Investigation of the Phenyl Ring of Imidazoquinolines

By

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Abstract

The 1H-Imidazo-[4,5-c]quinolines are a class of compounds that are agonists towards Toll-like receptor 7 and 8 (TLR7/8). For example, Imiquimod and Resiquimod have been shown to act as vaccine adjuvants, enhancing antigen-specific antibody production and skewing the immunity towards a Th1 response. Activation of TLR7/8 have been shown to stimulate dendritic cells to secrete cytokines, upregulate costimulatory molecule expression and enhance antigen presentation to T cells. Imidazoquinoline compounds have demonstrated vaccine adjuvant properties in several animal models. The adjuvant effects can be enhanced by measures that localize the drug with the vaccine without quickly entering the systemic circulation. Clinical studies demonstrate that local immune activation is useful; imiquimod is approved for the topical treatment of basal cell carcinomas. However, injection or oral routes of administration of imidazoquinolines are not therapeutically beneficial and possibly dangerous due to systemic and non-specific activation of the immune system. To take advantage of the adjuvant property of imidazoquinolines, they need to be formulated or designed to allow for local immune activation without induction of systemic cytokines. This study focuses on developing a new site on the phenyl ring to lead to better imidazoquinolines that could be easily formulated in future drug delivery studies.

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Chapter 1

Introduction

Chapter 1.1 Immunity

The immune system is a complex defense mechanism capable of differentiating and destroying harmful pathogens while causing minimal damage to the host. In all vertebrates, including humans, the immune system is classified into two separate subsystems, the innate and the adaptive. The innate immune system, the most primitive, is the first line of defense against harmful pathogens, it is nonspecific, rapidly activated, and without memory. Without specific memory, the innate immune system needs a way to recognize these pathogens. The innate immune system relies on germline-encoded pattern recognition receptors (PRRs) which consist of membrane-bound and unbound intracellular receptors that serve as guards of the host's immune system. These PRRs identify highly conserved pathogenic fragments referred to as pathogen-associated molecular patterns (PAMPs) commonly consisting of antigens commonly found from microbes and viruses, such as flagellin or specific RNA/DNA sequences. When a PAMP binds and activates a PRR this causes a proinflammatory response of cytokines and interferons (IFN). This response is critical for innate and adaptive immune responses. The innate immune system also acts to help stimulate the adaptive immune system allowing for long term protection. It is this specific memory of pathogens that builds the foundation for vaccination.

Chapter 1.2 Vaccines and Vaccine Adjuvants

Vaccines are our greatest defense in preventing infectious diseases.[1,2] Vaccines aid in significant functions within the healthcare setting. First, they are used as a prophylactic to protect healthy individuals, including those who do not have fully competent immune systems from diseases. Second, they protect those who are unable to receive vaccinations through the process of "herd immunity". Herd immunity is achieved when a majority of the population, usually between 75-94%, depending on the pathogen, have immunity and prevent colonization of the pathogen within the population.[3] And finally, vaccines can be therapeutic in already established disease, such as the Provenge® vaccine in hormone-resistant prostate cancer.[4,5]

The practice of vaccination was reported and widely accepted by Edward Jenner in 1798 when he inoculated an 8-year-old boy, James Phipps, with pus derived from a cowpox lesions from a milkmaid's hand. Edward then tried to infect James with the smallpox virus twice, but he was ultimately protected from the virus. Since the invention of vaccines, the global market of vaccines as of 2015 represent a 32.5 billion dollar market covering over 25 diseases. The market in 2024 is predicted to grow to 77.5 billion dollars according to Grand View Research, Inc.[6] With this increase in wealth, there will be a demand to develop vaccinations for unexplored diseases (such as malaria, HIV, flaviviruses, and cytomegaloviruses), as well as improve existing vaccines (such as the annual influenza, acellular pertussis, and tuberculosis) that remain suboptimal.[7,8]

Chapter 1.2.1 Live Attenuated and Inactivated Vaccines

The first vaccines were initially derived from whole inactivated or live attenuated pathogens. The smallpox and polio vaccines are perhaps the most recognizable and successful vaccines developed. The smallpox vaccine that Jenner had developed started from cowpox but more recently the vaccine was made from the vaccinia virus of the poxvirus family.[9] However, the polio vaccine has utilized both strategies of live attenuated oral poliovirus vaccine and the inactivated parenteral poliovirus vaccine.[10] The oral poliovirus vaccine contains all three serotypes of poliovirus that have been attenuated by serial passage though African green monkey kidney cells significantly reducing polio's pathogenicity.[11] This vaccine is widely used in the developing world due to low cost of manufacturing, ease of oral administration, and the strong induction of humoral and mucosal immunity. Most industrialized countries, including the United States, have adopted the inactivated parenteral poliovirus vaccine, using formalin to inactivate all three poliovirus serotypes. While the inactivated poliovirus vaccine is significantly safer than the attenuated vaccine, the inactivated vaccine does suffer from reduced efficacy at eliciting protective immunity in the lower intestinal tract, relative to the attenuated vaccine, and does not prevent intestinal

shedding of the virus. The inactivated vaccines do however provide protection equal to that of the oral vaccine in pharyngeal membranes.[12]

Chapter 1.2.2 Subunit Vaccines and Adjuvants

The tradeoffs between safety and efficacy between live attenuated and inactivated vaccines are relevant to many other diseases. Current trends in vaccine development have focused on use of highly purified protein antigens, called subunit vaccines. Some examples include Hepatitis B, Haemophilus influenza type B, human papillomavirus, and pertussis vaccines. Although subunit vaccines are extremely safe, the antigens are frequently poorly immunogenic and rely heavily on adjuvants to stimulate immunogenicity and direct adaptive immune responses to the antigen.[13,14]

Adjuvants according to Janeway are “immunologists’ dirty little secret” and their role in subunit vaccines are essential.[15] However, the FDA has only approved a few adjuvants, including AS03, AS04, MF59, and aluminum salts. The most commonly used adjuvants are aluminum salts commonly referred to as alum.[13] The adjuvantic activity of the aluminum salts were described in 1926 by Glenny *et al.*. In this work they suggested that an antigen alone was too rapidly eliminated to induce robust immune responses, thus the antigens required precipitation with alum in order to slow the rate of elimination of the antigen.[16] Many groups have expanded the understanding of the mechanisms of action of aluminum salts. Summarily, it has been observed that alum increases antigen uptake by dendritic cells (DCs) which leads to the recruitment of leukocytes to the injection site, indirectly activating the innate immune system by inducing necrosis and causing the release of uric acid, a danger signal.[17] Studies have been performed to see if alum has a receptor but nothing has been uncovered.[18,19]

While alum-adjuvanted vaccines are generally safe and well tolerated with over one billion doses administered world-wide, they are not without limitations.[20] Aluminum salt-adjuvanted vaccines are Th2 polarizing, providing limited benefit in vaccines targeting intracellular pathogens that rely on either Th1 or Th17 immunity. This was emphasized by Warfel et. al. in comparing the whole-

cell pertussis vaccine to the alum adjuvanted acellular pertussis vaccine.[21] Warfel et. al. demonstrate that baboons vaccinated with acellular, alum adjuvanted Infanrix® (GlaxoSmithKline) were able to prevent major disease upon challenge with *B. pertussis* in a similar manner to the whole-cell vaccine, but failed to prevent either colonization of the bacterium or transmission to naïve animals. They also noted that both natural infections and vaccinations with the whole-cell vaccine resulted in memory Th1 and Th17 cells, while the acellular vaccine yielded Th1 and Th2 responses. Thus, the acellular pertussis vaccine was able to protect animals from major disease, but it showed potential limitations in enabling "herd immunity".[21]

In addition to alum, the FDA has approved AS04 as a vaccine adjuvant. AS04 is a Th1-polarizing immune stimulator approved in use against human papilloma virus types 16 and 18. This builds upon the adjuvantic activity of alum immunogenicity through the adsorption of the TLR4 agonist monophosphoryl lipid A (MPLA) to alum.[22,23] Didierlaurent et. al. demonstrated that the activity of AS04 was driven primarily by the TLR4 component and resulted in enhancement of DC and monocyte antigen uptake and cytokine secretion both at the site of injection and draining lymph node. The alum component of AS04, on the other hand, served to prolong the APC stimulation observed at the injection site.[23] They also demonstrated that AS04 does not directly stimulate T cells through either CD69 upregulation or IFN-γ secretion, but only appeared to drive APC activation with strong expression of TNF-α, IL-6, and IL-1β.[23]

The latest generation of adjuvants to gain FDA approval are the oil-in-water emulsions MF59, squalene in water,[24] and AS03, squalene and α-tocopherol in water. The AS03 adjuvant is made up of the oily compounds, D,L-alpha-tocopherol (vitamin E) and squalene, and an emulsifier, polysorbate 80, and water containing some salts.[25] Early work on establishing mechanisms of action for these adjuvants proved that neither of the systems worked through the formation of micro-depots, but rather through leukocyte recruitment to the site of injection and the draining lymph nodes.[26,27] The oil-in-water emulsions differ from many TLR agonists, such as MPLA in

AS04, in that they act by inducing the release of chemokines in APCs, which increase the number of leukocytes that migrate to the site of injection and increase antigen internalization. However, they do not induce strong co-stimulatory molecule upregulation in APCs or activate T cells directly.[25,28,29] Within the leukocyte populations that respond to these adjuvants, monocytes serve as the primary antigen-internalizing cells at the site of injection, and appear to transport the antigen to draining lymph nodes for B cells and DC presentation of the antigen to T cells.[25,29]

Lately, adjuvants currently under development are generally comprised of ligands of novel targets such as the TLRs. AS04 contains the only TLR ligand currently approved by the FDA. However, there are several pure TLR agonists in clinical trials and many more in preclinical development, including some of the compounds described in this work. Some of the TLR agonists in current clinical trials include TLR9 active CpG DNA (phase III), TLR5/NLRC4 ligand flagellin (phase II), and TLR4 active glucopyranosyl lipid A (phase I).[30] Given that TLRs appear to be key to bridging the innate and adaptive immune systems, targeting these receptors to produce novel vaccine adjuvants have been a hot area of research the last decade. Depending on the TLR engaged, downstream responses include APC activation and maturation, leukocyte recruitment, as well as lymphocyte activation.

Chapter 1.3 Toll-Like Receptors

TLRs are ~90 kDa transmembrane protein receptors that detect various pathogens and their activation catalyzes innate and adaptive immune responses pathways. TLRs are predominantly expressed in tissues involved in immune function, such as the spleen and the peripheral blood leukocytes, as well as those exposed to the external environment such as the lung and the gastrointestinal tract.[31] Their expression profiles vary among tissues and cell types. TLRs are located on the plasma membrane except for TLR3, TLR7, TLR8, and TLR9 which are localized in the endosomal compartment.[32] Structurally, TLRs contain a Toll-interleukin receptor (TIR) domain for signal transduction, a single alpha helix spanning the membrane, and an extracellular

leucine-rich repeat (LRR) domain for ligand binding. TLRs can be divided into 5 subfamilies based on sequence similarity: TLR2, TLR3, TLR4, TLR5, and TLR9.[32] Antigen presenting cells (APC) such as monocytes, macrophages, and DC show broad, but not identical, TLR expression patterns.[33] Specifically, TLR7 is found in monocytes, macrophages, dendritic cells, and B cells, while TLR8's distribution is similar to TLR7 but with the addition of mast cells.[34] This thesis will focus on the TLR9 subfamily, specially TLR7 and TLR8.

Chapter 1.4 TLR9 Subfamily

The TLR9 subfamily consists of the endosomal receptors TLR7, -8, and -9. Within this family, TLR7 and TLR8 are the most closely related with 42% identity and 73% similarity in their amino acid sequences.[35,36] Upon ligand binding, the adapter protein myeloid differentiation primary response gene 88 (MyD88) is recruited to the TIR domains of the TLRs, and ultimately results in a signal transduction to the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), which leads to innate immune activation. The TLR9 subfamily has additional signal transduction to the interferon regulatory factors (IRFs), which exert transcription control of Type I and Type II IFNs.[37]

All three members of this family naturally recognize various forms of genetic material; TLR7 and TLR8 both recognize single stranded RNA, while TLR9 senses unmethylated deoxycytidyl-deoxyguanosine (CpG) motifs in DNA.[32] In addition to single stranded RNA, small agonists of TLR7 and -8 have been identified by many research groups while small agonists of TLR9 have remained elusive, with only discovery of small antagonists at TLR9.[38,39] While TLR7 and -8 share similarities in ligand recognition and both potentiate a skewed Th1 response, they do differ in specific cytokine responses. TLR7 stimulation results in activation of the transcription factors IRF3 and IRF7, resulting in the secretion of the Type I interferons IFN-β and IFN-α, respectively.[40] TLR8 activates IRF1, resulting in a Type II interferon response secreting IFN-γ.[41] The premise of the work is to advance the knowledge of TLR7/8 agonists towards development of better adjuvants.

Chapter 2

Investigation of the Phenyl Ring of Imidazoquinolines

Chapter 2.1 Introduction

The first class of synthetic small-molecule TLR7/8 agonists were substituted guanosine nucleosides, such as Loxoribine. These guanosine derivatives have shown to be effective at activating B cells and the enhancing the production of antibodies. They have also demonstrated that they enhance CTLs and NK cells. Despite this promising preclinical data, guanosine derivatives have not been developed as vaccine adjuvants.[42]

3M Pharmaceuticals in the 1980s were attempting to identify small molecules that would inhibit infection by herpes simplex virus (HSV)-2, a known causative agent of genital herpes. They identified a new structural class of molecules that demonstrated excellent antiviral activity, called imidazoquinolines, the most notable being Imiquimod and Resiquimod, **Figure 1**.[43,44] Further studies have demonstrated that imidazoquinolines were not directly active against HSV but instead induced IFN-α *in vivo*.[44] Imidazoquinolines have since also been shown to induce INF-ɣ, TNF- α, and several interleukins and well known TLR7/8 agonists.[45–48] Today, Imiquimod is a known TLR7 agonist and is the active ingredient in an FDA approved topical application, Aldara®, used to treat skin conditions such as superficial basal cell carcinoma and actinic keratosis.[49]

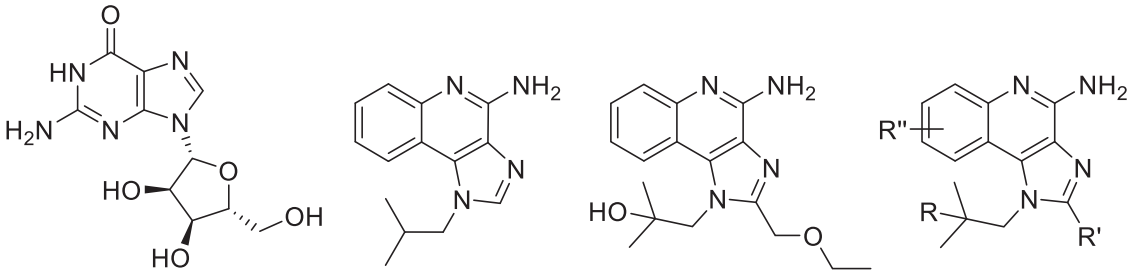


Figure 1. TLR7/8 agonists, Loxorabine and imidazoquinolines.

Since the discovery of imidazoquinolines by 3M Pharmaceuticals, many other groups including 3M have developed more agonists that target TLR7. Gerster *et. al.* of 3M Pharmaceuticals performed a structural activity relationship (SAR) of IFN producing imidazoquinolines. In Gerster'

s investigation, they found that simple alkyl or hydroxyalkyl substituents at N1 with unsubstituted C2 induced IFN. If the chain at N1 gets too bulky, activity is diminished or completely inactivated. Straight-chain alkyl substitution at the C2 position enhances IFN induction increasing up to butyl and maxing out. They also found that the amine at the C4 position appears to be the most crucial requirement for activity as all modifications at this position eliminated IFN production. Simple substitutions on the benzene ring diminished activity or eliminated it. When C2 was unsubstituted.[50]

Since 3M Pharmaceuticals' SAR study, others have progressed the knowledge of TLR7 agonists. Sunil David's group has developed agonists specific to TLR7 or -8 as well as mixed TLR7/8 agonists.[51–53] David Ferguson's group has recently developed new TLR7/8 agonists as well. Their work has shown that the C7 methyl ester regains activity when using substitutions at the N1 and C2 positions that Gerster discovered, with potency near to Resiquimod when using a N1 2-hydroxy-2-methylpropyl and a C2 n-butyl group.[54,55] When Gerster *et. al*. examined the IFN activity of a collection of C6−C9 substituted imidazoquinolines they did not examine the effect of combining N1 or C2 substitutions with C6−C9 aryl modified analogues.[50] Since the discovery of using the N1 and C2 combinations to restore C7 methyl ester activity, nobody has checked other substitutions at the C7 or other positions at the benzene ring.

In 2013 Tanji *et. al*. resolved the X-ray crystal structure of TLR8 with Resiquimod bound to the active site. As can be seen in **Figure 2,** Resiquimod sits deeply in the pocket with the N1 2-hydroxy-2-methylpropyl, and the C2 ethoxymethyl protrudes into a hydrophobic pocket.[56] The quinoline ring forms a pi-stacking with a Phe residue. The amidine group of the quinoline moiety forms hydrogen bonds with the side chain of Asp543 and The N atoms of the imidazole formed hydrogen bonds with the amide of Thr574. The C8-postion neighbors TYR353 providing additional pi-stacking interactions. From the C7 position, past the TYR353 is the surface of the receptor.[56]

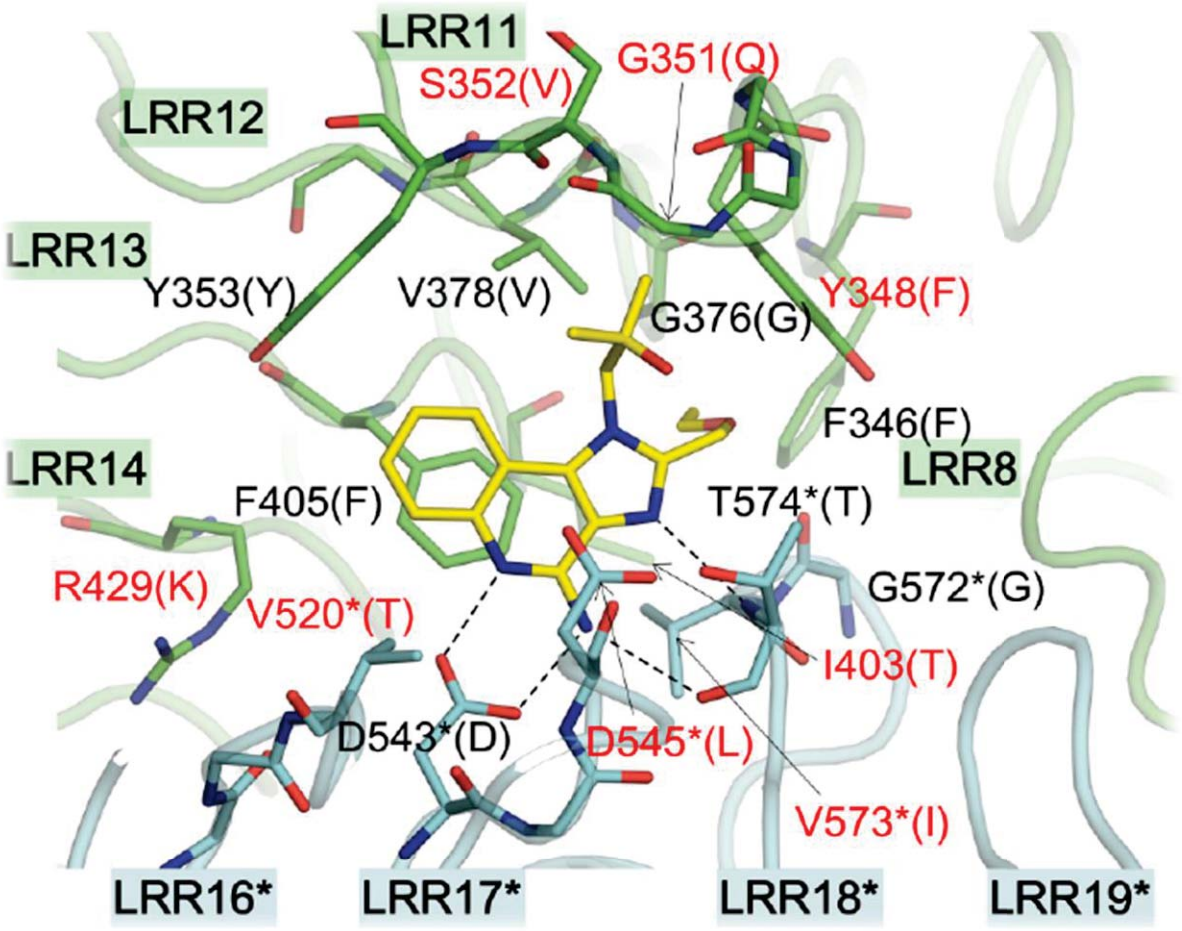


Figure 2. Possible TLR7 ligand binding site based on the TLR8 structure. Green from one monomer of TLR8 and blue is the other monomer of TLR8. Residues with * indicate residues from the blue monomer. Counterpart residues of human TLR7 are shown in parentheses. Nonconserved residues between TLR7 and TLR8 are highlighted as red colors. Image from Tanji *et al*.[56]

Although Imiquimod has been approved for topical use as a self-adjuvant, systemic use can be harmful due to systemic inflammation when concentrations get too high.[57] These small molecules imidazoquinolines are too rapidly cleared after subcutaneous or intramuscular administration; thus, they cannot be targeted locally except in the case of topical administration.[58] In an attempt to overcome this issue several groups have formulated imidazoquinolines using targeted delivery via covalently bonded carriers, lipophilic depots, or prodrugs.[59–65] This loss of activity with covalently bonded conjugates is most likely due to the disruption of binding as these positions are

pointed deep into the pocket as is observed from Tanji *et. al.* when Resiquimod is bounded to TLR8.[56] As for the depots and prodrugs, they do show activity when released, however, the released drug will still cause issues systemically if concentrations get too high. Thus, there is a need to develop new imidazoquinoline agonists that can have linkage sites pointing away from the pocket and out into the endosomal aqueous lumen. In this thesis we hypothesize that the C6-C9 positions may tolerate substitutions with C7 the most tolerant. These new substitutions could then be used as reactive sites to add linkers to a polymeric carrier such as hyaluronic acid.

Chapter 2.2 Results and Discussion

Chemistry

Imidazoquinolines are traditionally synthesized by a modified Traube reaction to cyclize the imidazole ring system from a quinoline outlined in Figure 3A.[50] This method allows for modifications of the N1 and C2 positions easily. To obtain functionalizations at the C6-C9 aryl positions the functional group needs to be already implemented into the precursor and must be able to tolerate the entire reaction sequence. This has made the development of a large synthetic library difficult and has left the SAR of these positions deficient in the literature.

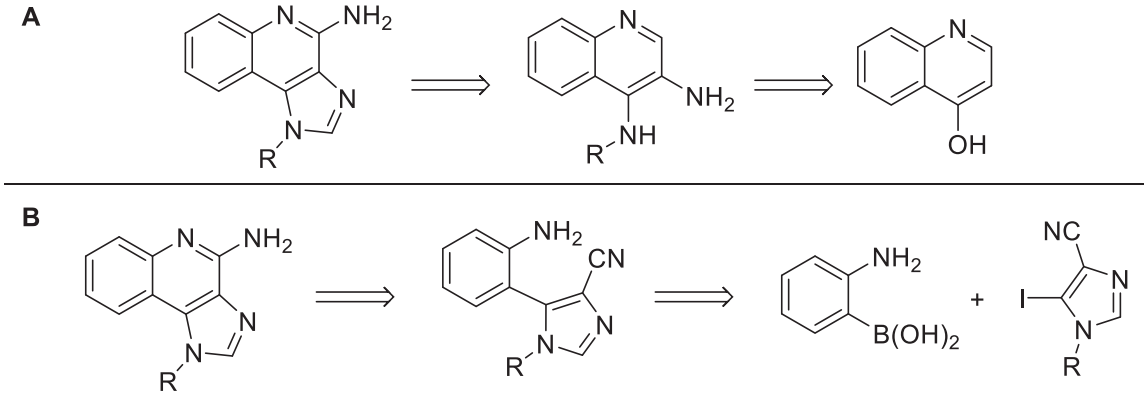


Figure 3. Retrosynthetic analysis showing the difference between to strategies to constructing imidazoquinolines. (**A**) The traditional route employing a Traube reaction by forming the imidazole ring from a diaminoquinoline. This forces substitutions on the phenyl ring to be implemented at the start of synthesis. (**B**) The route reported by Shi *et al.* utiziles a biaryl cross-coupling using highly substituted precursors.

The Ferguson group has developed a new strategy via highly substituted imidazoquinolines in approximately four steps outlined in **Figure 3B**.[54] This strategy utilizes a Suzuki coupling reaction to construct a substituted biaryl precursor that is readily cyclized to the final product under mild conditions. This lead to the development of TLR7 imidazoquinoline agonists with a C7 methyl ester. They observed that a C2 butyl chain regained TLR-7/8 activity for C7 methyl ester imidazoquinoline.[54,55] Recently, Larson *et al.*. recently utilized Shi's method to investigate the SAR of the N1 site when the C7 methyl ester is present.[66]

The synthesis of imidazoquinolines **7-19** are shown in **Figure 4**. The iodoimidazole precursors were synthesized through the multicomponent condensation of aminomalononitrile, orthoester, and alkyl amine. Unfortunately, this reaction sequence resulted in poor yields with several complications along several steps when trying to introduce new functionalities. In the paper describing the synthesis of the C7 methyl ester, the Ferguson group states that they used the amino malonitrile tosylate salt for the production of both substituted and unsubstituted C2 imidazoquinolines in 60% yields. [54] However, in our lab the best we could get was 20%. We found that using diaminomaleonitrile was best for synthesizing unsubstituted C2 imidazoles while amino malononitrile tosylate salt was best used for synthesizing C2-substituted imidazoles. Even with this finding, the first step only yielded 40% of the unsubstituted C2 imidazoles. Attempting to synthesize bromodiaminobenzenes resulted in spontaneous decomposition due to the aryl ring being too electron rich and prone to air autooxidation. The diortho-substituted bromoanilines were too sterically hindered to either synthesize the boronic esters or the Suzuki couplings. The Suzuki coupling was further limited to a few substrates and it seems to only work with weakly electron withdrawing groups (EWGs) of the 2-bromoanilines. If the aniline was substituted a nitro group around the ring, the Suzuki coupling would not proceed even though EWG on aryl boronic esters accelerate the oxidative addition step in the catalytic cycle. A reason why this reaction fails to proceed could be due the *o*-NO_2 and *p*-NO_2, with respect to the aniline, forms a tautomer that is

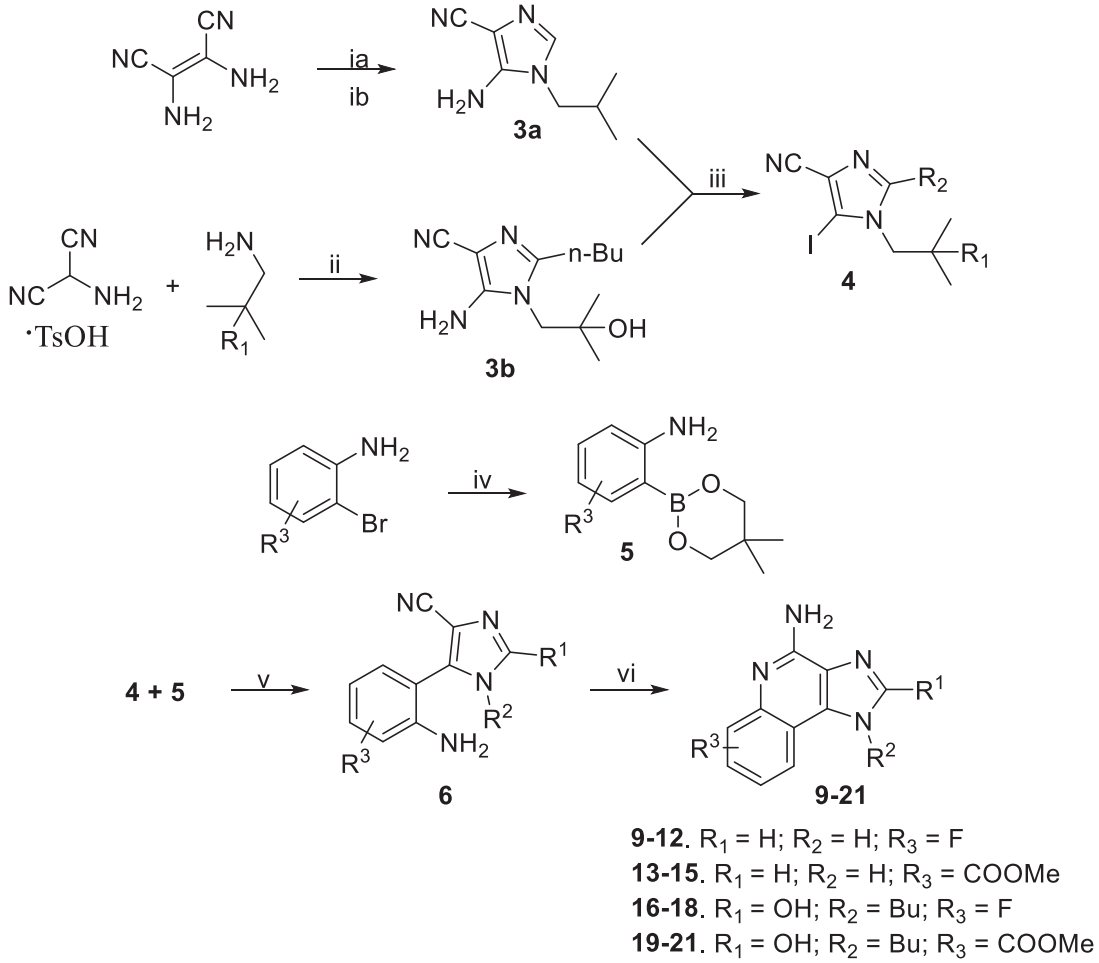
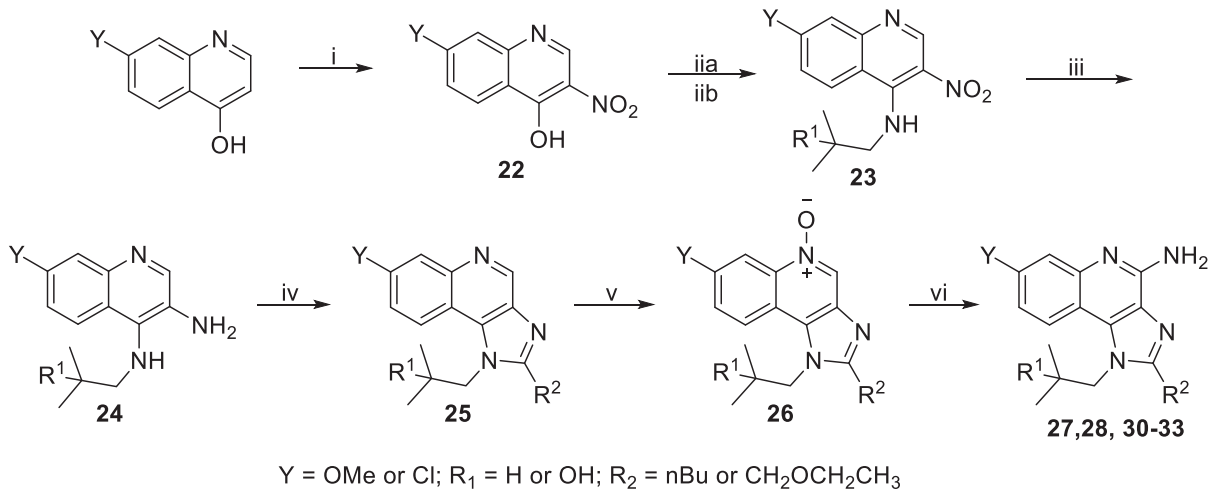


Figure 4. Synthesis of imidazoquinolines utilizing strategy by Shi *et al.* Reagents and conditions: (ia) $CH(OEt)_3$, 1,4-dioxane, reflux; (ib) anilinium chloride, EtOH, isobutylamine, 0 °C-rt, 5 h, 40% yield over two steps; (ii) $BuC(OEt)_3$, Et_3N, THF, reflux to rt, 15 h, 10-20%; (iii) CH_2I_2, isopentyl nitrite, $CHCl_3$, 80 °C, 30 min, 40-60%; (iv) $Pd(Cl)_2(dppf)$, KOAc, bis(neopentyl glycolato)diboron, DMSO, 90 °C, 5 h, 10-95%; (v) $Pd(PPh_3)_4$, Na_2CO_3, 1,4-dioxane-H_2O, 15 h; (vi) 4 N HCl in 1,4-dioxane, reflux, 4 h, 20-75% yield over two steps.

unstable in basic conditions. Also, when the NO_2 is para with respect to boronic ester, the C-B bond has double-bond like character and is not a good leaving group for the transmetallation step of the Suzuki reaction. Nitrile anilines were considered but they were not commercially available and also would most likely not survive the ring condensation step. With these substitution limitations and inability to make the imidazoquinolines on large scale, we opted to use the more traditional route to synthesize the TLR7/8 library. Using this route, we started with a two key

commercially available C7 substituted quinolin-4-ols, the methoxy and the chloro (**Figure 5**). These functional groups were chosen as they would be able to withstand the reaction sequence as the Imiquimod version had been made previously by Gerster *et al.* The desired imidazoquinolines were synthesized in large scale and were further derivatized after the completion of the amination step.



