocNHNHCOCF_3$ with butanoyl chloride as verified via NMR and IR spectroscopy, *Scheme 18* illustrating the pathway. The diacylated product is likely not forming due to the amplified steric hindrance and weaker reactivity of the acylating agent not allowing it to substitute the remaining free hydrogen in view of the instability of the tetrahedral intermediate.



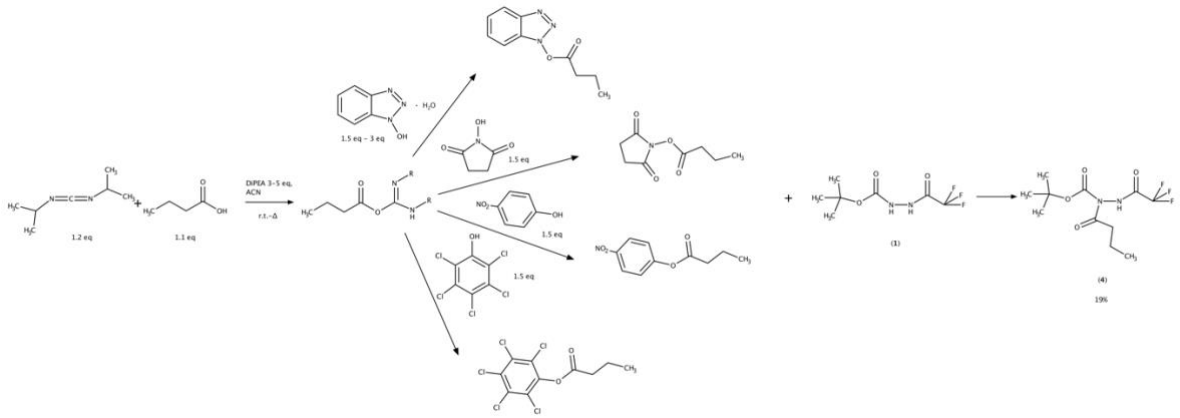
Scheme 18. Acylation of BocNHNHCOCF₃ via a HBTU-activated ester.

The esters generated from carbodiimides alone would prove inefficient acylating agents, to an extent owing to their low reactivity, and the reactions captured in *Scheme 19* would not take place. If the reaction of the formed O-acyl isourea ((**A**), *Scheme 19*) with sterically hindered BocNHNHCOCF₃ ((**1**), *Scheme 19*) is slow, then the irreversible rearrangement of the former into unreactive N-acyl urea ((**B**), *Scheme 19*) annihilates the majority of the acylating agent.



Scheme 19. Acylation of BocNHNHCOCF₃ via carbodiimide-activated esters.

The presence of catalytic amounts of nucleophilic additives suppresses the formation of N-acyl urea by rapidly reacting with O-acyl isourea to generate the activated ester [40]. In this work, several of such additives were employed with DIC, namely HOBt x H_2O, NHS, p-nitrophenol or pentachlorophenol, the reactions presented in *Scheme 20*, the most successful one being HOBt x H_2O. HOBt-activated ester is more reactive than the NHS one [41], and it may well be that its spatial geometry and leaving-group ability are the most favourable of the series. The steric hindrance of pentachlorophenol ester impedes its interaction with the hydrazine [42], whereas the enhanced conjugation within the molecule of p-nitrophenol ester decreases its nucleophilicity. Despite the formed ester being identical to the HBTU-activated one, the yields of (**4**) in those reactions were less than 20%. Several reasons for this might be theorized, such as solvent effects, the instability of the ester or its generation being slower than that of N-acyl urea.



Scheme 20. Acylation of BocNHNHCOCF₃ via DIC-activated ester intermediates and catalytic amounts of activating agents.

The addition of HOBt x H_2O to the activation reaction via HBTU was rather peculiarly found to almost hinder the reaction, with the percentage of the HPLC detected product being twice as low as in the reaction using HBTU exclusively. The result may be attributed to the hydrolysis of the activated ester on account of the increased water content in the system imparted by HOBt x H_2O.

Reaction	Acylating agent	Base	Conditions	Reaction time, h	Product by HPLC, 5-99% B	Yield of isolated products
5A	HBTU, 1.2eq butanoic acid, 1.1 eq	DiPEA, 3 eq	Room temperature, inert atmosphere	32	44.7% (**4**)	ω(**4**) = 49%
5B	DCC, 1.2 eq butanoic acid, 1.1 eq		Room temperature, dry and inert atmosphere	22	4% (**4**)	*

5C	DIC, 1.2 eq butanoic acid, 1.1 eq		r.t.- Δ, dry and inert atmosphere	72	4% (**4**)	*
5D	HOBt x H_2O, 1.5 eq DIC, 1.2 eq butanoic acid, 1.1 eq	DiPEA, 3 eq	Room temperature, dry and inert atmosphere	67.5	33.1% (**4**)	ω(**4**) = 15%
5E				69	35.1% (**4**)	ω(**4**) = 15%
5F		DiPEA, 5 eq		51	32.7% (**4**)	ω(**4**) = 19%
5G			Oil bath at 50°C, dry and inert atmosphere	50	0% (**4**)	*
5H	HOBt x H_2O, 3 eq DIC, 1.2 eq butanoic acid, 1.1 eq	DiPEA, 5 eq	Room temperature, dry and inert atmosphere	125	13.4% (**4**)	*
5I	HOBt x H_2O, 1.2 eq HBTU, 1.2 eq butanoic acid, 1.1 eq		Room temperature, inert atmosphere	51	21.4% (**4**)	*
5J	NHS, 1.5 eq DIC, 1.2 eq butanoic acid, 1.1 eq	DiPEA, 3 eq	Room temperature, dry and inert atmosphere	24	0% (**4**)	*
5K	*p*-nitrophenol, 1.5 eq	DiPEA, 3 eq		72	13.4% (**4**)	*

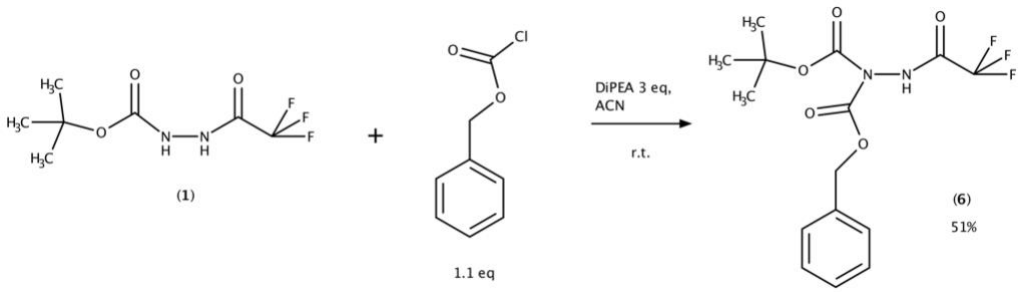
	DIC, 1.2 eq butanoic acid, 1.1 eq					
5L	Pentachlorophenol, 1.5 eq DIC, 1.2 eq butanoic acid, 1.1 eq	DiPEA, 3 eq		74	0.9% (**4**)	*

Table 5. Butanoylation of $BocNHNHCOCF_3$ via activated esters.

Reactions marked with "" were only monitored by HPLC.*

Generally, the activated ester approach is less effective, on account of prolonged reaction times and low reactivity of the acylating agents, and less convenient, in view of the necessity to maintain dry and inert conditions to ensure the stability of carbodiimides.

The Cbz-moiety was successfully incorporated into the N(Boc) site, furnishing N-Boc, N-Cbz-N'-$COCF_3$-hydrazine ((**6**), *Scheme 21*) in fair yields. On speculation, N-Boc, N-Cbz-N'-Cbz-hydrazine was present in chromatograms however isolated in minute quantities and its identity could not be confirmed by NMR. The manifest steric hindrance of the acylating agent and the resulting monoacylated product might have suppressed overacylation reactions.



Scheme 21. Acylation of $BocNHNHCOCF_3$ via CbzCl.

SUMMARY

The thesis aimed to elucidate the possibility and outcome of acylation of $BocNHNHCOCF_3$, the scope of the applicability of the method to various acylating agents including anhydrides, active esters, acyl chlorides and benzyl chloroformate.

Firstly, acylation of $BocNHNHCOCF_3$ with acetic anhydride had proven unsuccessful irrespective of the solvent, base, temperature or reaction time owing to the low reactivity of the species.

Subsequent experimentation with reactive benzoyl and butanoyl chlorides allowed for a swift conversion of the starting compound into a mono- (N-Boc, N-C(O)R-N'-$COCF_3$-hydrazine) and diacylated (N-Boc, N-C(O)R-N'-C(O)R-hydrazine) product, in very good and poor yields respectively. Increasing the amount of the acyl chloride would increase the yield of N-Boc, N-C(O)R-N'-C(O)R-hydrazine.

