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PII: S0040-4020(13)00461-4

DOI: 10.1016/j.tet.2013.03.072

Reference: TET 24163

To appear in: *Tetrahedron*

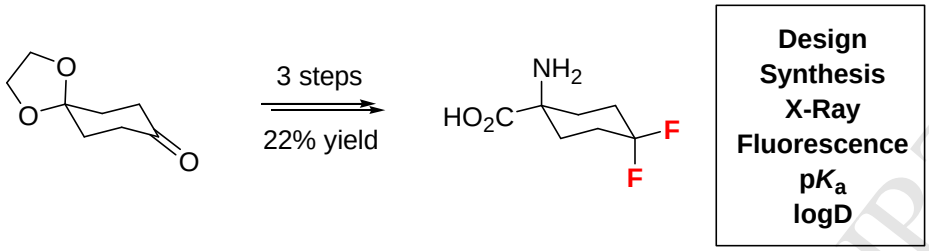
Received Date: 3 December 2012

Revised Date: 21 February 2013

Accepted Date: 18 March 2013

Please cite this article as: Mykhailiuk PK, Starova V, Iurchenko V, Shishkina SV, Shishkin OV, Khilchevskyi O, Zaporozhets O, 1-Amino-4,4-difluorocyclohexanecarboxylic acid as a promising building block for drug discovery: design, synthesis and characterization, *Tetrahedron* (2013), doi: 10.1016/j.tet.2013.03.072.

Graphical abstract



1-Amino-4,4-difluorocyclohexanecarboxylic acid as a promising building block for drug discovery: design, synthesis and characterization.

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Abstract

1-Amino-4,4-difluorocyclohexanecarboxylic acid has been designed as a fluorinated analogue of the pharmacologically relevant 1-aminocyclohexanecarboxylic acid. The synthesis has been performed in three steps from a commercially available material in 22% overall yield. An impact of fluorine atoms on conformation, lipophilicity, acidity and fluorescent properties of the amino acid has been studied. Various practical applications of the obtained compound are suggested.

Key words

Amino acids, 1-aminocyclohexanecarboxylic acid, fluorine, fluorination, morph-DAST.

Introduction

1-Aminocyclohexanecarboxylic acid (**1**) is a non-proteinogenic quaternary α-amino acid, which has gained much application in peptide chemistry[1] and drug discovery.[2,3] The interest to this compound is caused by several reasons. First, amino acid **1** is intrinsically conformationally restricted due to both the cyclic ring and the quaternary α-carbon atom.[4] Conformationally restricted compounds, due to fixation of functional groups in a biologically active conformation, are often more efficient and selective ligands for various targets compared to their non-restricted analogues.[5] Second, structure **1** is symmetric and therefore achiral, so that there is no need to additionally prepare the optically active compounds in the medicinal structure-activity relationship studies. Also, the saturated cyclohexane core ensures that derivatives of **1** are lipophilic, which is an important property of oral drugs.[6] It is not surprising therefore, that ~ 150 pharmacologically relevant derivatives of amino acid **1** are currently known.[3] Among them are the commercialized antibiotic Cyclacillin,[7] antineoplastic Spiromustine[8] and antiosteoporosis agents Balicatib[9] and L-006235.[10]

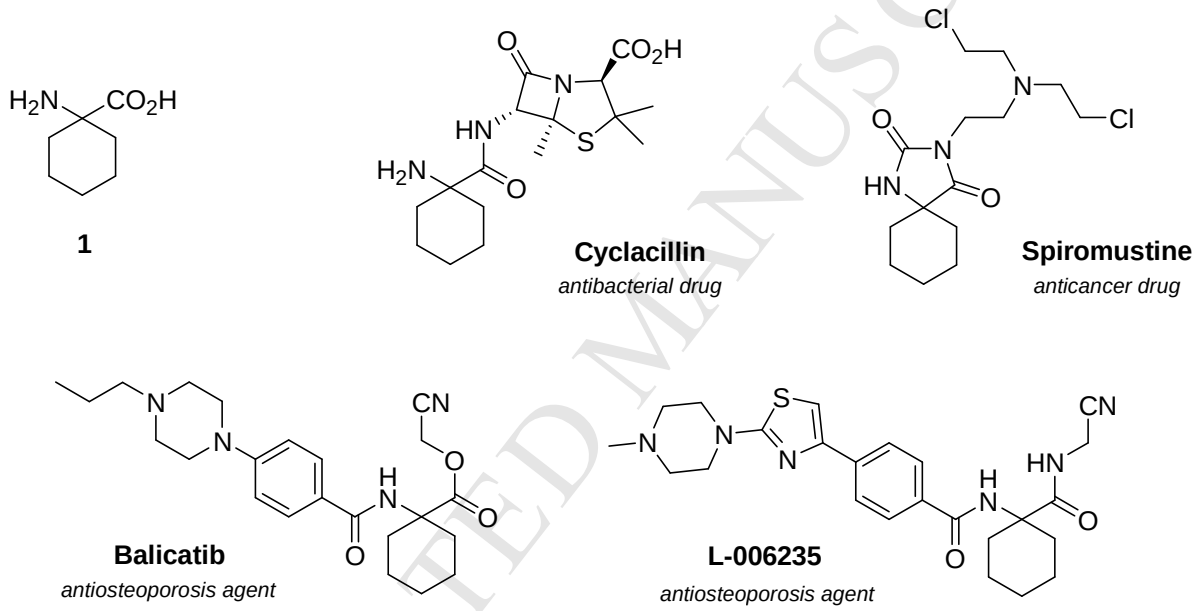


Figure 1. 1-Aminocyclohexanecarboxylic acid (**1**) and its bioactive derivatives: Cyclacillin (antibacterial drug, *Wyeth*, 1979), Spiromustine (anticancer drug, *National Cancer Institute*, 1987), Balicatib (antiosteoporosis agent, *Novartis*, 2006), L-006235 (antiosteoporosis agent, *Merck*, 2005).[7-10]

Substituted cyclohexanes are often metabolically labile due to rapid enzymatic hydroxylation at C(4) (Figure 2).[11] This problem, however, can be solved by replacing the 4-CH_2 group with a 4-CF_2 fragment. Incorporation of fluorine atoms into the cyclohexane ring prevents its oxidative degradation, since the C-F bond (116 kcal/mol) is significantly stronger than the C-H bond (99 kcal/mol).[12,13]

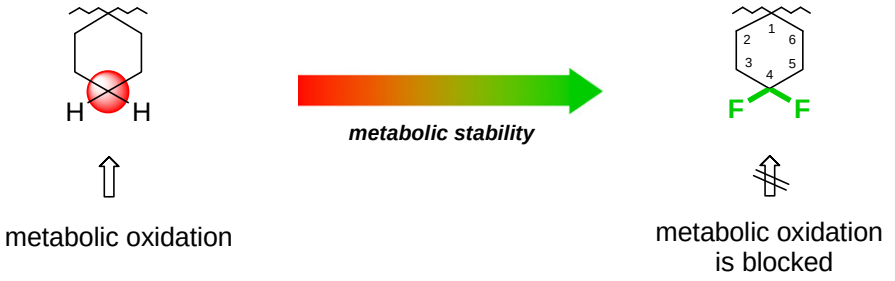


Figure 2. Comparison of metabolic stabilities of cyclohexane- and 4,4-difluorocyclohexane motifs.

Several examples of this concept are outlined in Figure 3. Neurokinin-2 antagonist **2**,[14] antiosteoporosis agent **3**[15] and bradykinin B_1 antagonist **4**[16] recently reached the preclinical/clinical trials. In all cases, incorporation of fluorine atoms into the cyclohexane ring prevented the initially observed metabolic hydroxylation at 4-CH_2.[17] It is also worth mentioning that introduction of the difluoro-unit into the launched antiretroviral drug Maraviroc, along with improving the metabolic profile, additionally reduced the compound`s toxicity.[18]

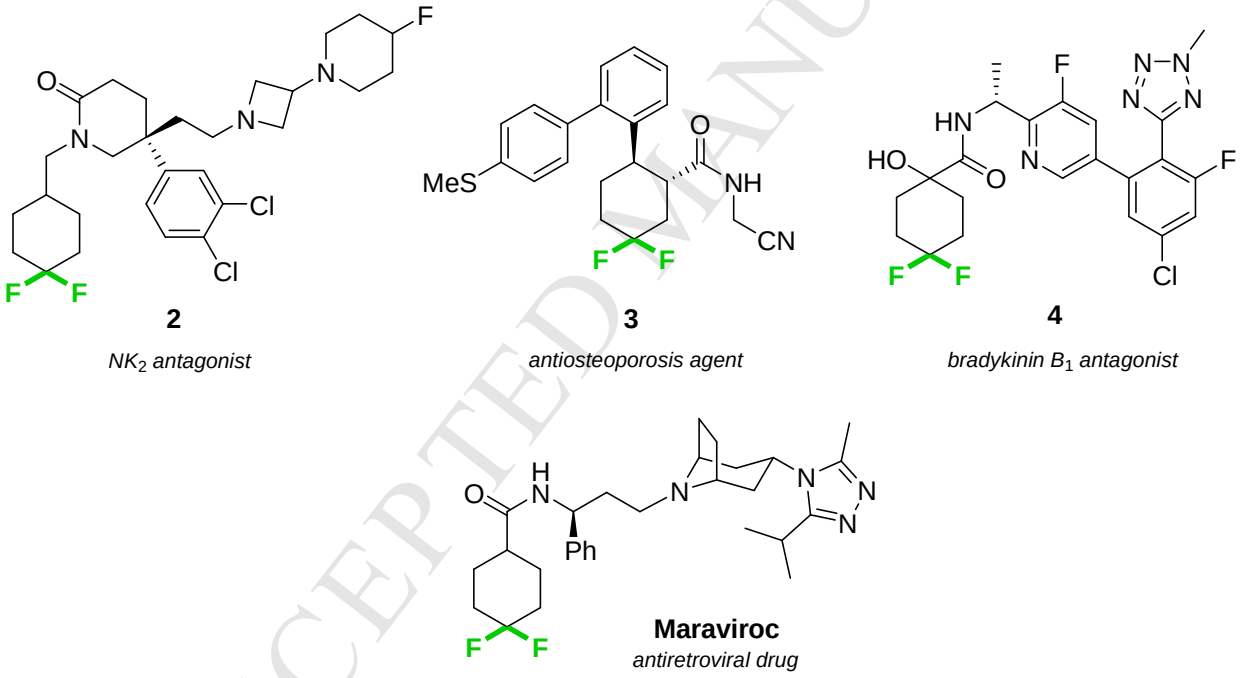
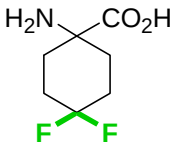


Figure 3. Pharmacologically relevant compounds with 4,4-difluorocyclohexane motif: neurokinin-2 antagonist **2** (*Pfizer*, 2005); antiosteoporosis agent **3** (*Merck*, 2006); bradykinin B_1 antagonist **4** (*Merck*, 2008); antiretroviral drug Maraviroc (*Pfizer*, 2007).

Given the high pharmaceutical potential of amino acid **1** and sensitivity of substituted cyclohexanes to metabolic hydroxylation at 4-CH_2, fluorinated building block **5** (Figure 4) seems to be conceptually useful for drug discovery projects. Importantly, incorporation of two fluorine atoms into amino acid **1** at C(4) preserves its achiral structure. Preparation of compound **5**, however, is not described in the open literature. Only the synthesis of a derivative of **5** is briefly mentioned in a recent patent[19] starting from a rather exotic non-commercially available starting material. Neither the detailed experimental procedures, nor the full compound characterizations are provided. In the present work, therefore, the practical synthesis of amino acid **5**[20] from a

commercially available starting compound has been developed. An impact of fluorine atoms on the conformation, lipophilicity, acidity and fluorescent properties of the amino acid has also been studied.



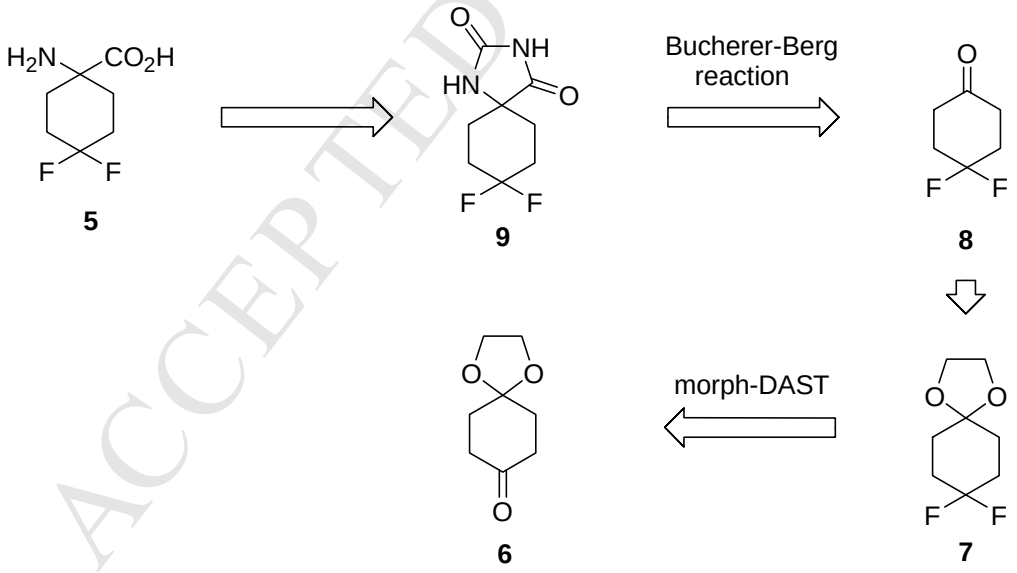
5

Figure 4. 1-Amino-4,4-difluorocyclohexanecarboxylic acid (**5**).

Results and Discussion

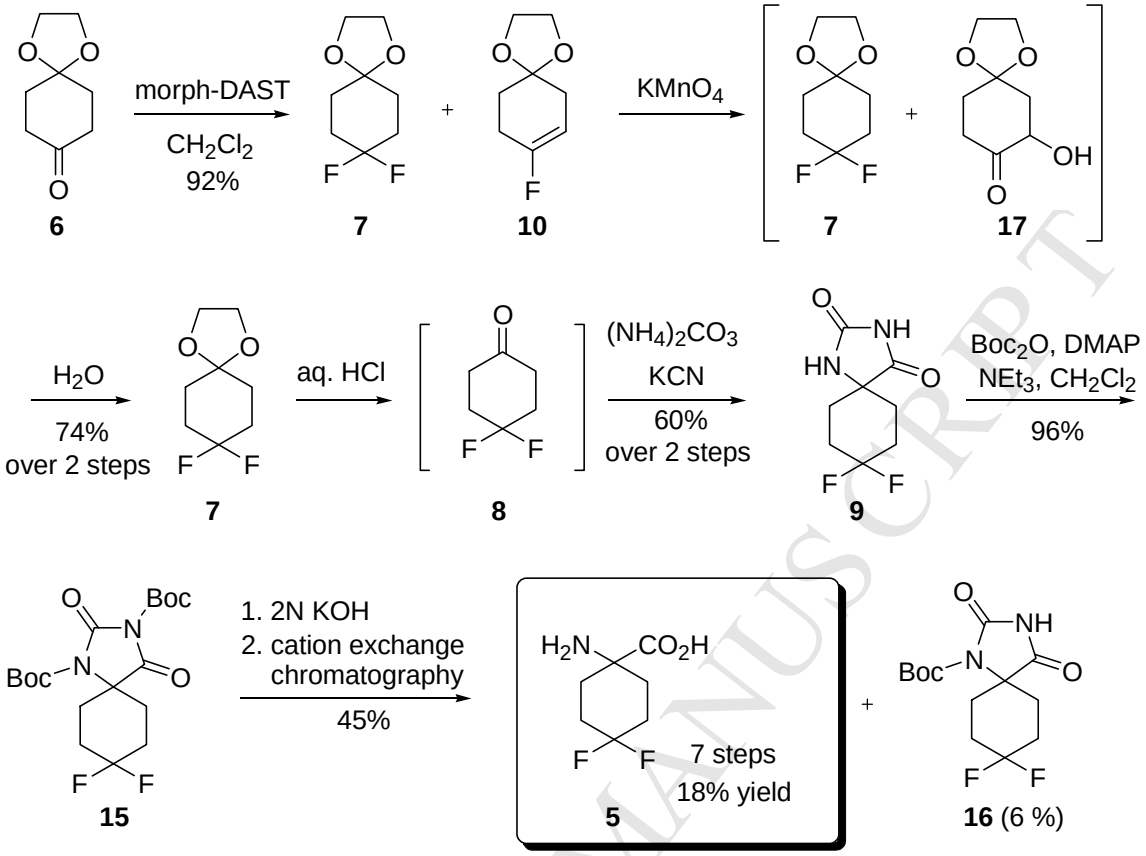
Retrosynthetic analysis. In a search for a convenient starting point for the synthesis of amino acid **5**, we pointed our attention to the substituted cyclohexanone **6**. This compound contained two carbonyl groups at the needed C(1) and C(4) positions of the cyclohexane ring. Moreover, one carbonyl group was already protected by the ketal moiety, allowing thus the stepwise transformation of the functional groups. Finally, ketone **6** was commercially available[21] and rather cheap.[22]

Incorporation of fluorine atoms into the cyclohexane skeleton (**7**) was planned to be performed by fluorination of the carbonyl group with morpholinosulfur trifluoride (morph-DAST, $O(CH_2CH_2)_2NSF_3$).[23,24] Subsequent construction of the aminocarboxylate moiety was expected to be realized by Bucherer-Berg reaction[25] at the other carbonyl group (**8**) followed by hydrolysis of the formed hydantoin **9**. Implementation of this strategy led to the retrosynthetic approach depicted in Scheme 1.



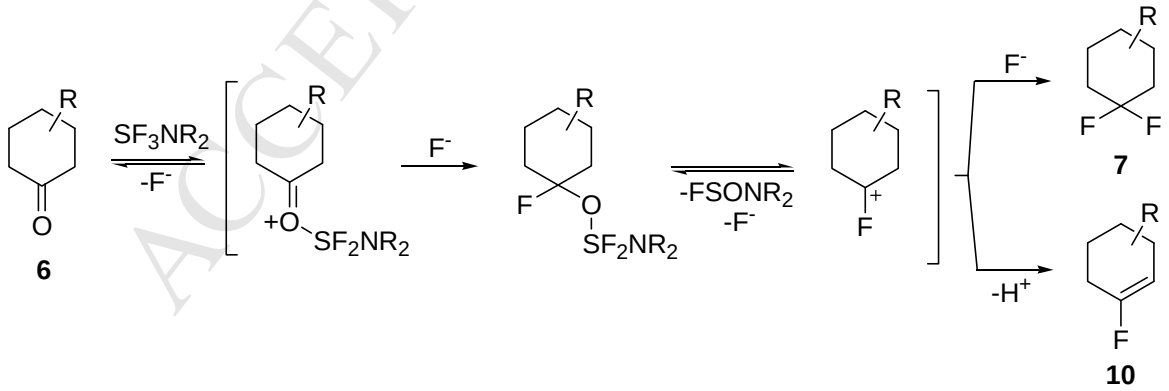
Scheme 1. Retrosynthetic analysis of amino acid **5**.

Synthesis. Fluorination of ketone **6** by morph-DAST in dry dichloromethane at room temperature afforded a 4:1 mixture of the required difluoro compound **7** and the by-product vinyl fluoride **10** in 92% combined yield (Scheme 2).[26] Practical separation of this mixture, however, was not effective. Optimization studies undertaken to influence the ratio of products by changing the reaction temperature and stoichiometry of the reagents were unsuccessful.