Y = OMe or Cl; R_1 = H or OH; R_2 = nBu or $CH_2OCH_2CH_3$

Figure 5. Synthesis of imidazoquinolines using Trabue strategy. Reagents and conditions: (i) 70% nitric acid, propionic acid, reflux 2h, 55-70%; (iia) $POCl_3$, DMF, 50 °C, 30 min; (iib) EtOH, Et_3N, R_1isobutylamine, reflux, 15 min, 70-90% yield over two steps; (iii) Iron (dust), NH_4Cl, EtOH-H_2O, reflux, 2 h, 70-100%; (iv) R_2COOH, 150 °C, 1 h, 6 N NaOH, 60-80%; (v) mCPBA, $CHCl_3$, rt, 4 h, 80%. (vi) NH_4OH, pTsCl, DCM, rt, 15 min, 70-85%.

Synthesizing C7 substituted imidazoquinolines using the Traube strategy was found to be more efficient and scalable than the Ferguson group strategy. The first four reactions can be completed in large scale with three of the four intermediates crashing out of solution to be collected by vacuum filtration with the fourth being easily purified by a Celite plug and extraction. The cyclization is completed by refluxing in neat valeric or ethoxyacetic acid. The oxidation product is generated in high yields and can be purified by chromatography. The final step is amination via ammonia hydroxide and tosyl chloride and is purified by chromatography. The 7-methoxy compound can be demethylated to the 7-phenol by refluxing in pyridine hydrochloride (**Figure 6**).

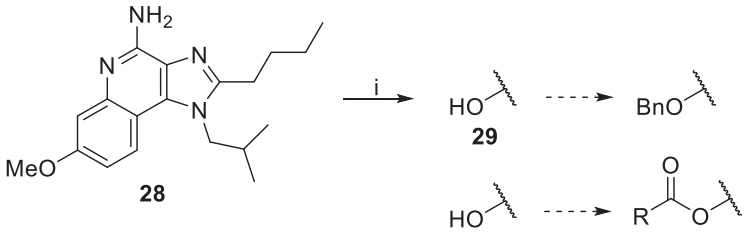


Figure 6. Derivitization of 7-methoxyimidazoquinolines. Reagents and conditions: (i) Pyr•HCl, reflux, 30 min, ____. Dashed arrows indicate future planned reactions for continuation of SAR studies.

The derivatization of 7-chloro compounds can be seen in **Figure 7**. The 7-chloro is converted into the nitrile by employing a method found by Littke *et al.*[67] Their method worked well on all our imidazoquinolines with yields around 80%. From the nitrile, several derivatives could be converted to several analogs. The nitrile was reduced the benzylamine by reduction with lithium aluminum hydride. The ketone was made via the Grignard reaction with benzyl magnesium bromide. The carboxylic acid via hydrolysis. The carboxylic acid could also be derivatized in several ways: amides, benzyl alcohols, esters, ethers, anilines, reverse amides etc.

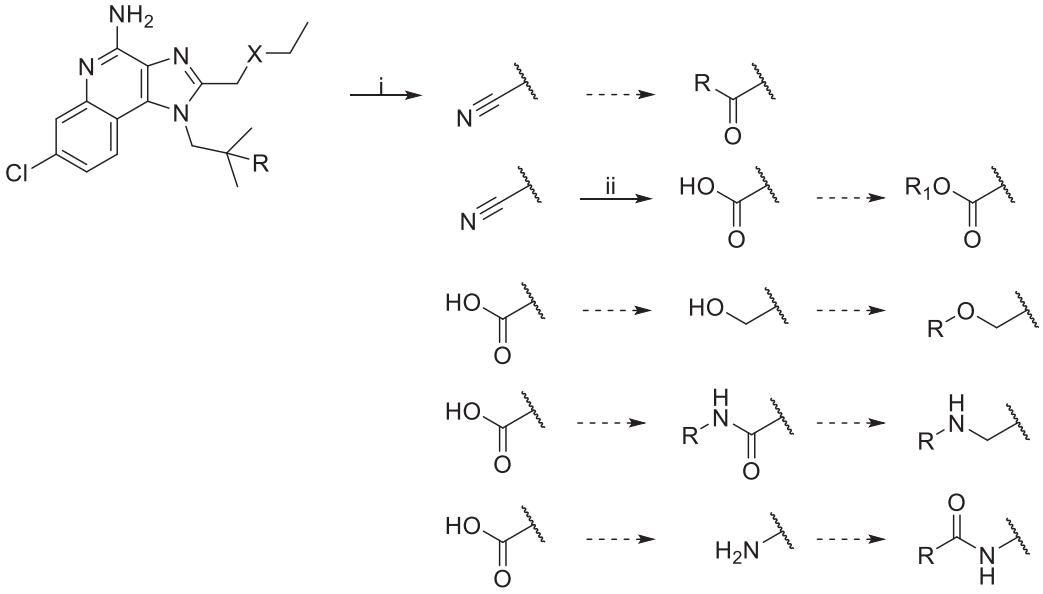


Figure 7. Derivitization of 7-Chloroimidazoquinolines. Reagents and conditions. (i) $Pd(TFA)_2$, Zn (dust), TrixiePhos, $Zn(CN)_2$, DMAC, 95 °C, 15 h, 70-90%. (ii) KOH, H_2O_2, EtOH, reflux, 4 h, 60%. Dashed arrows indicate future planned reactions for contiuation of SAR studies.

Biological Activity

The analogues (**7-21** and **27-38**) were screened for TLR-7 activity *in vitro* using HEK-293 cells that were transfected with the human TLR-7 gene along with the NF-κB inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene. This assay measures NF-κB mediated SEAP production spectrophotometrically following TLR-7 specific activation. It is important to point out this assay is not a direct measure of receptor activation or ligand binding affinity. In addition, some compounds could be cytotoxic, yielding low optical densities and incomplete dose−response curves.

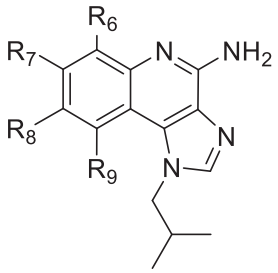


Table 1	Compound	R6	R7	R8	R9	EC_{50} (μM)
	1	H	H	H	H	6.10 ± 1.23
	7	H	H	NO_2	H	NA
	8	H	H	NH_2	H	NA
	9	F	H	H	H	NA
	10	H	F	H	H	5.98 ± 1.36
	11	H	H	F	H	N/A
	12	H	H	H	F	>100[a]
	13	H	COOMe	H	H	NA
	14	H	H	COOMe	H	NA[b]

HEK hTLR7 SEAP reporter assay of imidazoquinoline analogs. NA indicates no activity at 100 μM. [a]Activity detected at 100 uM but only the start of the sigmoidal curve was observed. [b]Compound was not pure by ^{1}H NMR.

A series of imidazoquinolines are reported in **Table 1**. Comparing Imiquimod (**1**) to the new compounds **7**-**14** shows that the only change tolerated was the replacement of the C7-H with a fluorine (**10**), all other changes caused a complete loss of activity. Note that **14** was not pure when evaluated, possible impurities are triphenylphosphine and uncyclized intermediate. Schiaffo *et al.* showed that the C2 butyl group (**20**) restored activity for the C7 ester (**15**) shown in **Figure 8**.[55]

With this in mind, adding butyl length chains could lead to new phenyl ring substituted imidazoquinolines.

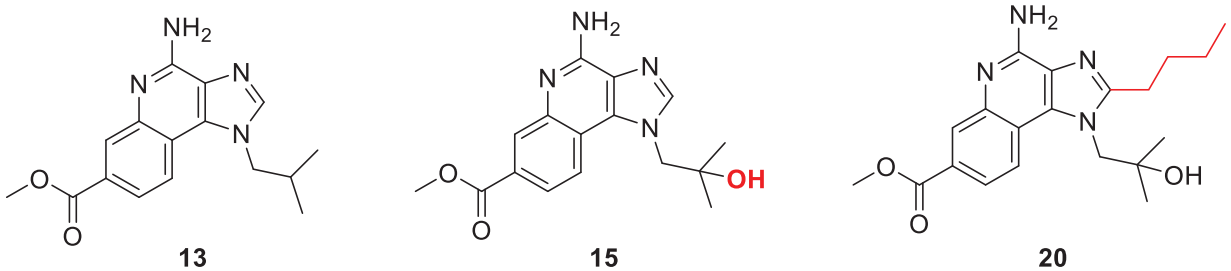
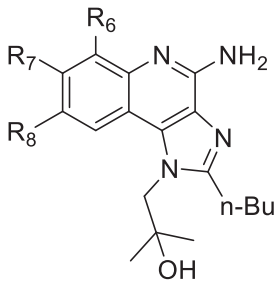


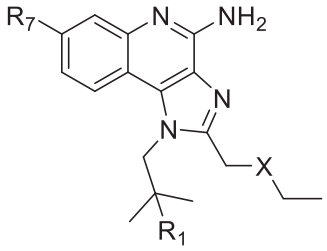
Figure 8. Comparing compounds **13** and **15** which have no activity to **20** suggests that the addition of C2 butyl group restores activity for C7 imidazoquinolines. **15** was synthesized and evaluated in Schiaffo *et al*. Compound **20** was evaluated by our lab and Schiaffo *et al.*[55]

Table 2	Compound	R6	R7	R8	EC_{50} (μM)
	16	F	H	H	2.90 ± 1.81[b]
	17	H	F	H	Active[c]
	18	H	H	F	0.379 ± 0.537
	19	COOMe	H	H	NS[d]
	20	H	COOMe	H	0.832 ± 0.158
	21	H	H	COOMe	Active[c]

HEK hTLR7 SEAP reporter assay of imidazoquinoline analogs. NA indicates no activity detected at 100 μM. [b]Compound was not pure by ^{1}H NMR. [c]Activity indicates compounds are active but exact EC_{50} values have not been fully determined due to lack of data. [d]Compound was not systhesized.

In order to elucidate if a C2-butyl imidazoquinolines could restore activity of phenyl ring substitutions, compounds **16** to **18** and **20** to **21** were synthesized and evaluated in the hTLR7 assay shown in **Table 2**. Compound **19** was attempted to be synthesized but only a small amount was produced and could not be separated from the mixture. Looking at this series, the activities of all synthesized compounds had activity. Unfortunately compounds **17** and **19** lack data to determine EC_{50} at the time of completing this thesis but did show activity. Compounds **16** was not

pure during evaluation. Impurity of **16** appears to be uncyclized intermediate Compounds **16**, **17**, and **19** will be resynthesized and reevaluated in the future.

Table 3	Compound	R_1	X	R_7	EC_{50} (μM)
	2	OH	O	H	0.29 ± 0.07
	27	OH	CH_2	OMe	0.058 ± 0.0058
	28	H	CH_2	OMe	0.098 ± 0.013
	29	H	CH_2	OH	0.068 ± 0.0093
	30	H	CH_2	Cl	0.29 ± 0.05
	31	H	O	Cl	0.59 ± 0.16
	32	OH	CH_2	Cl	0.046 ± 0.0050
	33	OH	O	Cl	0.55 ± 0.10
	34	H	CH_2	CN	0.52 ± 0.07[b]
	35	H	O	CN	16 ± 1.4[b]
	36	OH	CH_2	CN	0.89 ± 0.040
	37	OH	O	CN	4.5 ± 0.36
	38	H	O	COOH	2.6 ± 0.26[b]

HEK hTLR7 SEAP reporter assay of imidazoquinoline analogs. [b]Compound was not pure by ^1H NMR.

Table 3 shows that many compounds are in-between our two controls Imiquimod and Resiquimod, further suggesting that substitutions at the C7 position tolerates substitutions when the C2 is substituted. Comparing Resiquimod (**2**) to a C7-Cl (**33**) shows a very slight decrease in activity while C7-CN (**37**) resulted in a 16-fold decrease in activity. This decrease in activity of **37** could be due to the electron deficiency of the imidazoquinoline ring system or the C7-position favors a more lipophilic group as a 0.53 cLogP decrease according to ChemDraw is noted when changing -Cl to -CN. A similar trend of C7-Cl to C7-CN is observed when comparing compounds **30** to **34**, **31** to **35**, and **32** to **36** with a 2-, 27-, and 52-fold decreases respectively. A 4-fold decrease is observed when changing

the C7-Cl (**31**) to a C7-COOH (**38**). The C7-COOH of **38** could be binding as the ionized or unionized form depending on the stage of the endosome which can range from pH 4 to pH 6. Note that compounds **34**, **35**, and **38** are not pure. Impurities appear to be hydrolysis of the nitrile to the amide or carboxylic acid.

Some notable compounds are the C7-meothy compounds **27** and **28**, and the C7-OH, **29**, as these are very active suggesting that the increase of electron density of the imidazoquinoline ring system may be increasing the H-bonding interactions of the C4-NH_2 and N5 pyridine nitrogen. Looking between N1 substitutions, a huge increase in activity is noted for tertiary alcohols compared those without. When C2 contains the ether substitution a slight decrease is observed as well. Mixtures of C2 ethers and N1 tertiary alcohols lie in between those without. This data suggests that area near N1 may be a H-bonding interaction that is being exploited while C2 reaches into a very lipophilic pocket.

The strong activity of 7-Cl, **32**, is interesting and could be due to the Val near the C7 position helping with a hydrophobic interaction or the smaller substitution could fit the pocket better than the than the MeO. In the future we would like to synthesize a C7-OH with the tertiary alcohol to see if activity increases farther. The activity of **38** and **29** is exciting as they can be used in future studies to help deliver imidaquinolines safely to the endosome by allowing access to a cleavable ester or amide linkers to drug carriers which can be developed further in the near future. The development of the nitrile imidazoquinolines can be derivatized to several other functional groups such as the aniline, amides, reverse amides, esters, reverse esters, benzyl alcohols, and benzyl amines. These functionalities will further the knowledge of the SAR of the C7 position in the future.

Chapter 2.3 Conclusions

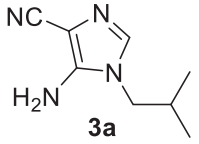
This study has found that the method developed by Shi *et al.* allow much access to new functional groups as one would hope due to several limitations. Utilizing the Traube reaction strategy proved more fruitful for making C7-substituted imidazoquinolines leading to the development of several active C7 imidazoquinoline agonists on gram scale. The most notable TLR-7 agonist developed from this study are **27**, **28**, **29**, and **32** which are the most active compounds reported using the HEK-293 hTLR7 assay to date. This study has also yielded an active carboxylic acid analog, **38**, which is of great interest in future studies of safely delivering TLR7 agonists as adjuvants. Now that the difference between N1 and C2 have been evaluated it has been concluded that both changes yield active compounds with different potencies, the study will narrow its focus to one type of substitution at the N1 and C2 positions to fully evaluate the SAR of the C7 position. Once the library is completed the C7-substituted imidazoquinolines will also be evaluated in HEK-293 hTLR8 cells for activity. The best compounds will be evaluated in human PBMCs and other immune response assays to determine specific immunological responses. In the distant future the best reactive handles will be used to explore linkers attached to imidazoquinolines and then will be attached to hyaluronic acid to help deliver our imidazoquinolines safely and effectively in order to develop a novel adjuvant that will be evaluated *in vivo*.

Chapter 2.4 Experimental

Chemistry

General Experimental Conditions

All solvents were used as received from commercial vendors. All starting chemicals were purchased from AK Scientific, Ark Pharm, AstraTech, Combi-Blocks, eNovation Chemicals, Fisher, Oakwood Chemical, Sigma-Aldrich, and Strem Chemicals or were synthesized using the cited literature protocol. Compounds 1-isobutyl-8-nitro-1H-imidazo[4,5-c]quinolin-4-amine (**7**) and 1-isobutyl-1H-imidazo[4,5-c]quinoline-4,8-diamine (**8**) were prepared according to the literature procedure according to Gerster *et al*. All reactions were conducted under an atmosphere of N_2 or Ar unless stated otherwise. Thin layer chromatography (TLC) was performed on 0.25 mm glass-backed silica GF plates from Analtech. Developed plates were visualized with a hand-held UV lamp. Flash chromatography was performed on a Combiflash system using pre-packed columns from Teledyne-Isco. ^{1}H and ^{13}C NMR spectra were recorded on a 500 MHz Bruker AVIII spectrometer equipped with cryogenically cooled carbon observe probe or a Bruker 400 MHz spectrometer in the noted solvent. Peaks are reported as chemical shift (δ) are reported in ppm, coupling constants reported in (*J*) are reported in Hz, and number of protons (H) are noted from integrations in MestReNova software. High resolution mass spectra (HRMS) were obtained on an LCT Premier (Micromass Ltd., Manchester UK) time-of-flight (TOF) mass spectrometer (MS) equipped with an ESI interface.

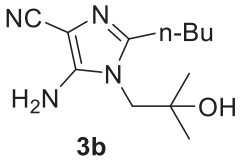


3a

5-amino-1-isobutyl-1H-imidazole-4-carbonitrile (3a)

3a was made using a modified procedure from Sun and Hosman.[68] A mixture of diaminomaleonitrile (6 g, 55.5 mmol) and triethyl orthoformate (9.2 mL, 55.5 mmol) in anhydrous dioxane (95 mL) was heated at reflux in a flask fitted with a short path jacketed distilling head with

a 100 mL receiver. Ethanol mixed with dioxane was collected continuously until ~60 mL had been collected in the receiver flask. The brown residue in the distillation flask was allowed to cool to room temperature. When hexanes (50 mL) was added, a dark brown crystalline solid formed and was filtered by vacuum filtration. The mother liquor was mixed with more hexanes and cooled to -20 °C to collect additional solid. The combined solid was dissolved in a minimum amount of hot diethyl ether and filtered to remove the dark brown solid impurity. The solvent was evaporated to obtain a white solid intermediate to be used in the next step without further purification. The intermediate (3.85 g, 23.5 mmol) and anilinium chloride (50 mg, 0.39 mmol) was dissolved in anhydrous ethanol (75 mL) and cooled to 0°C. Isobutyl amine (2.45 mL, 24.7 mmol) was added dropwise at 0°C and the reaction was stirred at room temperature for 5 h. The reaction mixture was added dropwise to a solution of sodium hydroxide (50 mL, 0.8 M) at 0 °C for five minutes. The reaction mixture was stirred overnight, filtered, washed with water (2 X 20 mL), and dried to obtain a copper-colored solid. (2.56 g, 66% yield). Spectral data matched previously reported data.[68]



3b

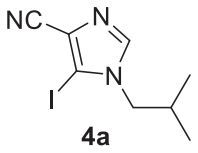
5-amino-2-butyl-1-(2-hydroxy-2-methylpropyl)-1H-imidazole-4-carbonitrile (3b)

3b was prepared from known procedure by Shi *et al*.[54] dried to obtain a light tan solid. (7 g, 55% yield). Spectral data matched previously reported data.[54]

General Procedure A for iodoimidazoles:

The procedure was adapted from Shi *et al*.[54] A solution of isoamyl nitrite (4 eq.) in $CHCl_3$ (8 mL per mmol) was added to a solution of imidazole-4-carbonitrile (1 eq.) in CH_2I_2 (1.6 mL per mmol) at 80 °C over 20 min. The mixture was heated for an additional 30 min and allowed to cool to 25

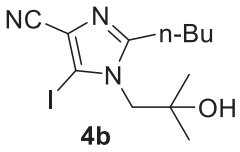
°C. The mixture was concentrated in vacuo, and the crude residue was purified by silica gel column chromatography.



4a

5-iodo-1-isobutyl-1H-imidazole-4-carbonitrile (4a)

The title compound was prepared according to the general procedure A using **3a**. **4a** was purified by Combiflash (70% EtOAc in hexanes) and was isolated as a light orange solid in 37% yield. Spectral data matched previously reported data.[54]



4b

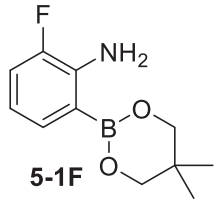
5-iodo-2-butyl-1-(2-hydroxy-2-methylpropyl)-1H-imidazole-4-carbonitrile (4b)

The title compound was prepared according to the general procedure A using **3b**. **4b** was purified by Combiflash (70% EtOAc in hexanes) and was isolated as a dark brown solid in 29% yield. Spectral data matched previously reported data.[54]

General Procedure B for the Synthesis of Arylboronates:

The procedure adapted from Fang *et al.* with modifications.[69] A mixture of $PdCl_2$(dppf) (0.03 eq), potassium acetate (3 eq), bis(neopentyl glycolato)diboron (1.2 eq), and arylbromide (1 eq) was added to a flask in a glove box filled with Argon under anhydrous conditions. After addition of anhydrous DMSO (8 mL per mmol of arylbromide), the mixture was stirred at 90 ºC for 5 h. The reaction solution was cooled to room temperature and poured into ice-water. The mixture was extracted with ethyl acetate and the combined organic layers were washed with saturated brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by Combiflash to give

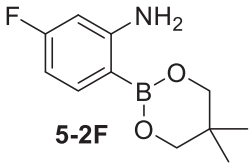
the corresponding arylboronates. The arylboronates were used in the next step without further purification.



5-1F

2-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)-6-fluoroaniline (5-1F)

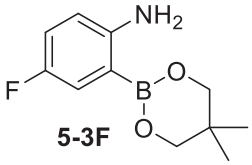
The title compound was prepared according to the general procedure B above using 2-bromo-6-fluoroaniline. **5-1F** was purified by Combiflash (10-40% EtOAc in hexanes) and was isolated as an off-white solid in 59% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.45 – 7.40 (m, 1H), 7.03 (ddd, *J* = 11.7, 7.9, 1.5 Hz, 1H), 6.61 (td, *J* = 7.7, 4.7 Hz, 1H), 4.90 (s, 2H), 3.82 (s, 4H), 1.06 (s, 6H). HRMS (m/z): [M+H] calcd for $C_{11}H_{16}BFNO_2$, 224.1156; found 224.1252.



5-2F

2-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)-5-fluoroaniline (5-2F)

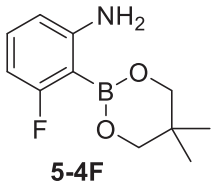
The title compound was prepared according to the general procedure B using 2-bromo-5-fluoroaniline. **5-2F** was purified by Combiflash (10-40% EtOAc in hexanes) and was isolated as a tan solid in 93% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.63 (dd, *J* = 8.4, 7.5 Hz, 1H), 6.38 (td, *J* = 8.6, 2.4 Hz, 1H), 6.27 (dd, *J* = 11.5, 2.3 Hz, 1H), 4.95 (s, 2H), 3.80 (s, 4H), 1.05 (s, 6H). HRMS (m/z): [M+H] calcd for $C_{11}H_{16}BFNO_2$, 224.1156; found 224.1258.



5-3F

2-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)-4-fluoroaniline (5-3F)

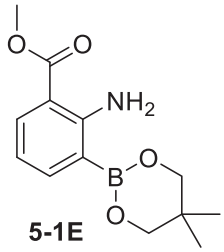
The title compound was prepared according to the general procedure B using 2-bromo-4-fluoroaniline. **5-3F** was purified by Combiflash (10-40% EtOAc in hexanes) and was isolated as a black solid in 27% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.31 (dd, *J* = 9.6, 3.2 Hz, 1H), 6.92 – 6.84 (m, 1H), 6.52 (dd, *J* = 8.8, 4.3 Hz, 1H), 4.64 (s, 2H), 3.77 (s, 4H), 1.03 (s, 6H). HRMS (m/z): [M+H] calcd for $C_{11}H_{16}BFNO_2$, 224.1156; found 224.1266.



5-4F

2-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)-3-fluoroaniline (5-4F)

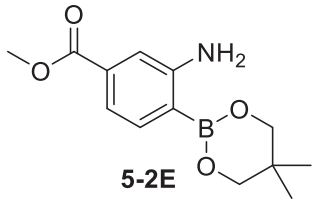
The title compound was prepared according to the general procedure B using 2-bromo-3-fluoroaniline. **5-4F** was purified by Combiflash (10-40% EtOAc in hexanes) and was isolated as a black oil in 10% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.07 (td, *J* = 8.1, 6.5 Hz, 1H), 6.38 – 6.31 (m, 2H), 4.86 (s, 2H), 3.81 (s, 4H), 1.05 (s, 6H). HRMS (m/z): [M+H] calcd for $C_{11}H_{16}BFNO_2$, 224.1156; found 224.1277.



5-1E

Methyl 2-amino-3-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)benzoate (5-1E)

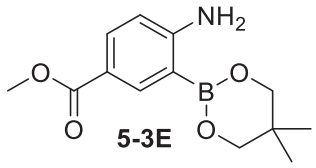
The title compound was prepared according to the general procedure B using methyl 3-amino-4-bromobenzoate. **5-1E** was purified by Combiflash (10-40% EtOAc in hexanes) and was isolated as an off-white fluffy crystalline solid in 57% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.69 (d, *J* = 7.7 Hz, 1H), 7.28 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.24 – 7.22 (m, 1H), 4.96 (s, 2H), 3.88 (s, 3H), 3.79 (s, 4H), 1.03 (s, 6H). HRMS (m/z): [M+H] calcd for $C_{13}H_{19}BNO_4$, 264.1407; found 264.1418.



5-2E

Methyl 3-amino-4-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)benzoate (5-2E)

The title compound was prepared according to the general procedure B using methyl 2-amino-3-bromobenzoate. **5-2E** was purified by Combiflash (10-40% EtOAc in hexanes) and was isolated as a brown solid in 11% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.69 (d, *J* = 7.8 Hz, 1H), 7.28 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.23 (dd, *J* = 1.5, 0.5 Hz, 1H), 4.89 (s, 2H), 3.88 (s, 3H), 3.79 (s, 4H), 1.03 (s, 6H). HRMS (m/z): [M+H] calcd for $C_{13}H_{19}BNO_4$, 264.1407; found 264.1399.



5-3E

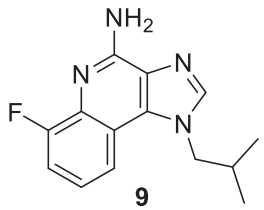
Methyl 4-amino-3-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)benzoate (5-3E)

The title compound was prepared according to the general procedure B using methyl 4-amino-3-bromobenzoate. **5-3E** was purified by Combiflash (10-40% EtOAc in hexanes) and was isolated as a light tan solid in 35% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.35 (d, *J* = 2.2 Hz, 1H), 7.87 – 7.78 (m, 1H), 6.53 (d, *J* = 8.5 Hz, 1H), 5.25 (s, 2H), 3.84 (s, 3H), 3.79 (s, 4H), 1.03 (s, 6H). HRMS (m/z): [M+H] calcd for $C_{13}H_{19}BNO_4$, 264.1407; found 264.1401.