Activated esters were generated *in situ* for butanoylation of $BocNHNHCOCF_3$. Without the implementation of a nucleophilic additive, HBTU allowed for the highest conversion of the substrate into the monoacylated product obtained in fair yield, whereas carbodiimides afforded none. Carbidiimides in combination with HOBt x H_2O generated the desired product in quantities sufficient for its isolation, yet the yields remained poor.

Acylation of $BocNHNHCOCF_3$ by benzyl chloroformate resulted in the incorporation of the Cbz group into the N(Boc) moiety, the product being obtained in fair yield. The steric hindrance of the tetrahedral intermediate affected the yield of the monoacylated product and did not allow for diacylation.

Overall, the method promises a selective pathway to monoacylated hydrazines via reactive acylating agents such as acyl halides, with the substitution occurring at the most nucleophilic nitrogen. Nearly equimolar proportions of such reagents with respect to the protected hydrazine are conducive to clean monoacylations. The reaction proceeding within the scope of an hour and at normal conditions, it could be readily extended to preparative procedures.

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APPENDIX

I. NMR supplement code table

Compound code	Compound name	NMR code
(**1**)	N-Boc-N'-$COCF_3$-hydrazine	KK64
		KK17
(**2**)	N-Boc, N-Bz-N'-$COCF_3$-hydrazine	KK35 6-10
(**3**)	N-Boc, N-Bz-N'-Bz-hydrazine	KK35 13-18
(**4**)	N-Boc, N-butanoyl-N'-$COCF_3$-hydrazine	KK46 (BnCl)
		KK55 (activated ester)
(**5**)	N-Boc, N-butanoyl-N'-butanoyl-hydrazine	KK49 7-13
(**6**)	N-Boc, N-Cbz-N'-$COCF_3$-hydrazine	KK53 7-12 7-9

II. NMR Spectra

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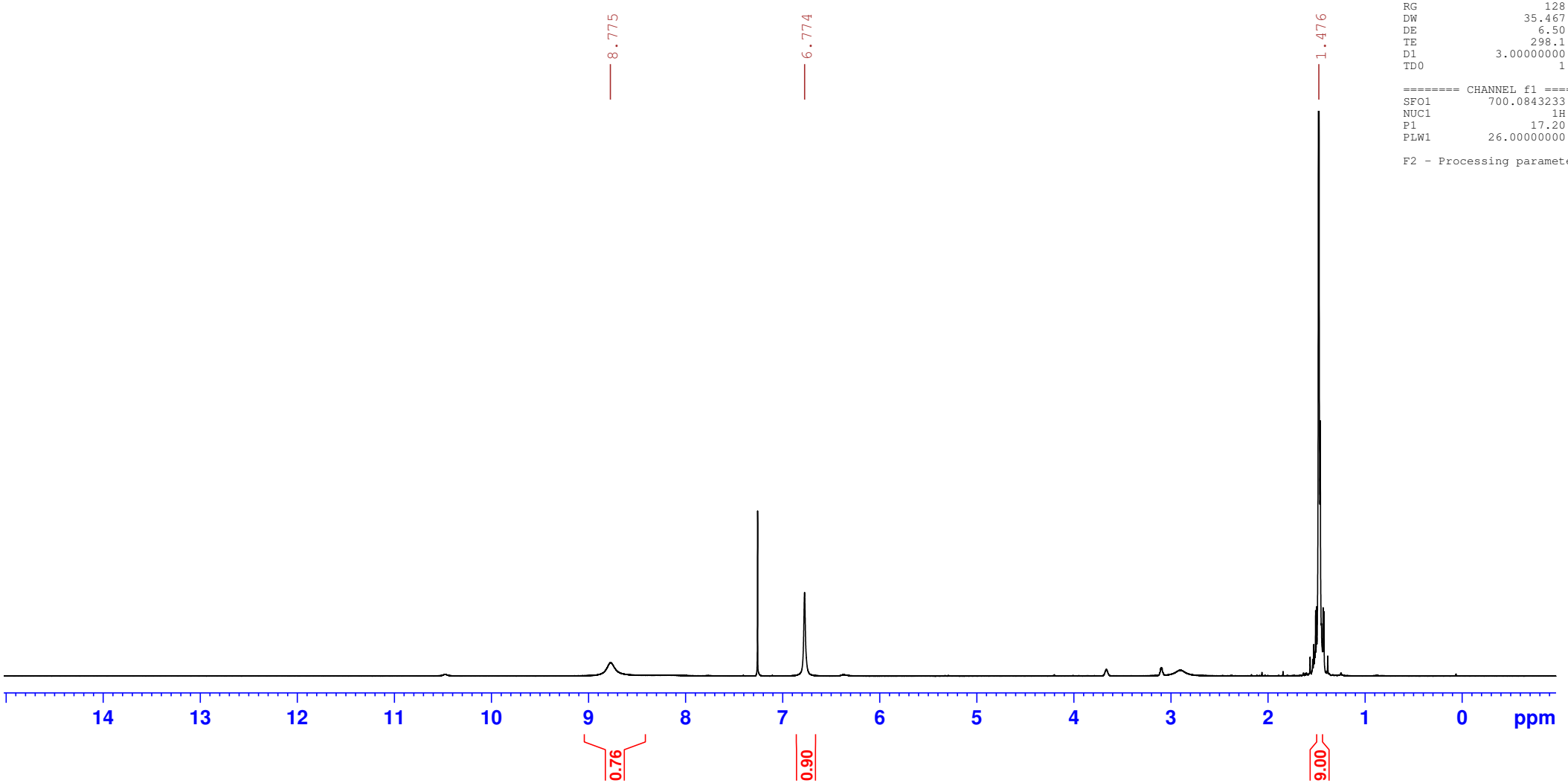


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TE                   298.1 K
D1               3.00000000 sec
TD0                      1

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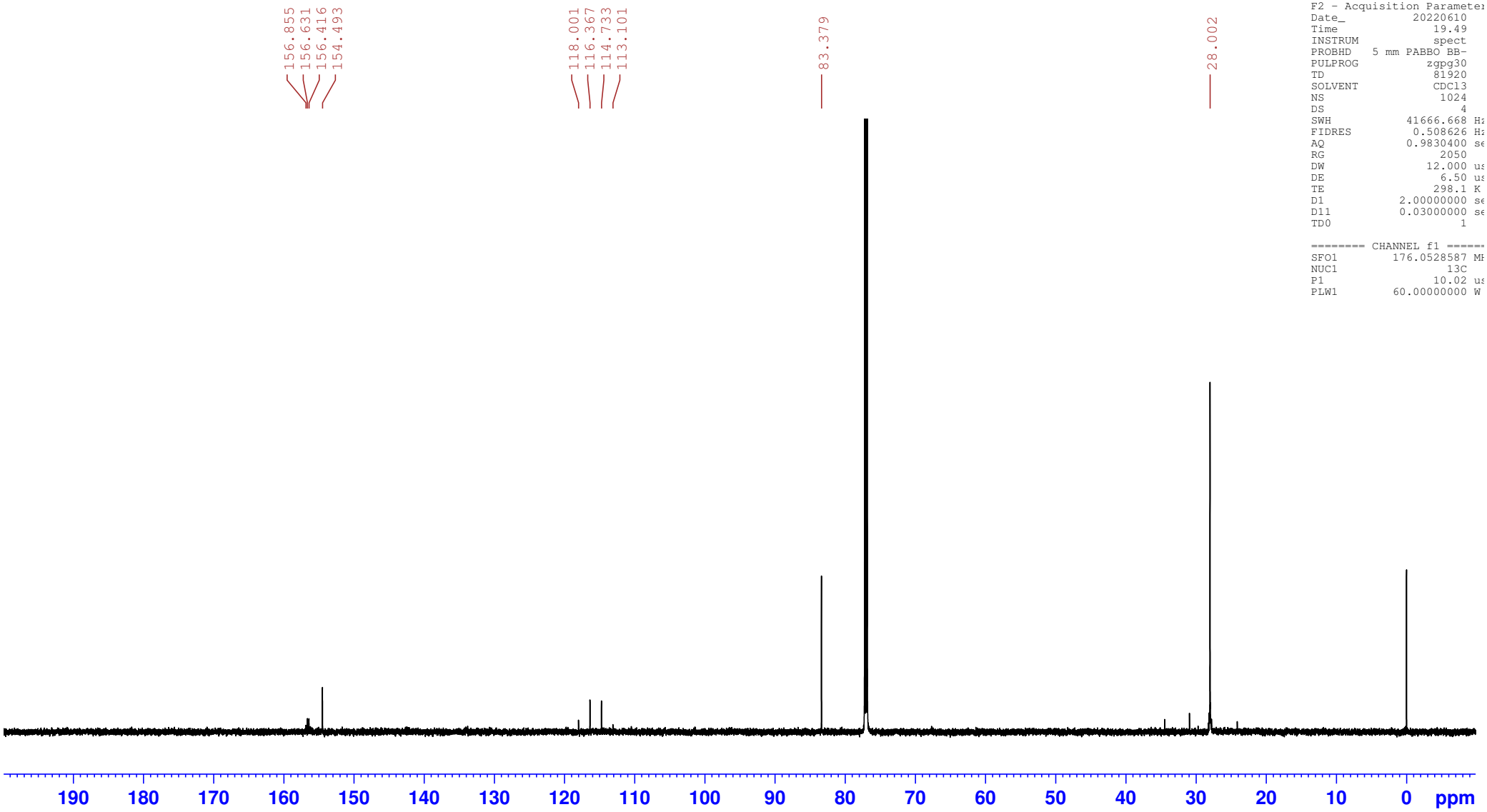