Scheme 2. Synthesis of amino acid **5**.

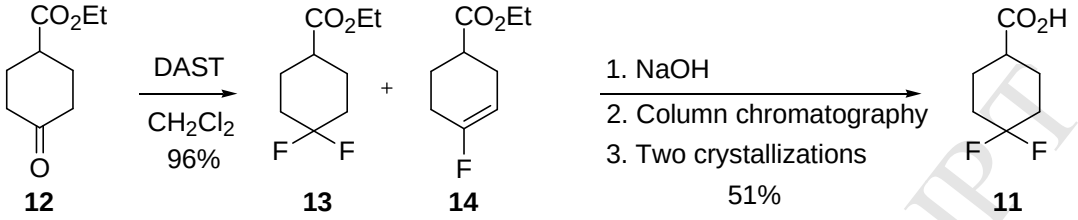
Formation of vinyl fluoride co-product from treatment of ketones with DAST and its analogues is described in the literature and appears difficult to control.[26,27] Fluorination of substituted cyclohexanones usually affords a mixture of products, which are difficult to separate (Scheme 3).[26,28] Moreover, it seems that no general method on the effective separation of such compounds is known.



Scheme 3. Mechanism of formation of fluorocyclohexene **10**.

In this context, a recent publication from *Pfizer* on the optimized industrial preparation of Maraviroc (Figure 3) is worth mentioning.[29] Acid **11** is a key intermediate in the synthesis of the drug (Scheme 4). The authors reported that fluorination of cyclohexanone **12** with DAST afforded a mixture of product **13** and fluorovinyl **14**. Hydrolysis of the ester group gave a

mixture of the corresponding acids, from which compound **11** was isolated in 51% yield after column chromatography followed by two crystallizations. The moderate yield of that optimized transformation aimed at bulk production of the launched drug, indicates that separation of difluorocyclohexane / fluorocyclohexene derivatives does still represent a significant challenge.



Scheme 4. Literature synthesis of acid **11**, a key component of Maraviroc (Ref. 29).

Since a direct separation of the mixture **7**/**10** was not effective, oxidation of alkene **10** into compound **17** was attempted next. Alcohol **17** seemed to be much more hydrophilic than **7**, so that their resolution was expected to be easier to perform. A solution of **7**/**10** in dichloromethane was treated with aq. solution of $KMnO_4$ at room temperature under vigorous stirring. The heterogeneous oxidation of the double C=C bond was very slow as monitored by ^{1}H-NMR spectroscopy. After 24 h, however, the reaction was completed. Unexpectedly, washing the crude reaction mixture with water afforded the pure crystalline product **7** in 74% yield, whereas the putative water-soluble alcohol **17** was completely extracted. It is important to note, that the developed simple protocol on the quantitative isolation of difluoromethyl compound **7** from the mixture **7**/**10** seems to be rather general and we believe that it will find further application in fluoroorganic chemistry, where formation of the by-product vinyl fluorides is common.

Acidic cleavage of the ketal group in compound **7** was next studied. The transformation smoothly proceeded by stirring a solution of **7** in aq. hydrochloric acid / isopropanol mixture at room temperature for 96 h. High volatility of compound **8**, however, hindered its quantitative isolation. Therefore, in order not to loose the product during the work-up process, the reaction mixture with crude ketone **8** was directly used in the next synthesis step. The pH value was adjusted first to 8-9 by aq. K_2CO_3 solution, followed by addition of KCN and $(NH_4)_2CO_3$ for the Bucherer-Berg transformation. Heating the reaction at reflux for 48 h afforded hydantoin **9** in 60% isolated yield after two steps.

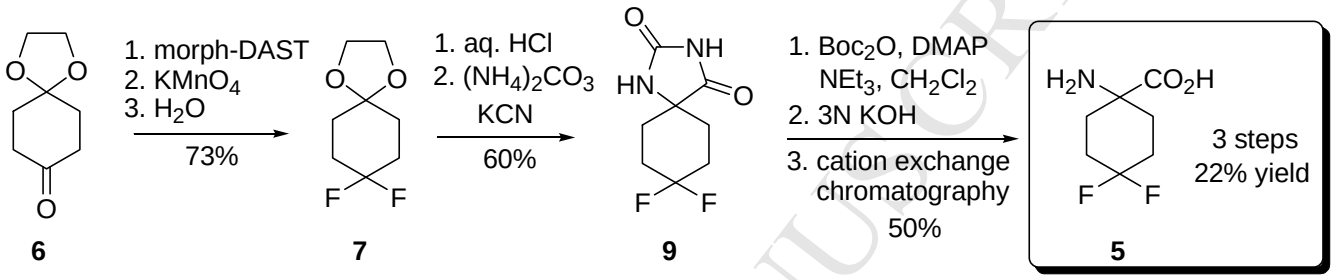
Hydrolysis of the hydantoin ring is usually performed under rather harsh conditions, e. g. conc. NaOH/$Ba(OH)_2$ at temperatures >100 °C, or refluxing conc. HBr, which is not compatible with many functional groups.[30] This problem was partially solved by Rebek and coworkers,[31] who showed that *N*,*N*'-bis(*tert*-butyloxycarbonyl)hydantoins could be hydrolyzed with dilute aqueous alkali solution at room temperature. Having this transformation in mind, hydantoin **9** was first converted into the *bis*-Boc-derivative **15** by treating with Boc_2O/NEt_3/DMAP. Hydrolysis of **15** with aq. 2N KOH at room temperature for 10 h followed by cation-exchange chromatography on CU-2 resin afforded the requested amino acid **5** in 45% yield. Interestingly, along with the target amino acid **5**, the side product **16** was also obtained in 6% yield.

Thus, the synthesis of amino acid **5** was performed in seven steps from ketone **6** in 18% overall yield.

Optimized synthesis. The practical usefulness of building blocks in drug discovery projects strongly depends on the availability of simple well-validated synthetic approaches to them.[32] The shorter and easier the synthesis of a building block is, the higher are chances of that compound to be involved in medicinal research. In this context, the synthesis of amino acid **5** was optimized to maximally reduce the number of synthetic manipulations.

First, synthesis of difluorocyclohexane **7** was performed one-pot from ketone **6** (Scheme 5). Products **7**/**10**, formed by fluorination of ketone **6**, were not isolated from the reaction mixture, but their solution in dichloromethane was directly treated with aq. $KMnO_4$. The pure crystalline product **7** was obtained in 73% yield without any purification. Next, one-pot synthesis of hydantoin **9** from ketal **7** was performed following the initially elaborated procedures. Finally, one-pot synthesis of amino acid **5** from hydantoin **9** was carried out. The key point was not to isolate compound **15**, but to treat the reaction mixture with aq. KOH directly. In order to prevent formation of the side-product **16**, the amount of KOH as well as the reaction time was increased twice. As a result, amino acid **5** was obtained in 50% yield.

The optimized synthesis of compound **5** from ketone **6** was performed in 22% overall yield in three steps.



Scheme 5. Optimized synthesis of amino acid **5**.

Influence of fluorine on conformation. Having developed a short and effective route to amino acid **5**, physico-chemical properties of that compound were studied next. Indeed, when replacing amino acid **1** by compound **5** in medicinal projects, one should always keep in mind, that incorporation of fluorine atoms into the cyclohexane ring might not only improve the compound metabolic stability, but it could also change its conformation, electronic properties, lipophilicity and pK_a of functional groups.[13]

Recently, O`Hagan et al. showed that the geometry of difluoromethylene-containing cyclododecanes differed much from that of the non-fluorinated analogues.[33] The observed difference was caused by the distortion of bond angles. The authors studied a set of structures with $CH_2CF_2CH_2$ fragment at Cambridge Crystal Structure Database (CCSD) and found that the average C-CF_2-C angle was 118°, whereas the average F-C-F angle was 104° (Figure 5), significantly wider and narrower, respectively, than the common tetrahedral angle of 109°.

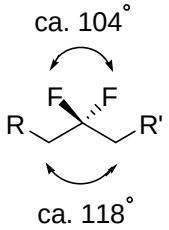


Figure 5. Average bond angles in $CH_2CF_2CH_2$ motif according to Ref 33.

Following this scenario, it was important to find out whether the fluorine atoms influenced the conformation of amino acid **5** and its derivatives. Since, only one cyclohexane with $CH_2CF_2CH_2$ unit was found in CCSD - 1,1,4,4-tetrafluorocyclohexane,[34] - single crystals of compounds **5***HCl, **7**, **9** and **16** suitable for X-Ray diffractional analysis were obtained (Figure 6).

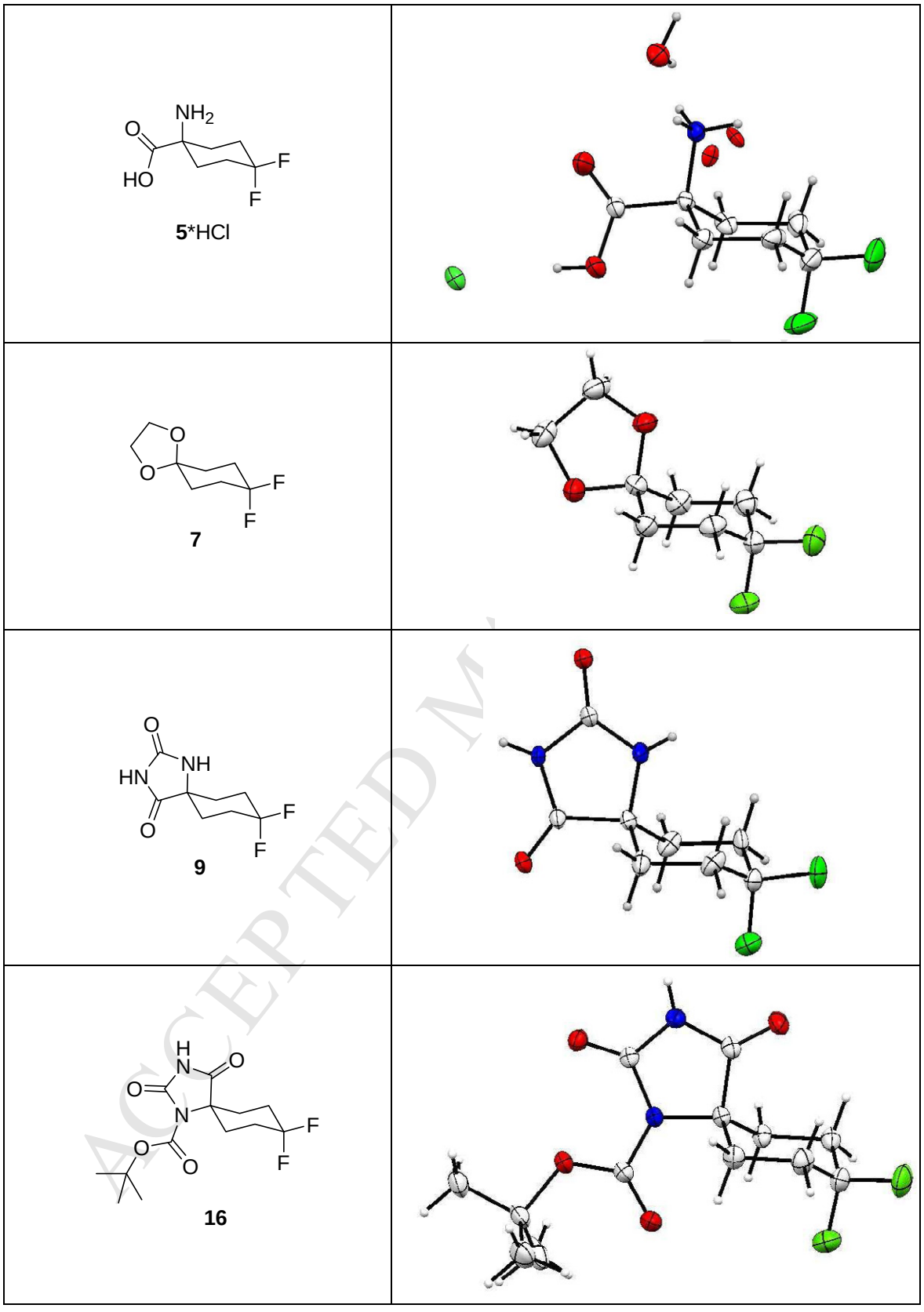
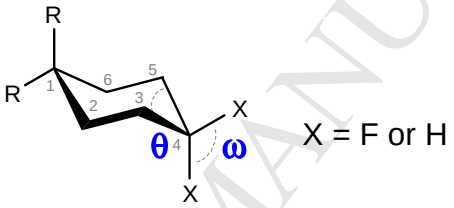


Figure 6. X-ray crystal structures of compounds **5***HCl, **7**, **9** and **16**.

Comparison of geometrical parameters of amino acid **5***HCl and its none-fluorinated analogue **1***HCl[35] in crystal phase revealed a chair conformation of the cyclohexane ring with an equatorial carboxylic group. In amino acid **5***HCl, angle ω = 105° was in a good agreement with previous by O`Hagans observation (Table 1). On the other hand, angle θ = 115° was larger than that of 111° in amino acid **1***HCl (Table 1), but significantly smaller than 118°, observed previously by O`Hagan et al. The possible explanation relies on intrinsic conformational stability of the cyclohexane ring, which hampers any valuable geometric deviations of the molecule imposed by different substituents. As a result, conformations of the cyclohexane ring in fluorinated compound **5***HCl and non-fluorinated one **1***HCl are very similar, as additionally confirmed by the characteristic puckering parameters[36] (Table 1). From the above data one can conclude that the presence of fluorine atoms in amino acid **5** does not influence the overall compound conformation.

Table 1. Geometric characteristics from X-ray diffraction data: C(3)-C(4) and C(4)-C(5) bond length (Å); X-C-X (ω, deg) and C-CX_2-C (θ, deg) bond angles; ring puckering parameters (S; Θ, deg; Ψ, deg) and deviations of C(1) and C(4) atoms from the mean plane of the remaining ring atoms in fluorinated compounds **5***HCl, **7**, **9**, **16** and none-fluorinated ones **1***HCl, **17**



Parameter	**5***HCl	**1***HCl[a]	**7**	**9**	**17**[a]	**16**
C(3)-C(4), Å	1.508(7)	1.505(3)	1.506(1)	1.488(6)	1.504(3)	1.494(3)
C(4)-C(5), Å	1.476(7)	1.505(3)	1.506(2)	1.486(6)	1.514(3)	1.485(2)
ω, deg	104.8(4)	99.0(3)	104.23(8)	103.9(2)	106.0(2)	104.3(1)
θ, deg	114.6(4)	111.0(2)	114.1(1)	114.9(3)	111.1(2)	114.6(1)
S	1.09	1.10	1.11	1.13	1.14	1.08
Θ, deg	0.9	2.4	0.2	2.8	0.6	1.9
Ψ, deg	7.9	0.0	9.0	19.4	21.0	18.7
dev. C(1), Å	-0.64	-0.61	-0.65	-0.71	-0.66	-0.61
dev. C(4), Å	0.60	0.66	0.62	0.59	0.66	0.61

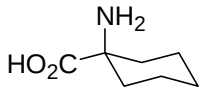
[a] Data for compounds **1***HCl and **17** are taken from refs. 35 and 37, respectively.

The above conclusion can also be made for compound **9**. Both hydantoin **9** and its non-fluorinated analogue **17**[37] (Table 2) had a chair conformation of the cyclohexane ring (Table 1) with carboxamide group at the equatorial position. Although θ angles in hydantoins **9**, **17** were rather different (Table 1), no distortion of the overall compound geometry was observed.

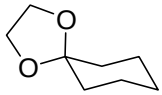
Comparison of the compound geometrical parameters based on only X-ray diffraction data may lead, however, to significant errors because conformational characteristics in the crystal phase are sensitive to packing effects. Moreover, in crystal phase, amino acid **5** existed as a hydrochloride salt, which might cause some conformational deformation of the cyclohexane ring due to strong electrostatic interactions. In order to separate these effects we have optimized geometry of compounds **5**, **7**, **9**, **16** and their non-fluorinated counterparts **1**, **18**, **17**, **19** using M06-2X/6-311G(d,p) method. The calculation clearly indicated an influence of fluorine atoms on the endocyclic C-C bond lengths at C(4) atom (Table 2). The presence of the fluorine

shortened C(3)-C(4) and C(4)-C(5) bonds compared to those of non-fluorinated analogues. Also, decrease of ω angle and considerable increase of θ angle were observed (Table 2). Nevertheless, replacement of hydrogen atoms for fluorine at C(4) did not influence the conformation of the saturated ring.

Table 2. Geometric characteristics from quantum-chemical calculations using M06-2X/6-311G(d,p) method: C(3)-C(4) and C(4)-C(5) bond length (Å); X-C-X (ω, deg) and C-CX_2-C (θ, deg) bond angles; ring puckering parameters (S; Θ, deg; Ψ, deg) and deviations of C(1) and C(4) atoms from the mean plane of the remaining ring atoms in compounds **5**, **7**, **9**, **16** and their non-fluorinated analogues **1**, **18**, **17**, **19**.



1*HCl



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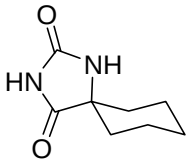

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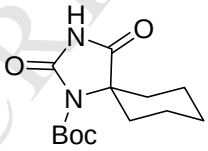

19

Parameter	**5**	**1**	**7**	**18**	**9**	**17**	**16**	**19**
C(3)-C(4), Å	1.511	1.530	1.512	1.531	1.513	1.530	1.511	1.529
C(4)-C(5), Å	1.513	1.531	1.512	1.531	1.513	1.530	1.511	1.529
ω, deg	106.0	107.0	105.8	107.0	106.2	107.0	105.9	107.1
θ, deg	114.0	111.1	113.9	110.8	114.0	111.0	113.8	110.8
S	1.15	1.16	1.14	1.15	1.14	1.15	1.12	1.12
Θ, deg	1.9	1.2	0.7	0.5	1.0	0.1	1.4	2.6
Ψ, deg	16.7	7.9	28.4	26.2	5.5	28.0	0.0	0.3
dev. C(1), Å	0.71	0.70	0.67	0.65	0.68	0.67	0.64	0.62
dev. C(4), Å	-0.63	-0.67	-0.64	-0.68	-0.63	-0.67	-0.64	-0.69

In summary, incorporation of fluorine atoms into amino acid **1** and its derivatives at C(4) did not have any valuable effect on their conformation. In particular, introduction of fluorine into **1** slightly changed the angle θ, but preserved the chain conformation of the cyclohexane ring, and its characteristic parameters. Therefore, one can safely replace amino acid **1** in bioactive compounds with the residue of **5** with no change of the compound geometry.

Impact of fluorine on fluorescence. With the aim to estimate the influence of fluorine atoms on amino acid **1** comprehensively, fluorescence measurements of the both **1** and **5** were performed. Excitation spectra of amino acids in aqueous solution (λ_{em} = 441 nm) had one maximum at 347 nm (*Supporting information*). Under excitation at 347 nm, the fluorescence emission spectra still had one identical maximum at 441 nm. Importantly, however, that though the fluorescence and emission spectra of both compounds had the same maxima, the spectra of **5** had ~30 times higher intensity than those of amino acid **1** under identical concentration. This difference clearly indicated the significant change of the polarity and lipophilic properties of the amino acid **1** upon incorporation of fluorine atoms. The determined quantum yield of compound **5** in aqueous solution was 0.10. Supplemented with blocked metabolism of **5** at C(4) atom, the above phenomena suggest the use of the amino acid as a fluorescent label to study, for example, the mechanism of translocation and/or metabolic degradation of the corresponding bioactive compounds.