General Procedure C for the Synthesis Imidazoquinolines:

The procedure was adapted from Shi *et al.* with modifications.[54] Inside of a glovebox filled with Argon $Pd(PPh_3)_4$ (5%), functionalized imidazole (1.0 eq), arylboronic esters (1.5 eq), and 2 M Na_2CO_3 aqueous solution (3.0 eq) were added to an oven dried scintillation vial. 1,4-dioxane was added to the scintillation vial and sealed with a PTFE coated cap. The vial was taken out of the glovebox and the mixture was heated to 80 °C overnight. The mixture was then allowed to cool

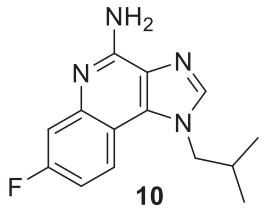
to room temperature and diluted with EtOAc and H_2O. The aqueous layer was extracted with EtOAc (3x). The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The resulting brown oil was loaded onto a short pad of silica gel, followed by flashing with CH_2Cl_2 (30 mL). The filtrate was concentrated in vacuo to provide the crude product which was used without further purification in the next step. To a round-bottom flask containing the crude product (1.0 eq), 4 N HCl in 1,4-dioxane (16 eq) was added. The mixture was heated at reflux for 4 h and then cooled to room temperature. The mixture was concentrated in vacuo and partitioned between 5% MeOH in DCM and saturated $NaHCO_3$ solution. The aqueous layer was extracted with 5% MeOH in DCM (3x). The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The crude residue was purified by flash column chromatography on silica gel.



9

6-fluoro-1-isobutyl-1H-imidazo[4,5-c]quinolin-4-amine (9)

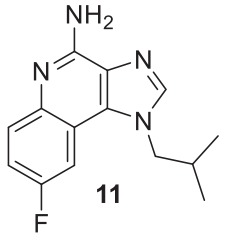
The title compound was prepared according to the general procedure C using **4a** and **5-1F**. **9** was purified by Combiflash (0-10% MeOH in DCM) and was isolated as a bright red solid in 45% yield over two steps. ^{1}H NMR (500 MHz, DMSO-d6) δ 8.24 (s, 1H), 7.82 (d, J = 8.1 Hz, 1H), 7.31 – 7.21 (m, 2H), 6.89 (s, 2H), 4.40 (d, J = 7.5 Hz, 2H), 2.51 (p, J = 1.8 Hz, 4H), 0.92 (d, J = 6.6 Hz, 6H); ^{13}C NMR (126 MHz, DMSO-d6) δ 156.78 (d, J = 247.6 Hz), 152.73, 144.30, 134.69 (d, J = 11.4 Hz), 131.97 (d, J = 4.2 Hz), 129.03, 121.14 (d, J = 8.4 Hz), 117.43 (d, J = 3.6 Hz), 116.88 (d, J = 4.1 Hz), 112.27 (d, J = 19.5 Hz), 53.87, 28.89, 19.76. HRMS (m/z): [M+Na] calcd for $C_{14}H_{15}N_4FNa$, 281.1178; found 281.1203.



10

7-fluoro-1-isobutyl-1H-imidazo[4,5-c]quinolin-4-amine (10)

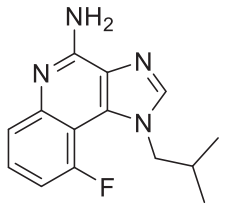
The title compound was prepared according to the general procedure C using **4a** and **5-2F**. **10** was purified by Combiflash (0-10% MeOH in DCM) and was isolated as a light red solid in 36% yield over two steps. ^{1}H NMR (500 MHz, DMSO-d6) δ 8.17 (s, 1H), 8.03 (dd, *J* = 9.0, 6.3 Hz, 1H), 7.30 (dd, *J* = 11.4, 2.7 Hz, 1H), 7.14 (td, *J* = 8.8, 2.8 Hz, 1H), 6.80 (s, 2H), 4.38 (d, *J* = 7.5 Hz, 2H), 2.14 (h, *J* = 13.8, 6.9 Hz, 1H), 0.91 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (126 MHz, DMSO-d6) δ 161.05 (d, *J* = 241.9 Hz), 153.03, 146.54 (d, *J* = 12.3 Hz), 143.30, 131.76, 127.54, 122.50 (d, *J* = 10.1 Hz), 111.94 – 111.79 (m), 110.21 (d, *J* = 20.4 Hz), 109.63 (d, *J* = 23.7 Hz), 53.27, 28.34, 19.31.HRMS (m/z): [M+H] calcd for $C_{14}H_{16}FN_4$, 259.1359; found 259.1334.



11

8-fluoro-1-isobutyl-1H-imidazo[4,5-c]quinolin-4-amine (11)

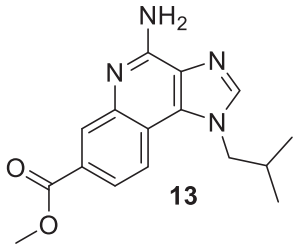
The title compound was prepared according to the general procedure C using **4a** and **5-3F**. **11** was purified by Combiflash (0-10% MeOH in DCM) and was isolated as a light orange solid in 42% yield over two steps. ^{1}H NMR (500 MHz, DMSO-d6) δ 8.22 (s, 1H), 7.71 (dd, *J* = 10.4, 2.9 Hz, 1H), 7.64 (dd, *J* = 9.2, 5.7 Hz, 1H), 7.33 (td, *J* = 8.8, 2.8 Hz, 1H), 6.63 (s, 2H), 4.40 (d, *J* = 7.5 Hz, 2H), 2.13 (hept, *J* = 6.8 Hz, 1H), 0.91 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (126 MHz, DMSO-d6) δ 156.81 (d, *J* = 237.1 Hz), 151.69, 143.83, 141.65, 131.81 – 130.79 (m), 128.75 (d, *J* = 12.9 Hz), 127.96 (d, *J* = 8.9 Hz), 115.22 (d, *J* = 23.9 Hz), 114.65 (d, *J* = 9.5 Hz), 105.26 (d, *J* = 24.1 Hz), 53.06, 28.51, 19.29. HRMS (m/z): [M+H] calcd for $C_{14}H_{16}N_4F$, 259.1359; found 259.1364.



12

9-fluoro-1-isobutyl-1H-imidazo[4,5-c]quinolin-4-amine (12)

The title compound was prepared according to the general procedure C using **4a** and **5-4F**. **12** was purified by Combiflash (0-10% MeOH in DCM) and was isolated as an orange solid in 23% yield over two steps. ^{1}H NMR (500 MHz, DMSO-d6) δ 8.10 (s, 1H), 7.58 (dddd, *J* = 36.0, 8.3, 6.6, 2.4 Hz, 3H), 3.83 (d, *J* = 7.5 Hz, 2H), 2.06 (dp, *J* = 13.9, 6.9 Hz, 1H), 0.85 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (126 MHz, DMSO-d6) δ 142.85, 132.71 (d, *J* = 102.4 Hz), 132.68 (d, *J* = 11.3 Hz), 132.01 (d, *J* = 2.7 Hz), 131.47 (d, *J* = 9.9 Hz), 129.75 (d, *J* = 6.2 Hz), 128.73 (d, *J* = 11.8 Hz), 120.19, 115.59, 88.23, 54.79, 28.91, 19.26. HRMS (m/z): [M+H] calcd for $C_{14}H_{16}FN_4$, 259.1359; found 259.1351.

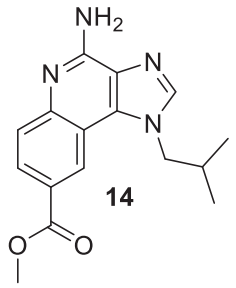


13

Methyl 4-amino-1-isobutyl-1H-imidazo[4,5-c]quinoline-7-carboxylate (13)

The title compound was prepared according to the general procedure C using **4a** and **5-2E**. **14** was purified by Combiflash (0-10% MeOH in DCM) and was isolated as a red solid in 59% yield over two steps. ^{1}H NMR (500 MHz, DMSO-d6) δ 8.28 (d, *J* = 1.5 Hz, 1H), 8.19 (t, *J* = 1.4 Hz, 1H), 8.10 (dd, *J* = 8.6, 1.8 Hz, 1H), 7.78 (dt, *J* = 8.5, 1.9 Hz, 1H), 6.85 (s, 2H), 4.42 (dd, *J* = 7.5, 1.7 Hz, 2H), 3.89 (d, *J* = 1.4 Hz, 3H), 2.24 – 2.09 (m, 1H), 0.91 (dd, *J* = 6.6, 1.7 Hz, 6H); ^{13}C NMR (126 MHz, DMSO-d6) δ 166.50, 152.91, 144.39, 144.37, 131.20, 129.44, 127.53, 127.49, 120.95,

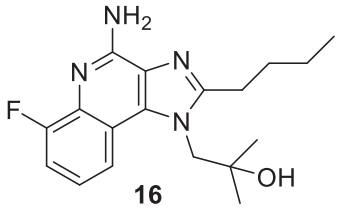
120.69, 118.12, 53.44, 52.16, 28.50, 19.30. HRMS (m/z): [M+H] calcd for $C_{16}H_{19}N_4O_2$, 299.1508; found 299.1535.



14

Methyl 4-amino-1-isobutyl-1H-imidazo[4,5-c]quinoline-8-carboxylate (14)

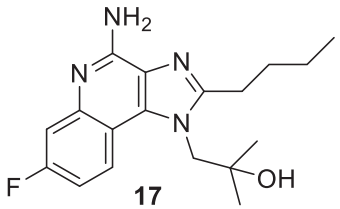
The title compound was prepared according to the general procedure C using **4a** and **5-3E**. **15** was purified by Combiflash (0-10% MeOH in DCM) and was isolated as a light tan solid in 31% yield over two steps. ^{1}H NMR (500 MHz, DMSO-d6) δ 8.63 (d, J = 2.0 Hz, 1H), 8.28 (s, 1H), 7.96 (dd, J = 8.7, 1.9 Hz, 1H), 7.65 (d, J = 8.7 Hz, 1H), 7.10 (s, 2H), 4.42 (d, J = 7.2 Hz, 2H), 3.90 (s, 3H), 2.24 – 2.08 (m, 1H), 1.00 (d, J = 6.7 Hz, 6H); HRMS (m/z): [M+H] calcd for $C_{16}H_{19}N_4O_2$, 299.1508; found 299.1527.



16

6-fluoro-1-(2-hydroxy-2-methylpropyl)-2-butyl-1H-imidazo[4,5-c]quinoline-4-amine (16)

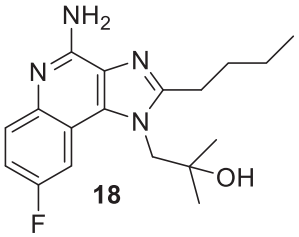
The title compound was prepared according to the general procedure C using **4b** and **5-1F**. **16** was purified by Combiflash (0-10% MeOH in DCM) and was isolated as a light tan solid in 41% yield over two steps. ^{1}H NMR (500 MHz, DMSO-d6) δ 8.63 (d, J = 2.0 Hz, 1H), 8.28 (s, 1H), 7.65 (d, J = 8.7 Hz, 1H), 7.10 (s, 2H), 4.42 (d, J = 7.2 Hz, 2H), 3.90 (s, 3H), 2.22 – 2.10 (m, 1H), 1.00 (d, J = 6.7 Hz, 6H); HRMS (m/z): [M+H] calcd for $C_{18}H_{24}FN_4O$, 331.1934; found 331.1960.



17

7-fluoro-1-(2-hydroxy-2-methylpropyl)-2-butyl-1H-imidazo[4,5-c]quinoline-4-amine (17)

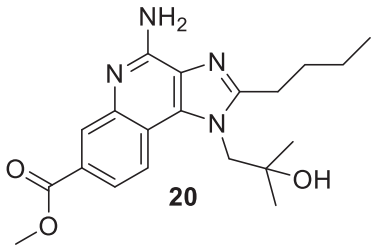
The title compound was prepared according to the general procedure C using **4b** and **5-2F**. **17** was purified by Combiflash (0-10% MeOH in DCM) and was isolated as a light tan solid in 36% yield over two steps. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.15 (dd, J = 9.2, 5.9 Hz, 1H), 7.40 (dd, J = 10.7, 2.7 Hz, 1H), 7.04 (ddd, J = 9.2, 8.0, 2.7 Hz, 1H), 5.73 (s, 2H), 4.54 (s, 2H), 3.01 – 2.76 (m, 2H), 1.89 – 1.74 (m, 2H), 1.45 (dp, J = 14.6, 7.4 Hz, 2H), 1.36 (s, 6H), 0.98 (t, J = 7.4 Hz, 3H). HRMS (m/z): [M+H] calcd for $C_{18}H_{24}FN_4O$, 331.1934; found 331.1954.



18

8-fluoro-1-(2-hydroxy-2-methylpropyl)-2-butyl-1H-imidazo[4,5-c]quinoline-4-amine (18)

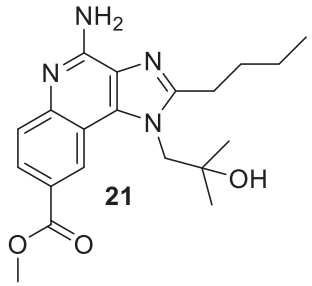
The title compound was prepared according to the general procedure C using **4b** and **5-3F**. **18** was purified by Combiflash (0-10% MeOH in DCM) and was isolated as a brown solid in 50% yield over two steps. ^{1}H NMR (500 MHz, DMSO-d6) δ 8.07 (d, *J* = 8.3 Hz, 1H), 7.24 – 7.16 (m, 1H), 7.13 (td, *J* = 8.1, 5.3 Hz, 1H), 6.67 (s, 2H), 4.78 (s, 1H), 4.53 (s, 2H), 3.03 (t, *J* = 7.8 Hz, 2H), 1.84 – 1.74 (m, 2H), 1.42 (h, *J* = 7.4 Hz, 2H), 1.16 (s, 6H), 0.94 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (126 MHz, DMSO-d6) δ 156.27 (d, *J* = 246.4 Hz), 155.60, 151.76, 134.13 (d, *J* = 10.2 Hz), 133.29 (d, *J* = 4.1 Hz), 126.63, 119.60 (d, *J* = 8.2 Hz), 117.59 (d, *J* = 3.3 Hz), 117.20 (d, *J* = 4.0 Hz), 110.90 (d, *J* = 19.5 Hz), 70.74, 54.54, 29.77, 26.97, 26.85, 22.05, 13.86. HRMS (m/z): [M+H] calcd for $C_{18}H_{24}FN_4O$, 331.1934; found 331.1935.



20

Methyl 4-amino-2-butyl-1-(2-hydroxy-2-methylpropyl)-1H-imidazo[4,5-c]quinoline-7-carboxylate (20)

The title compound was prepared according to the general procedure C using **4b** and **5-2E**. **20** was purified by Combiflash (0-10% MeOH in DCM) and was isolated as a light tan solid in 43% yield over two steps. Spectral data matched previously reported data.[54]



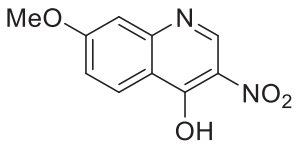
21

Methyl 4-amino-2-butyl-1-(2-hydroxy-2-methylpropyl)-1H-imidazo[4,5-c]quinoline-8-carboxylate (21)

The title compound was prepared according to the general procedure C using **4b** and **5-3E**. **21** was purified by Combiflash (0-10% MeOH in DCM) and was isolated as a brown solid in 37% yield over two steps. HRMS (m/z): [M+H] calcd for $C_{20}H_{27}N_4O_3$, 371.2083; found 371.2080.

Preparation of 7-methoxy and 7-chloroimidazoquinolones:

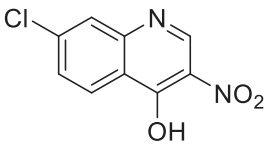
Step 1:



22a

7-methoxy-3-nitroquinolin-4-ol (22a)

The procedure was adapted from Gerster *et al.* with modifications.[50] In a round bottom flask, 7-methoxy-quinolin-4-ol was suspended in propionic acid and heated to reflux with a water cooled condenser opened to air. 70% nitric acid (1.8 eq.) was added dropwise over 15 minutes. The reaction was refluxed for 2 hours. The reaction was allowed to cool to room temperature and the solid was collected by vacuum filtration. The solid was washed with cold EtOH, followed by hexanes and **22a** was isolated as in 55% yield as a tan solid. Spectral data matched previously reported data.[50]



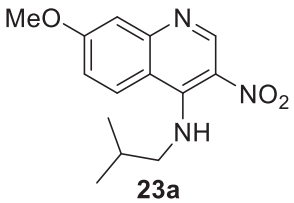
22b

7-chloro-3-nitroquinolin-4-ol (22b)

The procedure was adapted from Gerster *et al.* with modifications.[50] In a round bottom flask, 7-chloro-quinolin-4-ol was suspended in propionic acid and heated to reflux with a water cooled condenser opened to air. 70% nitric acid (2.2 eq.) was added dropwise over 15 minutes. The reaction was refluxed for 1 h. The reaction was allowed to cool to room temperature and diluted with EtOH and the solid was collected by vacuum filtration. The solid was washed with cold EtOH, followed by hexanes and **22b** was isolated in 70% yield as a tan solid. Spectral data matched previously reported data.[50]

General Procedure D (Step 2):

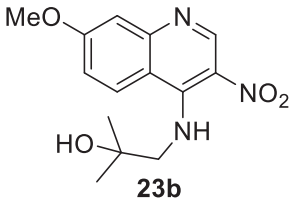
The procedure was adapted from Gerster *et al.* with modifications.[50] Phosphorus oxychloride (1.2 eq.) was added dropwise to a well stirred suspension of 7-substituted-nitroquinoline-4-ol in anhydrous DMF (1.8 mL per mmol) and an exothermic reaction was observed. After addition of $POCl_3$ was completed, the reaction was heated at 50 °C and stirred for 30 min. The resulting solution was cooled to room temperature and poured into ice water (7.5 mL per mmol). The resulting solid was collected by vacuum filtration, washed with water and pressed dry. The moist solid was added to a round bottom flask and suspended in EtOH (5 mL per mmol), Et_3N (2 eq.), and the appropriate alkyl amine (1.3 eq.) and refluxed for 15 min. Water was added to the solution and the solid was collected by vacuum filtration.



23a

7-methoxy-N-isobutyl-3-nitroquinolin-4-amine (23a)

The title compound was prepared according to the general procedure D using **22a** and isobutylamine to obtain a bright yellow solid in 80% yield. Spectral data matched previously reported data.[50]

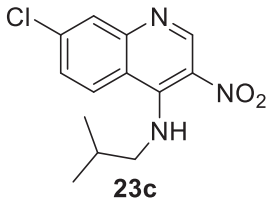


23b

1-((7-methoxy-3-nitroquinolin-4-yl)amino)-2-methylpropan-2-ol (23b)

The title compound was prepared according to the general procedure D using **22a** and 1-Amino-2-methyl-2-propanol to obtain a bright yellow solid in 80% yield. ^{1}H NMR (400 MHz, Methanol-d4) δ 9.20 (s, 1H), 8.38 (d, *J* = 9.5 Hz, 1H), 7.28 (d, *J* = 2.7 Hz, 1H), 7.19 (dd, *J* = 9.4, 2.7 Hz, 1H),

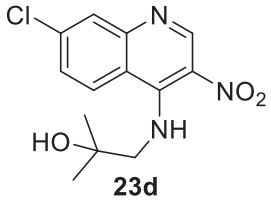
4.59 (s, 2H), 3.97 (s, 3H), 3.91 (s, 2H), 1.29 (s, 6H); HRMS (m/z): [M+H] calcd for $C_{14}H_{18}N_3O_4$, 292.1297; found 292.1309.



23c

7-chloro-N-isobutyl-3-nitroquinolin-4-amine (23c)

The title compound was prepared according to the general procedure D using **22b** and isobutylamine to obtain a bright yellow solid in 80% yield. Spectral data matched previously reported data.[50]



23d

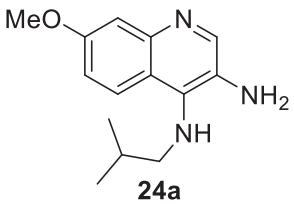
1-((7-chloro-3-nitroquinolin-4-yl)amino)-2-methylpropan-2-ol (23d)

The title compound was prepared according to the general procedure D using **22b** and 1-Amino-2-methyl-2-propanol to obtain a bright yellow solid in 80% yield. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.93 (s, 1H), 9.37 (s, 1H), 8.18 (d, *J* = 9.0 Hz, 1H), 7.99 (d, *J* = 2.4 Hz, 1H), 7.44 (dd, *J* = 9.1, 2.3 Hz, 1H), 3.85 (d, *J* = 4.9 Hz, 2H), 1.36 (s, 6H). HRMS (m/z): [M+H] calcd for $C_{13}H_{15}ClN_3O_3$, 296.0802; found 296.0813.

General Procedure E (Step 3):

A suspension of 7-substituted amino nitroquinoline in EtOH was heated to reflux in open air. Iron (dust 5 eq.) was added followed by aqueous NH_4Cl (2.3 M, 5eq.) and refluxed for 2 hours. The solution was allowed to cool and was filtered through a Celite plug and eluted with EtOAc. The volume of solvent was reduced on a rotovap and basified with saturated Na_2CO_3. The aqueous

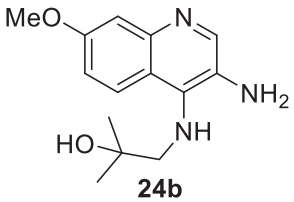
layer was extracted with EtOAc (3x). The combined organic layers were dried with Na_2SO_4, filtered, and evaporated on rotovap to dryness.



24a

7-methoxy-N^4-isobutylquinoline-3,4-diamine (24a)

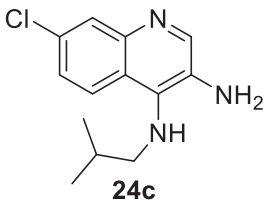
The title compound was prepared according to the general procedure E using **23a** to obtain an orange solid in 80% yield. Spectral data matched previously reported data.[50]



24b

1-((3-amino-7-methoxyquinolin-4-yl)amino)-2-methylpropan-2-ol (24b)

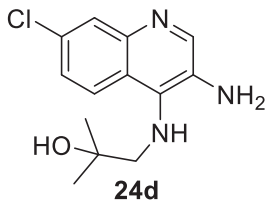
The title compound was prepared according to the general procedure E using **23b** to obtain an orange solid in 80% yield. ^{1}H NMR (400 MHz, Methanol-d4) δ 8.34 (d, *J* = 9.6 Hz, 1H), 8.01 (s, 1H), 7.20 (dd, *J* = 9.5, 2.6 Hz, 1H), 7.11 (d, *J* = 2.6 Hz, 1H), 3.97 (s, 3H), 3.94 (s, 2H), 1.31 (s, 6H). HRMS (m/z): [M+H] calcd for $C_{14}H_{19}N_3O_2$, 262.1555; found 262.168.f



24c

7-chloro-N^4-isobutylquinoline-3,4-diamine (24c)

The title compound was prepared according to the general procedure E using **23c** to obtain an orange solid in 80% yield. Spectral data matched previously reported data.[50]



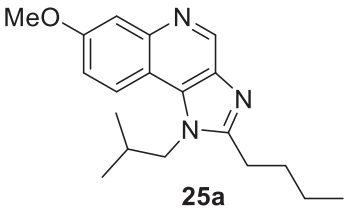
24d

1-((3-amino-7-chloroquinolin-4-yl)amino)-2-methylpropan-2-ol (24d)

The title compound was prepared according to the general procedure E using **23d** to obtain an orange solid in 80% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.84 (s, 1H), 8.16 (dd, *J* = 9.0, 0.5 Hz, 1H), 8.13 – 8.09 (d, 1H), 7.63 (dd, *J* = 9.0, 2.1 Hz, 1H), 4.60 (s, 2H), 3.90 (s, 2H), 1.28 (s, 6H); HRMS (m/z): [M+H] calcd for $C_{13}H_{14}ClN_3O_3$, 296.0802; found 296.0812.

General Procedure F (Step 4):

Valeric or ethoxy acetic acid (10 eq.) was added to diaminoquinolines in a small round bottom flask. The suspension was heated to reflux (~150 °C) in open air until water ceased to be released from the reaction. The reaction was allowed to cool and diluted with water then basified with 6M NaOH. The water was extracted with DCM (3x) and the combined organic layer was washed with brine and dried with Na_2SO_4, filtered, and concentrated on rotovap. The compound was purified by Combiflash (0-10% MeOH in DCM).

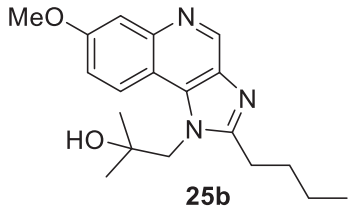


25a

2-butyl-7-methoxy-1-isobutyl-1H-imidazo[4,5-c]quinoline (25a)

The title compound was prepared according to the general procedure F using **24a** to obtain a brown solid in 80% yield. ^{1}H NMR (400 MHz, Chloroform-d) δ 9.21 (s, 1H), 7.98 (d, *J* = 9.2 Hz, 1H), 7.65 (d, *J* = 2.7 Hz, 1H), 7.29 – 7.26 (m, 1H), 4.27 (d, *J* = 7.6 Hz, 2H), 3.96 (s, 4H), 3.03 –

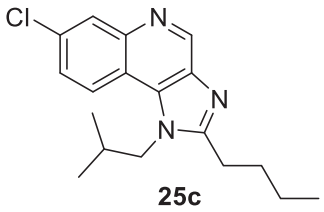
2.88 (m, 2H), 2.40 – 2.27 (m, 1H), 1.94 (dq, *J* = 9.2, 7.6 Hz, 2H), 1.50 (dt, *J* = 14.1, 7.2 Hz, 4H), 1.01 (m, *J* = 7.0, 5.6 Hz, 9H). HRMS (m/z): [M+H] calcd for $C_{19}H_{26}N_3O$, 312.2076; found 312.2086.



25b

1-(2-butyl-7-methoxy-1H-imidazo[4,5-c]quinolin-1-yl)-2-methylpropan-2-ol (25b)

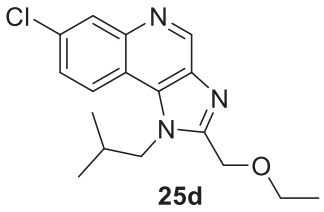
The title compound was prepared according to the general procedure F using **24b** to obtain a brown solid in 80% yield. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.94 (s, 1H), 8.53 (s, 1H), 7.81 (s, 1H), 7.33 (d, *J* = 9.4 Hz, 1H), 4.62 (s, 2H), 3.97 (s, 1H), 3.88 (s, 3H), 3.06 (t, 2H), 1.95 (p, *J* = 7.8 Hz, 2H), 1.52 (q, *J* = 7.5 Hz, 2H), 1.47 (s, 6H), 1.01 (t, *J* = 7.3 Hz, 2H). HRMS (m/z): [M+H] calcd for $C_{19}H_{26}N_3O_2$, 328.2025; found 328.2019.



25c

2-butyl-7-chloro-1-isobutyl-1H-imidazo[4,5-c]quinoline (25c)

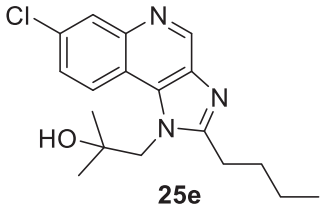
The title compound was prepared according to the general procedure F using **24c** to obtain a brown solid in 80% yield. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.27 (s, 1H), 8.38 (d, *J* = 2.2 Hz, 1H), 8.05 (d, *J* = 9.0 Hz, 1H), 7.62 (dd, *J* = 9.0, 2.2 Hz, 1H), 4.32 (d, *J* = 7.5 Hz, 2H), 2.99 – 2.93 (m, 2H), 2.33 (hept, *J* = 7.0 Hz, 1H), 1.96 (ddd, *J* = 15.5, 8.3, 6.8 Hz, 2H), 1.52 (dd, *J* = 15.1, 7.5 Hz, 2H), 1.03 (d, *J* = 6.7 Hz, 6H), 1.02 (t, *J* = 7.4 Hz, 3H).; ^{13}C NMR ($CDCl_3$, 126 MHz) δ 157.17, 136.50, 133.10, 127.57, 121.28, 116.03, 53.43, 52.83, 29.54, 29.18, 27.62, 22.64, 21.79, 19.84, 13.86. HRMS (m/z): [M+H] calcd for $C_{18}H_{26}NClN_3$, 316.1581; found 316.1590.



25d

7-chloro-2-(ethoxymethyl)-1-isobutyl-1H-imidazo[4,5-c]quinoline (25d)

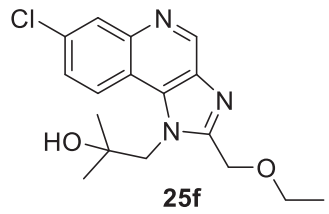
The title compound was prepared according to the general procedure F using **24c** to obtain a brown solid in 80% yield. ^{1}H NMR (400 MHz, Chloroform-d) δ 9.30 (s, 1H), 8.35 (s, 1H), 8.07 (d, *J* = 9.0 Hz, 1H), 7.64 (dd, *J* = 8.9, 2.2 Hz, 1H), 4.89 (s, 2H), 4.51 (d, *J* = 7.7 Hz, 2H), 3.61 (q, *J* = 7.0 Hz, 2H), 2.37 (tt, *J* = 13.3, 6.7 Hz, 1H), 1.25 (t, *J* = 7.0 Hz, 3H), 1.09 – 1.00 (m, 6H). HRMS (m/z): [M+H] calcd for $C_{17}H_{21}ClN_3O$, 318.1373; found 318.1366.



25e

1-(2-butyl-7-chloro-1H-imidazo[4,5-c]quinolin-1-yl)-2-methylpropan-2-ol (25e)

The title compound was prepared according to the general procedure F using **24d** to obtain a brown solid in 80% yield. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.99 (s, 1H), 8.52 (d, *J* = 9.0 Hz, 1H), 8.13 (s, 1H), 7.55 (dd, *J* = 9.1, 2.2 Hz, 1H), 4.62 (s, 2H), 3.06 (s, 2H), 1.95 (p, *J* = 7.7 Hz, 2H), 1.51 (dd, *J* = 15.2, 7.5 Hz, 2H), 1.46 (s, 6H), 1.01 (t, *J* = 7.3 Hz, 3H). HRMS (m/z): [M+H] calcd for $C_{18}H_{23}ClN_3O$, 332.1530; found 332.1545.



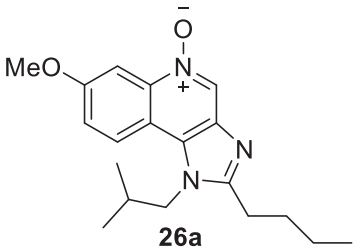
25f

1-(7-chloro-2-(ethoxymethyl)-1H-imidazo[4,5-c]quinolin-1-yl)-2-methylpropan-2-ol (25f)

The title compound was prepared according to the general procedure F using **24d** to obtain a brown solid in 80% yield. ^{1}H NMR (400 MHz, Chloroform-d) δ 9.14 (s, 1H), 8.41 (s, 1H), 8.25 (s, 1H), 7.60 (d, *J* = 8.9 Hz, 1H), 4.99 (s, 2H), 4.83 (s, 2H), 3.65 (q, *J* = 7.0 Hz, 2H), 1.40 (s, 6H), 1.25 (dd, *J* = 7.4, 6.7 Hz, 3H). HRMS (m/z): [M+H] calcd for $C_{17}H_{21}ClN_3O_2$, 334.1322; found 334.1328.