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D11 0.03000000 se
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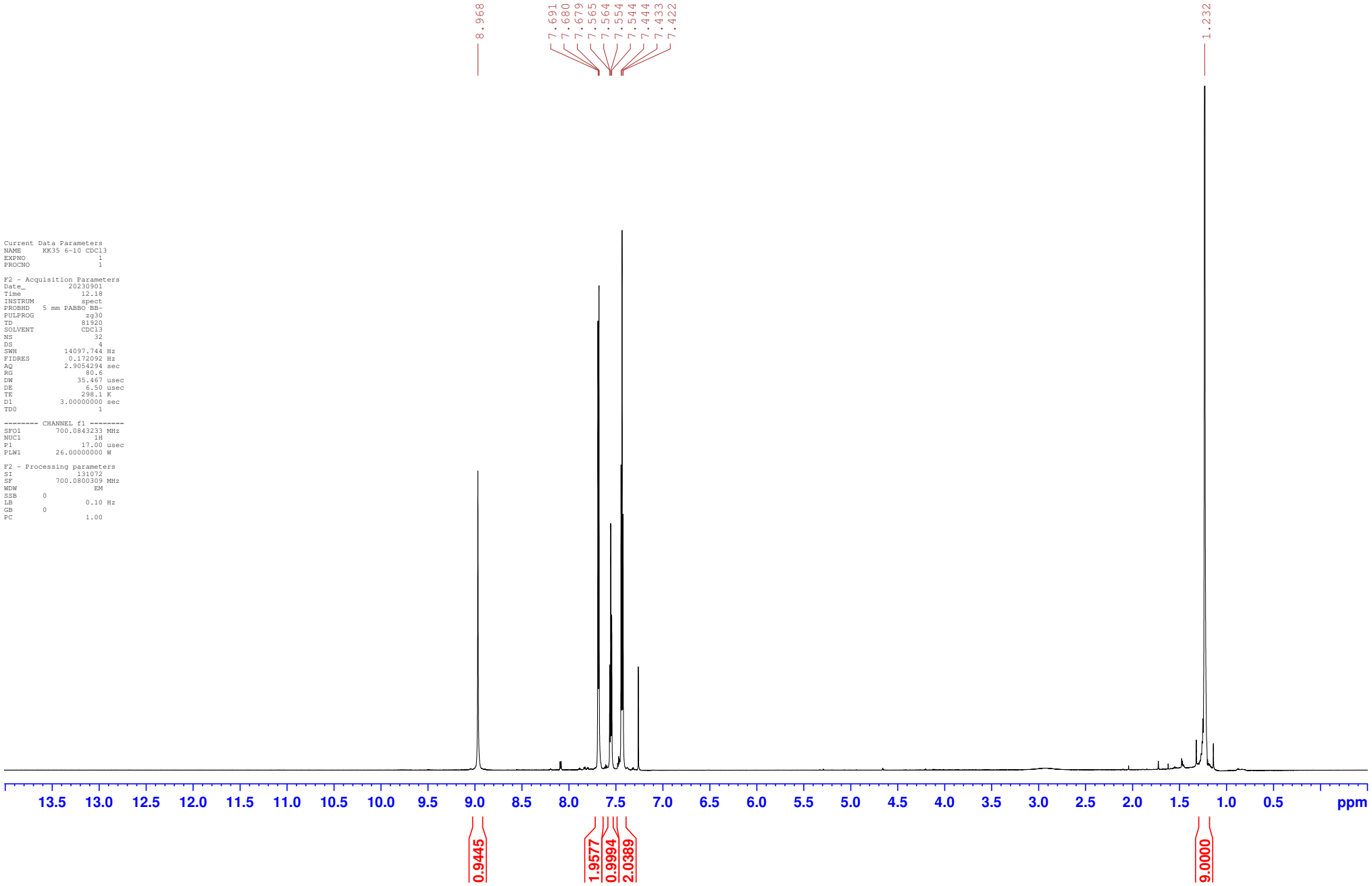


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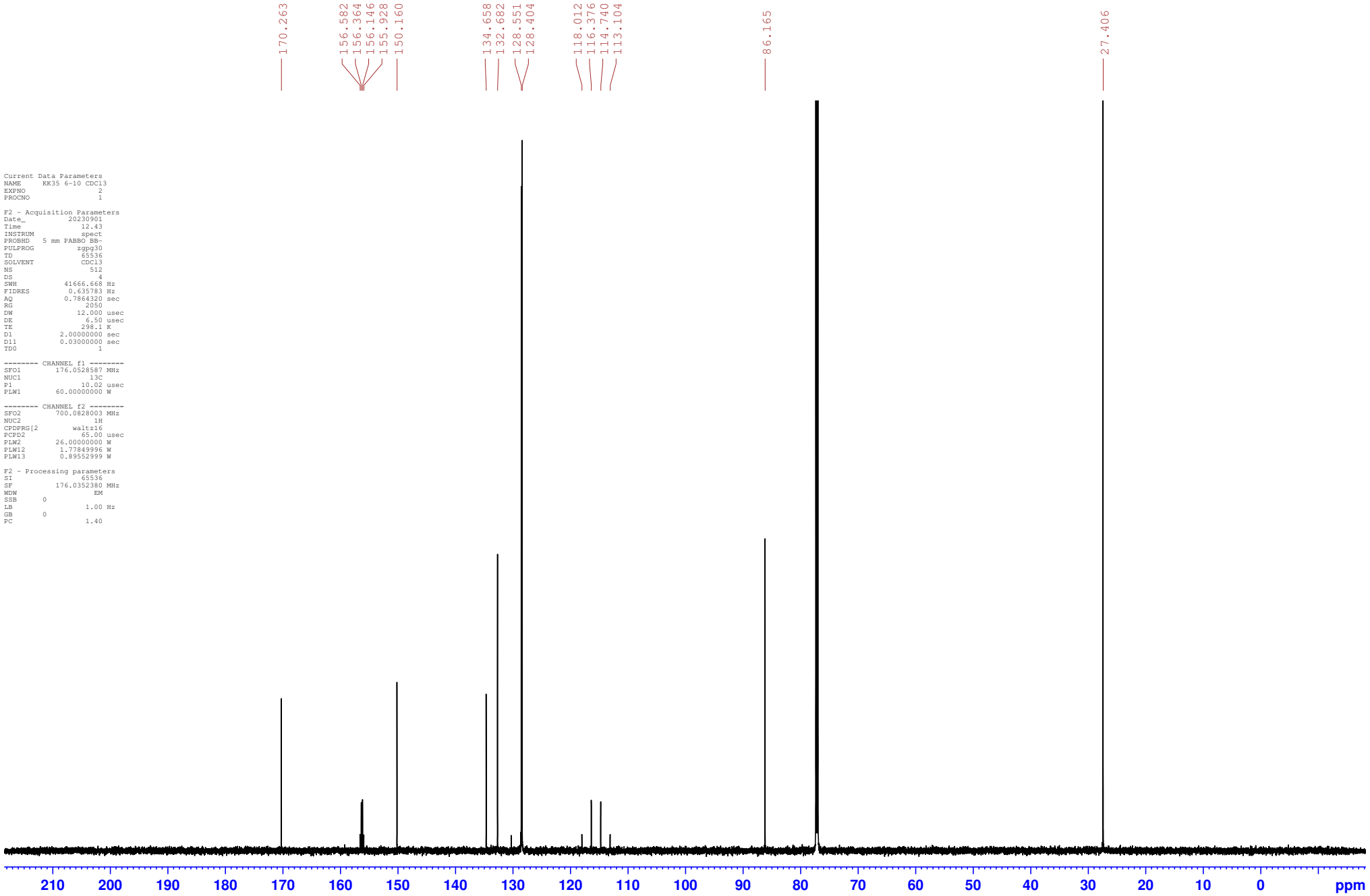