Impact of fluorine on pK_a. In medicinal chemistry, pK_a of functional groups is an important characteristic of drugs, as it strongly effects their binding affinity, selectivity, lipophilicity and toxicity.[38] An influence of fluorine atoms on pK_a of aminocarboxylate moiety in compound **5** was studied next.[39] Amino acid **1** was used as a reference point. pK_a measurements were performed by potentiometric titration (*Supporting Information*).

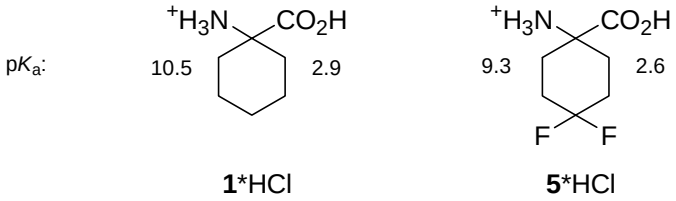

Figure 7. pK_a values for the both carboxylic and amino groups in **1***HCl, **5***HCl.

The measured pK_a values of amino acid **1** were 2.9 (CO_2H) and 10.5 (NH_3^+), while those in compound **5** were 2.6 (CO_2H) and 9.3 (NH_3^+) (Figure 7). As expected, incorporation of fluorine atoms slightly increased an acidity of the carboxylic function with ΔpK_a = -0.3. Basicity of the nitrogen atom, on the contrast, was significantly reduced by more than one magnitude of order with ΔpK_a = -1.2. The different impact of fluorine atoms on pK_a of amino and carboxylic groups is caused by the dependence of an inductive effect on the number of chemical bonds. In compound **5** hydrogen atoms at amino group are separated from fluorine by six chemical bonds, while that of carboxylic function – by seven.

Toxicity is frequently associated with basic nitrogen atoms.[40] In drug design therefore toxicity of bioactive compounds is often reduced by incorporating electronegative substituents near the basic center. Given the fact, that introduction of fluorine atoms into amino acid **1** does not have any valuable effect on the compound conformation, but does effectively reduce basicity of the nitrogen atom, amino acid **5** seems to be a promising replacement for compound **1**, once high toxicity of the corresponding derivatives is observed.

Impact of fluorine on lipophilicity. Lipophilicity is another key characteristic of drugs. Indeed, bioactive compounds must be lipophilic enough to effectively cross the biomembrane. On the other hand, highly lipophilic compounds have poor water solubility.[6] For oral drugs, therefore, it is important to have the right balance between these opposite effects.

To evaluate the impact of fluorine on lipophilicity; compounds **17** and **9** were compared. The measured logD (pH = 7.4) of compound **17** was 1.5, whereas the corresponding value of difluoro hydantoin **9** was 0.5, with a significant decrement of $\Delta logD_{7.4}$ = -1.0 (Figure 8). To understand this impressive effect, one should remember that LogD value depends on the both intrinsic lipophilicity (logP) of a compound and the number of neutral molecules at a given pH. Due to polarization of C-F bond, incorporation of two fluorine atoms into the cyclohexane ring brings polarity to molecule **9**, which decreases its intrinsic lipophilicity and hence the logD value.[41] Second, incorporation of fluorine obviously increases acidity of the imide N*H* proton. Therefore, at pH = 7.4, the number of none-dissociated molecules of hydantoin **9** is lower than that of compound **17**, which additionally reduces logD of **9**.[42]

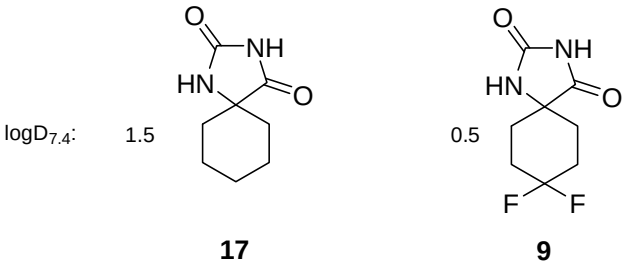

Figure 8. The measured $logD_{7.4}$ values of compounds **17** and **9**.

As previously mentioned, derivatives of amino acid **1** are highly lipophilic because of the saturated cyclohexane skeleton. Therefore, once a low solubility of bioactive derivatives of **1** is observed, amino acid **5** can be challenged as a replacement of **1** to decrease the compound LogD value, which might increase the water solubility.

Conclusion

1-Amino-4,4-difluorocyclohexanecarboxylic acid **5** was designed as an analogue of the pharmacologically relevant amino acid **1**. An optimized synthesis of **5** was performed in three steps from commercially available ketone **6** in 22% overall yield. Incorporation of fluorine atoms into amino acid **1** at C(4) did not change its geometry, but did have a profound effect on the fluorescent properties, pK_a and logD characteristics. Therefore, amino acid **5** can be very useful in those medicinal projects, where biologically active derivatives of amino acid **1** a) are metabolically unstable; b) toxic; c) have low water solubility. Also, we suggest the use of amino acid **5** as a fluorescent label to study, for example, the mechanism of translocation and/or metabolic degradation of the corresponding bioactive compounds. With rapid scalable synthesis and herein reported studies, we believe that building block **5** will find wide practical application in drug discovery projects in the future.

Experimental part

Solvents were purified according to standard procedures. Another starting materials were given by Enamine. Column chromatography was performed using Kieselgel Merck 60 (230-400 mesh) as the stationary phase. ^{1}H-, ^{19}F-, ^{13}C-NMR spectra were recorded either on Bruker Avance 500 spectrometer (at 500 MHz, 470 MHz and 125 MHz) or on Varian Unity Plus 400 spectrometer (at 400 MHz, 377 MHz and 101 MHz). Chemical shifts are reported in ppm downfield from TMS (^{1}H, ^{13}C) or $CFCl_3$ (^{19}F) as internal standards. Mass spectra were recorded on Agilent 1100 LCMSD SL instrument by chemical ionization (CI).

8,8-Difluoro-1,4-dioxaspiro[4.5]decane (7)

Method A:

A solution of ketone **6** (4.40 g, 28.2 mmol) in dry CH_2Cl_2 (100 mL) was cooled to 0 °C under argon atmosphere. Morph-DAST (11.85 g, 67.7 mmol, 2.4 equiv) was added dropwise upon stirring. The reaction mixture was allowed to warm to a room temperature and was stirred for 72 h. A saturated solution of $NaHCO_3$ in water (50 mL) was added and the reaction mixture was stirred for 10 min to quench the unreacted fluorinating agent. The organic layer was separated and the water phase was extracted with CH_2Cl_2 (2×50 mL). Combined organic phase was dried over Na_2SO_4 and evaporated under reduced pressure at 30 °C to afford the mixture of compounds **7**/**10** = 4/1 (4.61 g, 25.8 mmol, 92% yield) as a colourless oil. *Products are very volatile! Evaporation time must be minimized.* All amount of compounds **7**/**10** was dissolved in CH_2Cl_2 (100 mL). Solution of $KMnO_4$ (5.7 g, 36 mmol, 10 eq) in water (100 mL) was added to

the combined organic phases and the formed suspension was vigorously stirred for 24 h. The organic layer was separated and the water phase was extracted with CH_2Cl_2 (2×100 mL).The combined organic phases were dried over Na_2SO_4, and evaporated under vacuum to provide pure compound **7** (3.38 g, 19.0 mmol, 74% yield; 67% yield over two steps from ketone **6**) as an colourless oil, which crystallized upon storage. M.p. 48-49 °C.

Method B:

A solution of ketone **6** (4.40 g, 28.2 mmol) in dry CH_2Cl_2 (100 mL) was cooled to 0 °C under argon atmosphere. Morph-DAST (11.85 g, 67.7 mmol, 2.4 equiv) was added dropwise upon stirring. The reaction mixture was allowed to warm to a room temperature and was stirred for 72 h. A saturated solution of $NaHCO_3$ in water (50 mL) was added and the reaction mixture was stirred for 10 min to quench the unreacted fluorinating agent. The organic layer was separated and the water phase was extracted with CH_2Cl_2 (2×50 mL). Solution of $KMnO_4$ (5.7 g, 36 mmol, 10 eq) in water (100 mL) was added to the combined organic phase and the formed suspension was vigorously stirred for 24 h. The organic layer was separated and the water phase was extracted with CH_2Cl_2 (2×100 mL).The combined organic phases were dried over Na_2SO_4, and evaporated under vacuum at 30 °C to provide pure compound **7** (3.65 g, 20.5 mmol, 73% yield) as an colourless oil, which crystallized upon storage. The crystals of **7** were suitable for X-Ray diffractional study. M.p. 48-49 °C. *The product is very volatile! Evaporation time must be minimized in order to avoid sublimation of the product into a rotary evaporator.*

^{1}H NMR (500 MHz; $CDCl_3$; Me_4Si), δ: 3.98 (4H, s, OCH_2CH_2O), 2.07 (4H, tt, *J* = 13.5, 6.6 Hz, CH_2CF_2), 1.82 (4H, t, *J* = 6.6 Hz, CH_2CH_2CF_2).

^{13}C NMR (125 MHz; $CDCl_3$; Me_4Si), δ: 122.8 (q, ^1J(C,F) = 241.3 Hz, *C*F_2), 107.3 (s, *C*$(OCH_2)_2$), 64.5 (s, O*C*H_2), 31.4 (t, ^2J(C,F) = 25.1 Hz, *C*H_2CF_2), 31.0 (t, ^3J(C,F) = 6.3 Hz, *C*H_2CH_2CF_2).

^{19}F NMR (376 MHz; $CDCl_3$; $CFCl_3$), δ: -100.16 (s, C*F*$_2$).

MS (*m/z*): 178 $(M)^+$.

IR (KBr), cm^{-1}: 2975, 2889 (ν C_{sp3}-H), 1121 (ν C-O).

Anal. Calcd for $C_8H_{12}F_2O_2$: C, 53.93; H, 6.79. Found: C, 53.55; H, 6.44.

4,4-Difluorocyclohexanone (8)

A suspension of ketale **7** (20.0 g, 112.2 mmol) in 20% aq. HCl (16 ml) was vigorously stirred at 100 °C for 3 h. After cooling to a room temperature NaCl (ca. 3 g) was dissolved in the water phase. The water phase was then extracted with CH_2Cl_2 (3 * 50 ml). The combined organic phase was washed with water (50 ml), dried over Na_2SO_4 and slowly evaporated under reduced pressure at 20 °C. *The product is very volatile.* The formed material was a 1/1 mixture of product **8** and starting material **5**. After repeating the described above cycle, the obtained material was **8**/**5** = 7/3. The product of 95% purity (**8**) was obtained after repeating the described above procedure for five times in total. White crystalline (11.0 g, 71% yield). M. p. 31-32 °C.

^{1}H NMR (500 MHz; $CDCl_3$; Me_4Si), δ: 2.50 (4H, t, ^3J(H,H) = 6.5 Hz, CH_2CH_2CF_2), 2.28 (4H, tt, ^3J(H,F) = 13.0, ^3J(H,H) = 6.5 Hz, CH_2CF_2).

^{13}C NMR (125 MHz; $CDCl_3$; Me_4Si), δ: 207.0 (s, *C*=O), 122.8 (q, ^1J(C,F) = 240.0 Hz, *C*F_2), 36.5 (t, ^3J(C,F) = 3.0 Hz, *C*H_2CH_2CF_2), 32.6 (t, ^2J(C,F) = 25.0 Hz, *C*H_2CF_2).

^{19}F NMR (376 MHz; $CDCl_3$; $CFCl_3$), δ: -100.81 (p, ^3J(F,H) = 13.0 Hz, C*F*$_2$).

MS (*m/z*): 134 $(M)^+$.

IR (KBr), cm^{-1}: 2981 (ν C_{sp3}-H), 1719 (ν C=O).

Anal. Calcd for $C_6H_8F_2O$: C, 53.73; H, 6.01. Found: C, 53.42; H, 5.78.

8,8-Difluoro-1,3-diazaspiro[4.5]decane-2,4-dione (9)

1N aq. HCl (25 mL) was added to a solution of compound **7** (2.55 g, 14.3 mmol) in

isopropanol (50 mL). The reaction mixture was stirred at room temperature for 96 h. 2N aq. KOH solution was added to adjust pH value of the solution to 8-9. The reaction mixture was transferred into a glass tube followed by addition of KCN (1.40 g, 21.5 mmol) and $(NH_4)_2CO_3$ (4.10 g, 42.7 mmol). The tube was closed, and the reaction mixture was heated at 100 °C for 24 h. After cooling to a room temperature, the reaction mixture was transferred into a flask and the solvent was evaporated to ~ 30 mL. The formed solid was filtered off, washed with water (2×10 mL) on the filter and dried on air to provide hydantoin **9** (1.76 g, 8.6 mmol, 60% yield) as a white solid. M.p. > 220 °C.

The crystals of **9** suitable for X-Ray diffractional study were obtained by a slow evaporation of a diluted solution of **9** in isopropanol.

^{1}H NMR (500 MHz; DMSO-d_6; Me_4Si), δ: 8.51 (1H, br. s, N*H*), 2.18-2.00 (4H, broad m), 1.80 (2H, m), 1.74 (2H, m).

^{13}C NMR (125 MHz; DMSO-d_6; Me_4Si), δ: 178.2 (s, *C*O), 156.94 (s, *C*O), 123.5 (t, ^1J(C,F) = 240.0 Hz, *C*F_2), 60.6 (s, *C*(NH)CO), 30.7 (s, *C*H_2), 29.0 (t, ^2J(C,F) = 23.9 Hz, *C*H_2CF_2).

^{19}F NMR (377 MHz; DMSO-d_6; $CFCl_3$), δ: -91.31 (br. d, ^2J(F,F) = 233.7 Hz, C*F*F), -100.31 (br. d, ^2J(F,F) = 233.7 Hz, CF*F*).

MS (*m/z*): 204 $(M)^+$.

IR (KBr), cm^{-1}: 3199 (broad, ν N-H), 1769 (ν_{assym} C=O), 1734 (ν_{sym} C=O).

Anal. Calcd for $C_8H_{10}F_2N_2O_2$: C, 47.06; H, 4.94; N, 13.72. Found: C, 47.41; H, 4.74; N, 13.35.

1-Amino-4,4-difluorocyclohexanecarboxylic acid (5)

Method A:

A mixture of compound **9** (935 mg, 4.6 mmol), NEt_3 (834 mg, 9.2 mmol) and catalytic amount of DMAP (12 mg) in 1,2-dimethoxyethane (30 mL) was treated with Boc_2O (5.0 g, 22.9 mmol). The reaction mixture was vigorously stirred for 12 h. Another portion of DMAP (12 mg) was added, and the mixture was stirred for additional 12 h. The solvent was removed under reduced pressure and EtOAc (50 mL) / water (10 mL) were added to the residue. The mixture was stirred for 10 min. Organic phase was separated and washed with 1N aq. HCl (2×10 mL) and with water (10 mL). Organic phase was dried over Na_2SO_4 and evaporated under reduced pressure. The residue was additionally dried at 50 °C at 0.1 mm Hg for 1 h to afford di(*tert*-butyl)-8,8-difluoro-2,4-dioxo-1,3-diazaspiro[4.5]decane-1,3-dicarboxylate (**15**) (1.78 g, 4.4 mmol, 96% yield). The obtained compound was of ~ 90% purity and was used in the next synthesis step without additional purification. Yellowish oil. ^{1}H NMR (500 MHz; DMSO-d_6; Me_4Si), δ: 2.90 (2H, m, C*H*$_2$), 2.55 (2H, m, C*H*$_2$), 2.11 (2H, m, C*H*$_2$), 1.83 (2H, m, C*H*$_2$), 1.61-1.55 (18H, 2 s, C(C*H*$_3$)$_3$+ C(C*H*$_3$)$_3$). All amount of compound **15** was dissolved in 1,2-dimethoxyethane (30 mL) followed by addition of 2N aq. KOH (23 mL, 46 mmol, 10 eq). The reaction mixture was vigorously stirred at room temperature for 12 h. The solvents were removed under vacuum, and water (30 mL) was added. The water phase was washed with CH_2Cl_2 (2×5 mL). Combined organic phases were discarded, and the water phase was acidified with aq. HCl to pH ~ 4. The formed precipitate was filtered off to provide pure compound **16** (84 mg, 0.3 mmol, 6% yield). The crystals of **16** suitable for X-Ray diffractional study were obtained by slow evaporation of a diluted solution of **16** in isopropanol. M.p. > 220 °C.

^{1}H NMR (500 MHz; DMDO-d_6; Me_4Si), δ: 2.63 (2H, t, *J* = 5.0 Hz, C*H*$_2$), 2.01 (2H, m, C*H*$_2$), 1.92 (2H, m, C*H*$_2$), 1.47 (s, 9H, C(CH$_3$)$_3$).

^{13}C NMR (125 MHz; DMDO-d_6; Me_4Si), δ: 177.2 (s, *C*O), 156.9 (s, *C*O), 148.4 (s, *C*O, Boc), 123.0 (t, ^1J(C,F) = 241.3 Hz, *C*F_2), 83.1 (s, *C*(CH$_3$)$_3$), 62.9 (s, *C*(NH)CO), 29.7 (t, ^2J(C,F) = 25.0 Hz, *C*H_2CF_2), 28.2 (s, C(*C*H$_3$)$_3$), 26.9 (d, ^3J(C,F) = 6.4 Hz, *C*$H_2CH_2CF_2$).

^{19}F NMR (376 MHz; DMDO-d_6; $CFCl_3$), δ: -91.77 (d, ^2J(F,F) = 236.9 Hz, C*F*F), -100.83 (dtt, *J* = 236.9, 37.7, 11.3 Hz).

MS (*m/z*): 304 $(M)^+$.

IR (KBr), cm^{-1}: 3247 (broad, ν N-H), 2987, 2944 (ν C_{sp3}-H), 1826, 1791, 1744, 1739 (ν C=O), 1146, 1109 (ν C-O).
Anal. Calcd for $C_{13}H_{18}F_2N_2O_4$: C, 51.31; H, 5.96; N, 9.21. Found: C, 51.01; H, 5.65; N, 8.89.

pH value of the collected water phase was again adjusted to ~ 9 with aq. KOH. The solution was evaporated under reduced pressure to ~ 5 mL, followed by purification on cation-exchange resin Cu-2. Elution with water was first performed to remove all inorganic salts. Subsequent elution with 25% NH_3 afforded pure amino acid **5** (360 mg, 2.0 mmol, 45% yield from **15**. 43% yield over two steps from **9**) as a white solid. M.p. > 220 °C.
^{1}H NMR (500 MHz; D_2O + CF_3COOD; Me_4Si), δ: 2.21 (2H, m), 2.17-2.05 (2H, broad m), 2.05-1.85 (4H, broad m).
^{13}C NMR (125 MHz; D_2O + CF_3COOD; Me_4Si), δ: 172.28 (s, *C*O_2H), 122.29 (t, ^1J(C,F) = 238.8 Hz, *C*F_2), 57.44 (s, *C*(NH_2)CO_2H), 28.49 (t, ^2J(C,F) = 25.0 Hz, *C*H_2CF_2), 28.30 (s, *C*H_2).
^{19}F NMR (470 MHz; D_2O; $CFCl_3$), δ: -95.95 (br. d, ^2J(F,F) = 233.0 Hz, C*F*F), -99.56 (br. d, ^2J(F,F) = 233.0 Hz, CF*F*).
Anal. Calcd for $C_7H_{11}F_2NO_2$: C, 46.93; H, 6.19; N, 7.82. Found: C, 46.63; H, 6.01; N, 7.54.
The crystals of **5***HCl suitable for X-Ray diffractional study were obtained by a slow evaporation of a diluted solution of **5** in aq. hydrochloric acid.
IR (KBr), cm^{-1}: 3383 (broad, ν NH_3^+), 2944 (ν C_{sp3}-H), 1748 (ν C=O).