General Procedure G (Step 5):

The procedure was adapted from Gerster *et al.* with modifications.[50] In a round bottom flask, imidazoquinoline was dissolved in $CHCl_3$ and stirred at room temperature. *m*CPBA (1 eq + 1eq) was added portion wise 1 h apart. After 3 hours the reaction was diluted with DCM, washed with saturated Na_2CO_3 aqueous solution, extracted with DCM (3x), dried with Na_2SO_4, filtered, and concentrated on rotovap. The compound was purified by Combiflash (0-10% MeOH in DCM).

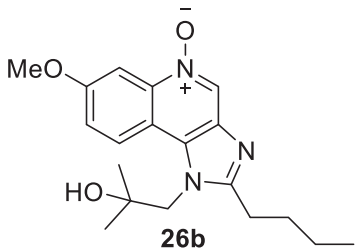


26a

2-butyl-7-methoxy-1-isobutyl-1H-imidazo[4,5-c]quinoline 5-oxide (26a)

The title compound was prepared according to the general procedure G using **25a** to obtain a brown solid in 80% yield. ^{1}H NMR (DMSO-d6, 500 MHz) δ 8.99 (s, 1H), 8.24 (d, *J* = 9.0 Hz, 1H), 8.22 (d, *J* = 2.8 Hz, 1H), 7.47 (dd, *J* = 9.2, 2.9 Hz, 1H), 4.37 (d, *J* = 7.6 Hz, 2H), 2.91 (t, *J* = 7.6 Hz, 2H), 2.13 (dt, *J* = 13.5, 6.8 Hz, 1H), 1.82 (p, *J* = 7.5 Hz, 2H), 1.44 (h, *J* = 7.4 Hz, 2H), 0.94 (t,

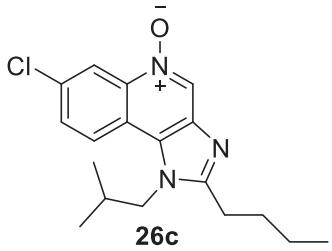
J = 7.5 Hz, 3H), 0.91 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (DMSO-d6, 126 MHz) δ 158.67, 157.18, 139.13, 134.35, 129.30, 126.50, 123.02, 120.00, 112.15, 100.90, 55.61, 51.30, 28.95, 28.60, 26.40, 21.84, 19.15, 13.79. HRMS (m/z): [M+H] calcd for $C_{19}H_{26}N_3O_2$, 328.2025; found 328.2034.



26b

2-butyl-7-methoxy-1-(2-hydroxy-2-methylpropyl)-1H-imidazo[4,5-c]quinoline 5-oxide (26b)

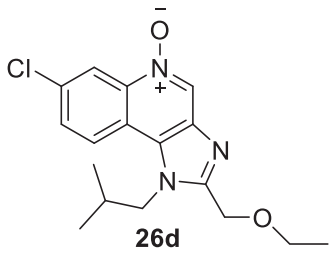
The title compound was prepared according to the general procedure G using **25b** to obtain a brown solid in 80% yield. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.67 (d, *J* = 9.3 Hz, 1H), 8.02 (s, 1H), 7.52 (d, *J* = 2.7 Hz, 1H), 7.19 (dd, *J* = 9.3, 2.8 Hz, 1H), 4.10 (s, 1H), 3.83 (s, 3H), 3.43 (d, *J* = 3.8 Hz, 1H), 2.92 (s, 3H), 1.86 (q, *J* = 7.7 Hz, 2H), 1.30 (d, *J* = 2.0 Hz, 2H), 0.98 (t, *J* = 7.4 Hz, 3H). HRMS (m/z): [M+H] calcd for $C_{19}H_{26}N_3O_3$, 344.1974; found 344.1986.



26c

2-butyl-7-chloro-1-isobutyl-1H-imidazo[4,5-c]quinoline 5-oxide (26c)

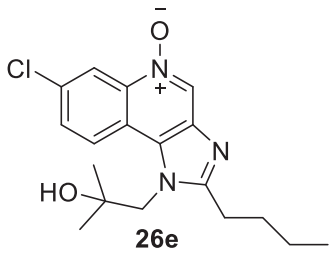
The title compound was prepared according to the general procedure G using **25c** to obtain a brown solid in 80% yield. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.58 (s, 1H), 8.88 (d, *J* = 2.0 Hz, 1H), 8.20 (d, *J* = 9.0 Hz, 1H), 7.86 (dd, *J* = 9.0, 2.2 Hz, 1H), 4.39 (d, *J* = 7.6 Hz, 2H), 3.04 – 2.97 (m, 2H), 2.30 (dt, *J* = 13.8, 6.9 Hz, 1H), 1.97 (p, *J* = 7.6 Hz, 2H), 1.52 (ddd, *J* = 14.2, 7.2, 5.4 Hz, 3H), 1.07 (d, *J* = 6.7 Hz, 6H), 1.03 (t, *J* = 7.3 Hz, 3H); HRMS (m/z): [M+Na] calcd for $C_{18}H_{22}ClN_3ONa$, 354.1349; found 354.1362.



26d

7-chloro-2-(ethoxymethyl)-1-isobutyl-1H-imidazo[4,5-c]quinoline 5-oxide (26d)

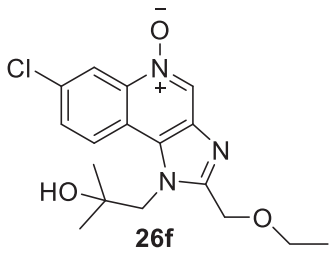
The title compound was prepared according to the general procedure G using **25d** to obtain a brown solid in 80% yield. ^{1}H NMR (400 MHz, DMSO-d6) δ 9.12 (d, *J* = 9.9 Hz, 1H), 8.83 – 8.73 (m, 1H), 8.40 (dd, *J* = 20.5, 9.0 Hz, 1H), 7.92 (ddd, *J* = 10.9, 8.9, 2.4 Hz, 1H), 4.80 (s, 2H), 4.55 – 4.43 (m, 2H), 3.59 (q, *J* = 7.0 Hz, 2H), 2.22 (p, *J* = 7.1 Hz, 1H), 1.16 (t, *J* = 7.0 Hz, 3H), 0.92 (d, *J* = 6.7 Hz, 6H). HRMS (m/z): [M+Na] calcd for $C_{17}H_{20}ClN_3O_2Na$, 372.1091; found 372.1099.



26e

2-butyl-7-chloro-1-(2-hydroxy-2-methylpropyl)-1H-imidazo[4,5-c]quinoline 5-oxide (26e)

The title compound was prepared according to the general procedure G using **25e** to obtain a brown solid in 80% yield. ^{1}H NMR (400 MHz, Chloroform-d) δ 9.04 (s, 1H), 8.72 (d, *J* = 9.1 Hz, 1H), 8.48 (s, 1H), 7.65 (d, *J* = 8.5 Hz, 1H), 4.48 (s, 2H), 3.01 (s, 2H), 1.93 (p, *J* = 7.7 Hz, 2H), 1.51 (dt, *J* = 15.0, 7.4 Hz, 2H), 1.44 (s, 6H), 1.23 (d, *J* = 16.9 Hz, 1H), 1.01 (t, *J* = 7.3 Hz, 3H). HRMS (m/z): [M+H] calcd for $C_{18}H_{23}ClN_3O_2$, 348.1479; found 348.1468.



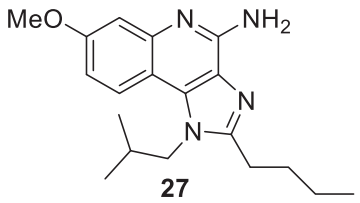
26f

7-chloro-2-(ethoxymethyl)-1-(2-hydroxy-2-methylpropyl)-1H-imidazo[4,5-c]quinoline 5-oxide (26f)

The title compound was prepared according to the general procedure G using **25f** to obtain a brown solid in 80% yield. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.18 (s, 1H), 8.66 (d, *J* = 8.9 Hz, 1H), 8.64 – 8.60 (m, 1H), 7.73 (d, *J* = 8.3 Hz, 1H), 4.97 (s, 2H), 4.80 (s, 2H), 3.61 (q, *J* = 7.0 Hz, 2H), 1.41 (s, 6H), 1.24 (t, *J* = 7.0 Hz, 3H). HRMS (m/z): [M+Na] calcd for $C_{17}H_{20}ClN_3O_2Na$, 372.1091; found 372.1085.

General Procedure H (Step 6):

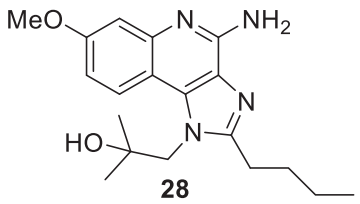
The procedure was adapted from Gerster *et al.* with modifications.[50] Concentrated NH_4OH (1 mL per mmol) was added to a round bottom flask containing N-oxide imidazoquinoline (1 eq) in anhydrous DCM (1 mL per mmol) stirring vigorously at room temperature. *p*-TsCl (1.1 eq) was added to an addition funnel and dissolved in anhydrous DCM (1 mL per mmol). The *p*-TsCl in DCM was added dropwise slowly over 15 min minutes and more DCM was added to help push residual *p*-TsCl into the flask to the round bottom. An exotherm was observed during the addition. The reaction was allowed to stir for another 15 min after addition of *p*-TsCl was completed. Water was added and if precipitate formed, the solid was isolated by vacuum filtration. The mother liquor or biphasic mixture was extracted with DCM, washed with brine, dried with Na_2SO_4, and concentrated *in vacuo* and purified by silica gel flash chromatography.



27

2-butyl-7-methoxy-1-isobutyl-1H-imidazo[4,5-c]quinolin-4-amine (27)

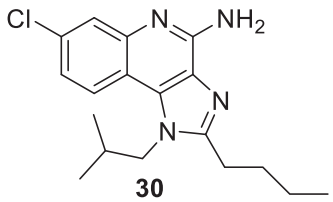
The title compound was prepared according to the general procedure H using **26a** to obtain a brown solid in 80% yield. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.76 (d, *J* = 9.0 Hz, 1H), 7.30 (d, *J* = 2.7 Hz, 1H), 7.00 (dd, *J* = 9.0, 2.6 Hz, 1H), 6.43 (s, 2H), 4.20 (d, *J* = 7.6 Hz, 2H), 3.92 (s, 3H), 2.90 – 2.83 (m, 2H), 2.31 (dp, *J* = 13.4, 6.6 Hz, 1H), 1.86 (p, *J* = 7.6 Hz, 2H), 1.49 (h, *J* = 7.4 Hz, 2H), 1.02 – 0.97 (m, 9H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 159.49, 154.41, 150.80, 134.77, 124.73, 121.28, 114.23, 108.69, 105.40, 55.70, 52.71, 30.01, 29.28, 27.58, 22.73, 19.92, 19.79, 13.99. HRMS (m/z): [M+H] calcd for $C_{19}H_{27}N_4O$, 327.2185; found 327.2196.



28

1-(4-amino-2-butyl-7-methoxy-1H-imidazo[4,5-c]quinolin-1-yl)-2-methylpropan-2-ol (28)

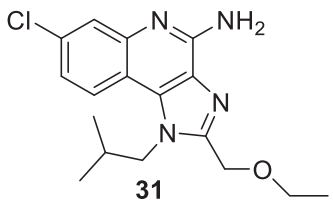
The title compound was prepared according to the general procedure H using **26b** to obtain a brown solid in 80% yield. ^{1}H NMR (500 MHz, DMSO-d6) δ 9.12 (d, *J* = 10.7 Hz, 1H), 8.80 (dd, *J* = 5.0, 2.3 Hz, 1H), 8.40 (dd, *J* = 20.5, 9.1 Hz, 1H), 7.91 (ddd, *J* = 11.1, 9.0, 2.4 Hz, 1H), 4.80 (s, 1H), 4.49 (s, *J* = 21.9, 7.7 Hz, 2H), 3.59 (q, *J* = 7.0 Hz, 2H), 2.22 (p, *J* = 7.0 Hz, 1H), 1.16 (t, *J* = 7.0 Hz, 3H), 0.92 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d6) δ 157.65, 154.34, 151.79, 146.23, 134.09, 124.72, 122.33, 110.62, 109.58, 106.88, 70.79, 54.87, 54.46, 48.59, 29.80, 26.76, 22.05, 13.87. HRMS (m/z): [M+H] calcd for $C_{17}H_{21}ClN_3O_2$, 350.1271; found 350.1267.



30

2-butyl-7-chloro-1-isobutyl-1H-imidazo[4,5-c]quinolin-4-amine (30)

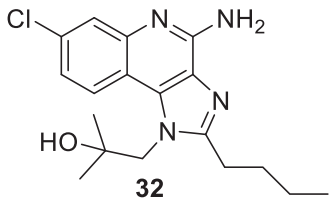
The title compound was prepared according to the general procedure H using **26c** to obtain a brown solid in 81% yield. ^{1}H NMR (DMSO-d6, 500 MHz) δ 7.97 (d, *J* = 8.9 Hz, 1H), 7.56 (d, *J* = 2.3 Hz, 1H), 7.26 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.69 (s, 2H), 4.33 (d, *J* = 7.6 Hz, 2H), 2.93 – 2.87 (m, 2H), 2.11 (dq, *J* = 13.8, 6.9 Hz, 1H), 1.85 – 1.76 (m, 2H), 1.44 (dt, *J* = 14.7, 7.4 Hz, 2H), 0.95 (t, *J* = 7.4 Hz, 3H), 0.91 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (DMSO-d6, 126 MHz) δ 153.99, 152.59, 145.83, 132.08, 130.51, 126.58, 124.81, 121.97, 120.90, 113.57, 51.31, 29.65, 28.82, 26.43, 21.93, 19.16, 13.81. HRMS (m/z): [M+] calcd for $C_{18}H_{23}ClN_4$, 330.1611; found 330.1630.



31

7-chloro-2-(ethoxymethyl)-1-isobutyl-1H-imidazo[4,5-c]quinolin-4-amine (31)

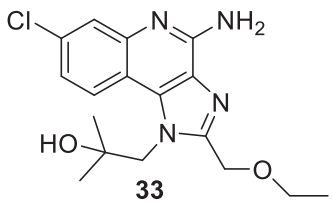
The title compound was prepared according to the general procedure H using **26d** to obtain a brown solid in 78% yield. ^{1}H NMR (500 MHz, DMSO-d6) δ 8.01 (d, *J* = 8.8 Hz, 1H), 7.58 (d, *J* = 2.3 Hz, 1H), 7.27 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.88 (s, 2H), 4.75 (s, 2H), 4.42 (d, *J* = 7.7 Hz, 2H), 3.56 (q, *J* = 7.0 Hz, 2H), 2.20 (hept, *J* = 6.9 Hz, 1H), 1.15 (t, *J* = 7.0 Hz, 3H), 0.91 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (126 MHz, DMSO-d6) δ 152.93, 149.72, 146.29, 132.82, 131.12, 126.48, 124.82, 122.43, 121.00, 113.45, 65.41, 64.16, 51.77, 28.61, 19.25, 14.92. HRMS (m/z): [M+H] calcd for $C_{17}H_{22}ClN_4O$, 333.1482; found 333.1475.



32

1-(4-amino-2-butyl-7-chloro-1H-imidazo[4,5-c]quinolin-1-yl)-2-methylpropan-2-ol (32)

The title compound was prepared according to the general procedure H using **26e** to obtain a brown solid in 73% yield. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.29 (d, *J* = 8.9 Hz, 1H), 7.53 (d, *J* = 2.3 Hz, 1H), 7.17 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.63 (s, 2H), 4.77 (s, 1H), 4.51 (s, 2H), 3.00 (t, 2H), 1.78 (ddd, *J* = 15.4, 8.9, 7.1 Hz, 2H), 1.42 (h, *J* = 7.4 Hz, 2H), 1.16 (s, 6H), 0.94 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 126z MHz) δ 155.39, 152.57, 145.93, 133.30, 130.16, 126.28, 124.58, 123.17, 120.10, 114.26, 70.76, 54.49, 48.59, 29.78, 26.80, 22.03, 13.86. HRMS (m/z): [M+H] calcd for $C_{18}H_{24}ClN_4O$, 347.1639; found 347.1649.



33

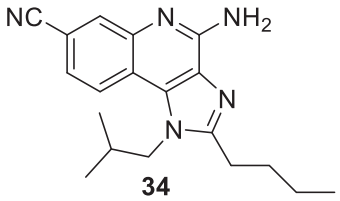
1-(4-amino-7-chloro-2-(ethoxymethyl)-1H-imidazo[4,5-c]quinolin-1-yl)-2-methylpropan-2-ol (33)

The title compound was prepared according to the general procedure H using **26f** to obtain a brown solid in 84% yield. ^{1}H NMR (DMSO-d6, 500 MHz) δ 8.30 (d, *J* = 8.9 Hz, 1H), 7.54 (d, *J* = 2.3 Hz, 1H), 7.19 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.79 (s, 2H), 4.88 (s, 1H), 4.64 (s, 2H), 3.51 (q, *J* = 7.0 Hz, 2H), 1.16 (s, 6H), 1.13 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (DMSO-d6, 126 MHz) δ 153.38, 151.61, 146.91, 134.40, 131.26, 126.67, 125.14, 123.86, 120.75, 114.52, 71.11, 65.80, 65.28, 55.25, 15.47. HRMS (m/z): [M+H] calcd for $C_{17}H_{22}ClN_4O_2$, 349.1431; found 349.1425.

Preparation of Aryl Nitriles:

General Procedure I

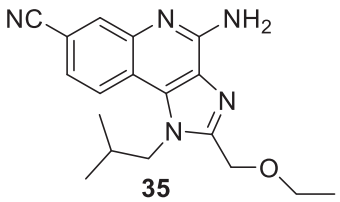
The procedure was adapted from Littke *et al.* with modifications.[67] Aryl chloride (1 eq), $Pd(TFA)_2$ (5%), Zn (dust, 20%), TrixiePhos (10%), $Zn(CN)_2$ (56%) were added to an oven-dried 40 mL vial and vacuum flushed with nitrogen three times. Anhydrous dimethylacetamide (DMAC) (0.19 M) was added via syringe to the sealed vial. The vial was shaken and placed on a heating mantel at 95 °C and stirred overnight. The mixture was allowed to cool to room temperature and filtered through a plug of Celite using either 100% EtOAc or 20% MeOH in DCM to elute product. The solvent was then concentrated down and redissovled in EtOAc and washed with water (4-5x by 10x the volume of DMAC), washed with brine and dried with sodium sulfate. Solvent was concentrated down on rotovap and purified by Combiflash (0-10% MeOH in DCM).



34

4-amino-2-butyl-1-isobutyl-1H-imidazo[4,5-c]quinoline-7-carbonitrile (34)

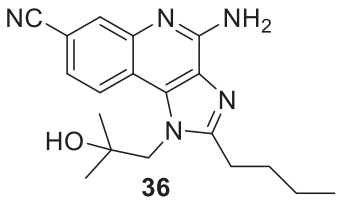
The title compound was prepared according to the general procedure I using **30** to obtain a brown solid in 80% yield. ^{1}H NMR (500 MHz, DMSO-d6) δ 8.13 (d, J = 8.5 Hz, 1H), 7.98 (t, J = 1.5 Hz, 1H), 7.56 (dt, J = 8.3, 1.5 Hz, 1H), 6.90 (s, 2H), 4.38 (d, J = 7.6 Hz, 2H), 2.94 (t, J = 6.3 Hz, 2H), 2.18 – 2.06 (m, 1H), 1.83 (t, J = 7.7 Hz, 2H), 1.46 (q, J = 7.6 Hz, 2H), 0.98 – 0.94 (t, 3H), 0.93 (d, J = 6.6 Hz, 7H); ^{13}C NMR (500 MHz, DMSO-d6) δ 155.29, 152.90, 144.07, 131.51, 130.47, 128.12, 122.36, 121.70, 119.40, 118.00, 108.27, 51.38, 29.60, 28.90, 26.48, 21.91, 19.13, 13.80. HRMS (m/z): [M+H] calcd for $C_{19}H_{24}N_5$, 322.2032; found 322.2034.



35

4-amino-2-(ethoxymethyl)-1-isobutyl-1H-imidazo[4,5-c]quinoline-7-carbonitrile (35)

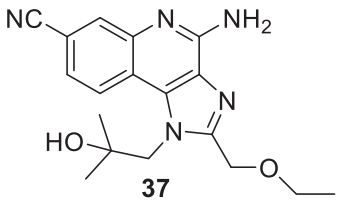
The title compound was prepared according to the general procedure I using **31** to obtain a brown solid in 80% yield. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.22 (d, J = 1.7 Hz, 1H), 8.01 (d, J = 8.5 Hz, 1H), 7.61 (dd, J = 8.5, 1.7 Hz, 1H), 6.42 (s, 2H), 4.86 (s, 2H), 4.48 (d, J = 7.7 Hz, 2H), 4.15 (q, J = 7.1 Hz, 1H), 3.65 (q, J = 7.0 Hz, 2H), 2.36 (dt, J = 13.9, 7.0 Hz, 1H), 1.31 – 1.24 (m, 3H), 1.07 (d, J = 6.7 Hz, 6H); ^{13}C NMR (126 MHz, DMSO-d6) δ 151.85, 149.18, 143.94, 132.94, 132.40, 129.13, 124.57, 122.81, 119.48, 119.06, 109.77, 66.15, 64.69, 52.50, 29.12, 19.74, 15.45. HRMS (m/z): [M+H] calcd for $C_{18}H_{22}N_5O$, 324.1824; found 324.1816.



36

4-amino-2-butyl-1-(2-hydroxy-2-methylpropyl)-1H-imidazo[4,5-c]quinoline-7-carbonitrile (36)

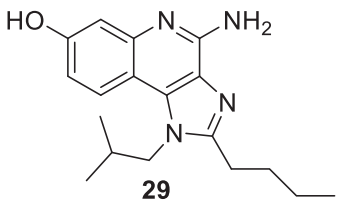
The title compound was prepared according to the general procedure I using **32** to obtain a brown solid in 80% yield. ^{1}H NMR (500 MHz, DMSO-d6) δ 8.45 (d, *J* = 8.6 Hz, 1H), 7.94 (d, *J* = 1.8 Hz, 1H), 7.46 (dd, *J* = 8.6, 1.8 Hz, 1H), 6.85 (s, 2H), 4.78 (s, 1H), 4.52 (s, 2H), 3.02 (t, *J* = 7.8 Hz, 2H), 1.80 (ddd, *J* = 15.3, 8.9, 7.1 Hz, 2H), 1z.42 (h, *J* = 7.4 Hz, 2H), 1.17 (s, 6H), 0.94 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (126 MHz, DMSO-d6) δ 156.64, 152.92, 144.16, 132.82, 130.29, 127.82, 123.04, 121.50, 119.52, 118.79, 107.94, 70.75, 54.55, 29.75, 26.88, 22.02, 13.86. HRMS (m/z): [M+H] calcd for $C_{19}H_{24}N_5O$, 338.1981; found 338.1981.



37

4-amino-2-(ethoxymethyl)-1-(2-hydroxy-2-methylpropyl)-1H-imidazo[4,5-c]quinoline-7-carbonitrile (37)

The title compound was prepared according to the general procedure I using **33** to obtain a brown solid in 80% yield. ^{1}H NMR (500 MHz, DMSO-d6) δ 8.47 (d, *J* = 8.6 Hz, 1H), 7.96 (d, *J* = 1.8 Hz, 1H), 7.48 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.01 (s, 2H), 4.89 (s, 1H), 4.68 (s, 2H), 3.52 (q, *J* = 7.0 Hz, 2H), 1.19 – 1.15 (m, 2H), 1.13 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, DMSO-d6) δ 153.22, 152.28, 144.67, 133.41, 130.35, 127.66, 123.24, 121.66, 119.38, 118.59, 108.61, 70.61, 65.43, 64.75, 54.86, 27.58, 14.98. HRMS (m/z): [M+H] calcd for $C_{18}H_{22}N_5O_2$, 340.1774; found 340.1778.

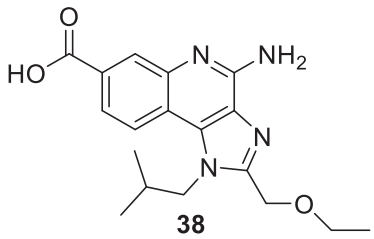


29

4-amino-2-butyl-1-isobutyl-1H-imidazo[4,5-c]quinolin-7-ol (29)

28 (1 eq) was added to a melt of pyridine hydrochloride (10 eq) and heated at 210 °C for 30 minutes. The reaction was allowed to cool for a few minutes and diluted with water and acidified with 6 N HCl to pH 1. The solid was collected by vacuum filtration and washed with hexanes and dried in air to obtain **29** in 75% yield as a light brown solid. ^{1}H NMR (500 MHz, DMSO-d6) δ 14.00 (s, 1H), 10.69 (s, 1H), 8.60 (s, 3H), 7.98 (d, *J* = 9.1 Hz, 1H), 7.19 (d, *J* = 2.4 Hz, 1H), 7.09 (dd, *J* = 9.0, 2.4 Hz, 1H), 5.10 (s, 4H), 4.35 (d, *J* = 7.6 Hz, 2H), 2.92 (t, *J* = 7.6 Hz, 2H), 2.09 (dq, *J* = 13.8, 6.9 Hz, 1H), 1.81 (p, *J* = 7.6 Hz, 2H), 1.44 (h, *J* = 7.4 Hz, 2H), 0.97 – 0.90 (m, 9H); ^{13}C NMR (126 MHz, DMSO-d6) δ 158.73, 155.98, 148.63, 135.85, 135.55, 123.38, 122.04, 115.11, 105.18,

102.76, 51.49, 29.25, 28.73, 26.38, 21.84, 19.11, 13.81. HRMS (m/z): [M+H] calcd for $C_{18}H_{25}N_4O$ 313.2028; found 313.2041



38

4-amino-2-(ethoxymethyl)-1-isobutyl-1H-imidazo[4,5-c]quinoline-7-carboxylic acid (38)

35 was suspended in EtOH with KOH. H_2O_2 was added and the reaction was stirred at reflux for 4 h diluted with water, and acidified with 6 M HCl. The water layer was extracted 5x with DCM and evaporated to dryness. Crude product was purified by Combiflash (5%-10% MeOH in DCM) to obtain a white solid in 52% yield. ^{1}H NMR (500 MHz, DMSO-d6) δ 14.43 (s, 1H), 8.37 (s, 1H), 8.26 (s, 2H), 8.05 – 8.00 (d, J = 8.2 Hz, 1H), 7.68 (s, 1H), 4.82 (s, 2H), 4.56 (d, J = 7.6 Hz, 2H), 3.61 (q, J = 6.9 Hz, 2H), 2.21 (dt, J = 14.0, 7.0 Hz, 1H), 1.17 (t, J = 7.0 Hz, 3H), 0.94 (d, J = 6.6 Hz, 6H); ^{13}C NMR (126 MHz, DMSO-d6) δ 167.02, 153.34, 150.29, 135.11, 134.35, 125.92, 123.65, 122.60, 118.78, 114.89, 106.65, 66.21, 64.33, 52.63, 29.26, 19.67, 15.37. HRMS (m/z): [M-] calcd for $C_{18}H_{21}N_4O_3$, 341.1614; found 341.1628.

Biology:

NFκB-Inducible SEAP Reporter Assay for TLR-7 Activity:

TLR-7/8 analog activities were assessed using HEK-Blue hTLR7 and Null1-k cell lines from InvivoGen. HEK-Blue hTLR7 cells are HEK293 cells co-transfected with the hTLR7 gene and an optimized secreted embryonic alkaline phosphatase (SEAP) reporter gene. HEK-Blue Null1-k cells are transfected with the SEAP reporter gene but do not express the hTLR7 gene and are used as a control.

The experiments followed manufacturer protocol as follows:

SEAP levels were measured via absorbance at 637 nm using HEK-Blue Detection cell culture medium (InvivoGen), which contains an SEAP specific colorimetric substrate. Compound stock solutions were first diluted in sterile DMSO at a concentration of 100 μM. Lower concentrations were prepared by serial dilution of the initial 100 μM stock samples into sterile DMSO and these solutions (22 μL) were subsequently diluted into sterile H_2O (198 μL) using a BioTek Precision XS instrument. HEK-Blue hTLR7 and Null1-k cell suspensions in sterile HEK-Blue Detection medium were prepared at a density of ~280,000 cells per mL. Corning 3603 clear bottom, 96-well tissue culture treated plates were seeded with hTLR7 and Null1-k cell suspensions (180 μL/well), after which 20μL of the various sample concentrations in 10% v/v DMSO/H_2O were added (20 μL) to yield the desired testing concentration in 1% v/v DMSO. The plate was incubated at 37 °C and 5% CO_2 in the dark. Absorbance measurements were taken at 637 nm at 12 and 17-hour time points, 4 replicates per concentration point, per cell line (hTLR7, Null1-k).

The positive control used was Imiquimod and Resiquimod at concentrations of 0.02 μM, 0.2 μM, 2 μM, 20 μM, and 50 μM. The negative control (or vehicle control) used was water. Compounds 6-10 were tested at concentrations of 0.02 μM, 0.2 μM, 2 μM, 20 μM, and 100 μM with 6 replicates each.