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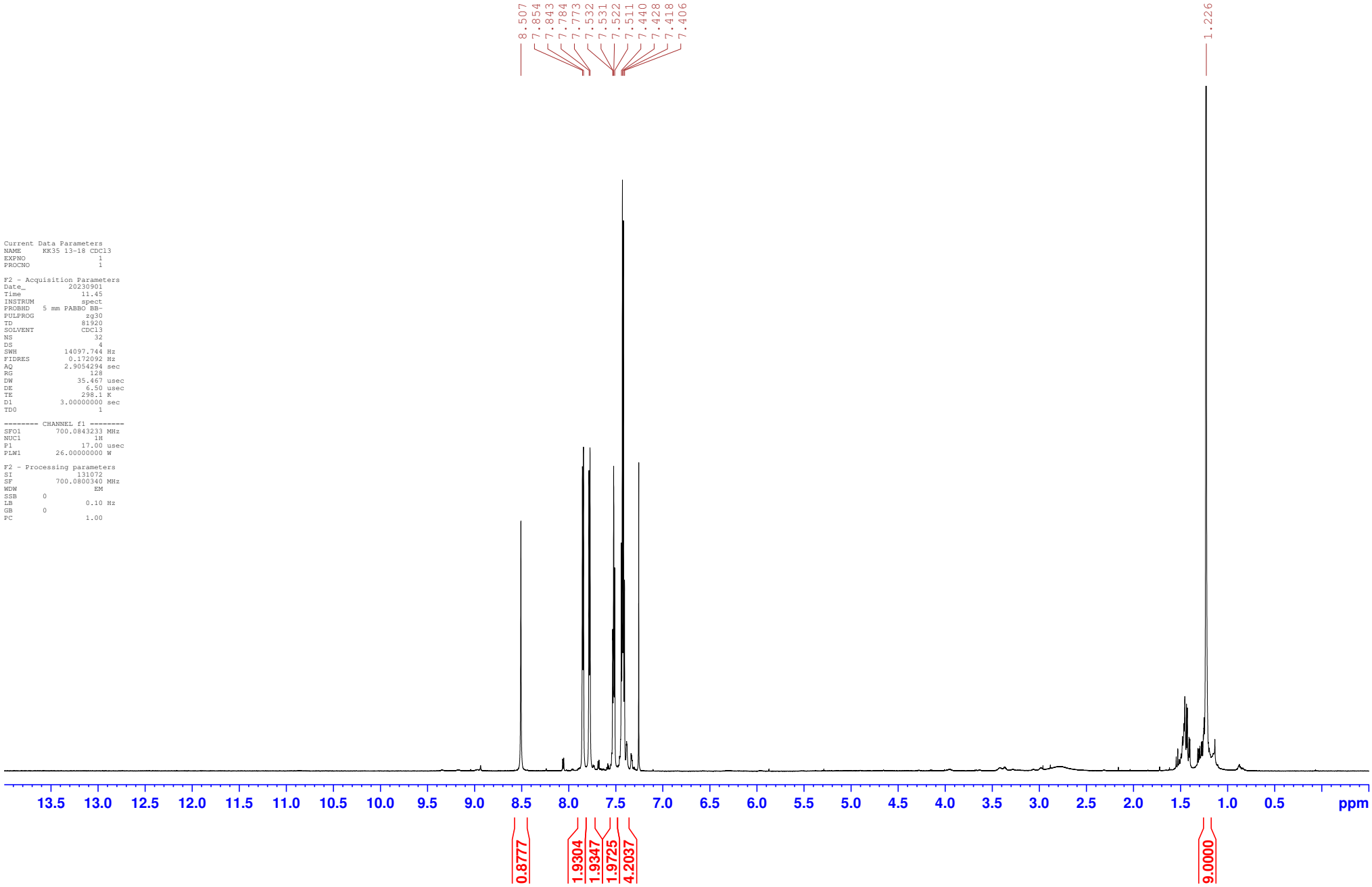


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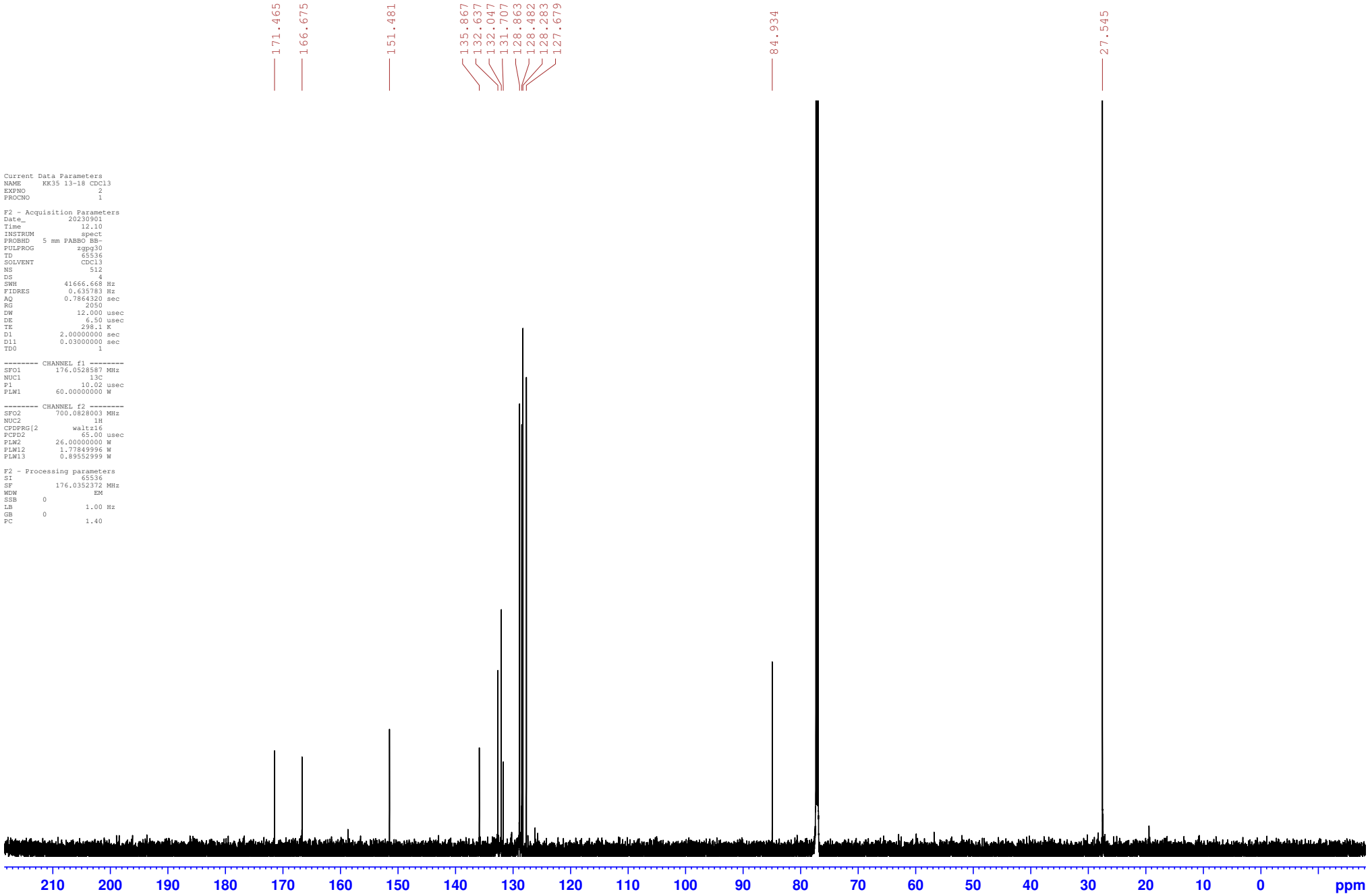