Method B:

A mixture of compound **9** (935 mg, 4.6 mmol), NEt_3 (834 mg, 9.2 mmol) and catalytic amount of DMAP (12 mg) in dimethoxyethane (30 mL) was treated with Boc_2O (5.0 g, 22.9 mmol). The reaction mixture was vigorously stirred for 12 h. Another portion of DMAP (12 mg) was added, and the mixture was stirred for additional 24 h. 2N aq. KOH (34 mL, 69 mmol, 15 eq) was added. The reaction mixture was vigorously stirred at room temperature for 24 h. The solvents were removed under vacuum, and water (50 mL) was added. The water phase was washed CH_2Cl_2 (2×10 mL). Combined organic phases were discarded, and the water phase was evaporated under reduced pressure to ~ 10 mL. The obtained solution was purified on cation-exchange resin Cu-2. Elution with water was first performed to remove all inorganic compounds. Subsequent elution with 25% NH_3 afforded pure amino acid **5** (410 mg, 2.3 mmol, 50% yield) as a white solid.

Acknowledgement

This work was supported by Enamine Ltd. PM is very grateful to Dr. Andrei Bezdudny for the generous gift of morph-DAST; and to Dr. Sergey Zozulya for the help with determination of logD parameters, to Dr. Olena Litsis for IR measurements, and to Dr. Karen Tarasenko for the help in the preparation of manuscript.

Supporting Information

Crystallographic data for compounds **5***HCl, **7**, **9**, **16** and structure description; determination of pK_a and logD values; fluorescence measurements.

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SUPPORTING INFORMATION

1-Amino-4,4-difluorocyclohexanecarboxylic acid as a promising building block for drug discovery: design, synthesis and characterization

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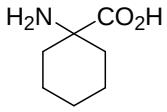
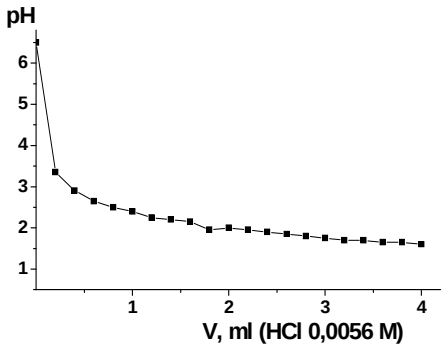
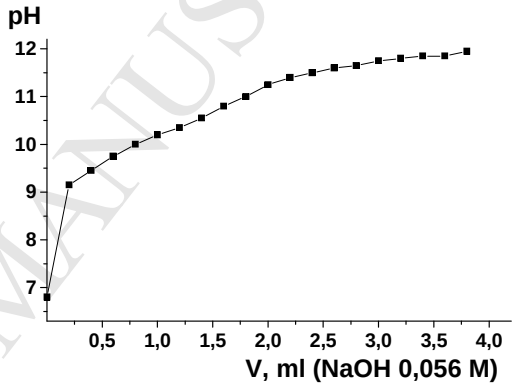
Table of contents

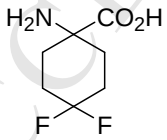
1) Determination of pK_a values for amino acids 1 and 5.

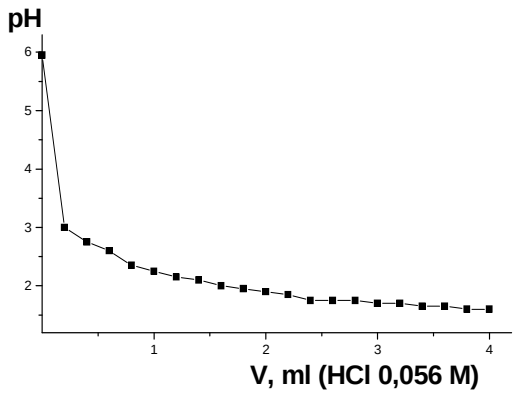
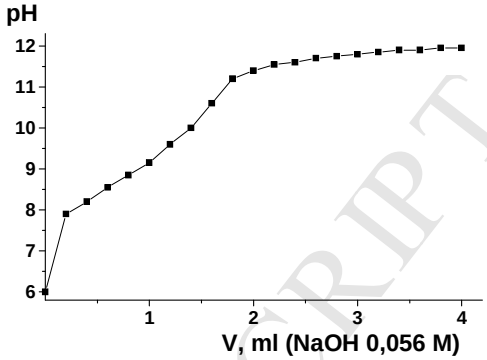
pK_a values were obtained from the pH-metric titration curves using "Hyperquad 2000" program.

Amino acid	Obtained data	
	pK(COOH)	pK(NH_3^+)
 **1**	2,92	10,48

a	b
	

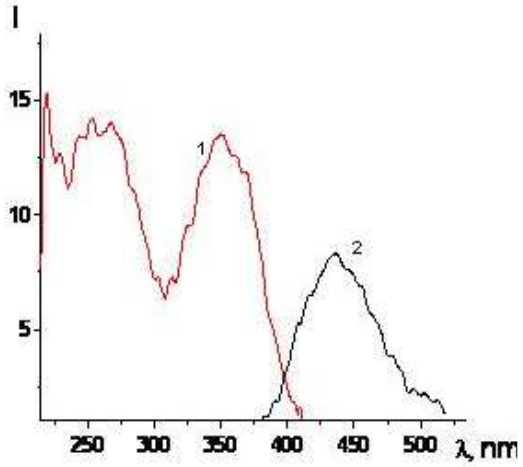
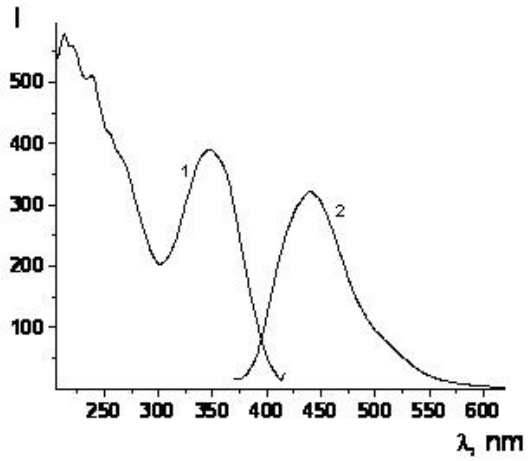
Titration curves of amino acid **1** with 0,056M HCl (a) and with 0,056M NaOH (b). V_0= 10 mL. Ve.p.= 1,6 mL (a); Ve.p.= 1,4 mL (b).

Amino acid	Obtained data	
	pK(COOH)	pK(NH_3^+)
 **5**	2,56	9,31

a	b
	

Titration curves of amino acid **5** with 0,056M HCl (a) and with 0,056M NaOH (b). V_0= 10 mL. Ve.p.= 1,4 mL (a); Ve.p.= 1,6 mL (b).

2) Fluorescent properties of amino acids 1 and 5.

a (**1**)	b (**5**)
	

Excitation (1a, 1b) and fluorescence (2a, 2b) spectra of aqueous solution of amino acids **1** (a) and **5** (b). $C_{amino\ acid}$ = 0,001 M, λ_{em}= 441 нм (1), λ_{exi}=347 нм (2), interval 10, 20. l= 1 см. The spectra are referenced excluding the signal of water.

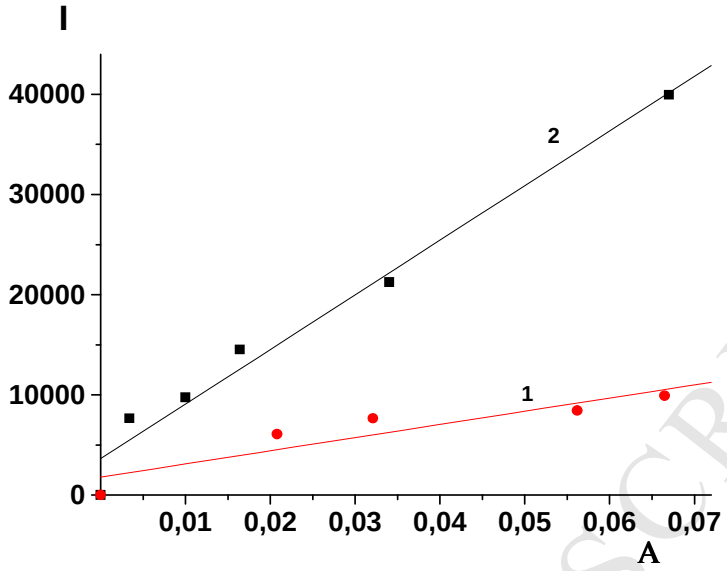
Fluorescence Quantum yield

For determination of quantum yield of compound **5**, *L*-phenylalanine was chosen as a standard (ST), with the known quantum yield in aqueous solution.[1]

The solutions were prepared by dissolving the compounds in bi-distilled water (pH ≈ 5,5). pI (phenylalanine) = (2.2 + 9.1) / 2 = 5.6; pI (**5**) = (2.56+9.31) / 2 = 5.9. Under these conditions the compounds exist in a zwitter-ionic form. Changing the pH value of the solution by adding bases or acids decreased the fluorescent intensity; emission and excitation maximum were shifted to a long wavelength region.

The quantum yield was calculated on the basis of dependence of integrated fluorescence intensity (I) on the optical density (A):

[1] Lakowicz, J. R.; Brouwer, A. M. *Pure Appl. Chem.*, **2011**, *83*, 2213.



Linear plots for standard sample of phenylalanine (**1**) and substance **5**. The data of fluorescence intensity were obtained under excitation wavelength 258 nm.

$\Phi_X = \Phi_{ST}(Grad_X/Grad_{ST})(\eta^2_X/\eta^2_{ST}) = 0{,}024*(545027/131488)= 0{,}099$

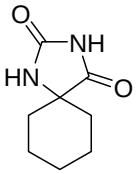
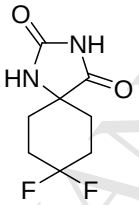
where,
Φ_X - quantum yield of unknown substance
Φ_{ST} - quantum yield of standard
$Grad_{ST}$ – gradient = tgα of curve 1
$Grad_X$ - gradient = tgα of curve 2

Similar results were also obtained under excitation at 230 and 347 nm wavelengths. The average measured quantum yield for **5** in aqueous solution was 0,100±0,005.

3) Determination of LogD of compounds 9 and 17

Partition ratio (or **LogD**) is a logarithm of the ratio of drug concentrations in two immiscible solvents, typically pH-buffered water and n-octanol. It is a measure of hydrophobic/hydrophilic properties of a given molecule. The partition ratio is determined using shake-flask method, which consists of dissolving some of the solute of interest in a volume of n-octanol and an aqueous buffer of choice, then measuring the concentration of the solute in each solvent, by using LC-MS.

Compounds:

Compound	Structure	MW
17		168.20
9		204.18

Materials and reagents:
DMSO (SIGMA-ALDRICH 34869 - CHROMASOLV® Plus, for HPLC, ≥99.7%)
PBS buffer (Helicon, Am-E404-100, pH 7.4)
n-Octanol (SIGMA-ALDRICH 472328 - ACS reagent, ≥99%)
Acetonitrile (SIGMA-ALDRICH 34998 - CHROMASOLV® Plus, for HPLC, ≥99.9%)

Equipment:
Multi Mix MTR 22 rotator
Eppendorf 5417R centrifuge
Shimadzu VP HPLC system and AB Sciex API3000 mass-detector.

Procedure:
Aqueous phase: n-octanol mix was pre-saturated during 24 hours.
10 mM compound DMSO stock was dissolved in aqueous phase to a final concentration of DMSO of 1%. Equal volume of n-octanol was added and mixed in rotator for 60 minutes. Phase separation was assured by centrifugation for 3 minutes @14000rpm. Both phases were sampled. Each sample was analyzed by LC-MS and the peak area ratios calculated to produce LogD values.

Results:

Compound ID	Aq_Analyte Peak Area (counts)	Oct_Analyte Peak Area (counts)	D	LogD, pH7.4	Mean LogD, pH7.4	SD	RSD,%
17	43400	1516600*	34.94	1.54	1.51	0.035	2.3
17	48200	1437800*	29.83	1.48			
17	44100	1451900*	32.92	1.52			
9	258500	974500	3.77	0.58	0.52	0.045	8.7
9	298000	933000	3.13	0.50			
9	286000	904000	3.16	0.50			

4) X-Ray diffraction study of the compounds 5*HCl, 7, 9, 16

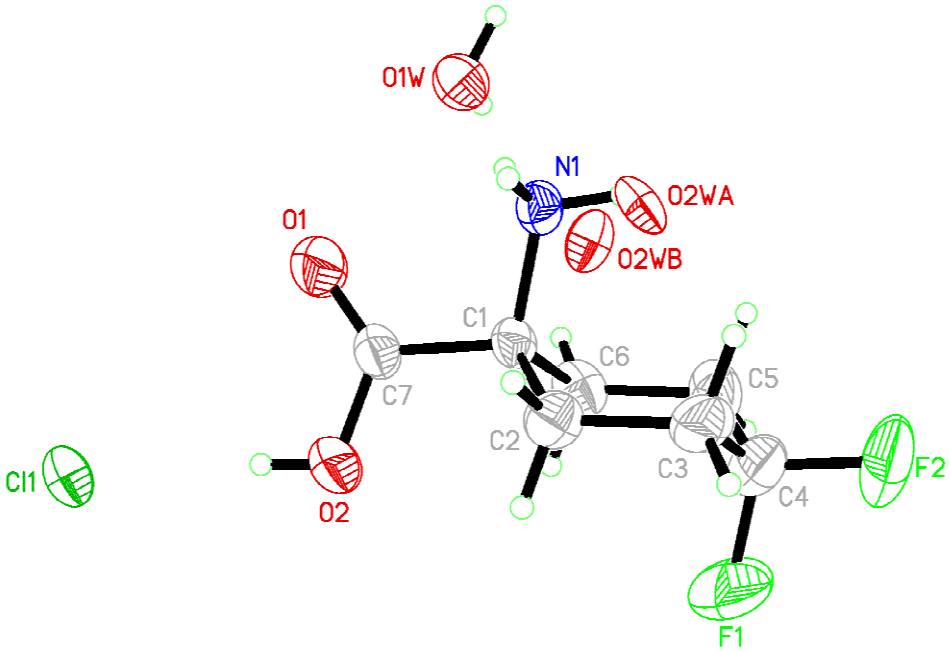


Figure S1. Structure of compound **5** according to X-ray diffraction data. Thermal ellipsoids are shown at the 50 % probability level.

The compound **5** is chloride of organic cation (Fig. S1) which exists in the crystal phase as hydrate in the ratio 1:1.25. The water molecule with partial occupancy is disordered over two positions (A and B) with equal populations what does not allow to determine the positions of the hydrogen atoms at it. The positive charge of the cation is localized on the nitrogen atom. It is confirmed by protonation of this atom (positions of hydrogen atoms were located from electron density difference maps and the N1-C1 bond length (1.503(4) Å) which is elongated as compared with mean value [1] for the Csp^3-N bond (1.469 Å)).

The saturated ring adopts a chair conformation (the puckering parameters [2] are: S= 1.09, Θ = 0.9°, Ψ = 7.9°). Deviations of the C1 and C4 atoms from the mean plane of the remaining atoms of the ring are -0.64 Å and 0.60 Å, respectively. The protonated amino group has axial orientation (the C5-C6-C1-N1 torsion angle is 69.7(4)°) what causes the appearance of the shortened intramolecular contact H5b…H1Nb 2.25 Å (the van der Waals radii sum [3] is 2.34 Å). The carboxyl group is located in equatorial position and it is almost orthogonal to the C6-C1 endocyclic bond (the C5-C6-C1-C7 and C6-C1-C7-O1 torsion angles are -175.2(3)° and -101.8(5)°, respectively).

In the crystal phase the cations of **5** are bonded with anions and water molecules by intermolecular hydrogen bonds: N(1)-H(1Na)…O(1w)' (1-x, 0.5+y, -z) H…O 2.00 Å N-H...O 171°; N(1)-H(1Nb)...Cl(1)' (1+x, 1+y, z) H...Cl 2.39 Å N-H...Cl 162°; N(1)-H(1Nc)...O(1w)' H...O 1.98 Å N-H...O 170°; O(1w)-H(1wa)...Cl(1)' (1+x, y, z) H...Cl 2.43 Å O-H...Cl 149°; O(1w)-H(1wb)...Cl(1)' (1-x, 0.5+y, -z) H...Cl 2.24 Å O-H...Cl 176°; O(2)-H...Cl(1)' H...Cl 2.22 Å O-H...Cl 173°; C(5)-H(5a)...F(2)' (2-x, -0.5+y, 1-z) H...F 2.50 Å C-H...F 169°; C(5)-H(5b)...Cl(1)' (1+x, 1+y, z) H...Cl 2.80 Å C-H...Cl 148°; C(6)-H(6a)...Cl(1)' (1+x, y, z) H...Cl 2.95 Å C-H...Cl 166°; C(2)-H(2a)...Cl(1)' (x, 1+y, z) H...Cl 2.86 Å C-H...Cl 159°; C(3)-H(3a)...Cl(1)' (1+x, 1+y, z) H...Cl 2.89 Å C-H...Cl 146°; C(3)-H(3b)...F(1)' (1-x, 0.5+y, 1-z) H...F 2.38 Å C-H...F 165°.

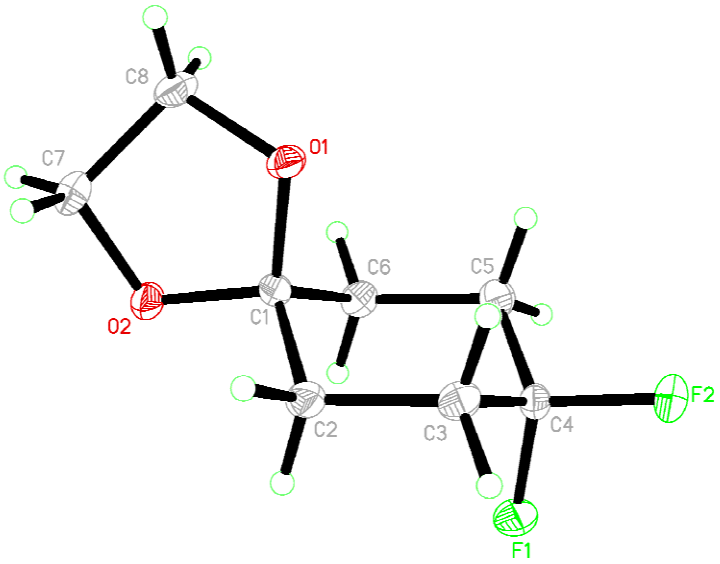


Figure S2. Structure of compound **7** according to X-ray diffraction data. Thermal ellipsoids are shown at the 50 % probability level.

The five-memebered ring of the compound **7** (Fig. S2) adopts an envelope conformation. The C8 atom deviates from the mean plane of remaining atoms of the ring by -0.53 Å. The cyclohexane ring adopts a chair conformation (the puckering parameters are: S= 1.11, Θ = 0.2°, Ψ = 9.0°). Deviations of the C1 and C4 atoms from the mean plane of remaining atoms of the ring are -0.65 Å and 0.62 Å, respectively. Joined cycles are turned in such way that mean planes through-passing the O1, C1, O2, C7 atoms and the C2, C3, C5, C6 atoms are almost orthogonal (the angle between two planes is 85°).

In the crystal phase the intermolecular hydrogen bond C8-H8b…F2’ (1-x, 1-y, -z) H…F 2.53 Å C-H…F 131° is observed.