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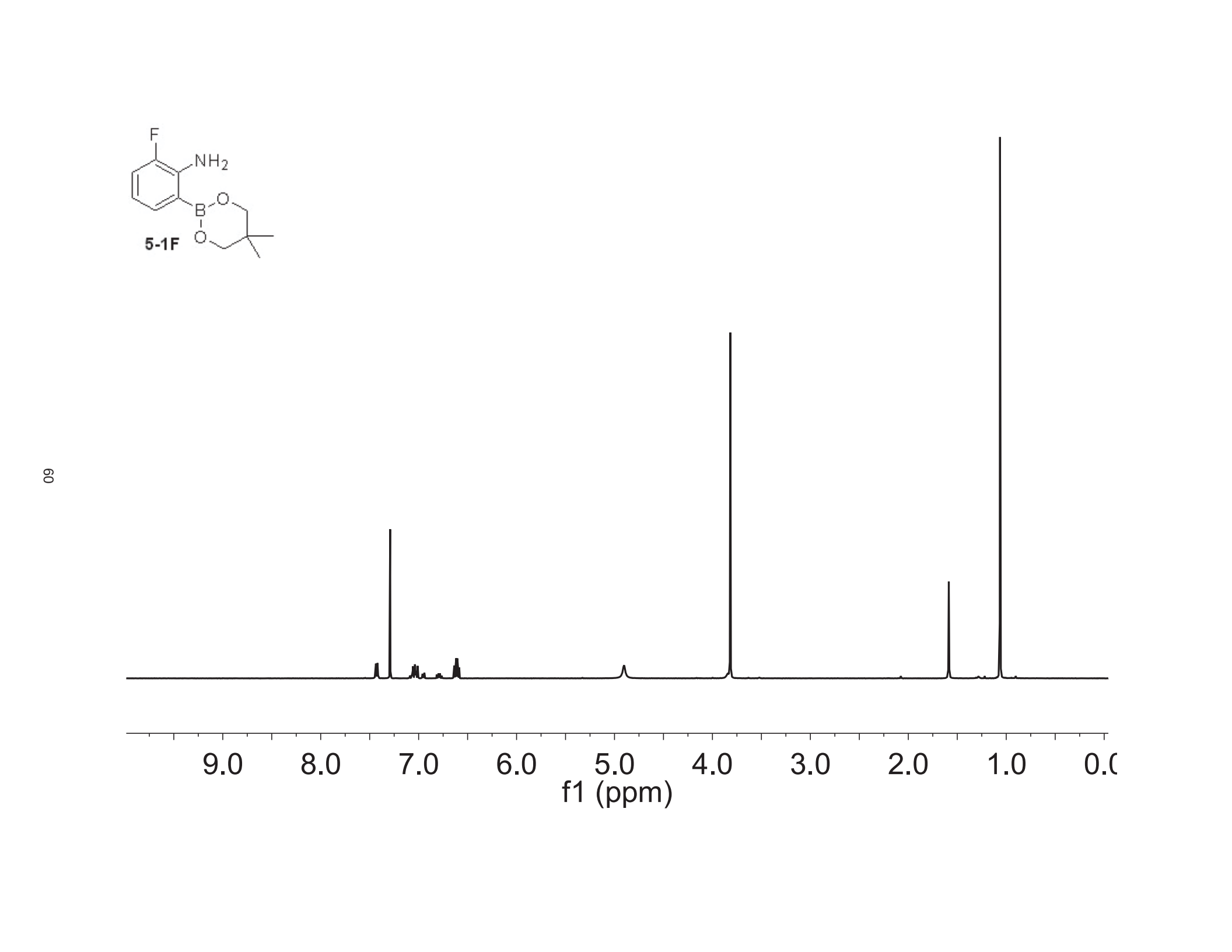
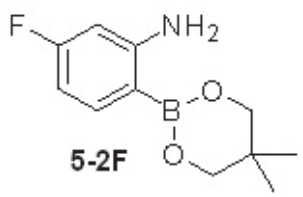
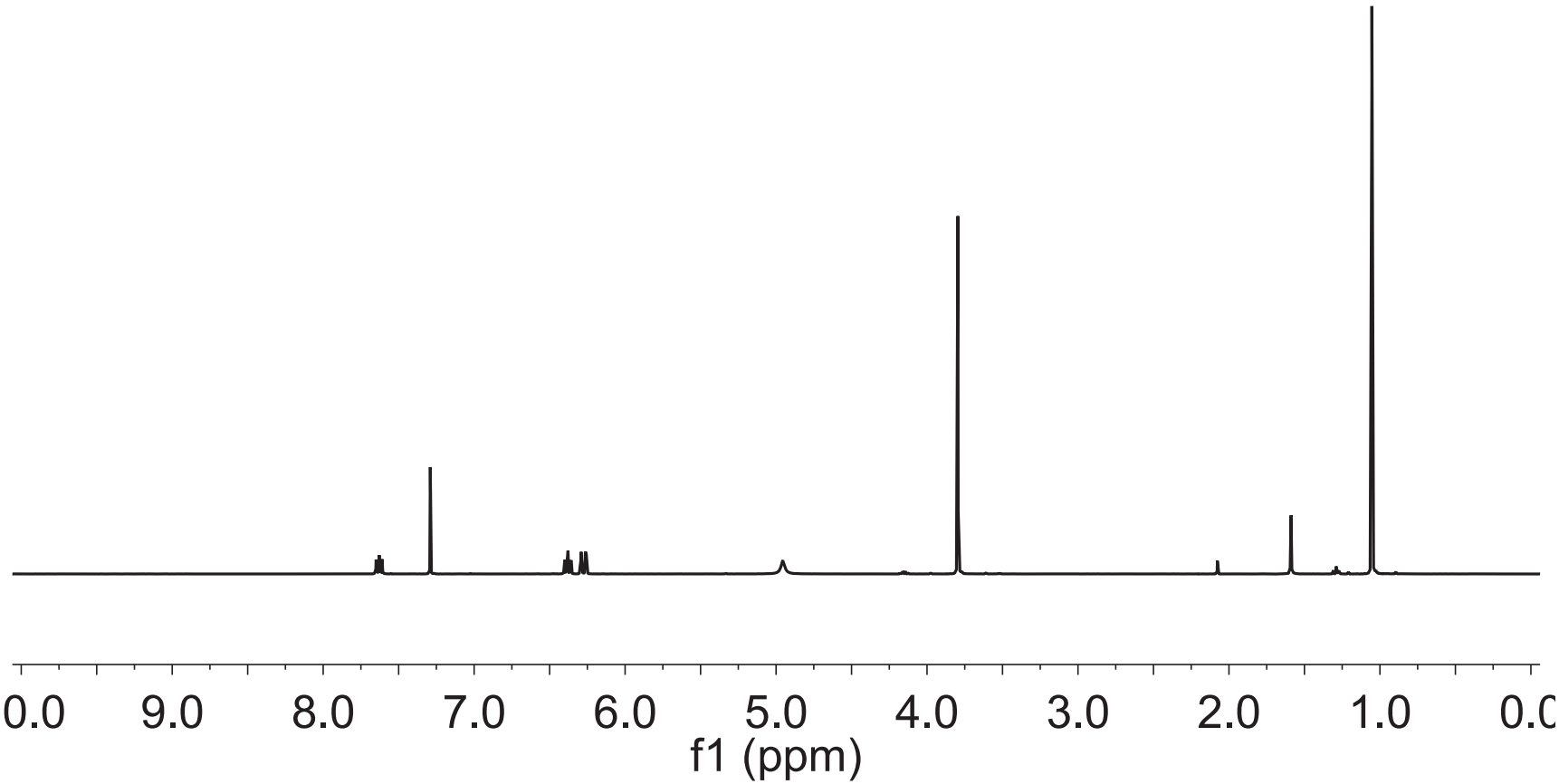
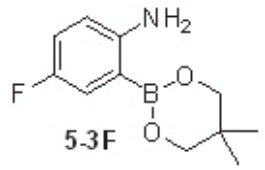
Appendix A: ^{1}H and ^{13}C Spectra


F
NH2
B
O
O
5-1F
60
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f1 (ppm)

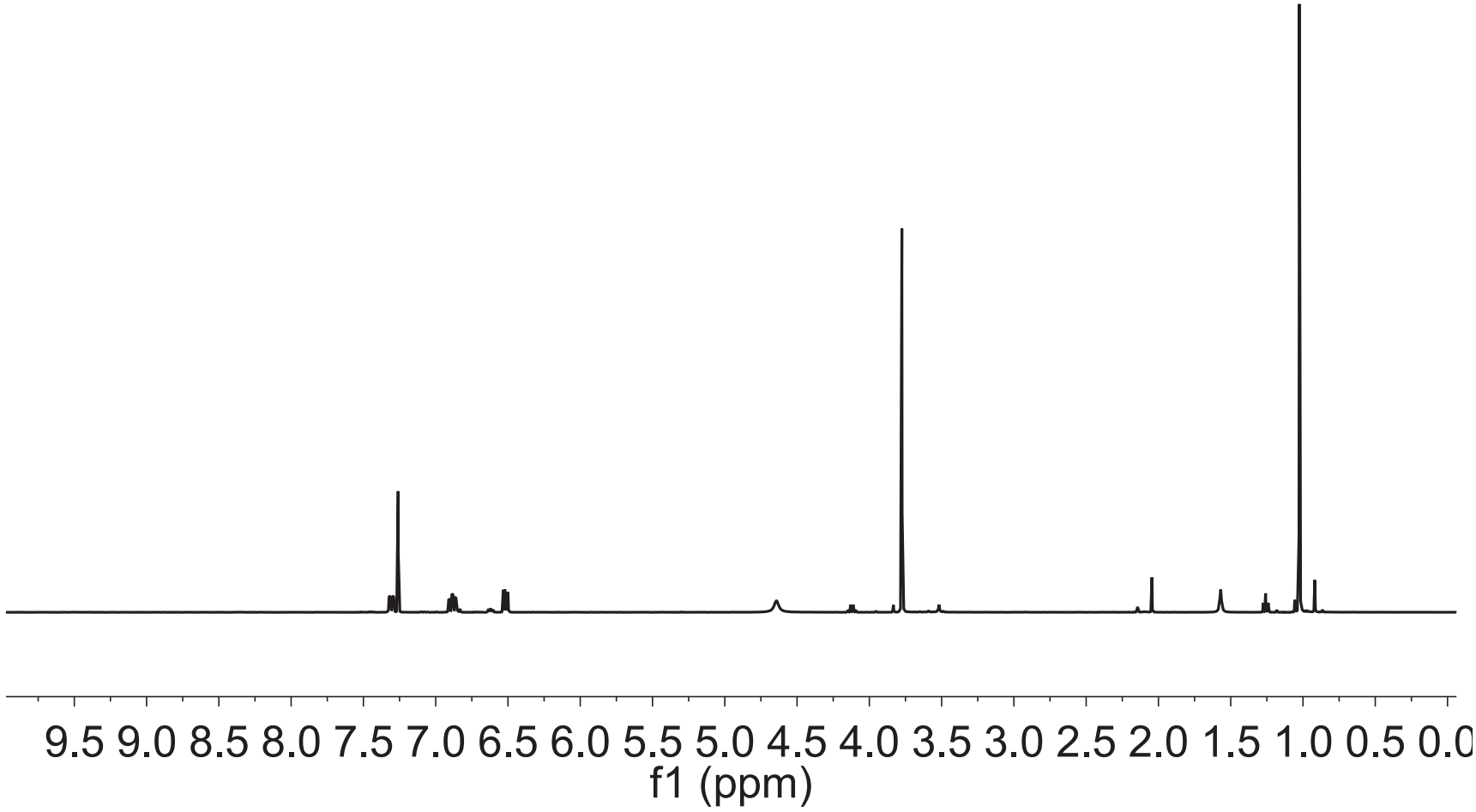


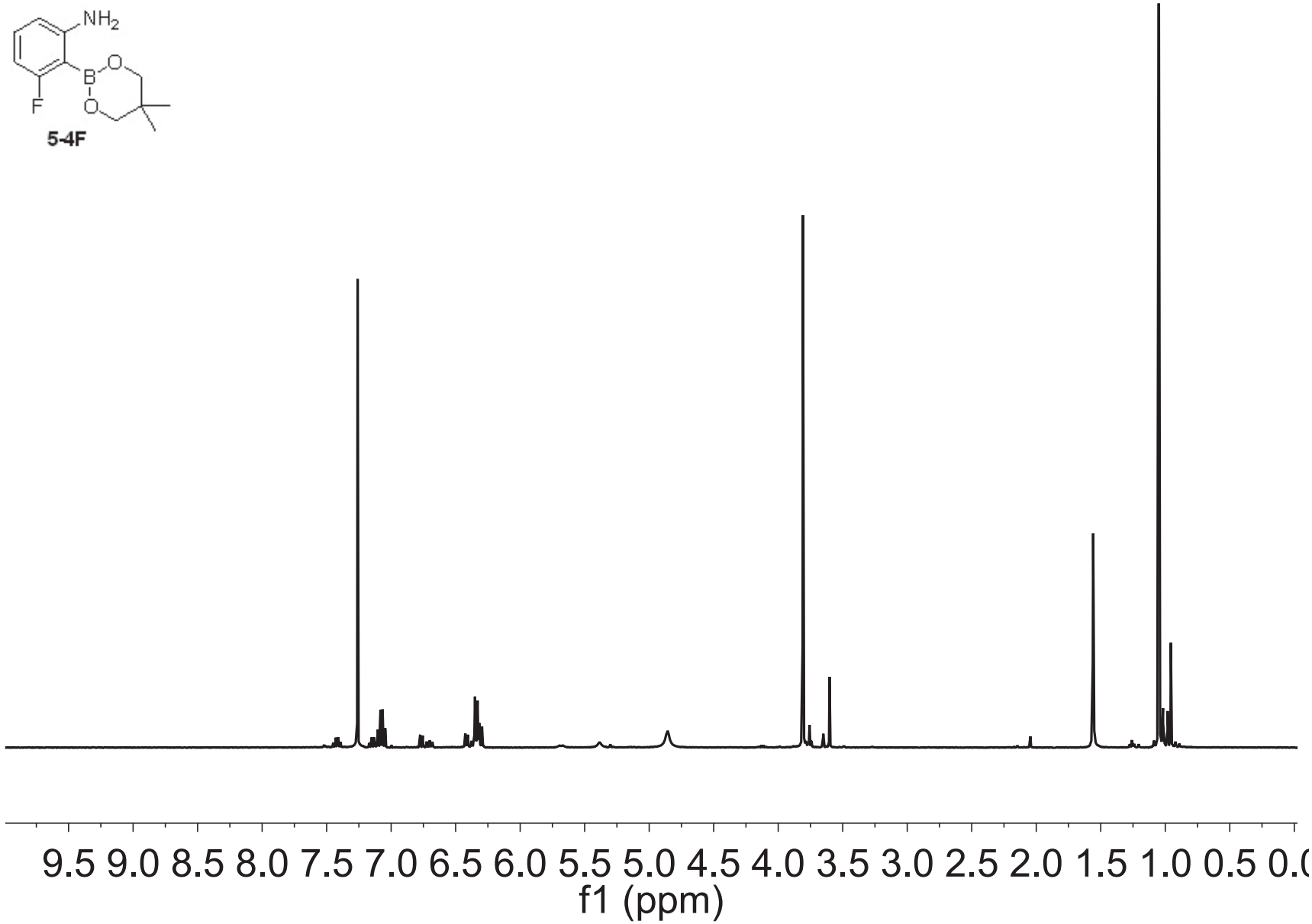
5-2F

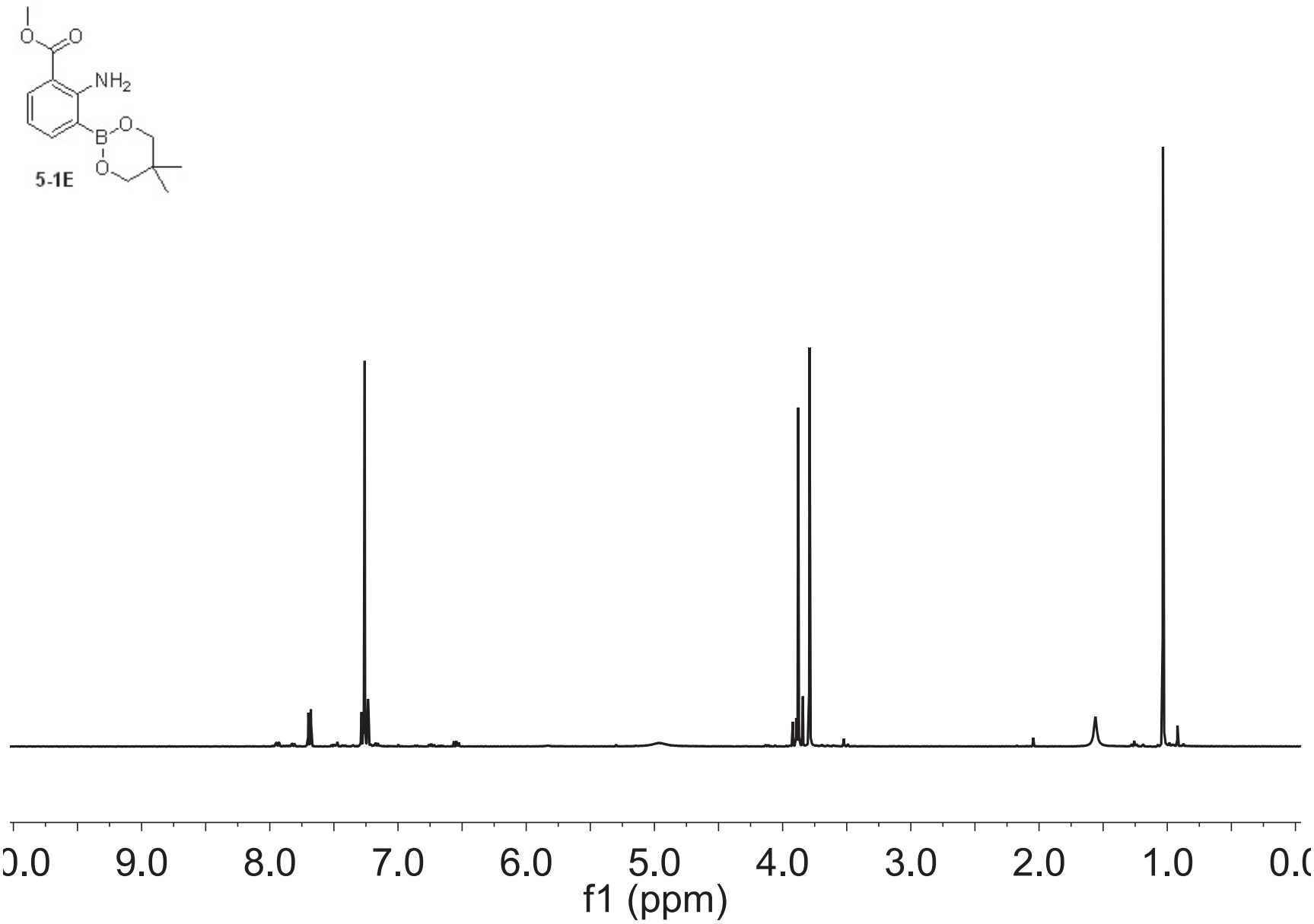


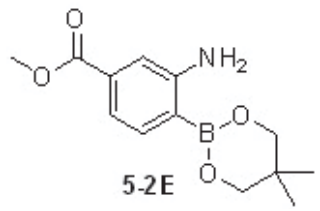

NH2
F
B
O
O

5-3F


9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

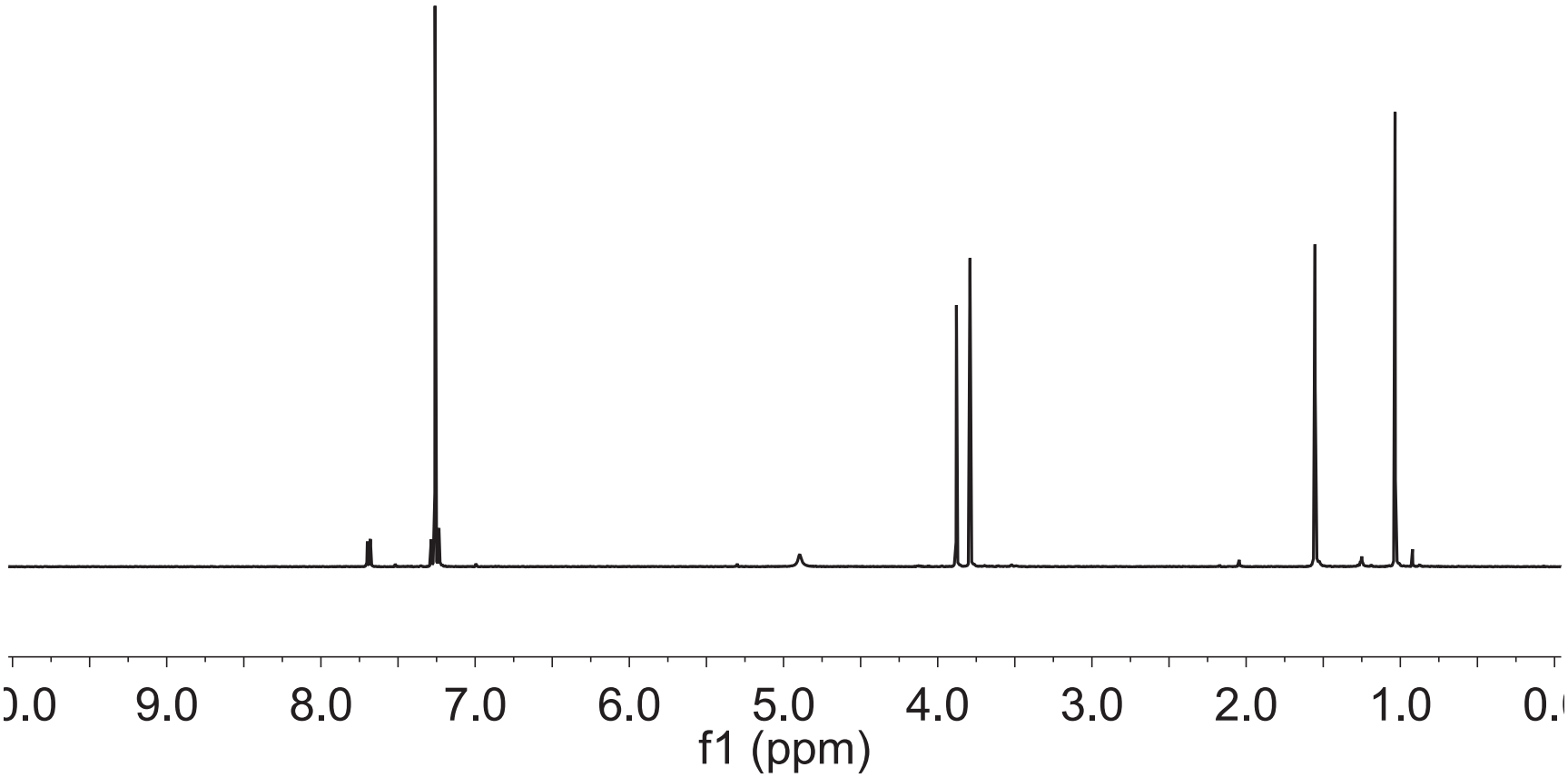

NH2
B
O
O
F
5-4F
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

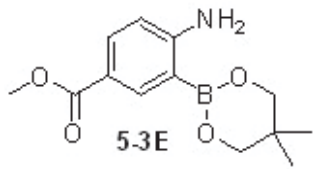

O
O
NH2
B
O
O
5-1E
0.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f1 (ppm)



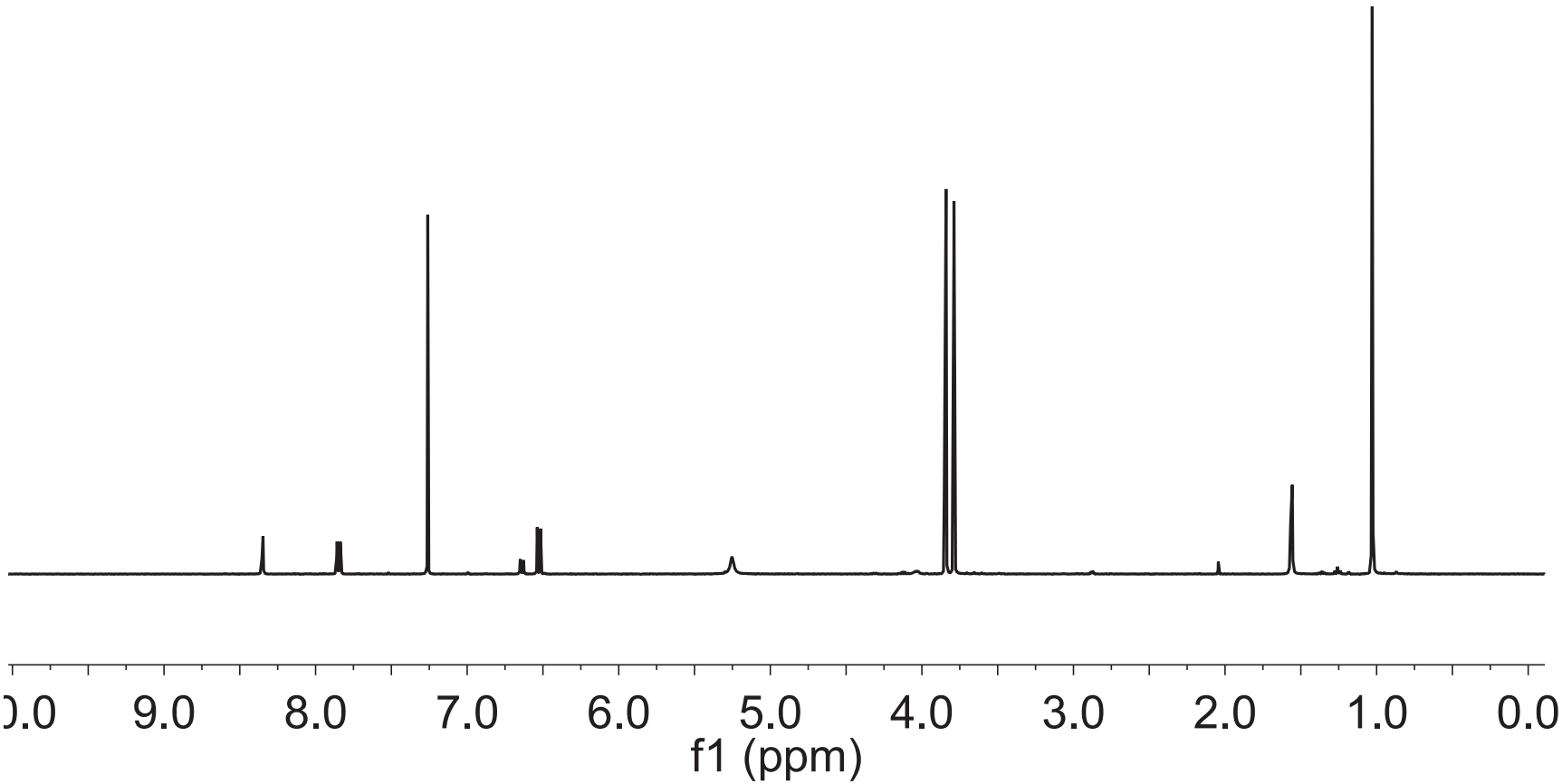
5-2E

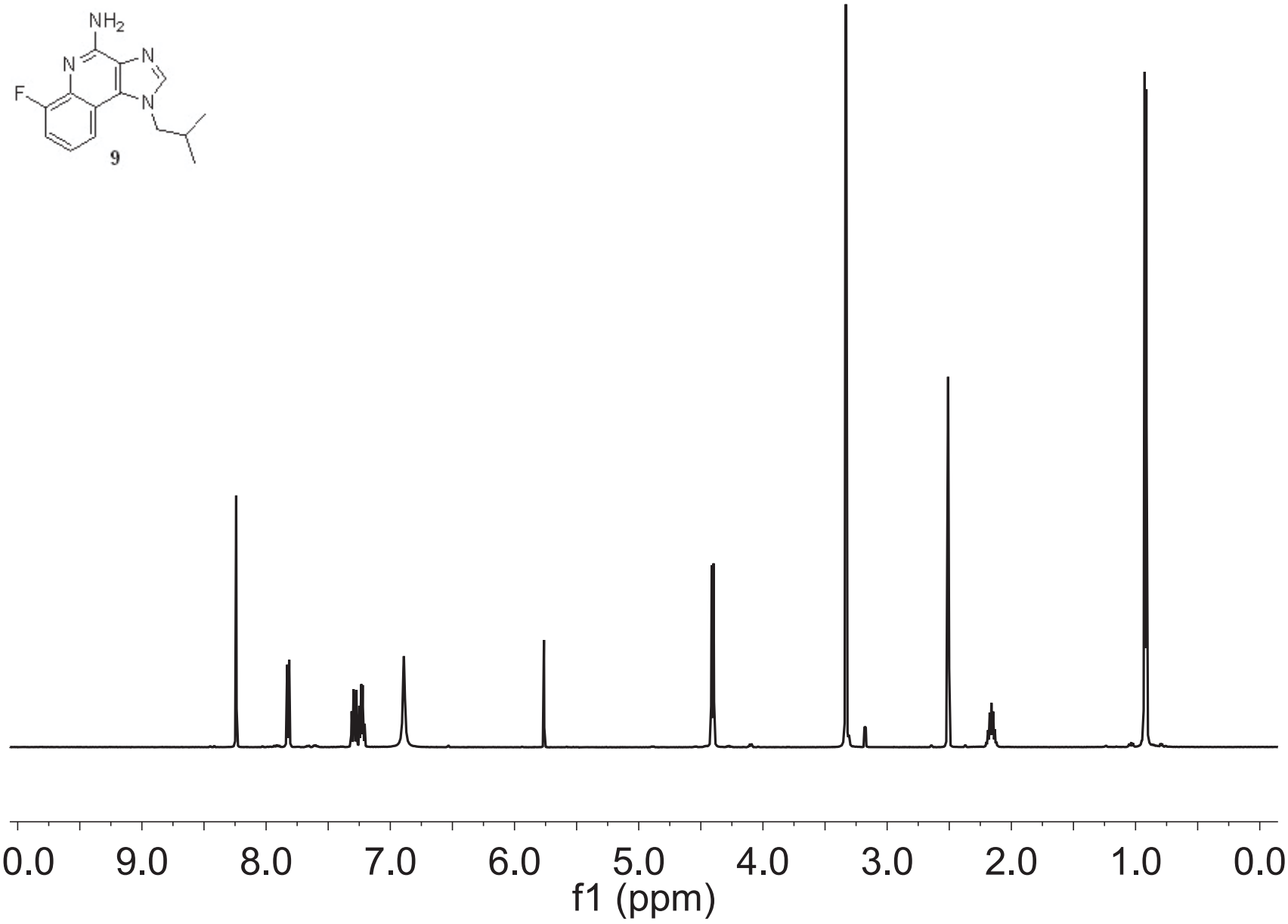
65

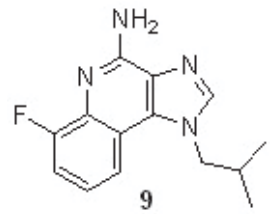




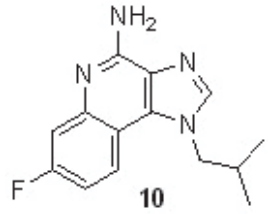
5-3E




NH2
N
N
F
N
9
0.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f1 (ppm)