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PROCNO                   1

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FIDRES         0.635783 Hz
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DW               12.000 usec
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F2 - Processing parameters
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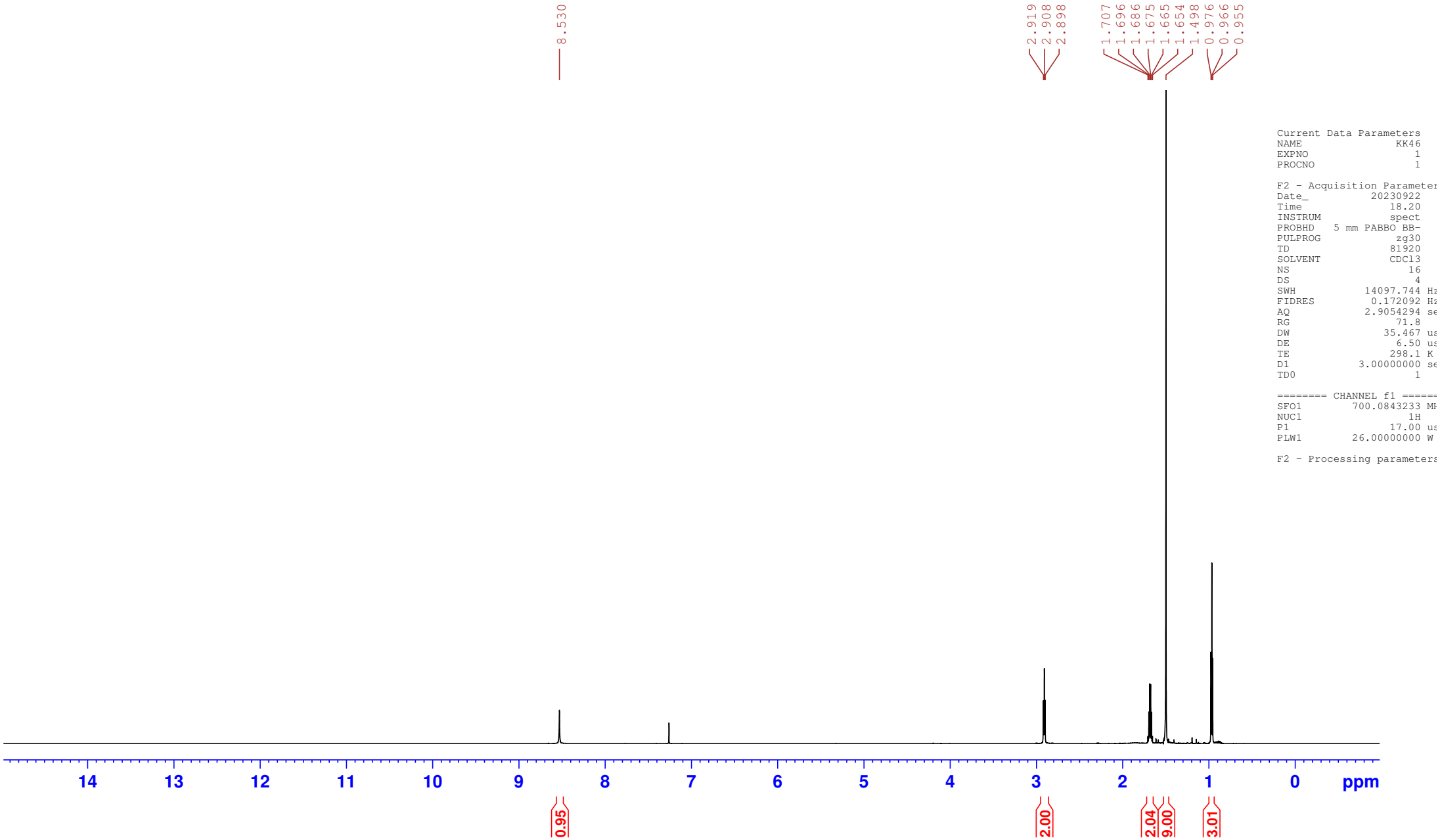
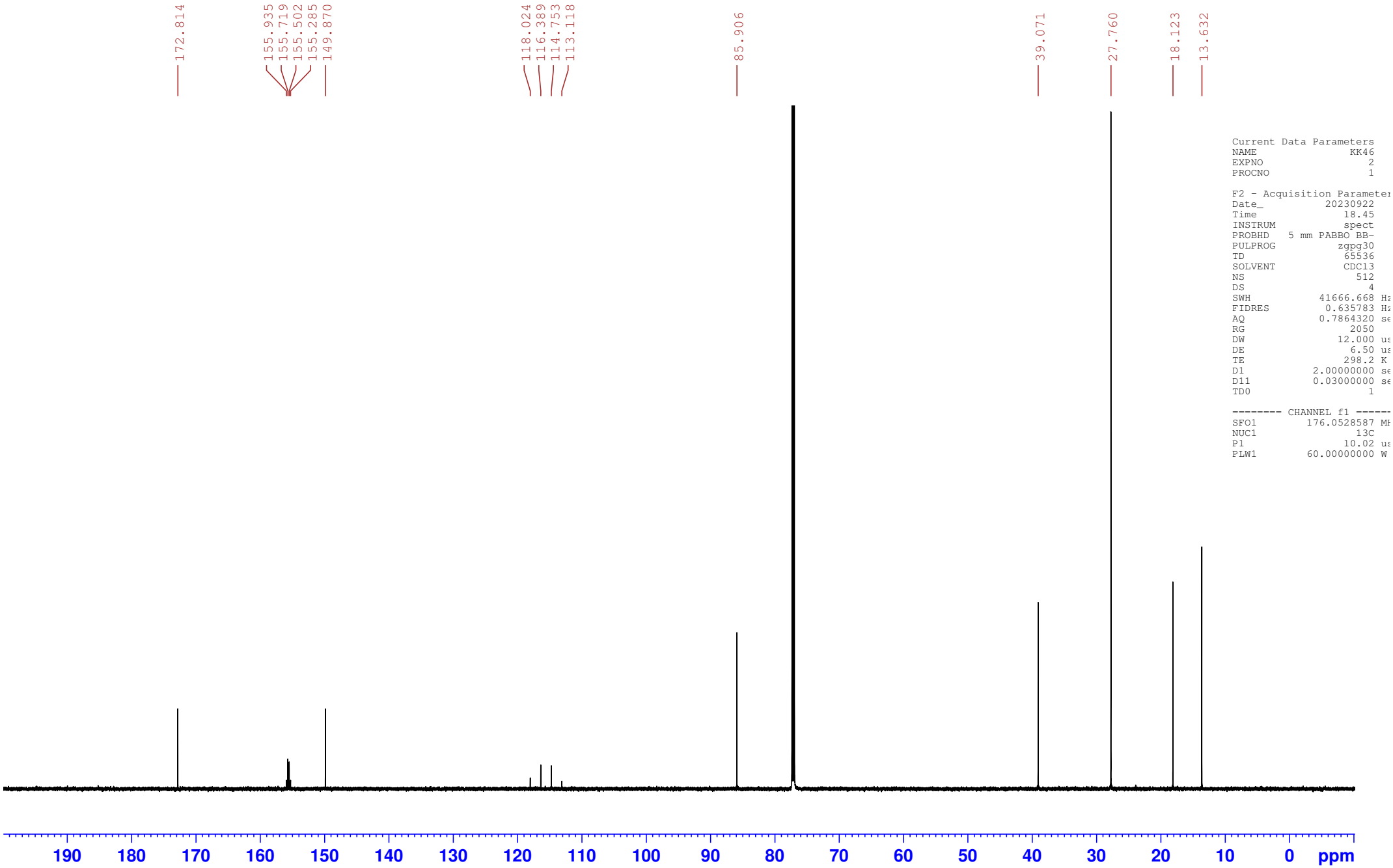