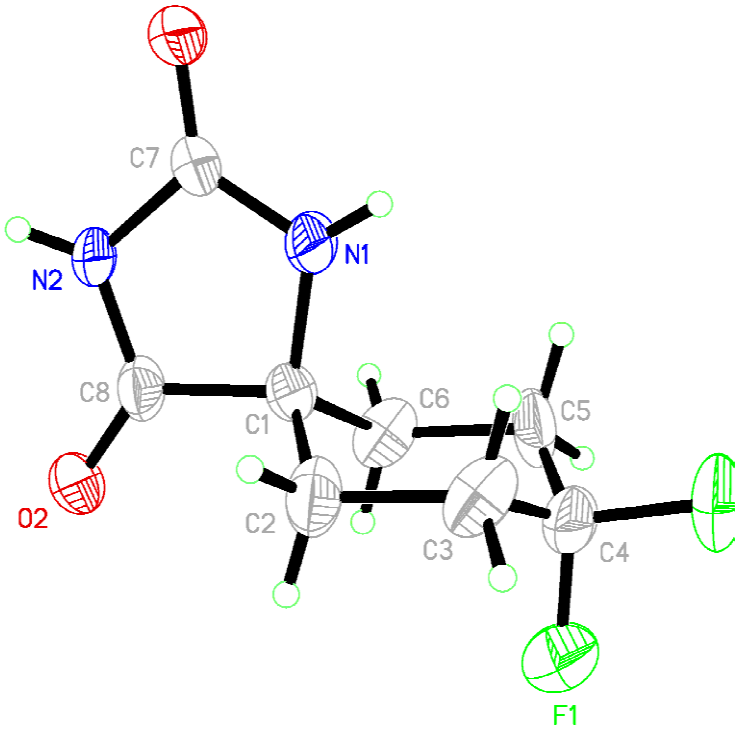


Figure S3. Structure of compound **9** according to X-ray diffraction data. Thermal ellipsoids are shown at the 50 % probability level.

The saturated ring of the compound **9** (Fig. S3) adopts a chair conformation (the puckering parameters are S= 1.13, Θ = 2.8°, Ψ = 19.4°). Deviations of the C1 and C4 atoms from the mean plane of the remaining atoms of the cycle are -0.71 Å and 0.59 Å, respectively. The five-membered heterocycle is joined with cyclohexane ring and is turned almost orthogonal to the plane of the C2, C3, C5, and C6 atoms (the angle between planes is 90°).

In the crystal phase the molecules **9** form the infinite chains (Fig. S4) along the [0 0 1] crystallographic direction due to intermolecular hydrogen bonds N(1)-H…O(1)' (1-x, 1-y, -z) H…O 2.09 Å N-H…O 170° and N(2)-H…O(2)' (1-x, 1-y, 1-z) H…O 2.17 Å N-H…O 163°.

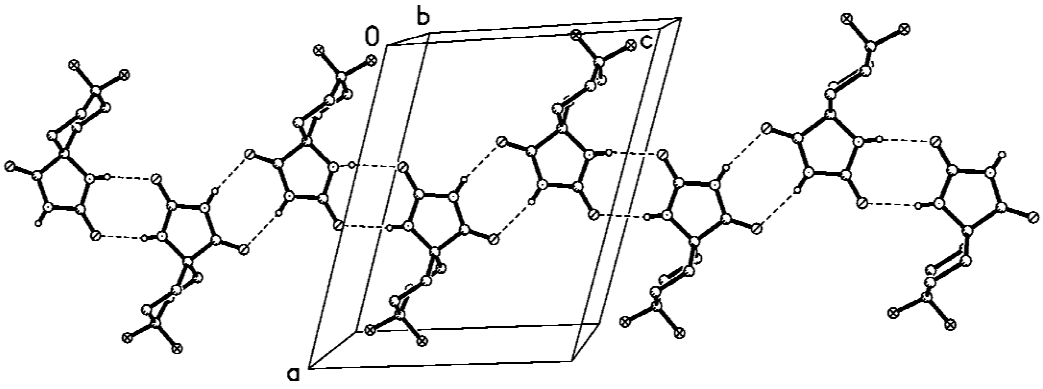


Figure S4. The infinite chains of the molecules **9** along the [0 0 1] crystallographic direction.

The formation of the hydrogen bonds causes the elongation of the C7-O1 (1.228(2) Å) and C8-O2 (1.225(2) Å) bonds as compare with mean value 1.210 Å.

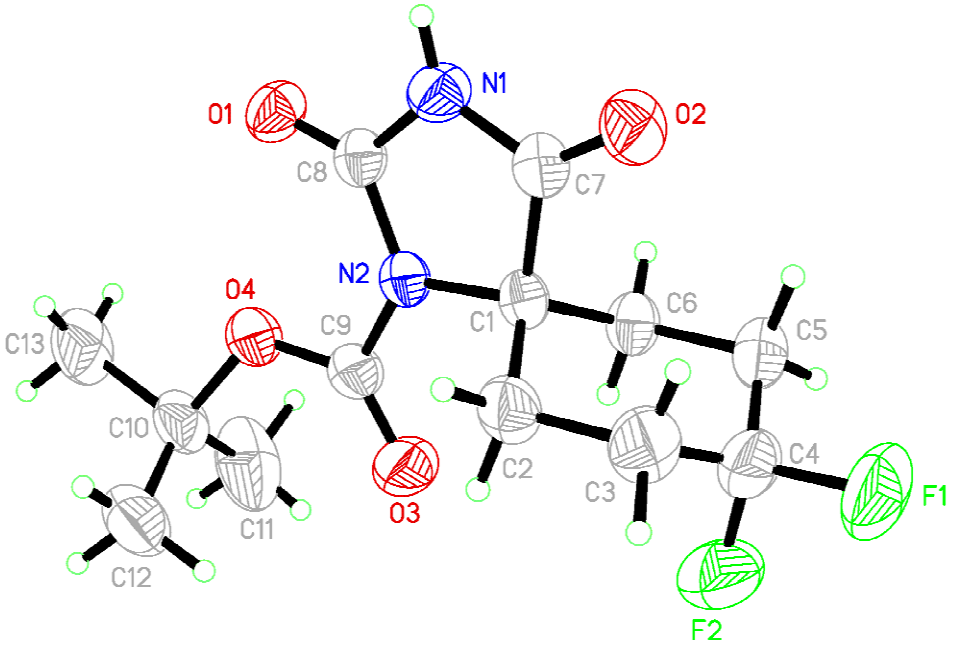


Figure S5. Structure of compound **16** according to X-ray diffraction data. Thermal ellipsoids are shown at the 50 % probability level.

Partially saturated heterocycle of the compound **16** (Fig. S5) is planar within 0.02 Å. Spirojoined cyclohexane ring adopts a chair conformation (the puckering parameters are: S= 1.08, Θ = 1.9°, Ψ = 18.7°) and is turned in such way that the mean plane of the C2, C3, C5 and C6 atoms is orthogonal to the heterocycle plane (the angle between planes is 89°). Deviations of the C1 and C4 atoms from the mean plane of remaining atoms of the ring are 0.61 Å and -0.61 Å, respectively. Such orientation of joined rings is stabilized additionally by the C6-H6b…O3 intramolecular hydrogen bond (H…O 2.39 Å C-H…O 123°) and leads to the appearance of the H5a…C7 shortened intramolecular contact (the distance between atoms is 2.84 Å as compared with van der Waals radii sum 2.87 Å). The carboxyl fragment of the substituent at the N2 atom is almost coplanar to the plane of the heterocycle (the C1-N2-C9-O3 torsion angle is -4.7(2)°). The tert-butyl group is located in *ap*-conformation relatively the N2-C9 bond and it is turned in such way that the C10-C13 bond is anti-periplanar to the C9-O4 bond (the C10-O4-C9-N2 and C9-O4-C10-C13 torsion angles are -169.2(1)° and 178.4(2)°, respectively).

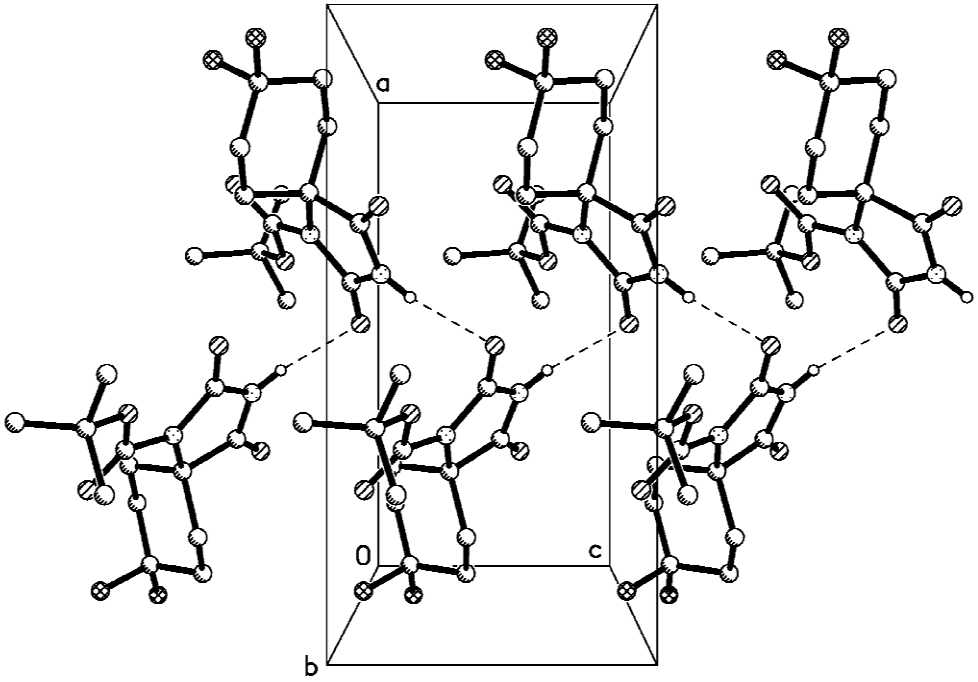


Figure S6. The packing of the molecules **16** in the crystal phase.

In the crystal phase the molecules **16** form the staggered chains (Fig. S6) along the [0 0 1] crystallographic direction due to the formation of the N1-H…O1' (1-x, 1-y, 0.5+z) intermolecular hydrogen bond (H…O 2.15 Å N-H…O 154°).

Experimental part.

X-Ray diffraction studies were performed on an automatic «Xcalibur 3» diffractometer (graphite monochromated MoK_α radiation, CCD-detector, ω-scanning). The structures were solved by direct method using SHELXTL package [4]. Positions of hydrogen atoms were located from electron density difference maps and refined using riding model with $U_{iso} = nU_{eq}$ (n = 1.5 for protonated aminogroup and water molecule and 1.2 for other hydrogen atoms) of the carrier atom in the structures **5** and **9**. The hydrogen atoms of molecules **7** and **16** and hydrogen atom of the molecule **9** which take part in the formation of hydrogen bond were refined in isotropic approximation. The crystallographic data and experimental parameters are listed in Table S1. Final atomic coordinates, geometrical parameters and crystallographic data have been deposited with the Cambridge Crystallographic Data Centre, 11 Union Road, Cambridge, CB2 1EZ, UK (fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk). The deposition numbers are given in Table S1.

Table S1. The crystallographic data and experimental parameters for compounds **5, 7, 9,** and **16**.

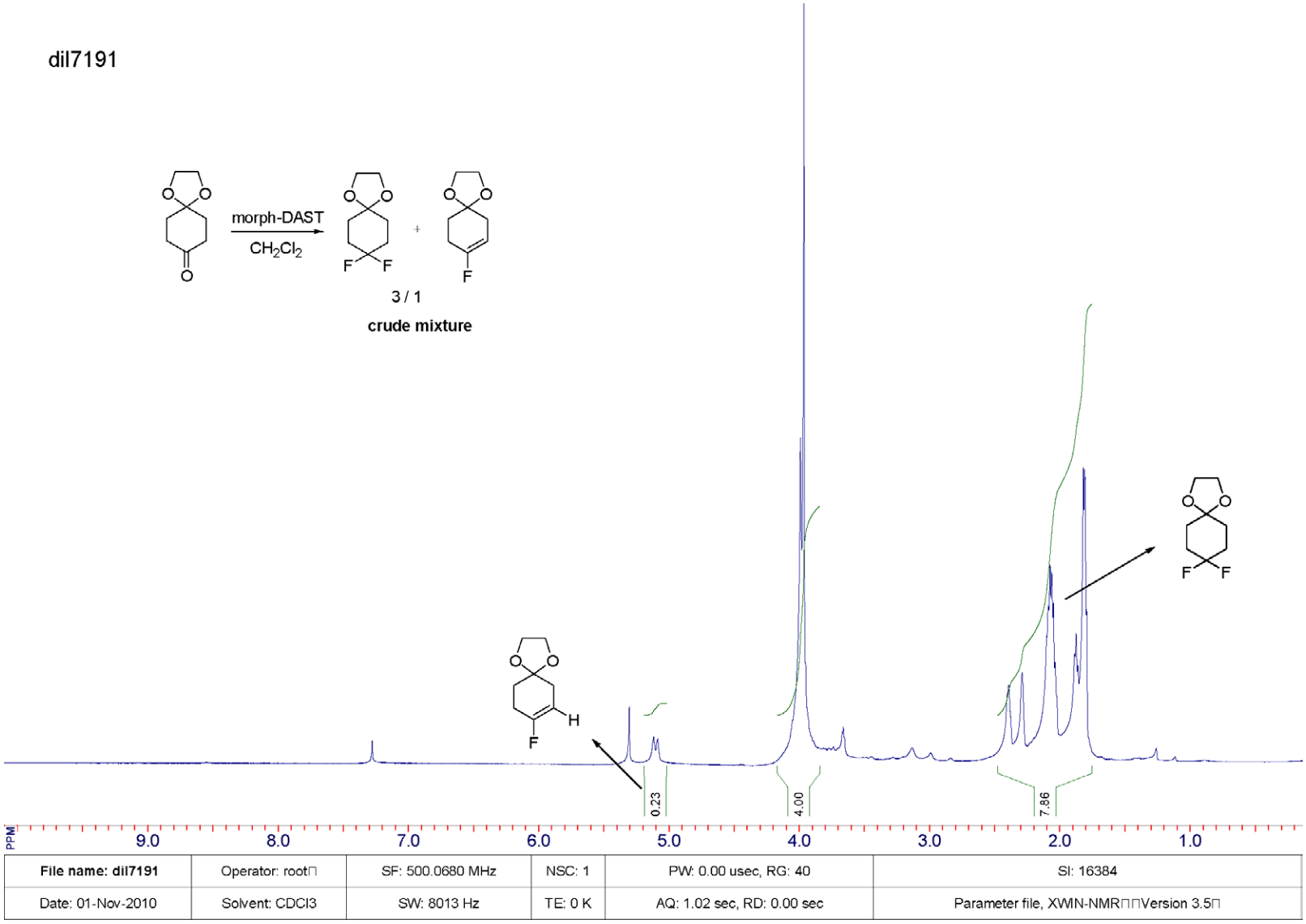
Parameter	**5**	**7**	**9**	**16**
Unit cell dimensions				
a, Å	6.6436(6)	9.8810(5)	12.730(2)	11.892(1)
b, Å	6.4536(5)	8.9680(4)	7.093(1)	20.951(1)
c, Å	12.682(1)	10.2254(6)	10.315(2)	5.9494(4)
α, deg.	90.0	90.0	90.0	90.0
β, deg.	96.694(9)	118.536(7)	108.68(1)	90.0
γ, deg.	90.0	90.0	90.0	90.0
V, $Å^3$	540.02(8)	796.03(7)	882.4(2)	1482.3(2)
F(000)	248	376	424	640
Crystal system	monoclinic	monoclinic	monoclinic	orthorhombic
Space group	$P2_1$	$P2_1/n$	$P2_1/c$	$Pna2_1$
Z	2	4	4	4
T, K	293	100	293	293
μ, mm^{-1}	0.369	0.134	0.138	0.117
D_{calc}, g/cm^3	1.461	1.487	1.537	1.364
$2\Theta_{max}$, grad	60	50	60	60
Measured reflections	4858	3233	8018	7712
Independent reflections	2542	1307	2572	3789
R_{int}	0.033	0.013	0.086	0.025
Reflections with F>4σ(F)	2107	1214	760	3079
Parameters	147	157	135	262
R_1	0.061	0.028	0.044	0.057
wR_2	0.160	0.089	0.088	0.083
S	1.046	1.377	0.718	0.994
CCDC number	903595	903596	903597	855776

Literature

1. Zefirov,N.S., Palyulin,V.A., Dashevskaya,E.E.// J.Phys.Org.Chem., 1990, v.3, p.147-154.
2. Burgi H.-B., Dunitz J.D. Structure correlation. Vol.2. VCH. Weinheim. 1994. p.741-784.
3. Zefirov Yu. V. // Kristallographiya (Russian) , 1997, v.42, p.936-958.
4. Sheldrick G.M. *Acta Crystallogr., Sect. A* **2008**, *A64*, 112.