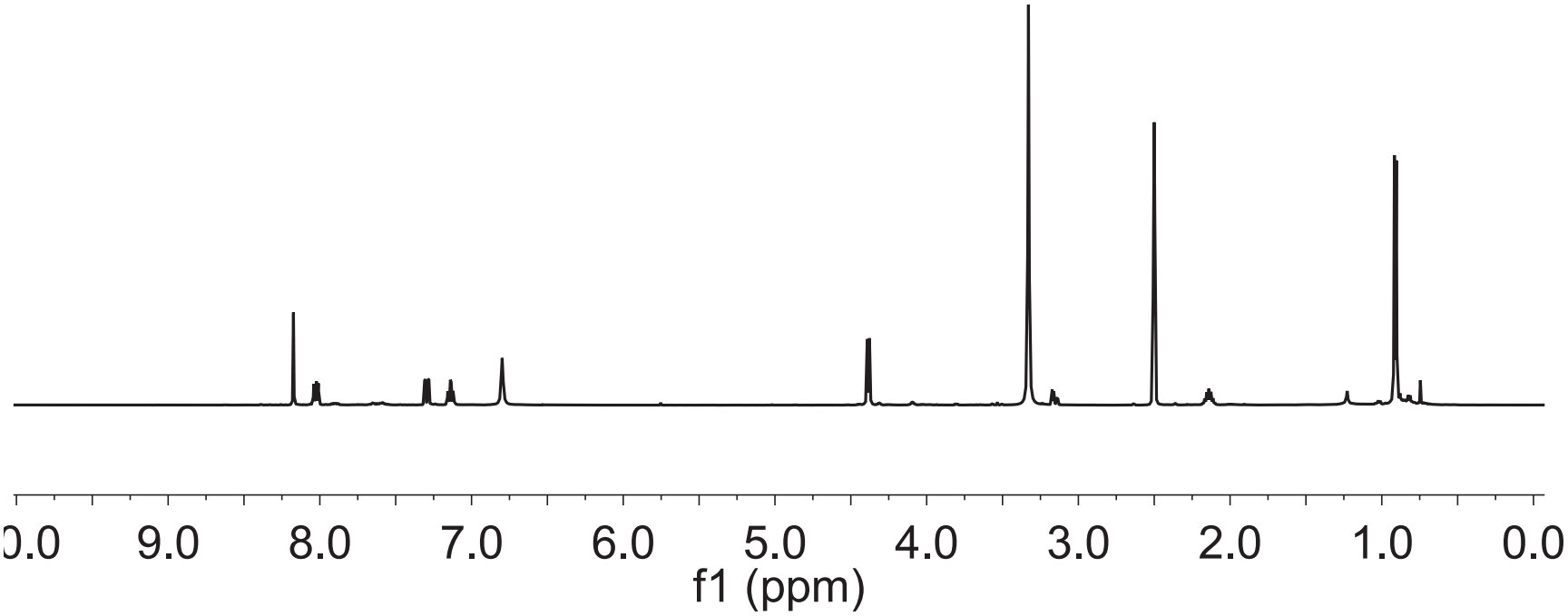


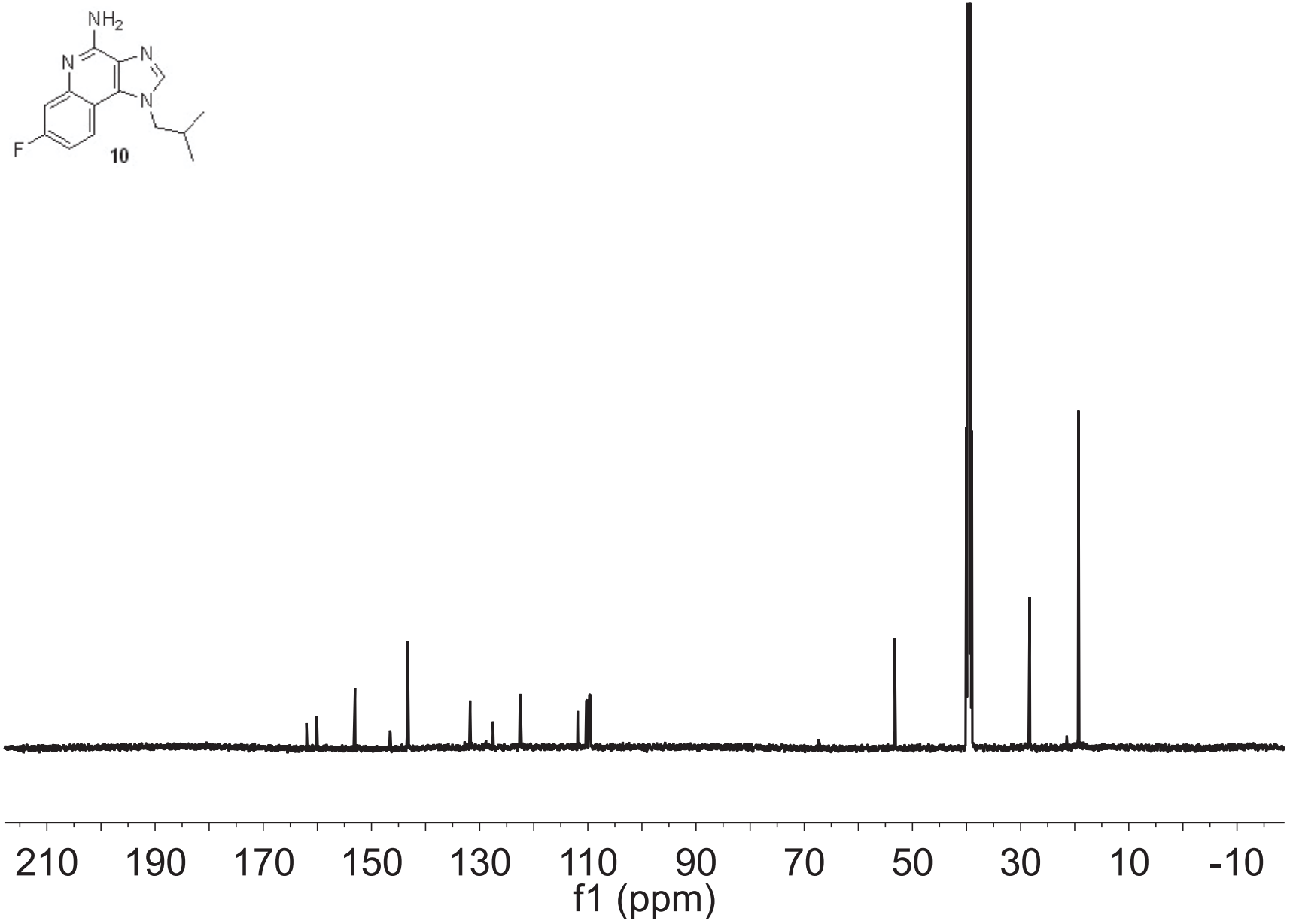
9

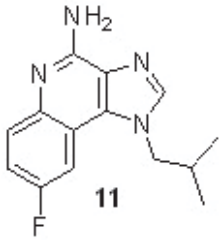


10

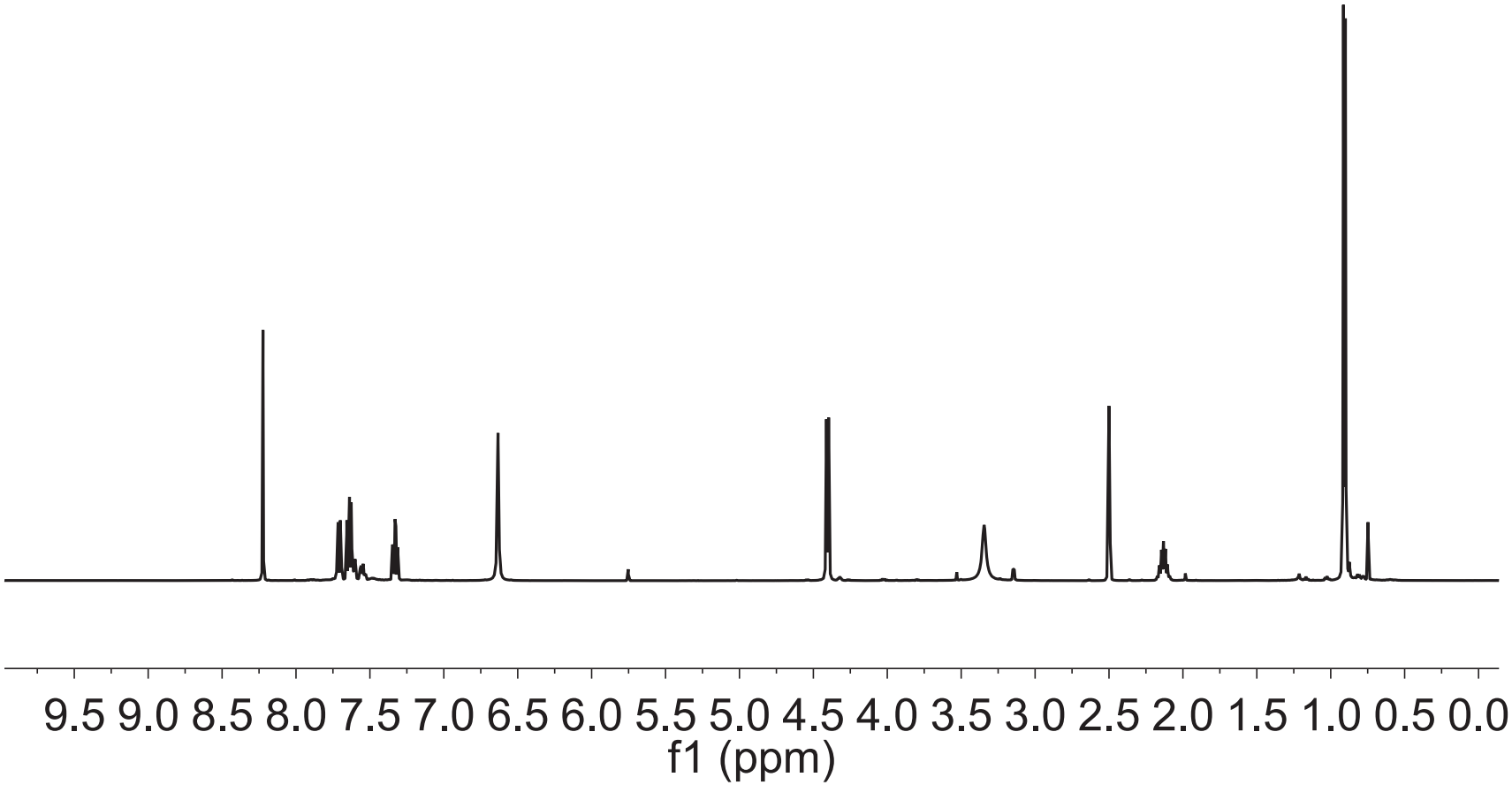
69




NH2
N
N
N
F
10
70
210
190
170
150
130
110
90
70
50
30
10
-10
f1 (ppm)


NH2
N
N
N
F

11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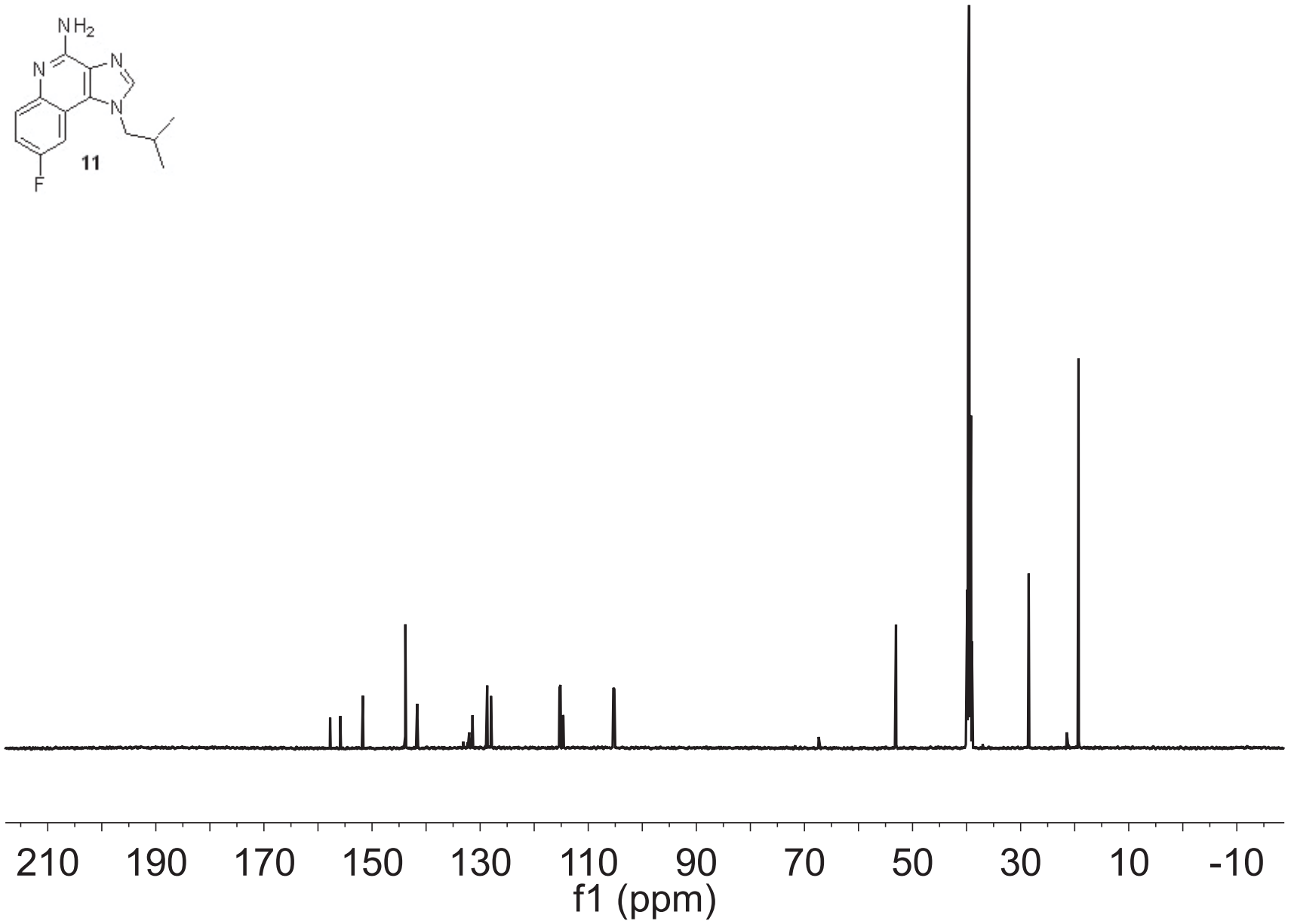
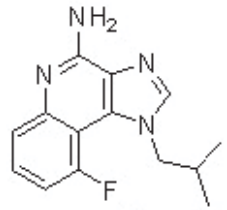
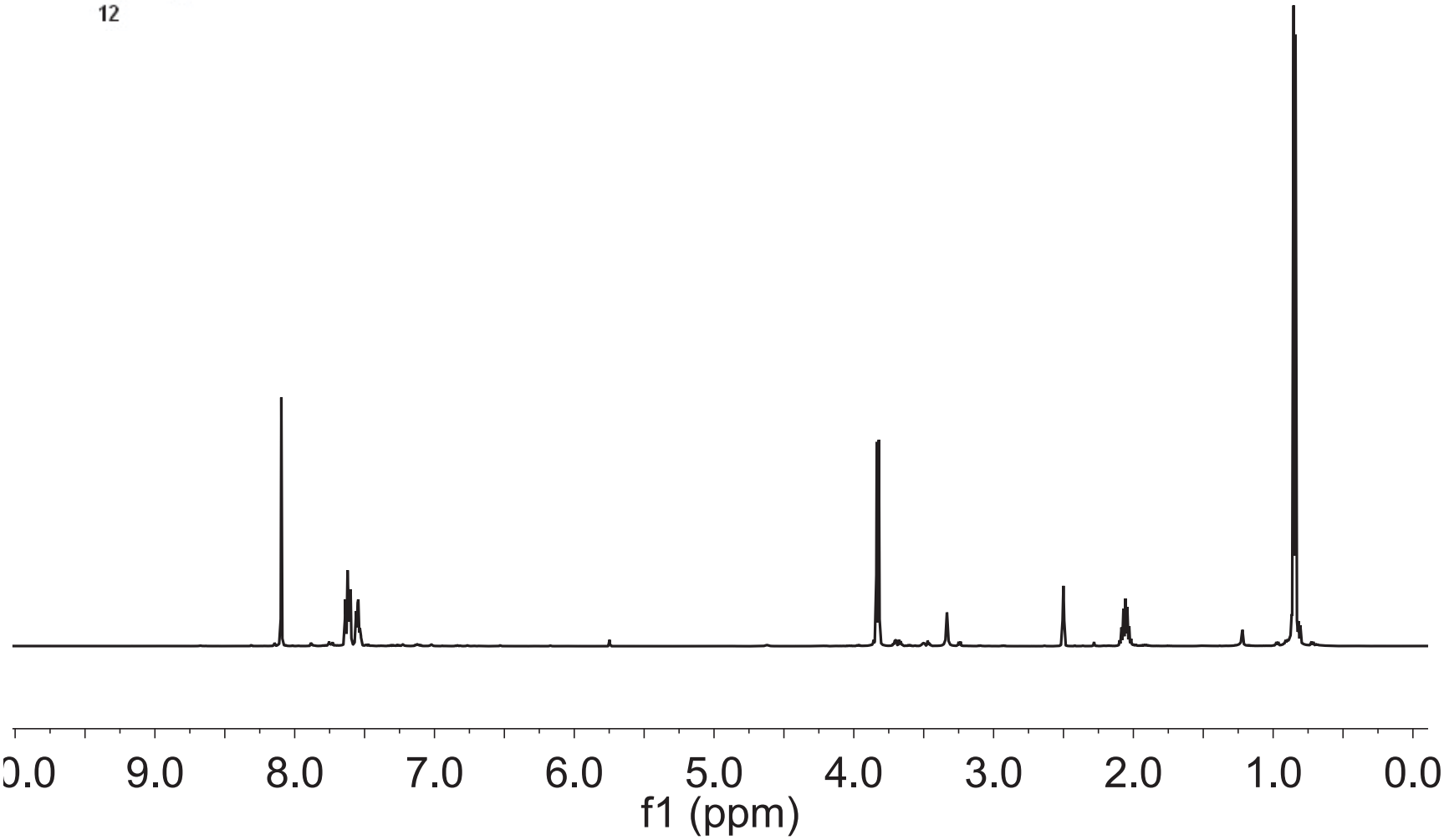
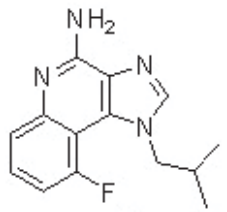
f1 (ppm)


NH2
N
N
N
F
11
210
190
170
150
130
110
90
70
50
30
10
-10
f1 (ppm)



12





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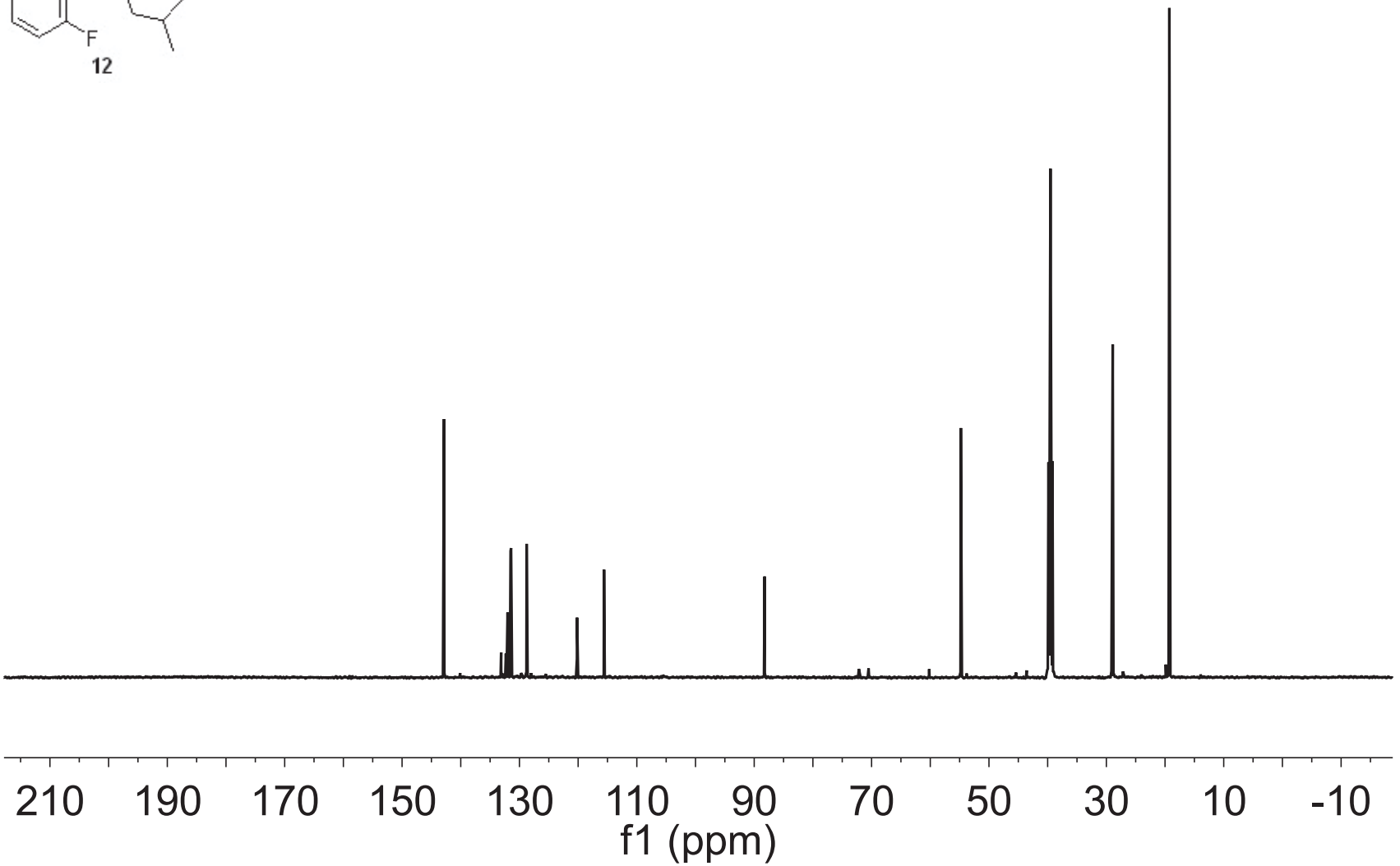
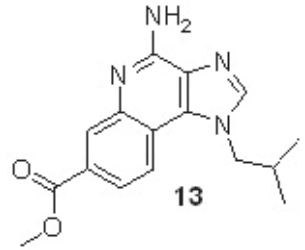
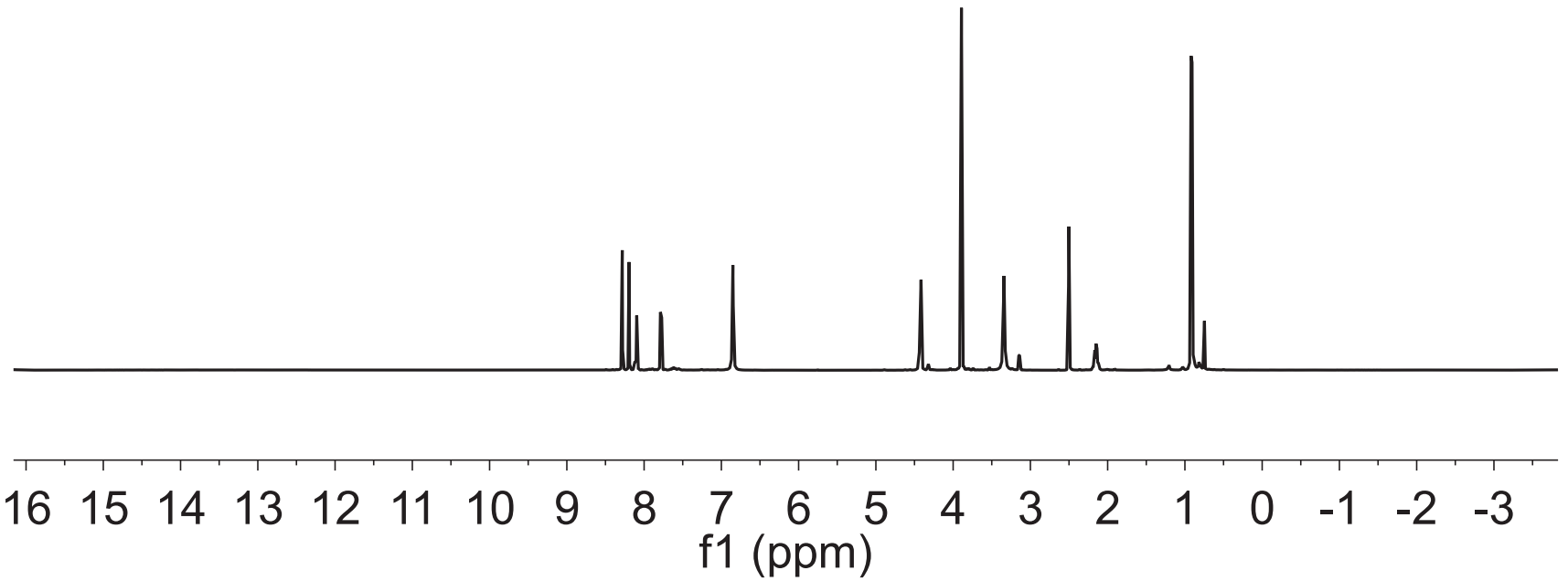



NH2
N
N
N
O
O
13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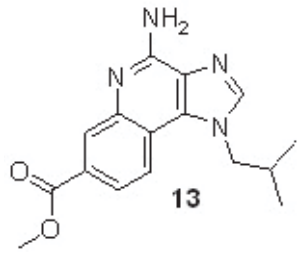
f1 (ppm)