KK46 13C CDCl3
C13CPD CDCl3 {C:\Spectra\data\AntonM\nmr} AntonM 7

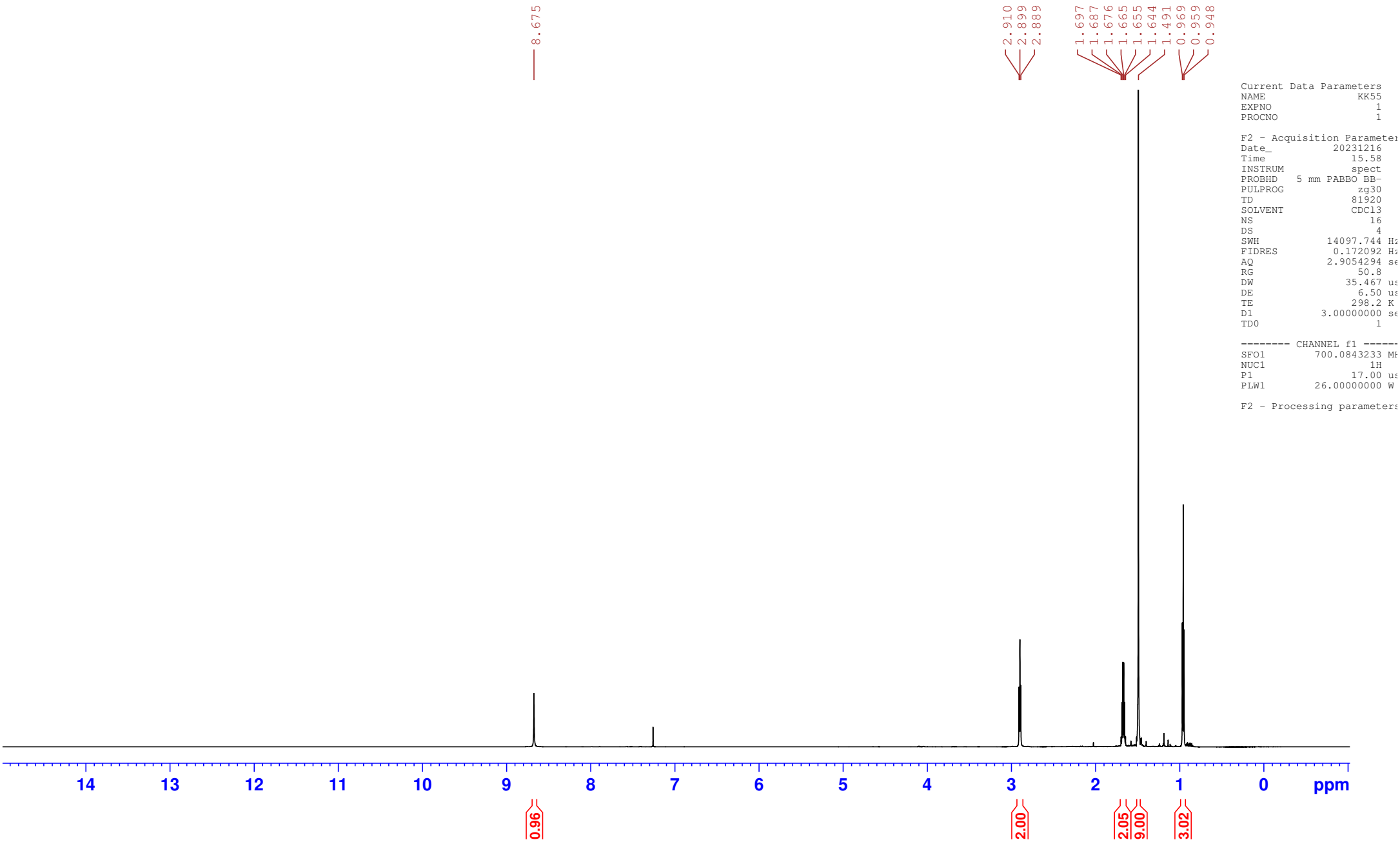


Current Data Parameters
NAME KK46
EXPNO 2
PROCNO 1

F2 - Acquisition Parameter
Date_ 20230922
Time 18.45
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 512
DS 4
SWH 41666.668 Hz
FIDRES 0.635783 Hz
AQ 0.7864320 se
RG 2050
DW 12.000 us
DE 6.50 us
TE 298.2 K
D1 2.00000000 se
D11 0.03000000 se
TD0 1

======== CHANNEL f1 ======
SFO1 176.0528587 MH
NUC1 13C
P1 10.02 us
PLW1 60.00000000 W

KK55 CDCl3 1H
PROTON CDCl3 {C:\Spectra\data\AntonM\nmr} AntonM 56



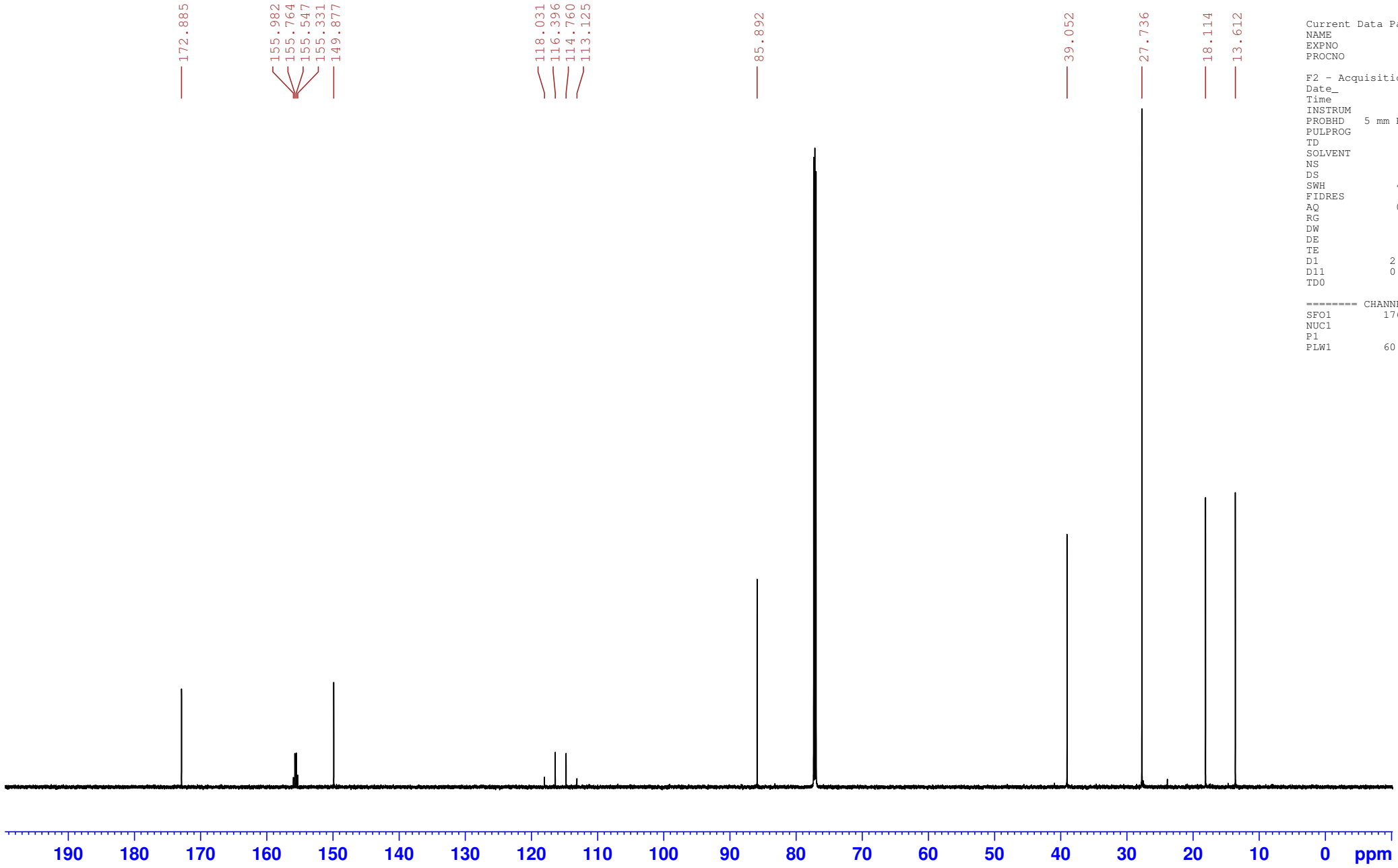
Current Data Parameters
NAME KK55
EXPNO 1
PROCNO 1

F2 - Acquisition Parameter
Date_ 20231216
Time 15.58
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 81920
SOLVENT CDCl3
NS 16
DS 4
SWH 14097.744 Hz
FIDRES 0.172092 Hz
AQ 2.9054294 se
RG 50.8
DW 35.467 us
DE 6.50 us
TE 298.2 K
D1 3.00000000 se
TD0 1