5) Copies of ^{1}H NMR, ^{13}C NMR and IR spectra

Crude mixture **7**+**10**



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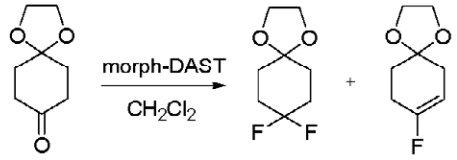
dil7191-C13



3 / 1

crude mixture

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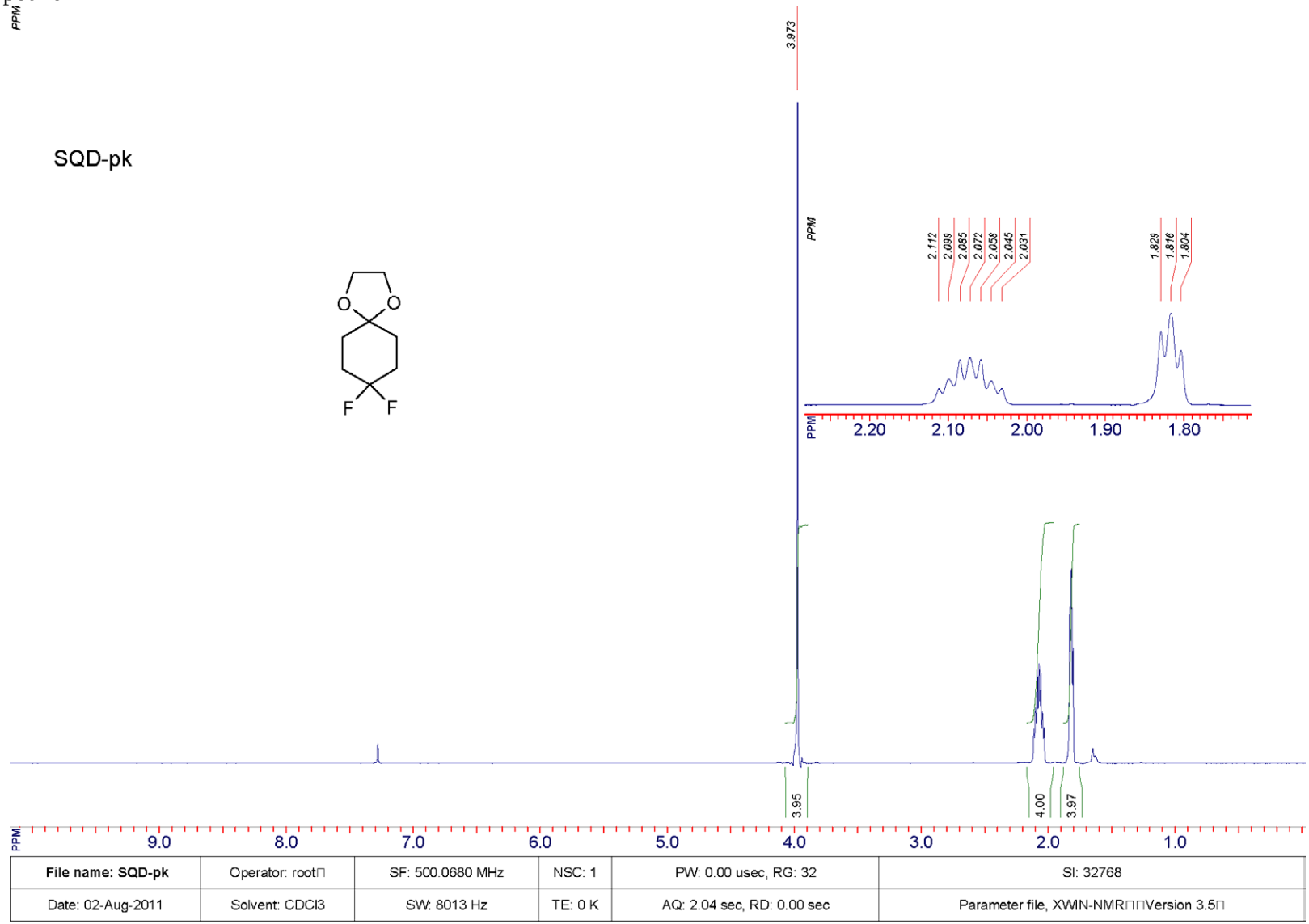
Compound **7**



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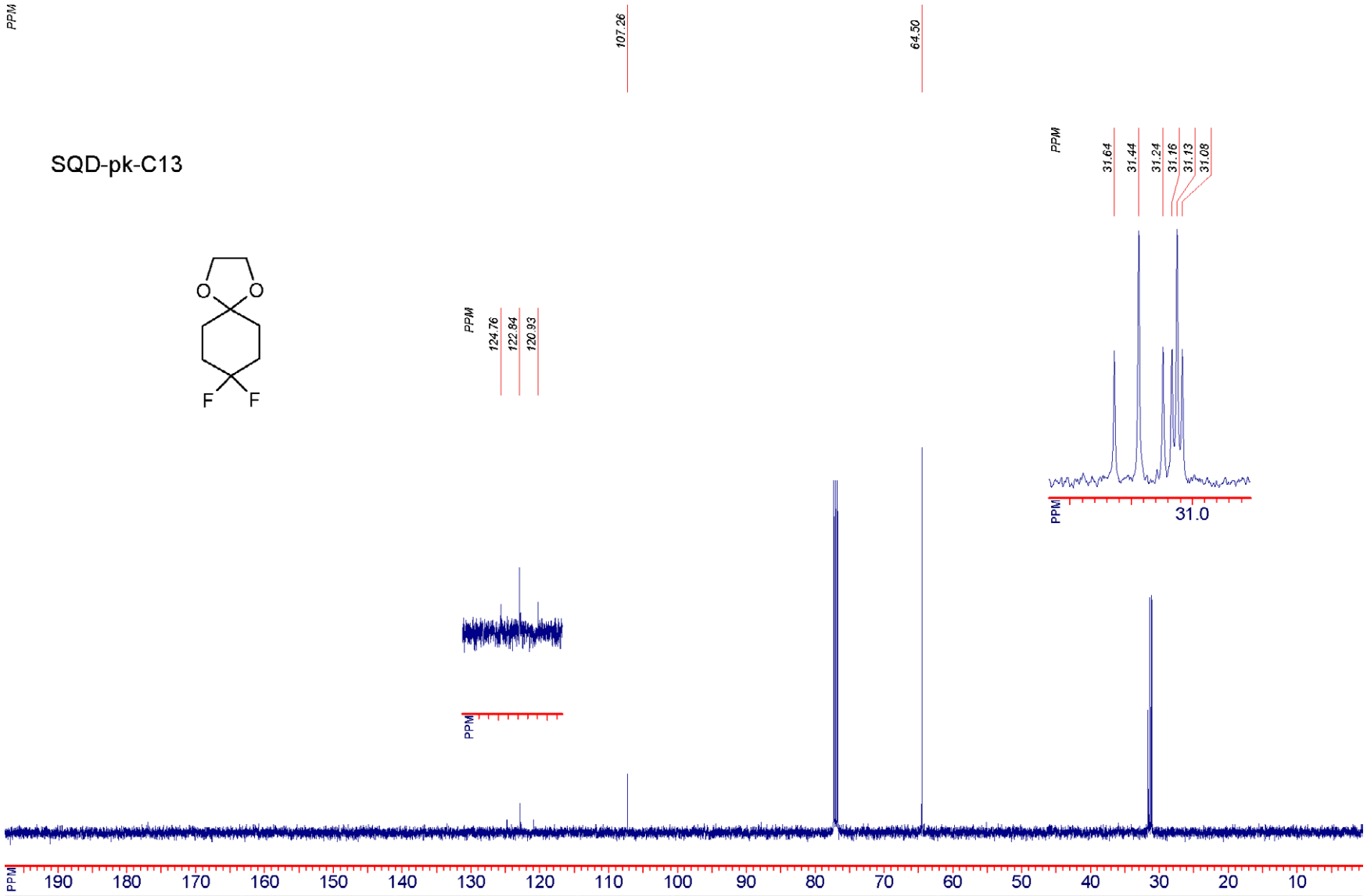

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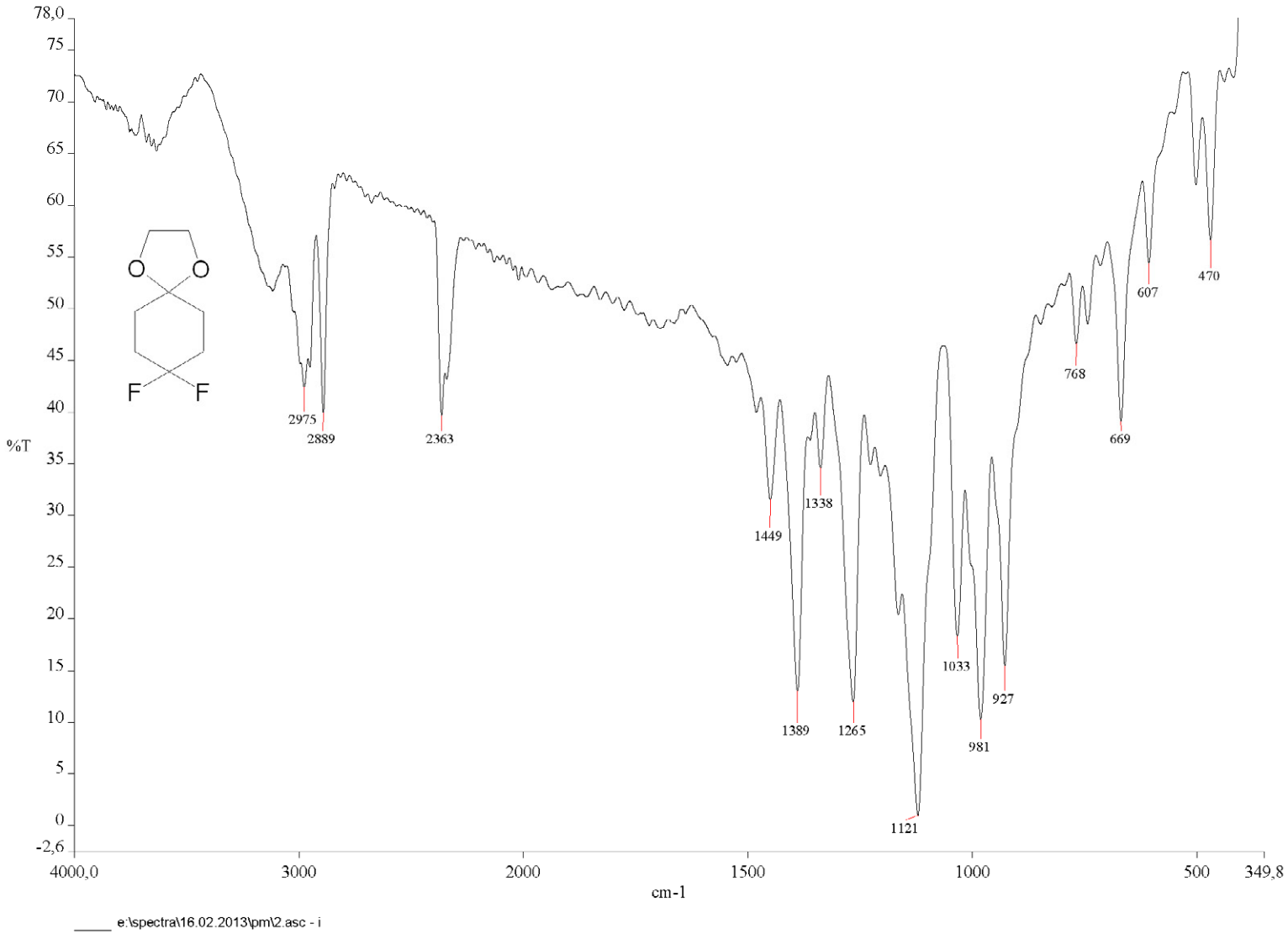
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Compound **8**

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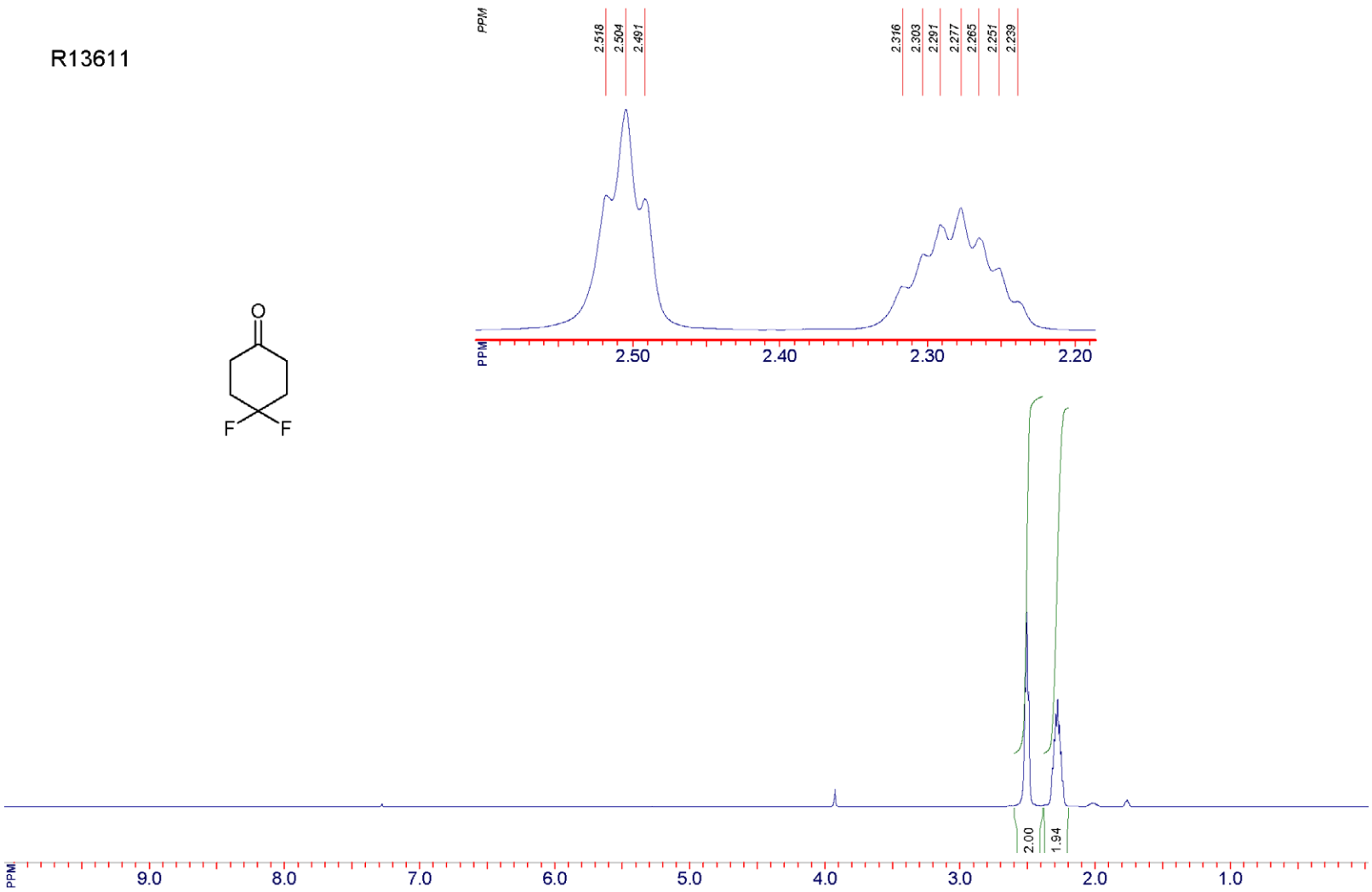

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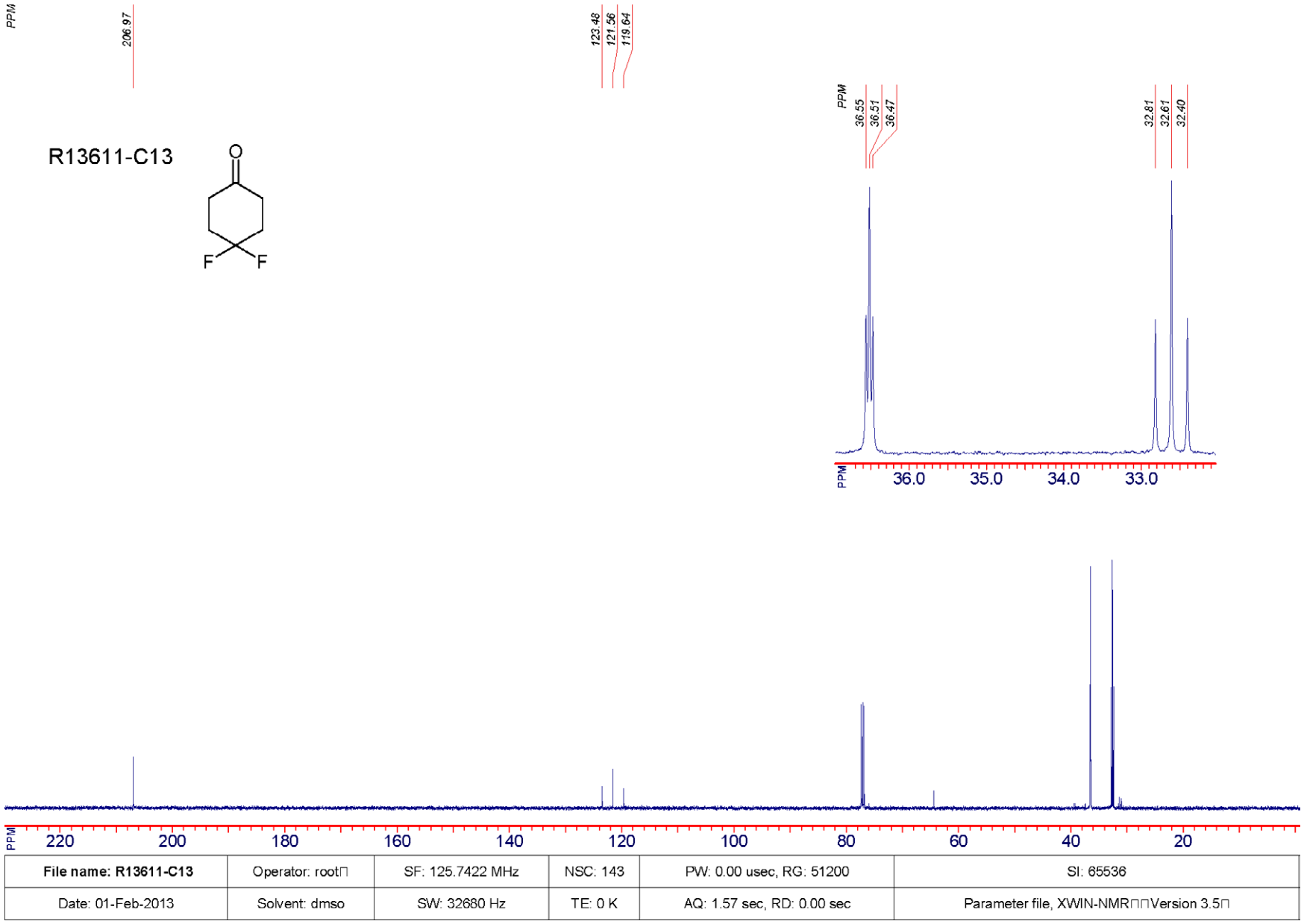