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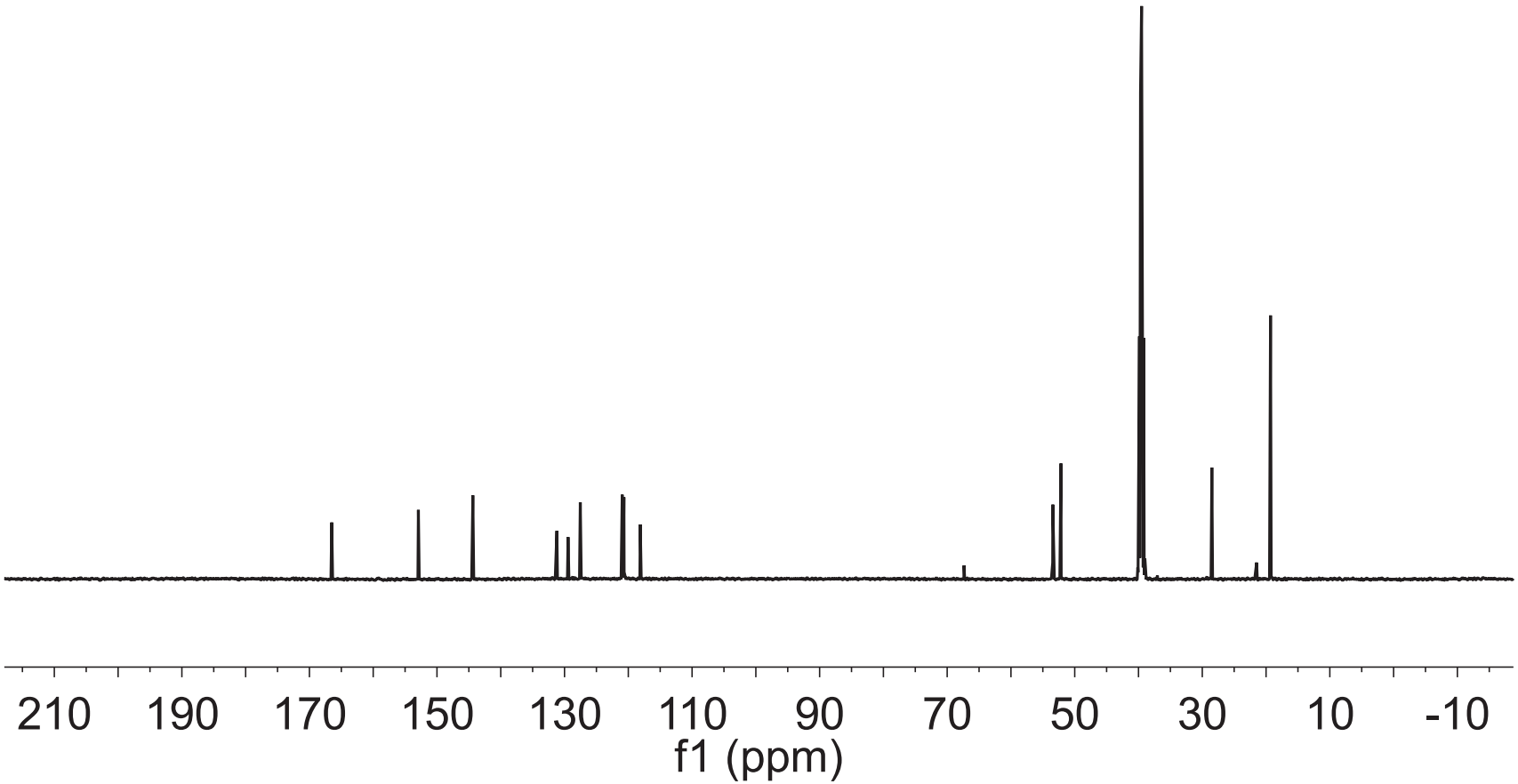
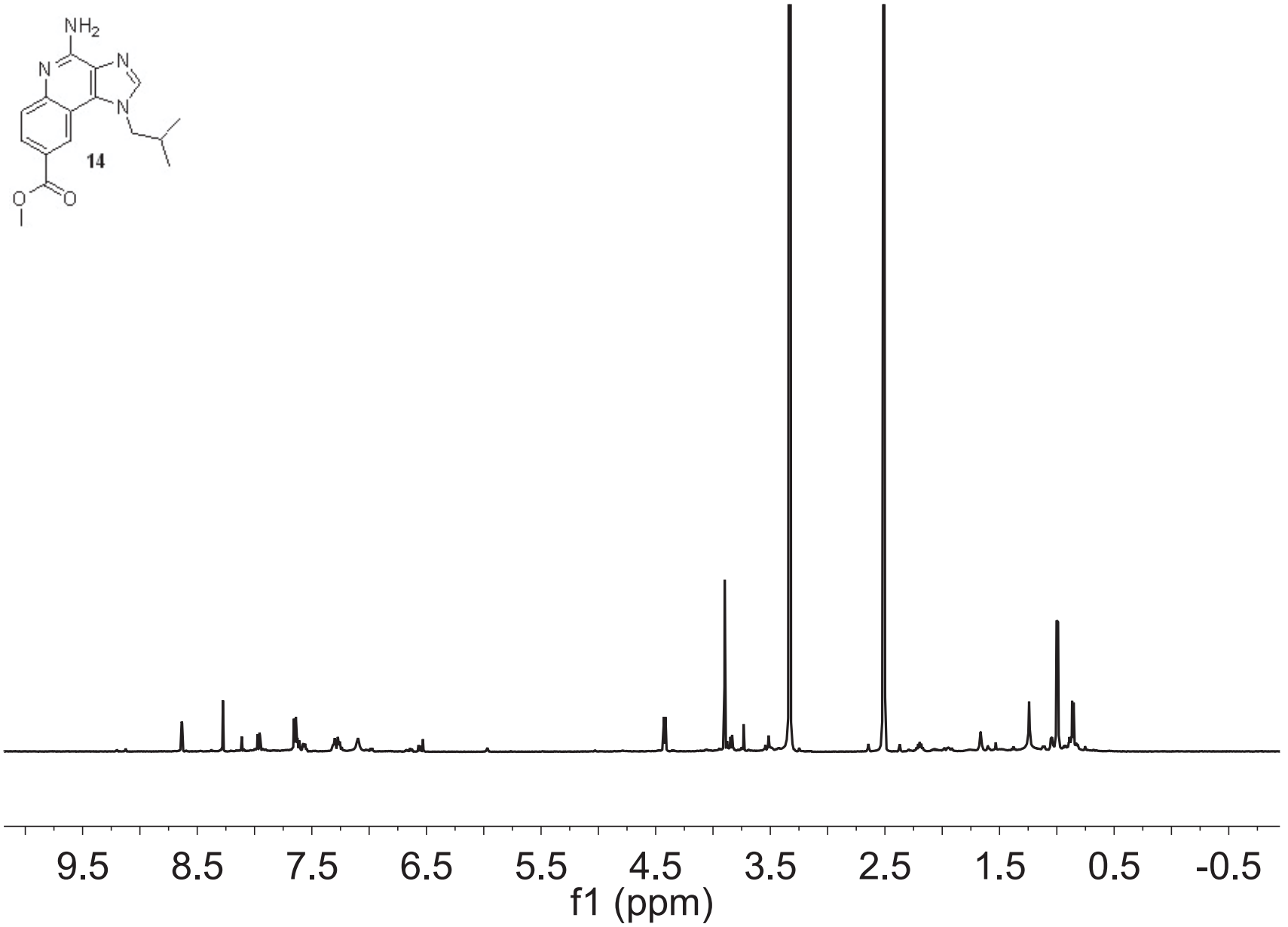
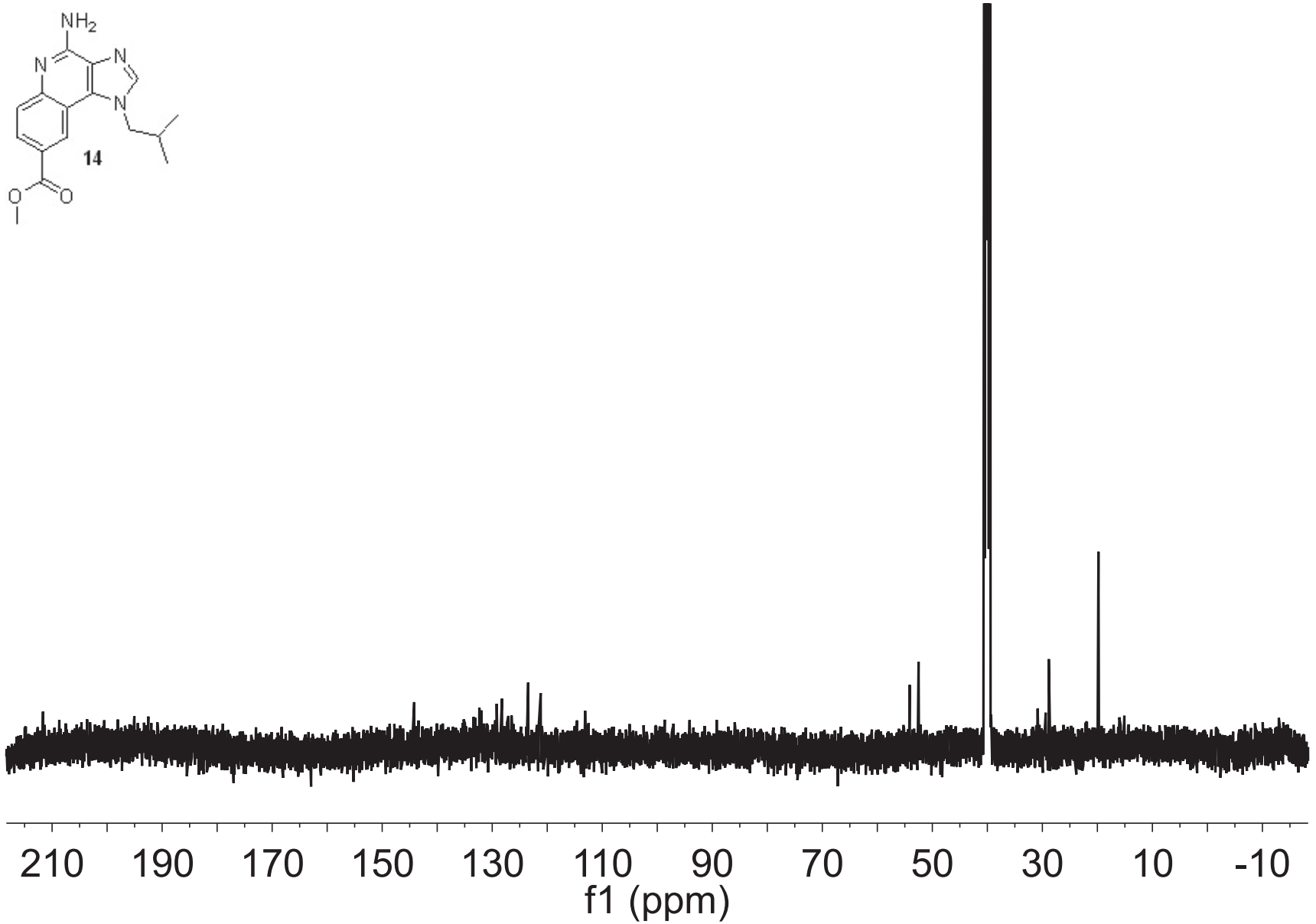



NH2
N
N
N
O
O
14
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
-0.5
f1 (ppm)


NH2
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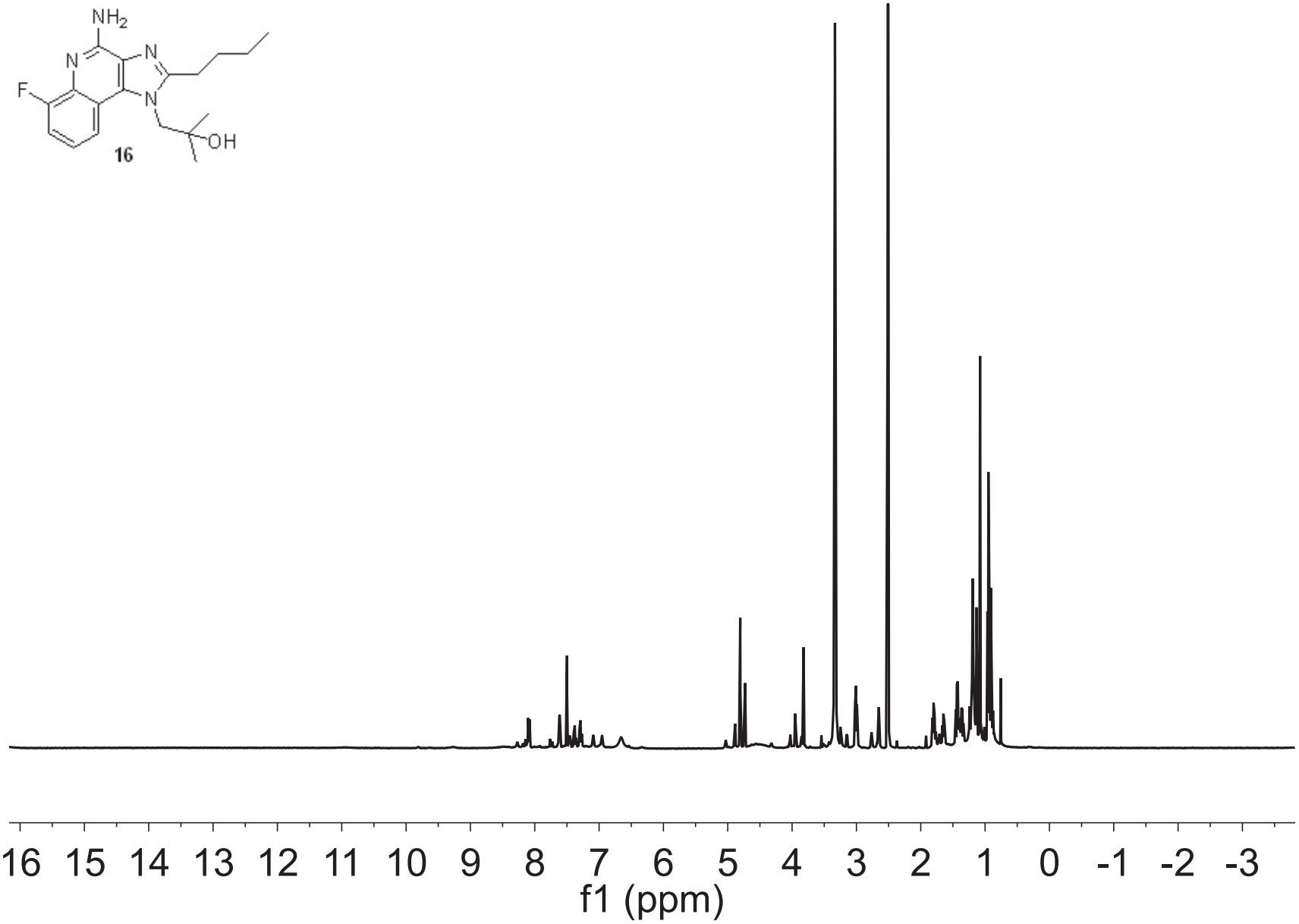
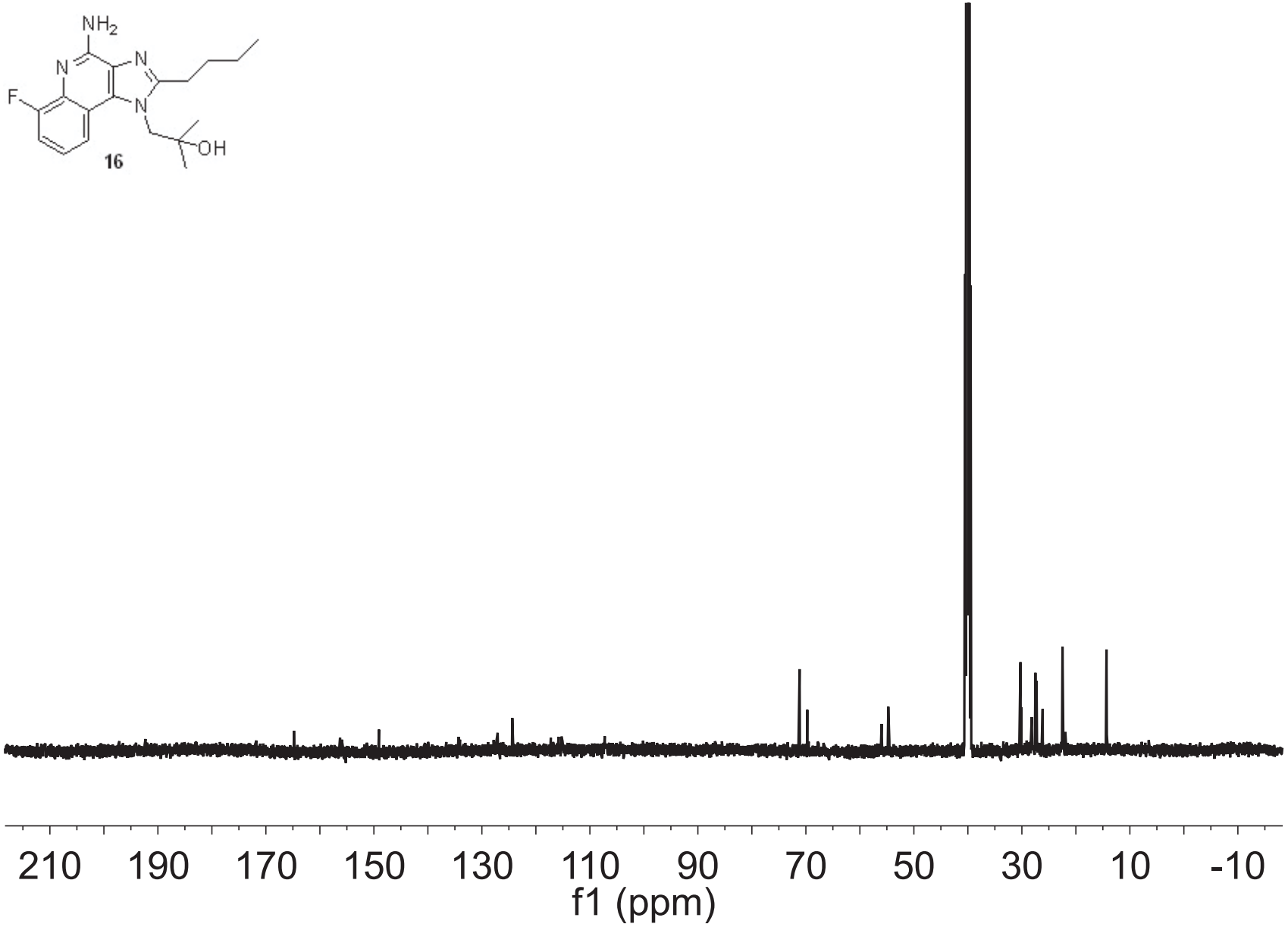
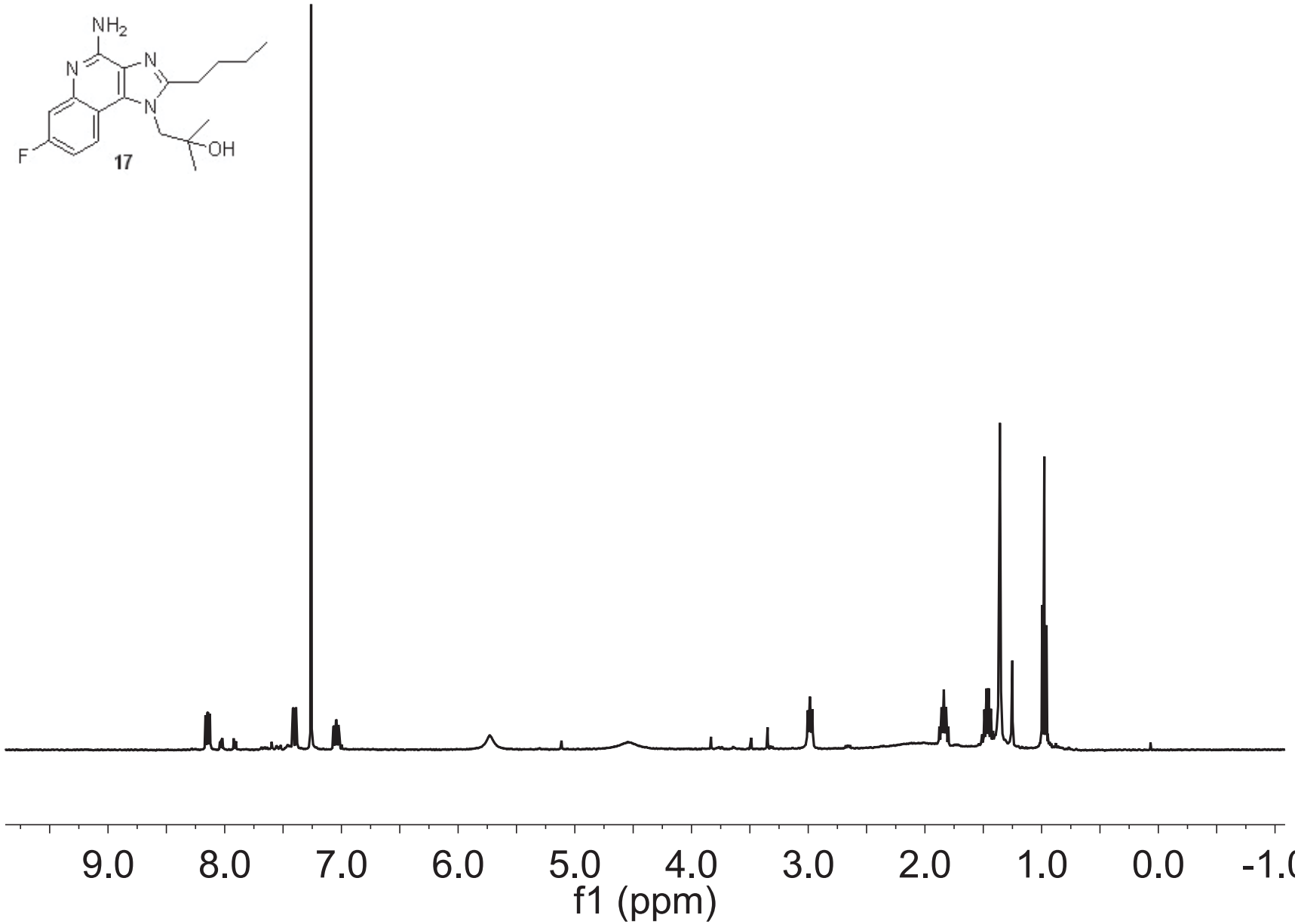
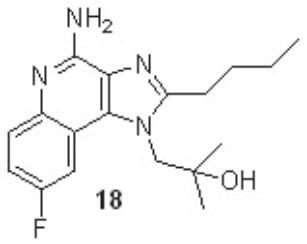
N
N
14
O
O
210
190
170
150
130
110
90
70
50
30
10
-10
f1 (ppm)

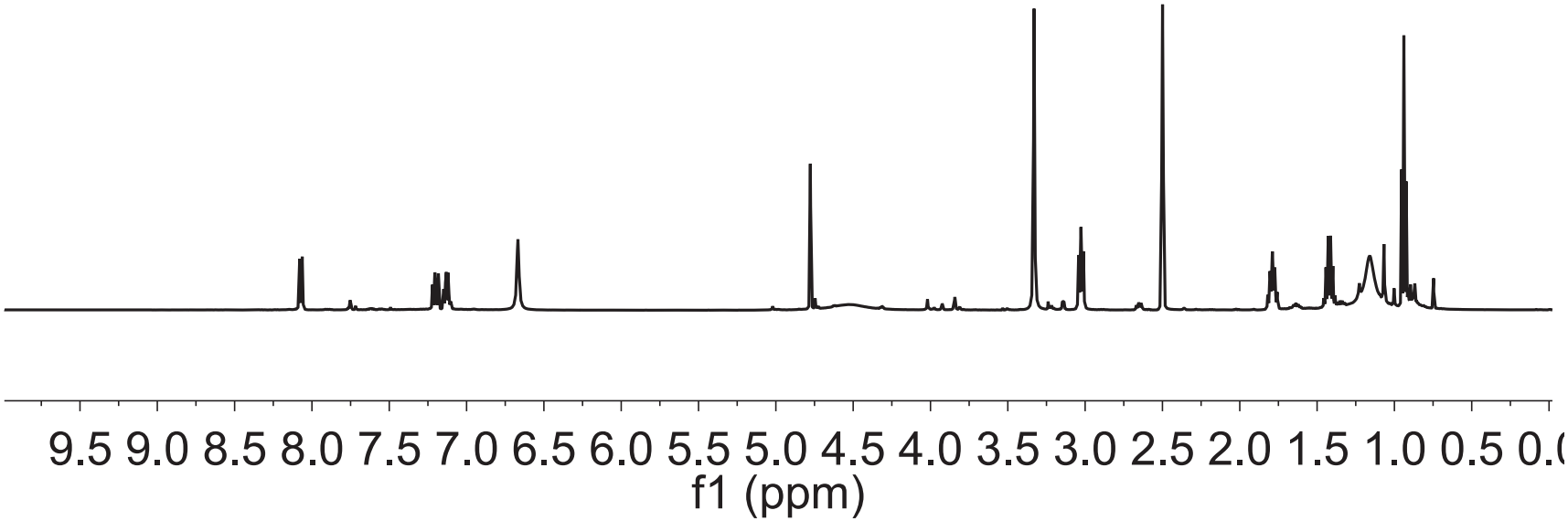

NH2
N
N
F
N
OH
16
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3
f1 (ppm)

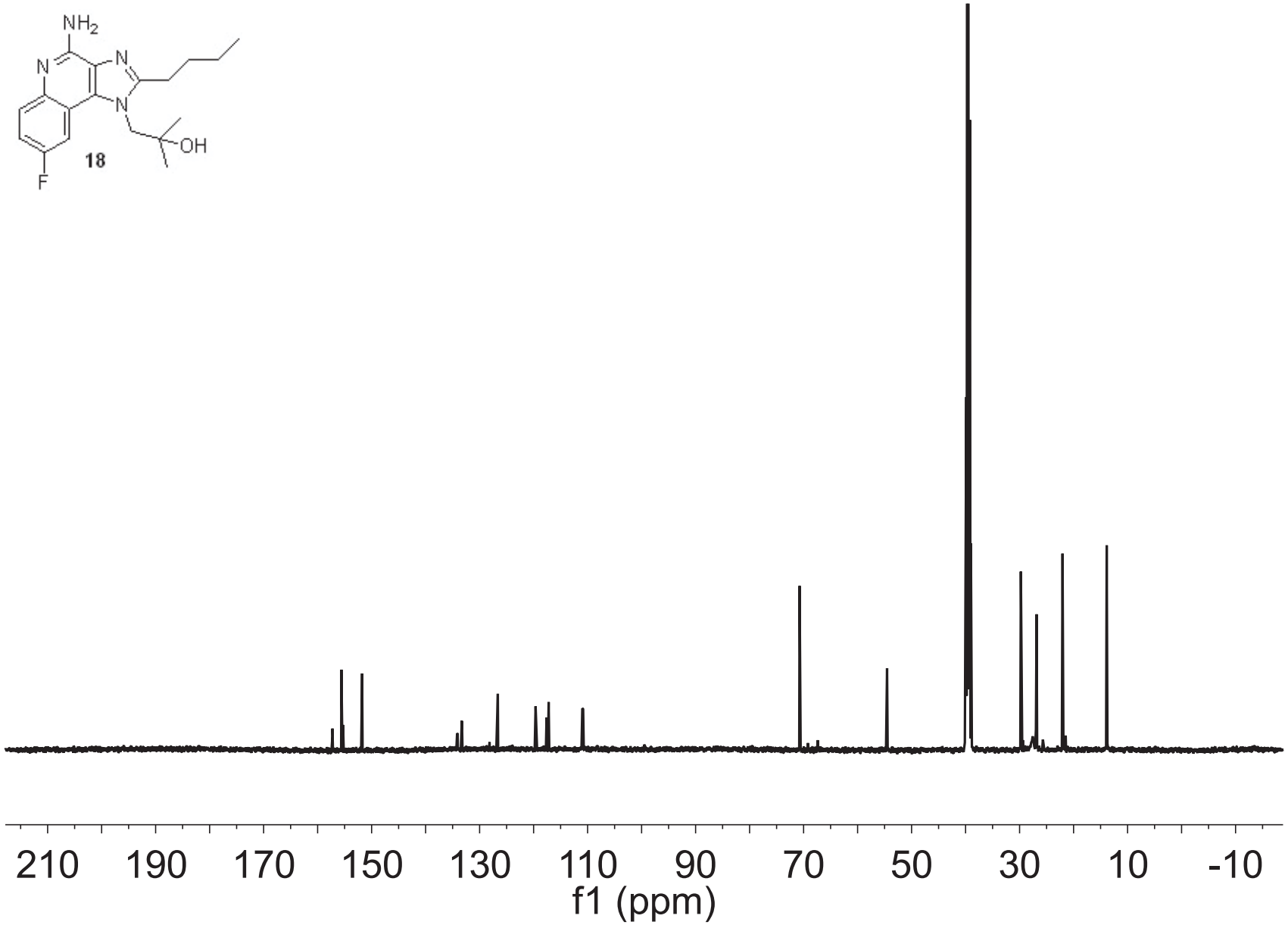

NH2
N
N
F
N
OH
16
210
190
170
150
130
110
90
70
50
30
10
-10
f1 (ppm)

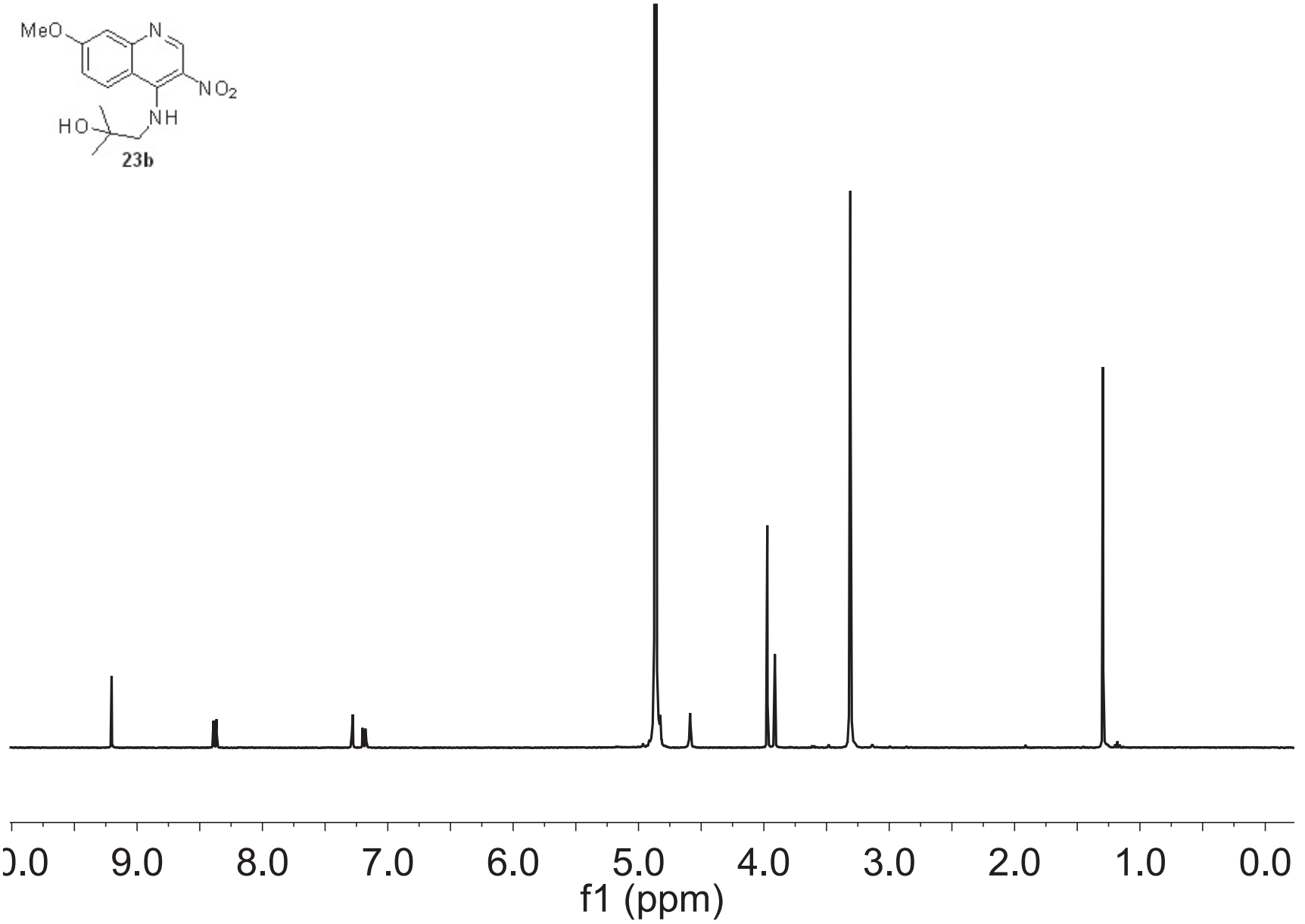

NH2
N
N
N
F
OH
17
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f1 (ppm)