======== CHANNEL f1 ======
SFO1 700.0843233 MH
NUC1 1H
P1 17.00 us
PLW1 26.00000000 W

F2 - Processing parameters

KK55 CDCl3 13C
C13CPD CDCl3 {C:\Spectra\data\AntonM\nmr} AntonM 56

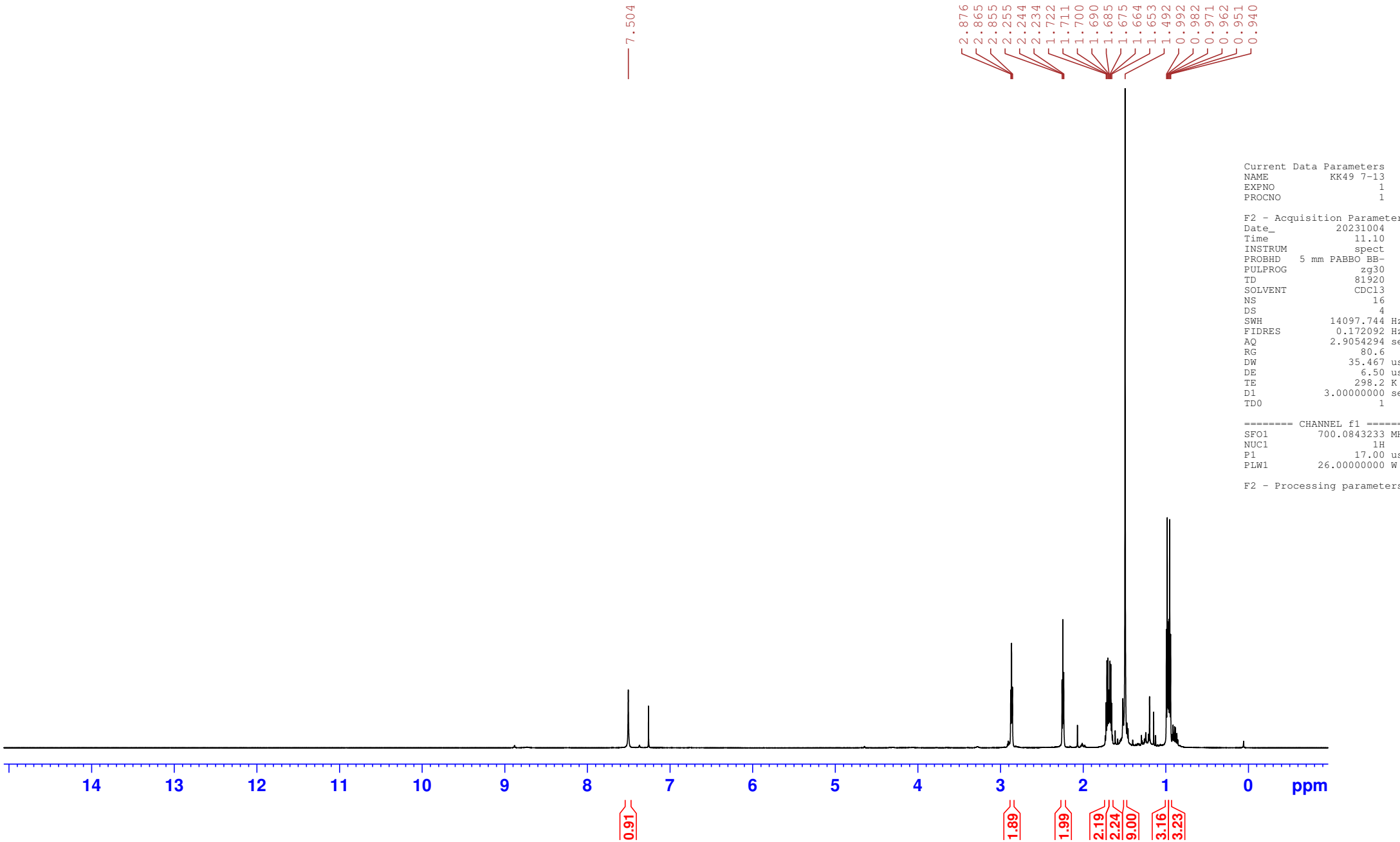


Current Data Parameters
NAME KK55
EXPNO 2
PROCNO 1

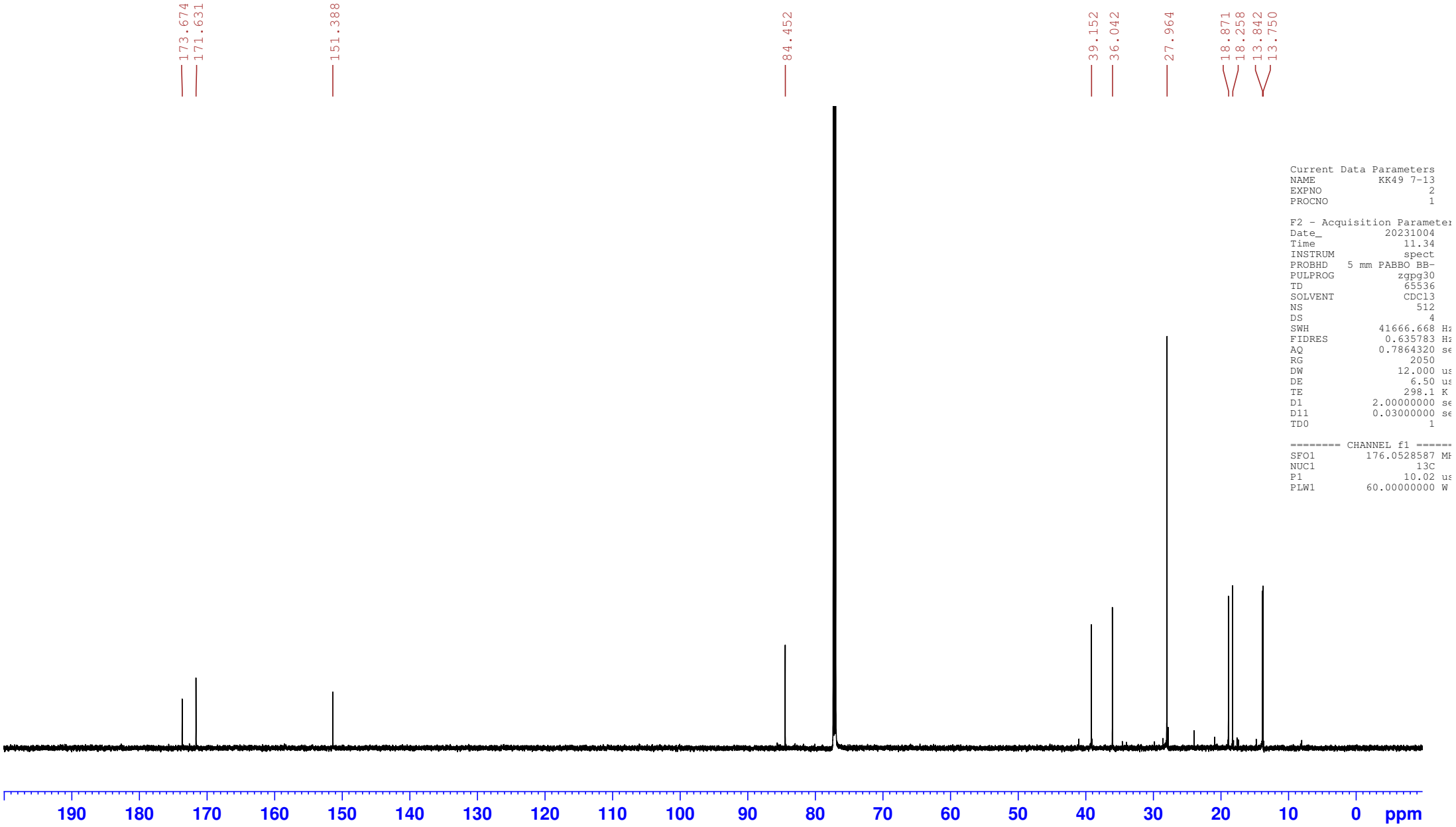
F2 - Acquisition Parameter
Date_ 20231216
Time 16.24
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 512
DS 4
SWH 41666.668 Hz
FIDRES 0.635783 Hz
AQ 0.7864320 se
RG 2050
DW 12.000 us
DE 6.50 us
TE 298.1 K
D1 2.00000000 se
D11 0.03000000 se
TD0 1