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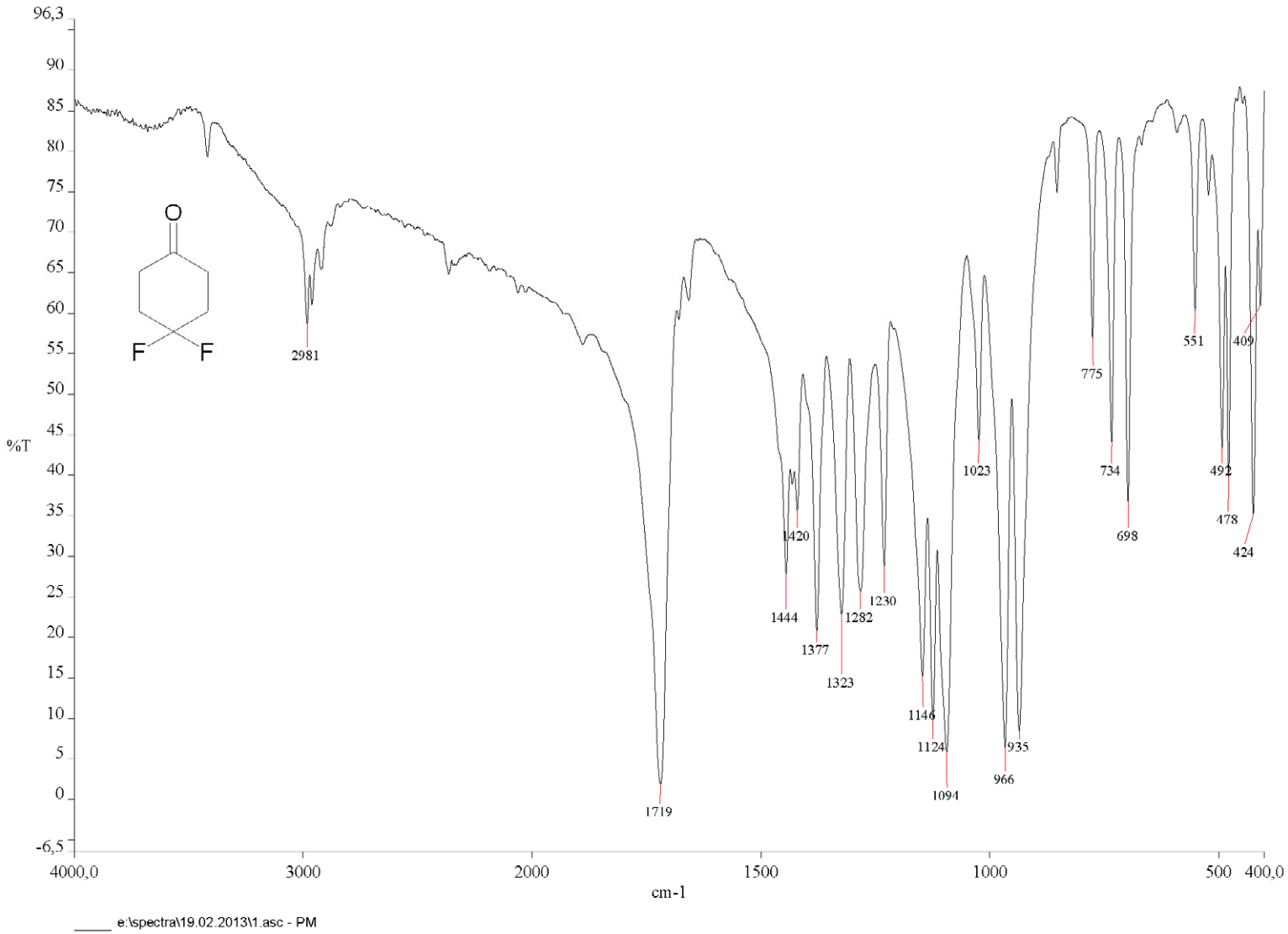
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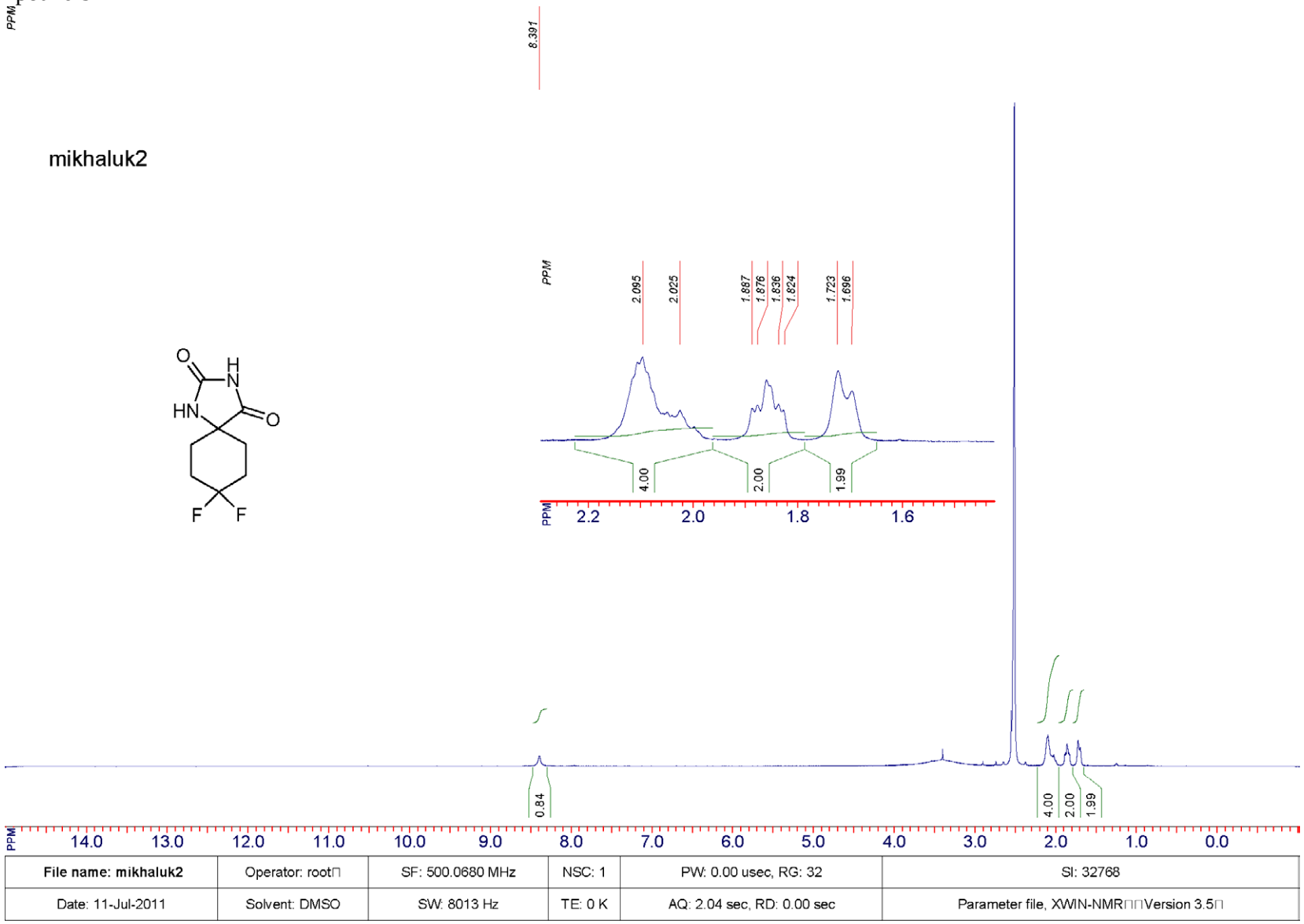

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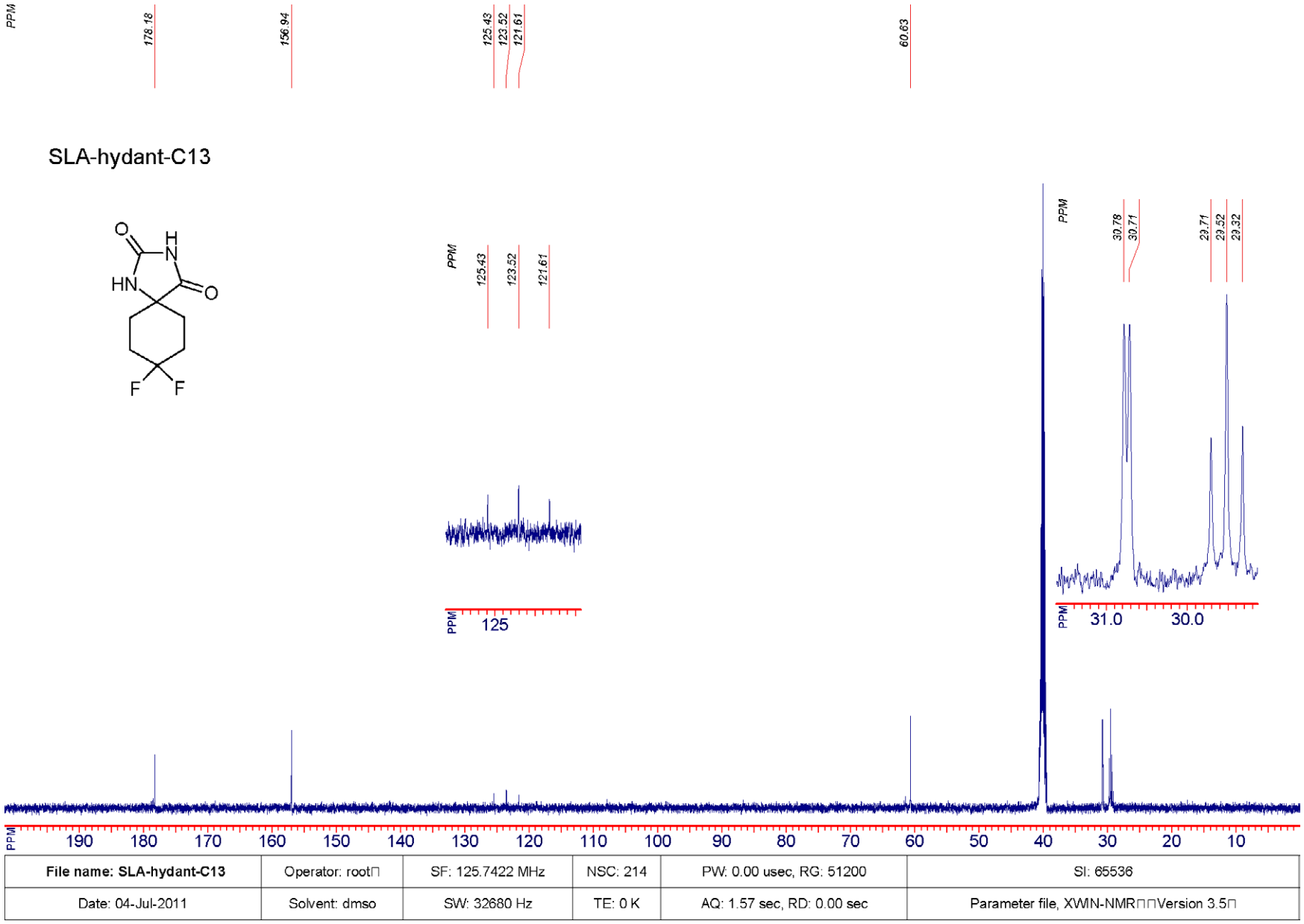

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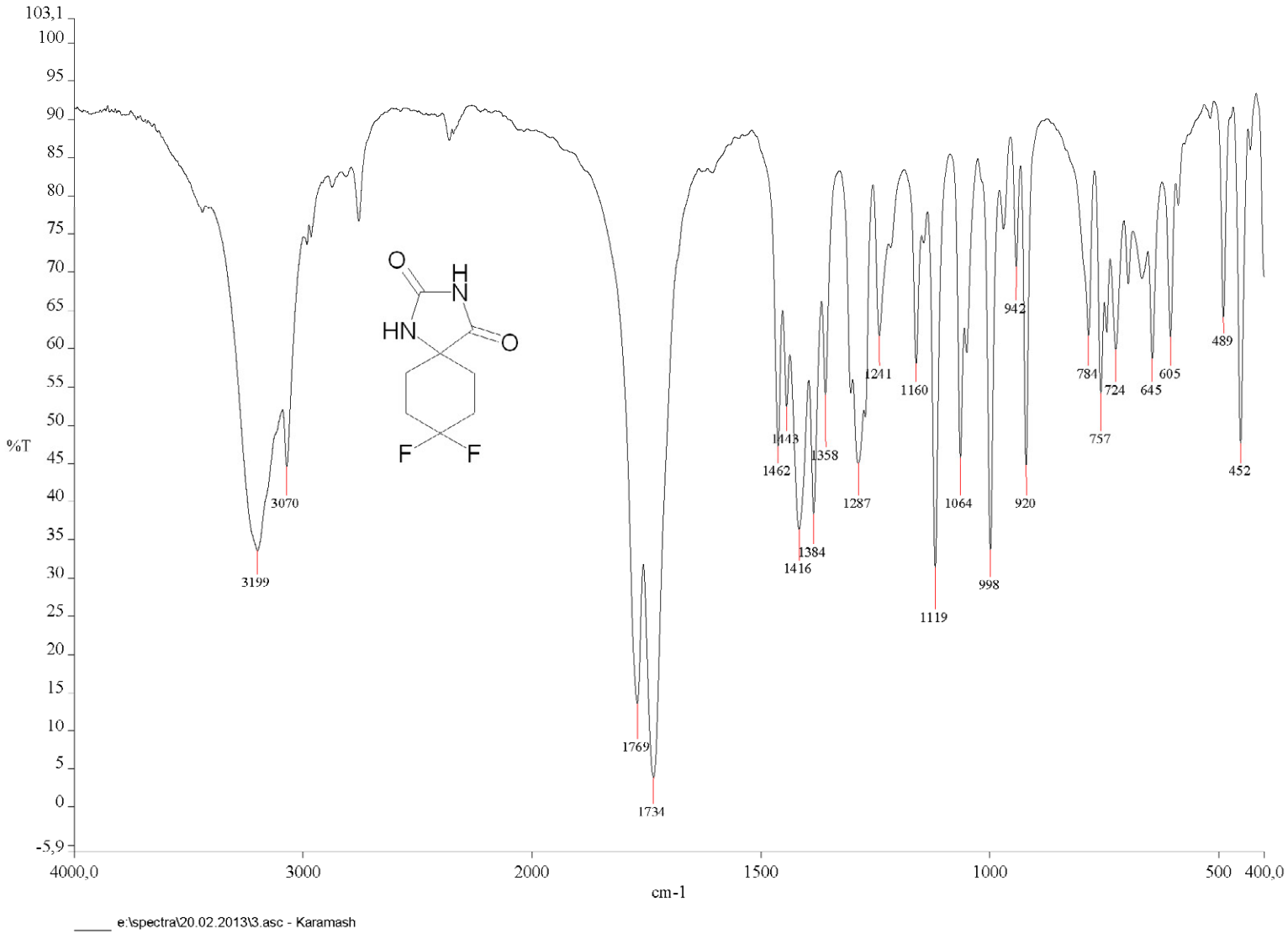
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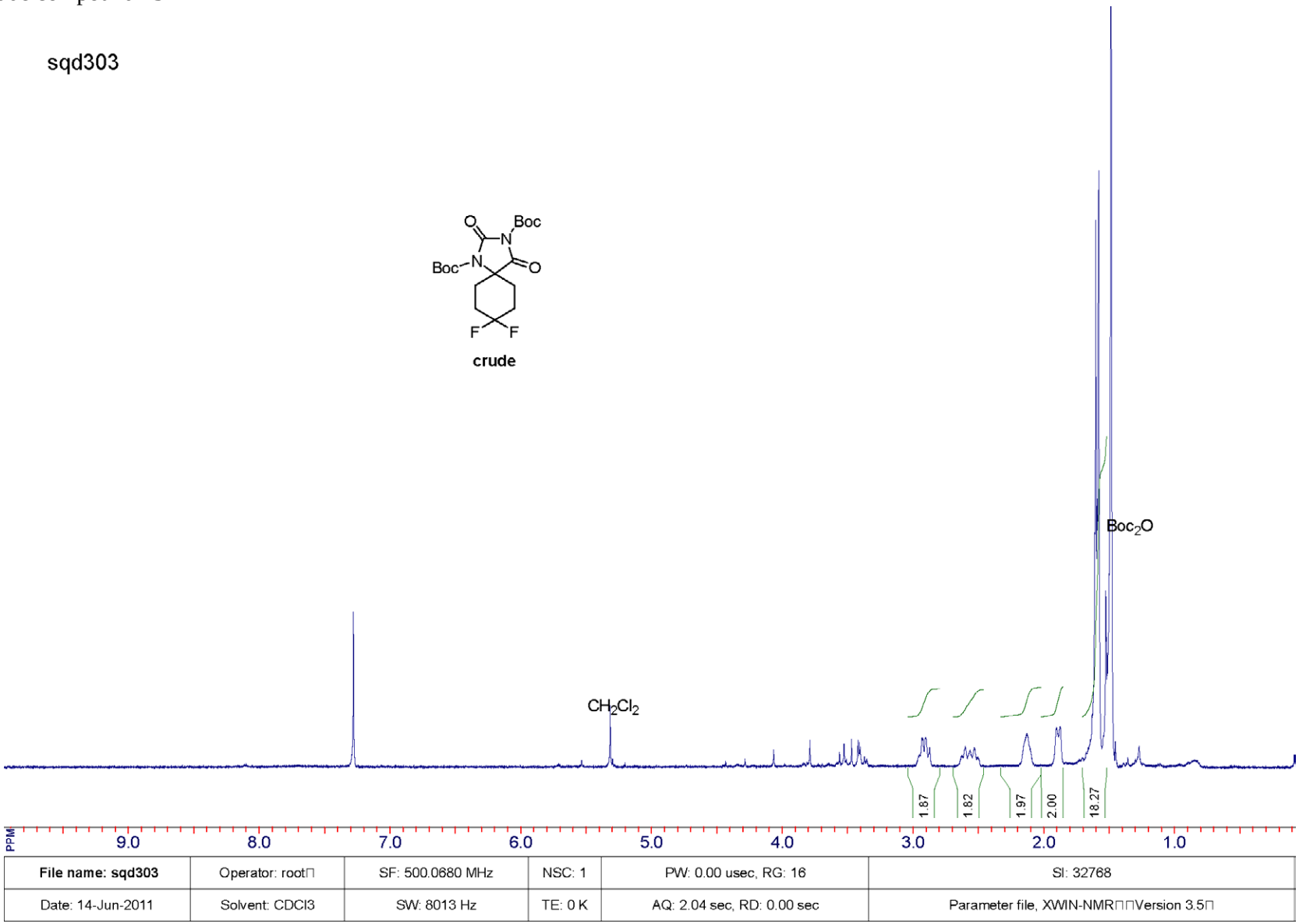
Crude compound **15**



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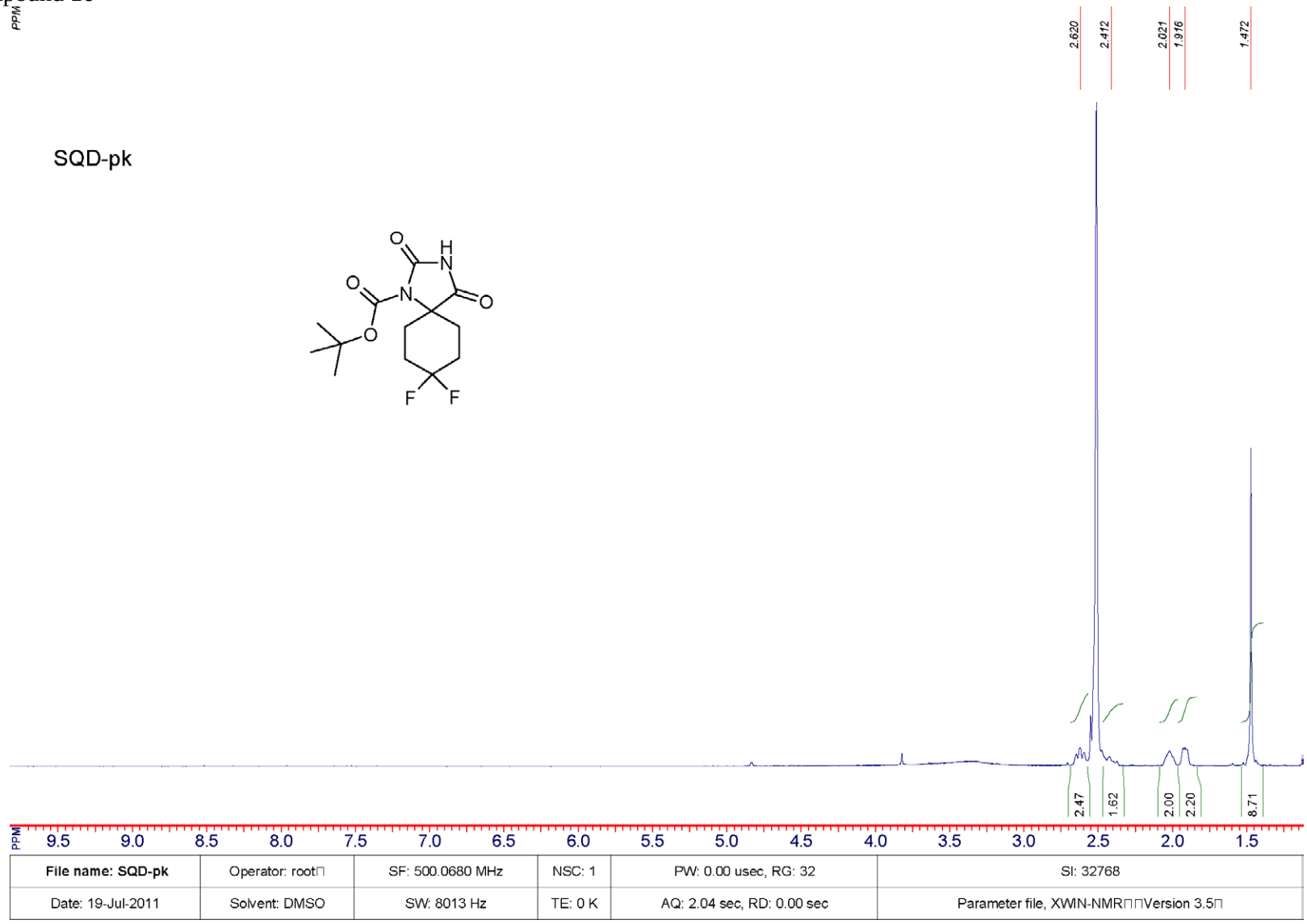
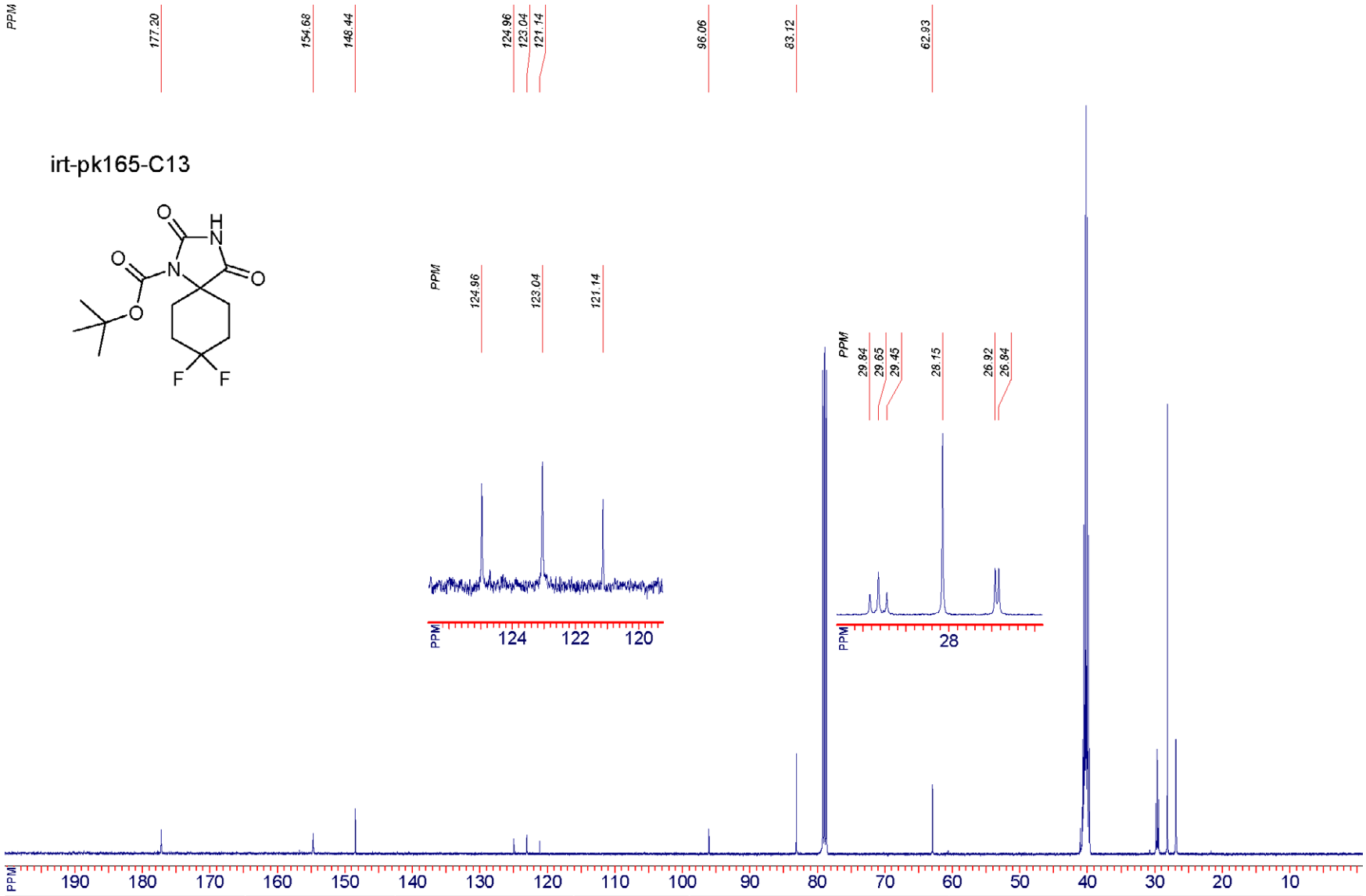
Date: 14-Jun-2011	Solvent: CDCl3	SW: 8013 Hz	TE: 0 K	AQ: 2.04 sec, RD: 0.00 sec	Parameter file, XWIN-NMR⊓⊓Version 3.5⊓

Compound **16**



File name: SQD-pk	Operator: root⊓	SF: 500.0680 MHz	NSC: 1	PW: 0.00 usec, RG: 32	SI: 32768
Date: 19-Jul-2011	Solvent: DMSO	SW: 8013 Hz	TE: 0 K	AQ: 2.04 sec, RD: 0.00 sec	Parameter file, XWIN-NMR⊓⊓Version 3.5⊓



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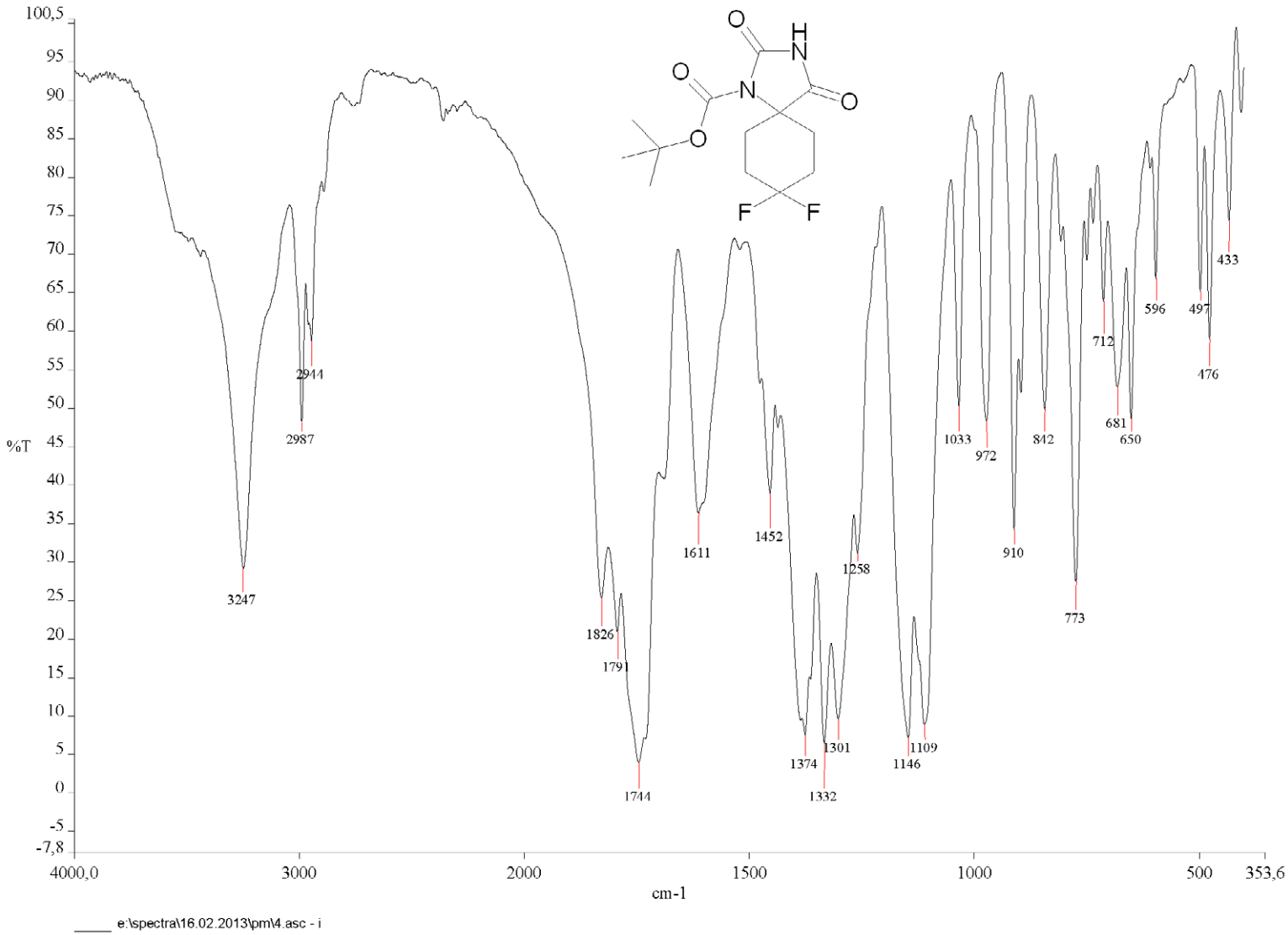
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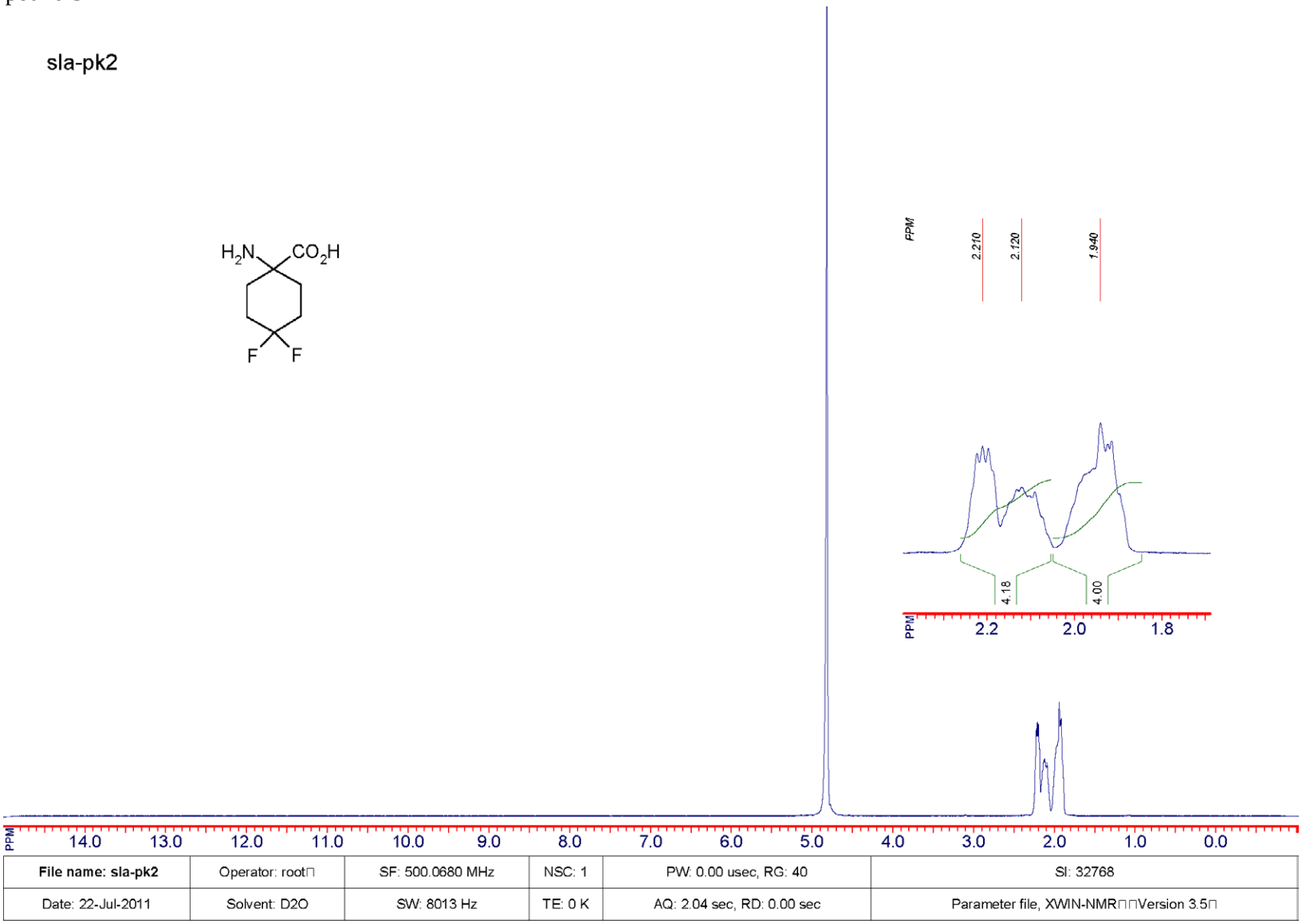
Compound **5**



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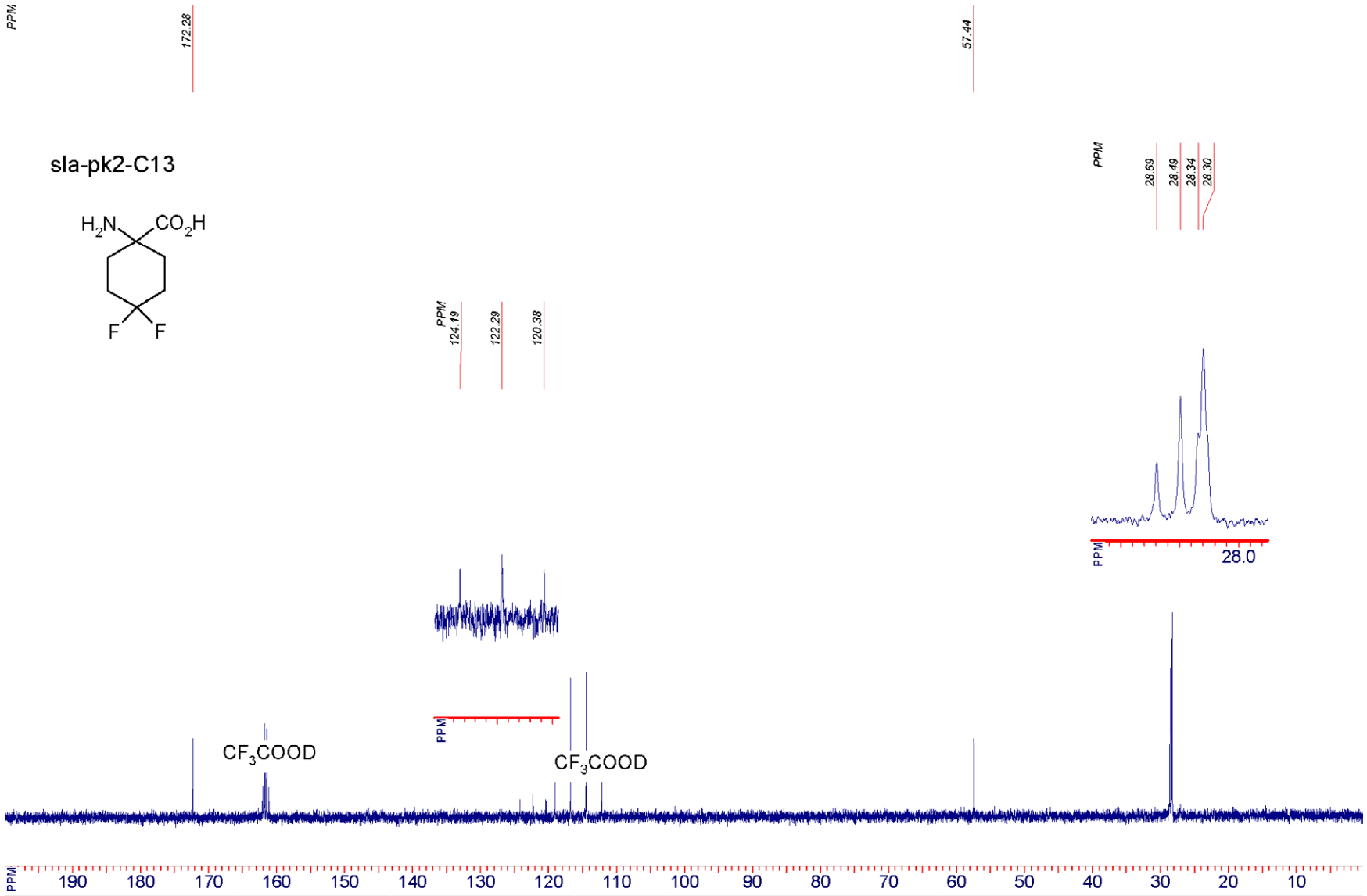

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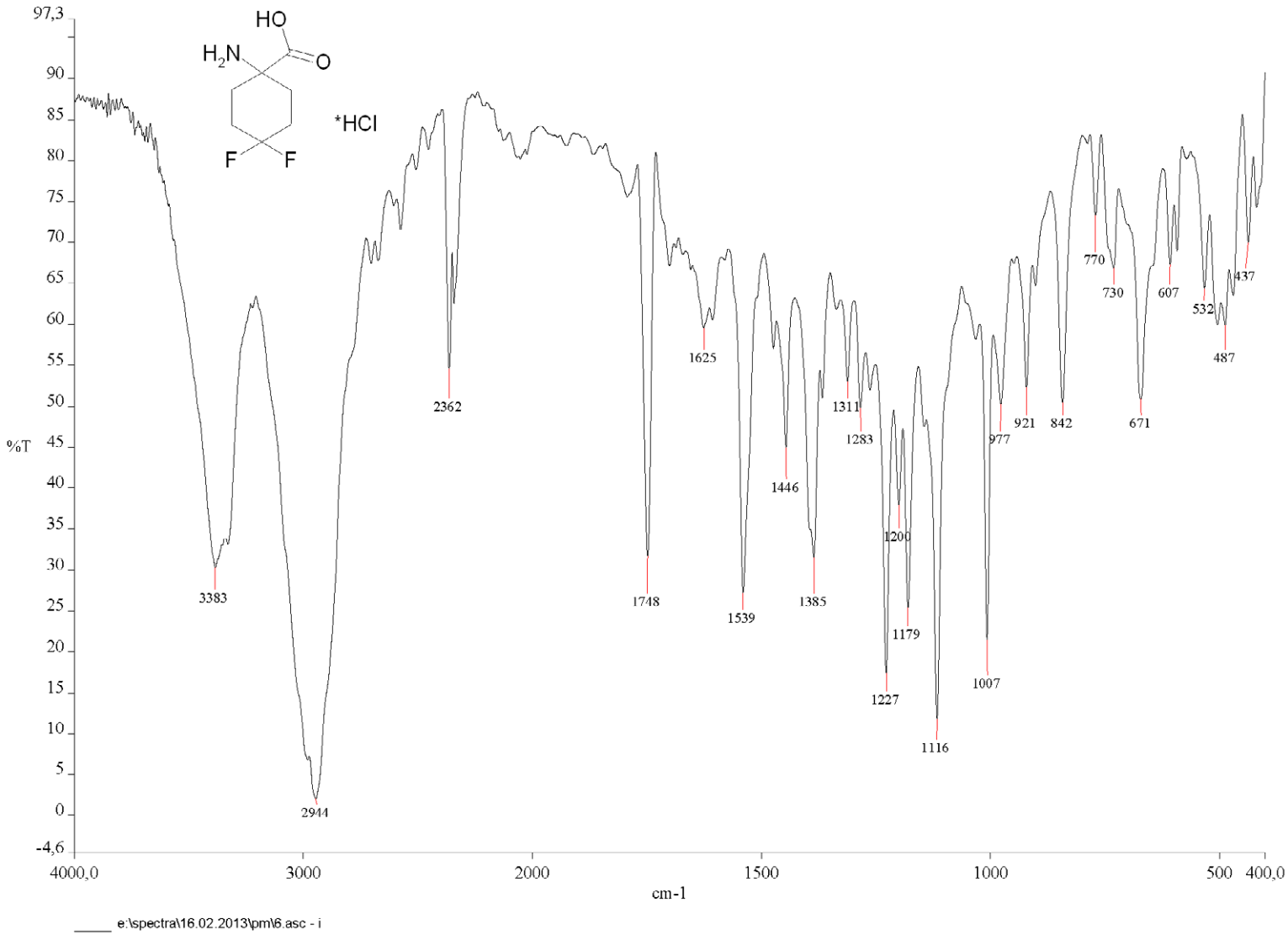
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