======== CHANNEL f1 ======
SFO1 176.0528587 MH
NUC1 13C
P1 10.02 us
PLW1 60.00000000 W

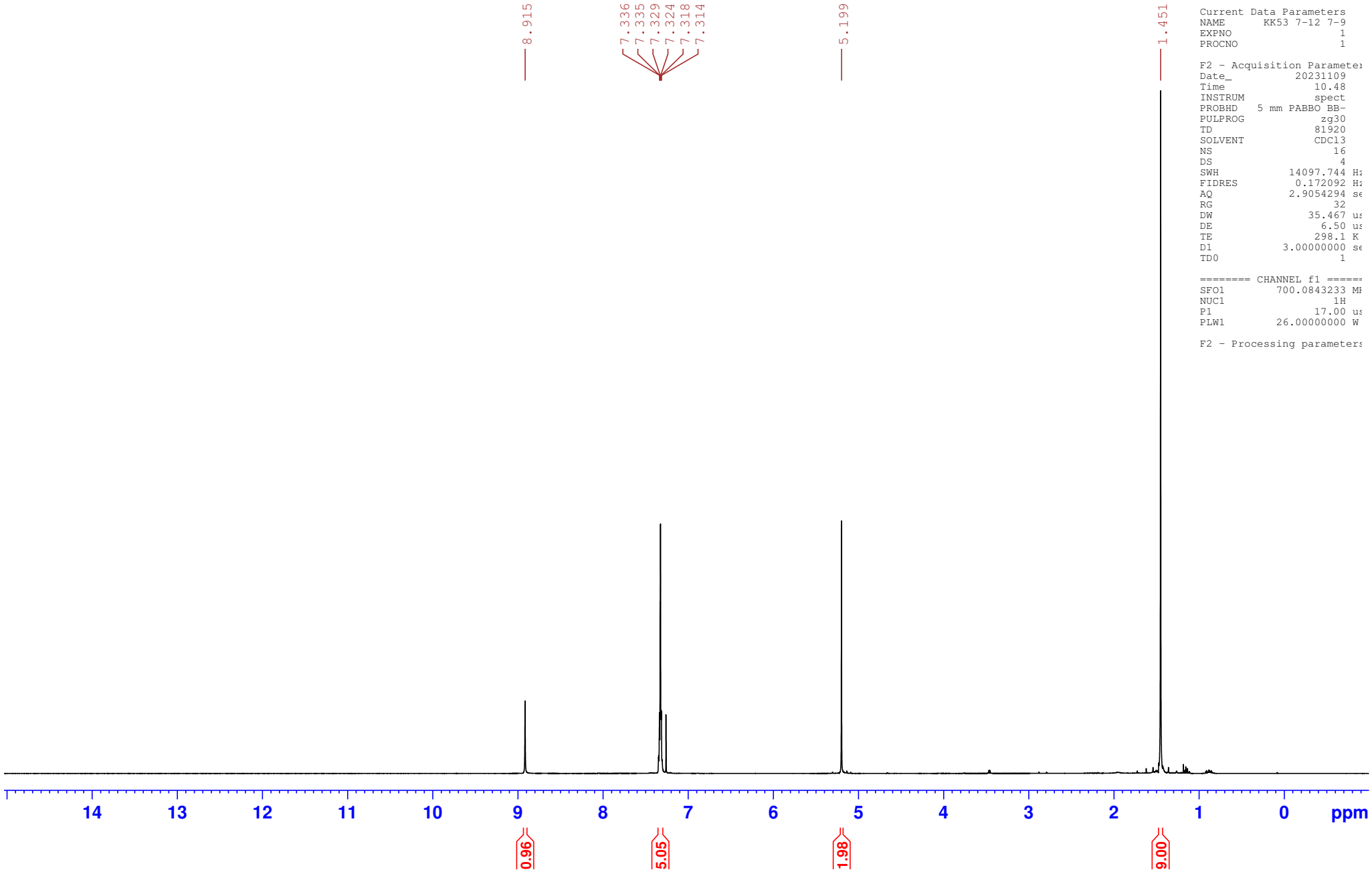
KK49 7-13 CDCl3 1H
PROTON CDCl3 {C:\Spectra\data\AntonM\nmr} AntonM 19



KK49 7-13 CDCl3 13C
C13CPD CDCl3 {C:\Spectra\data\AntonM\nmr} AntonM 19



KK53 7-12 7-9 CDCl3 1H
PROTON CDCl3 {C:\Spectra\data\AntonM\nmr} AntonM 8



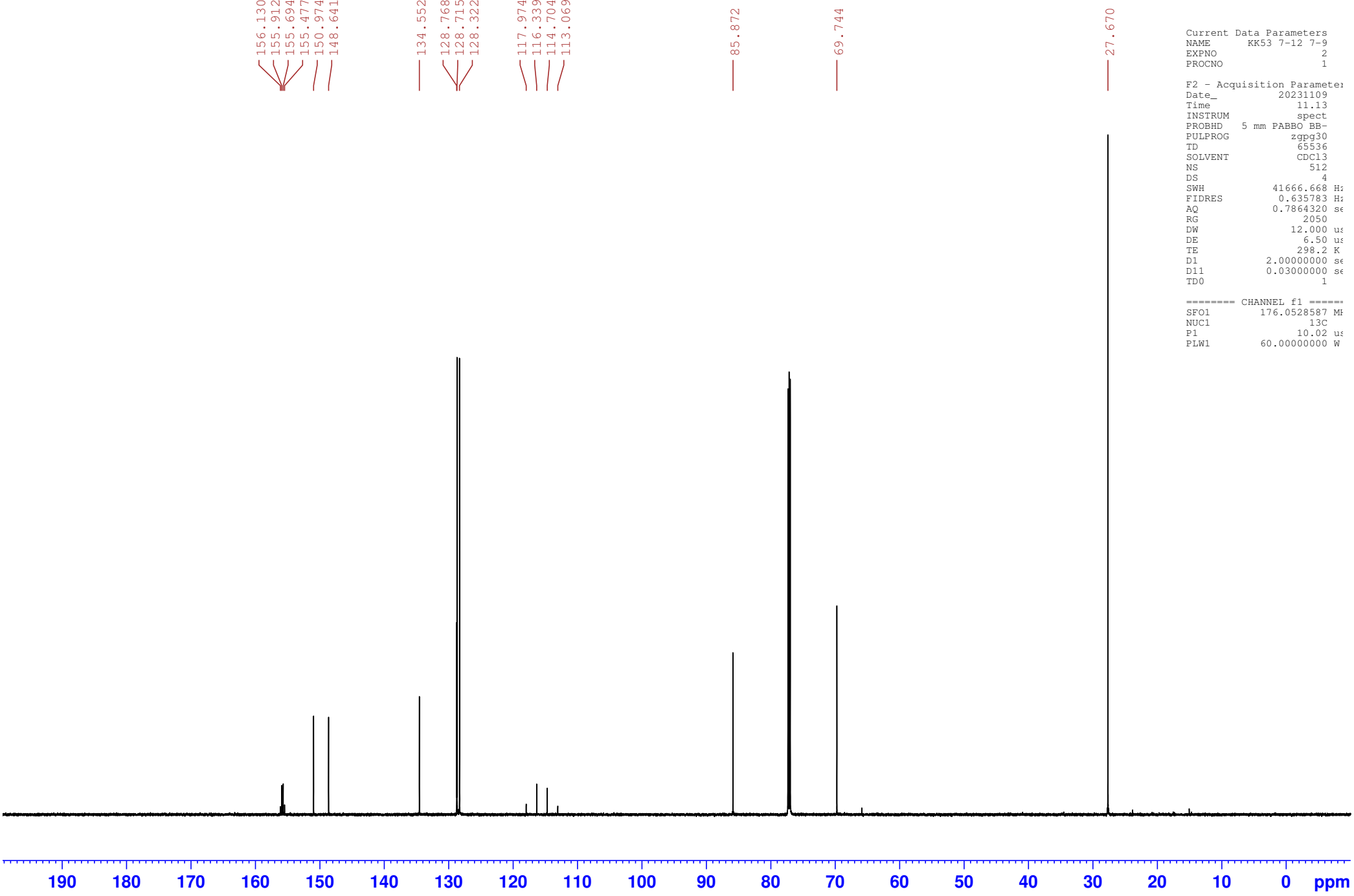
Current Data Parameters
NAME KK53 7-12 7-9
EXPNO 1
PROCNO 1

F2 - Acquisition Parameter
Date_ 20231109
Time 10.48
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 81920
SOLVENT CDCl3
NS 16
DS 4
SWH 14097.744 Hz
FIDRES 0.172092 Hz
AQ 2.9054294 se
RG 32
DW 35.467 us
DE 6.50 us
TE 298.1 K
D1 3.00000000 se
TD0 1

======== CHANNEL f1 ======
SFO1 700.0843233 MH
NUC1 1H
P1 17.00 us
PLW1 26.00000000 W

F2 - Processing parameters

KK53 7-12 7-9 CDCl3 13C
C13CPD CDCl3 {C:\Spectra\data\AntonM\nmr} AntonM 8



Current Data Parameters
NAME KK53 7-12 7-9
EXPNO 2
PROCNO 1

F2 - Acquisition Parameter
Date_ 20231109
Time 11.13
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 512
DS 4
SWH 41666.668 Hz
FIDRES 0.635783 Hz
AQ 0.7864320 se
RG 2050
DW 12.000 us
DE 6.50 us
TE 298.2 K
D1 2.00000000 se
D11 0.03000000 se
TD0 1

======== CHANNEL f1 ======
SFO1 176.0528587 MH
NUC1 13C
P1 10.02 us
PLW1 60.00000000 W

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22/05/2024