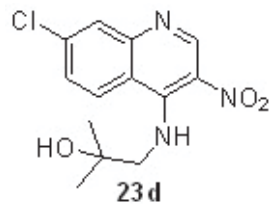

NH2
N
N
N
OH
F

18

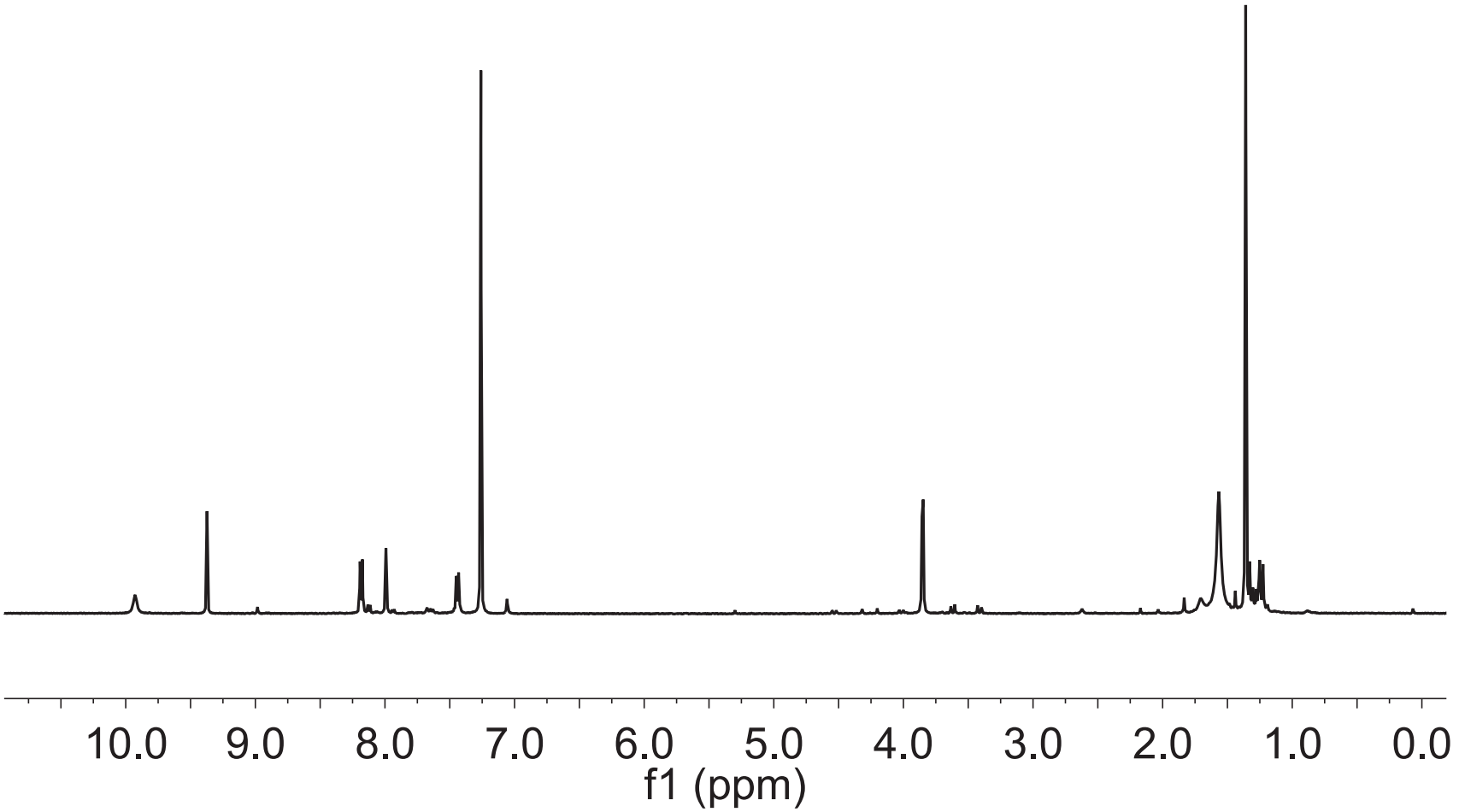

9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.(
f1 (ppm)

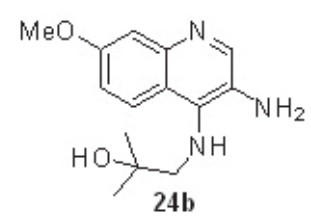

NH2
N
N
N
OH
F
18
210
190
170
150
130
110
90
70
50
30
10
-10
f1 (ppm)


MeO
N
NO2
NH
HO
23b
0.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f1 (ppm)



23 d

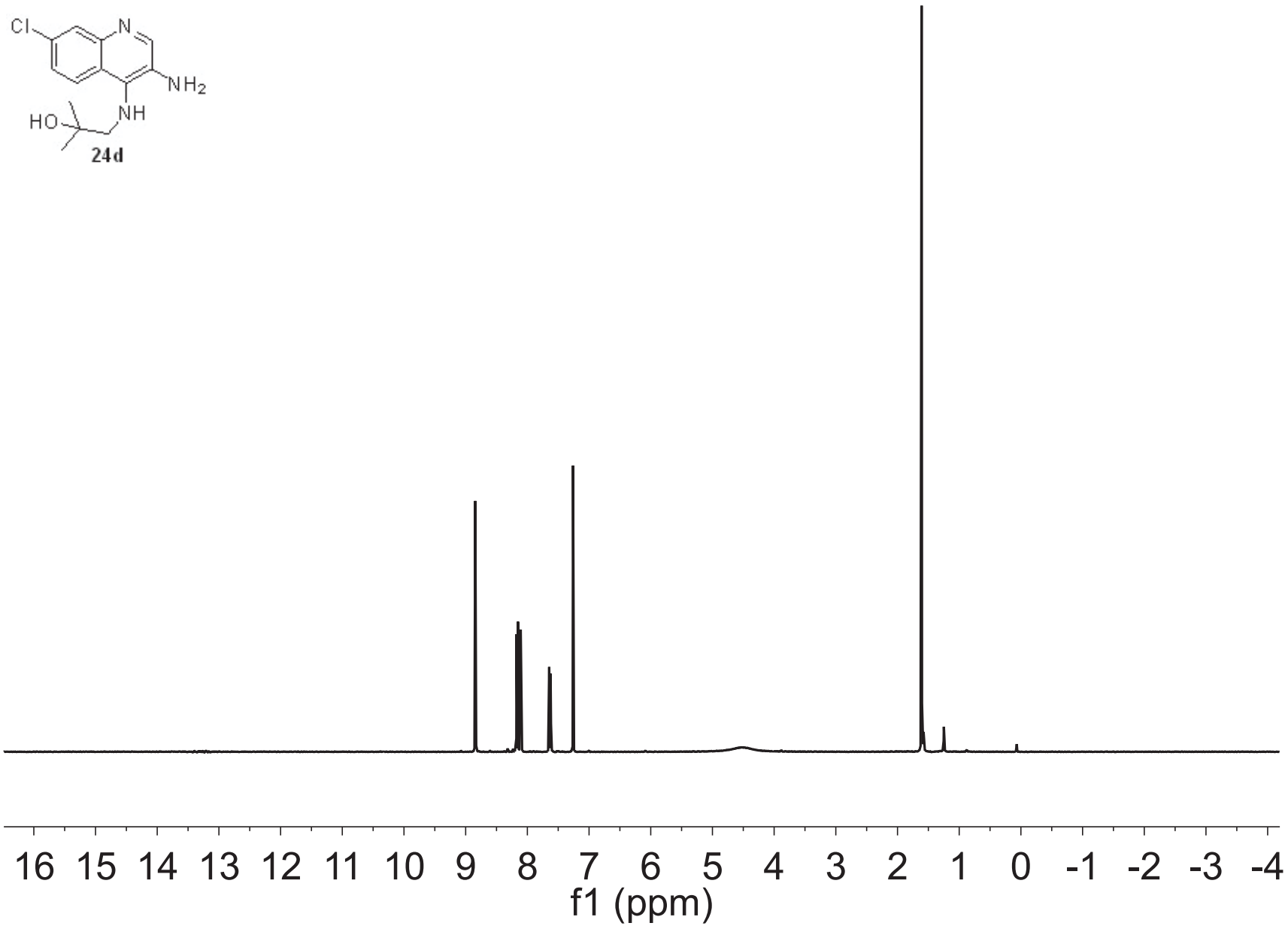


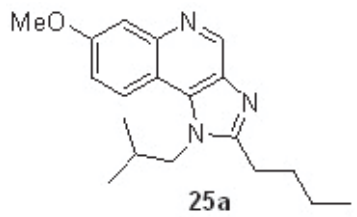


24b

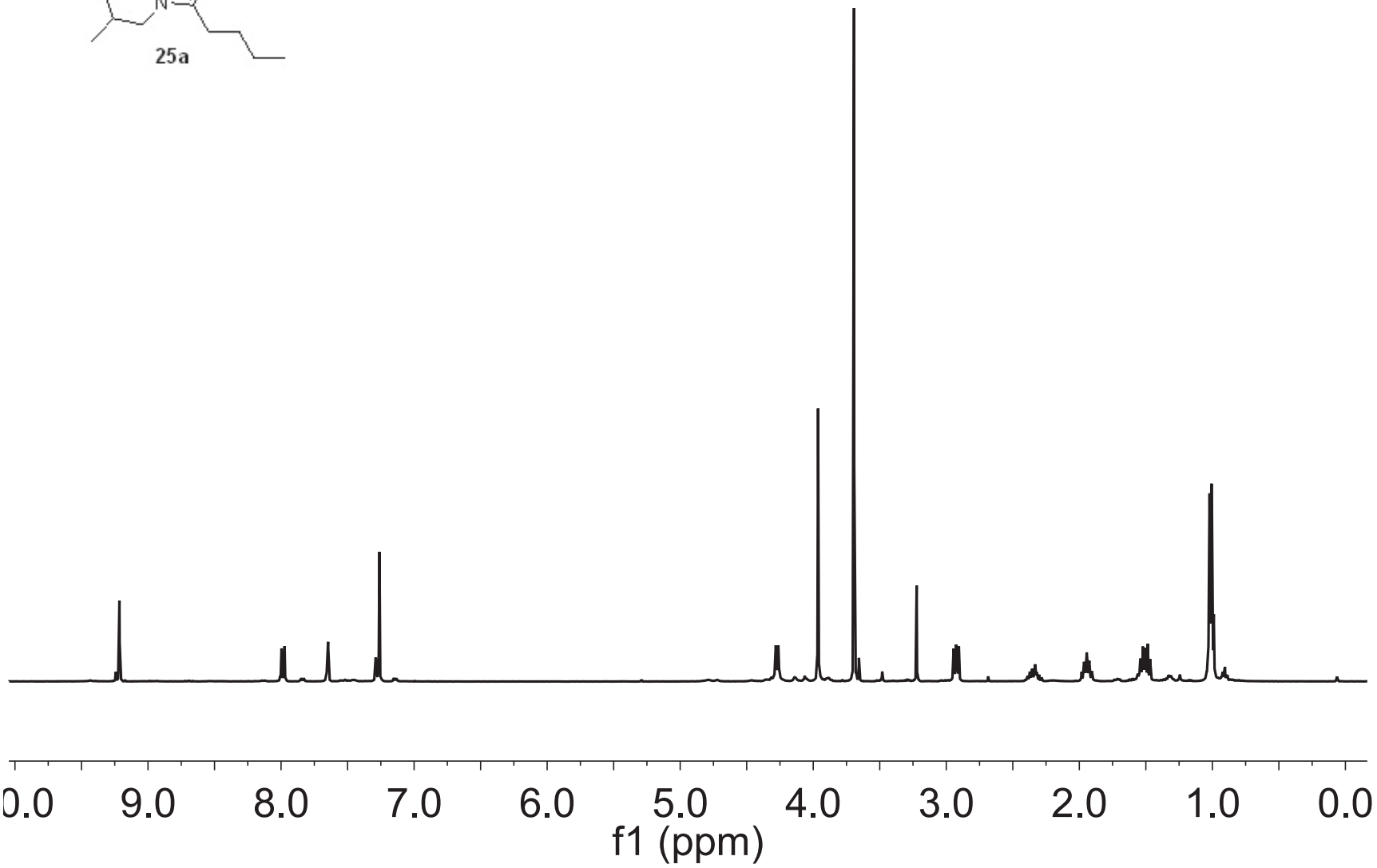
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

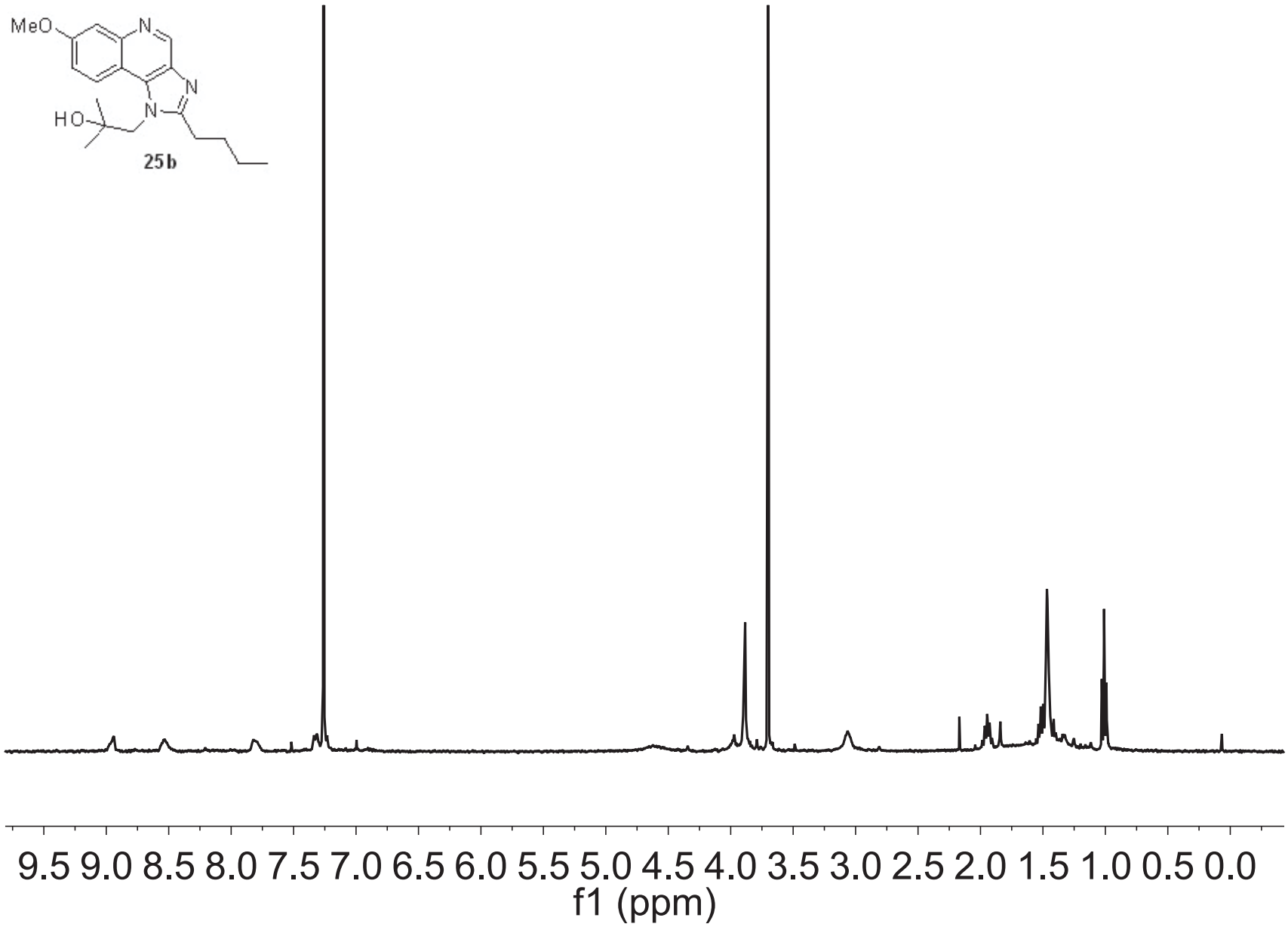
f1 (ppm)

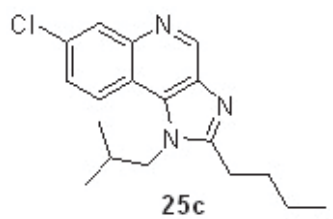

Cl
N
NH2
NH
HO
24d
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 -4
f1 (ppm)



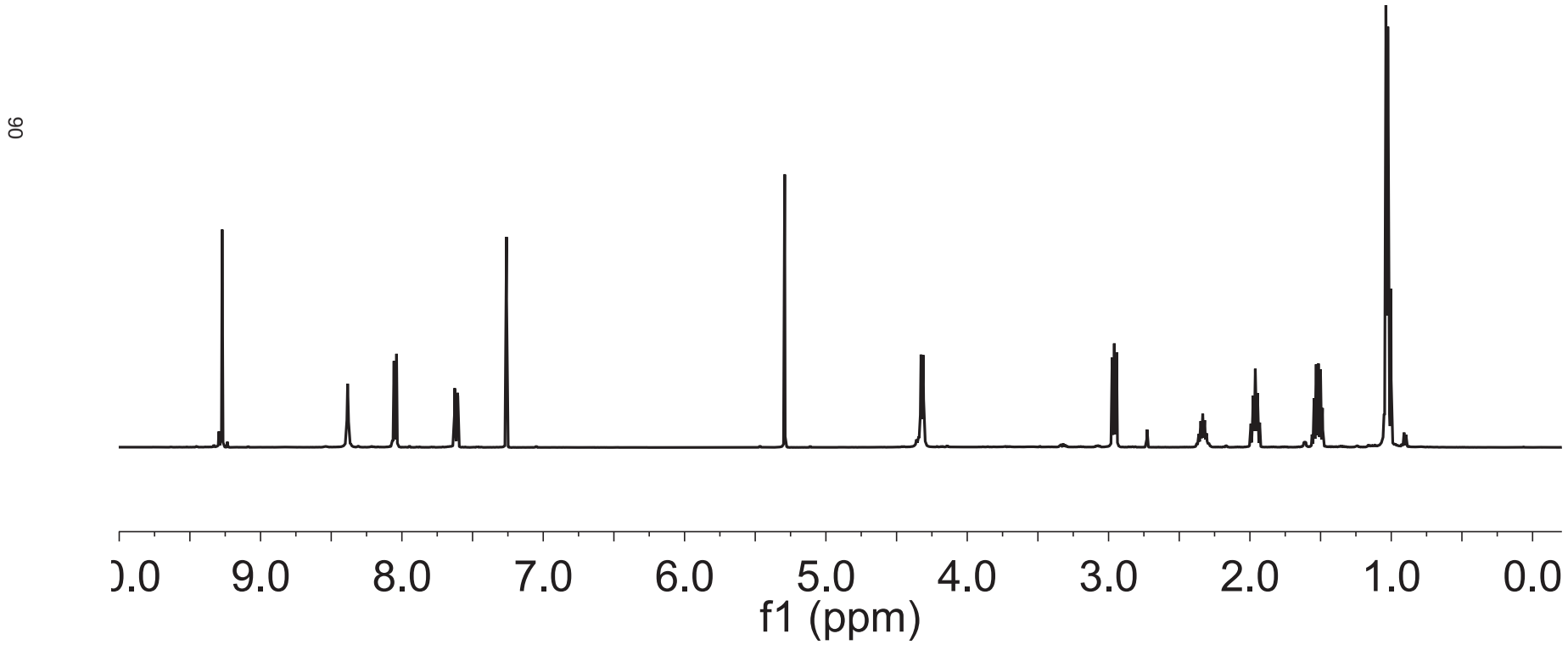
25a

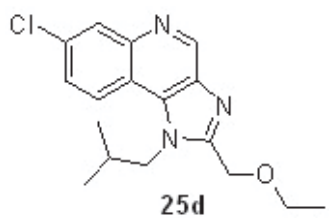



MeO
N
N
N
HO
25b
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

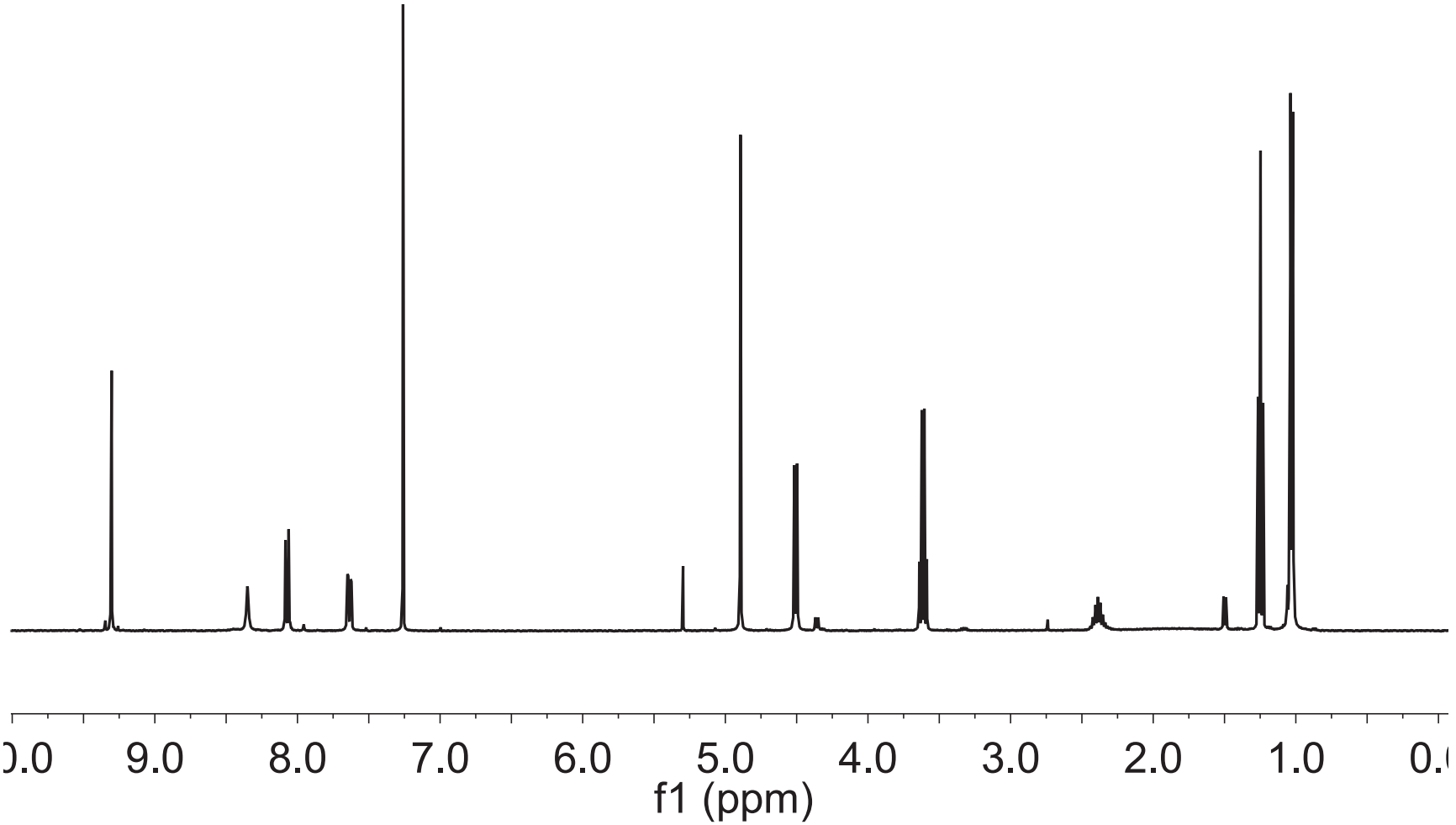


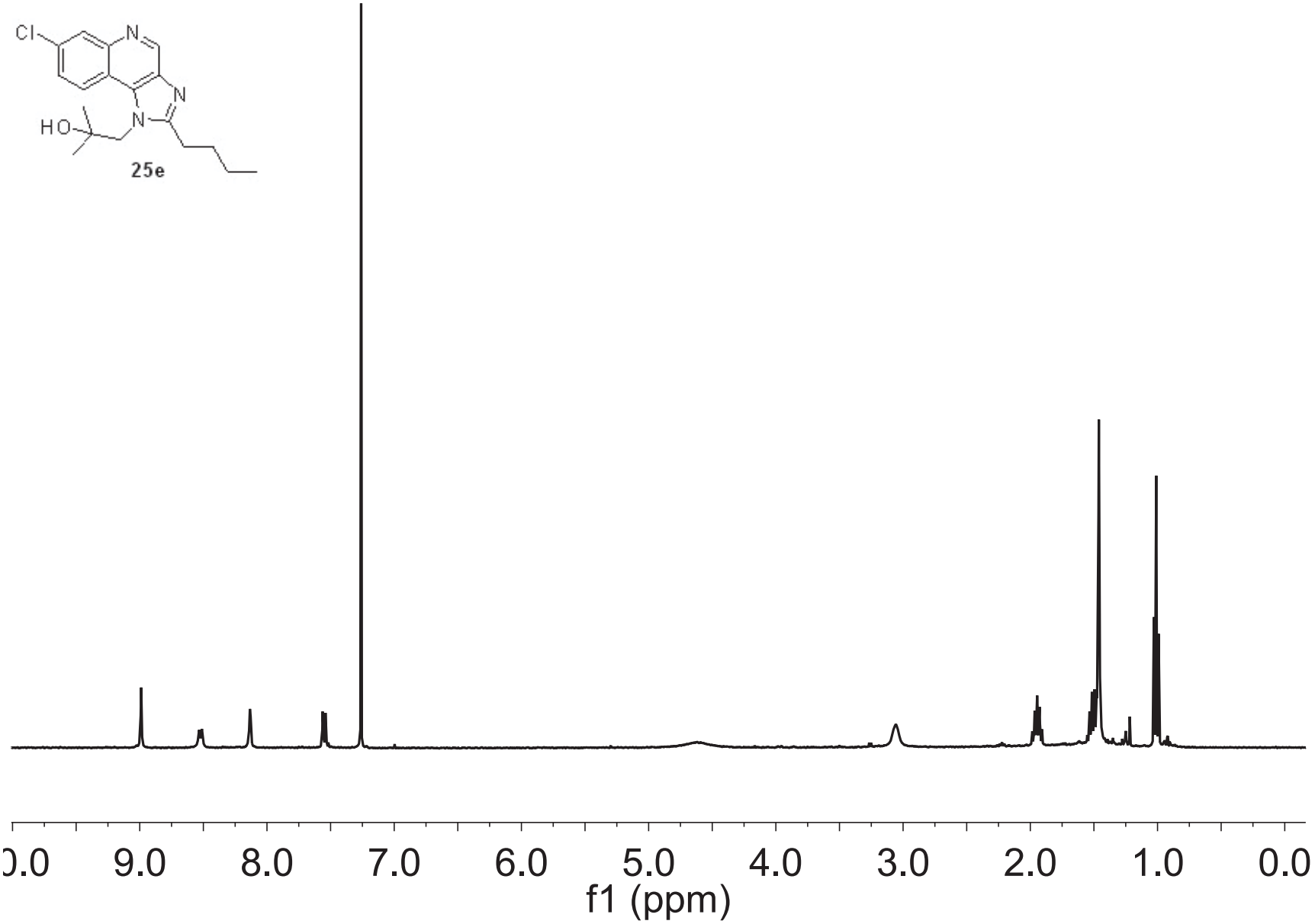
25c

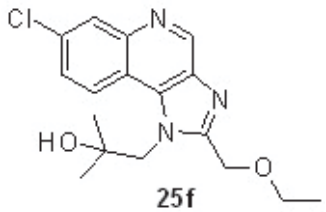




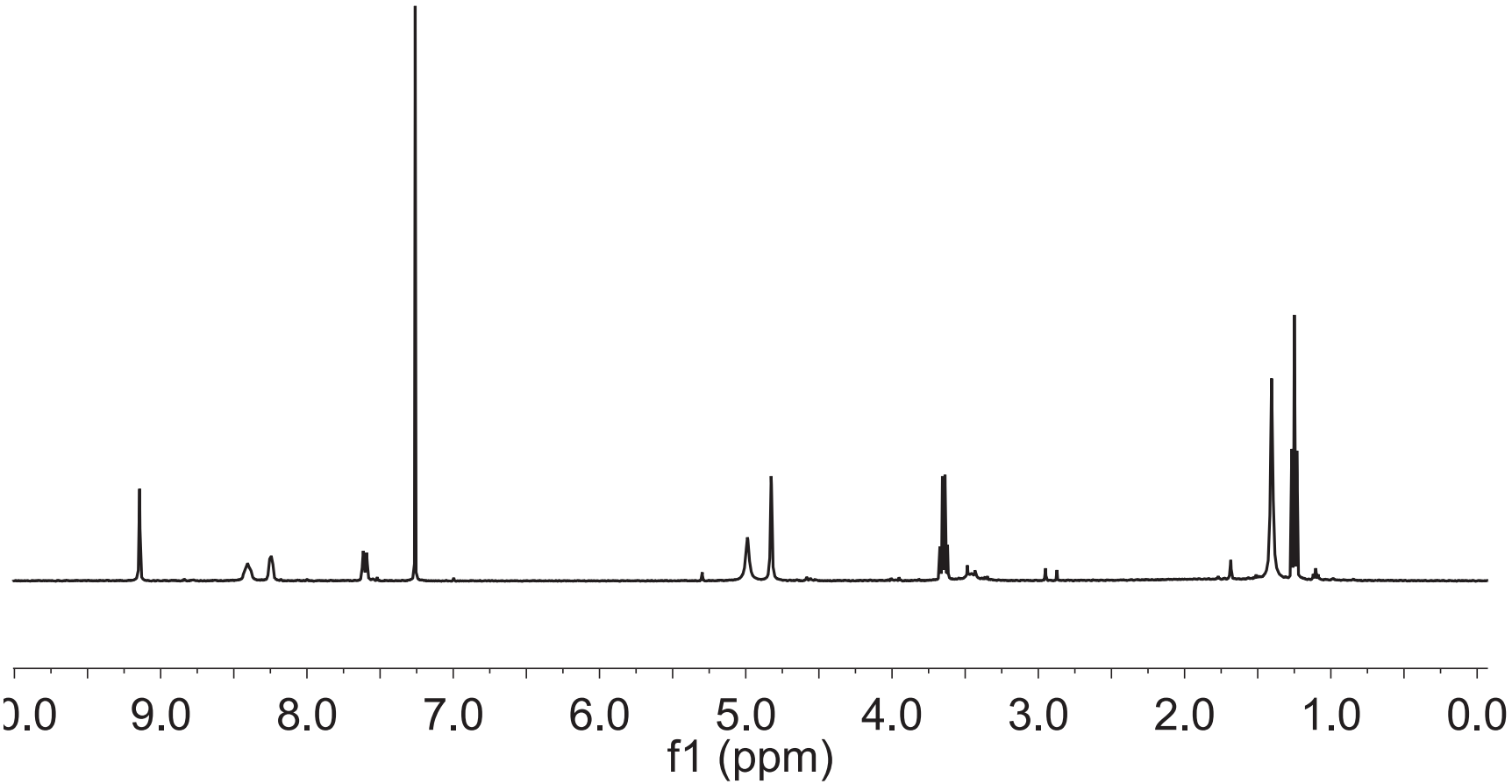
25d

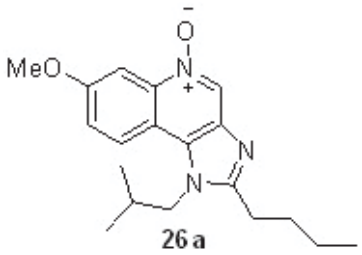



Cl
N
N
N
HO
25e
92
0.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f1 (ppm)

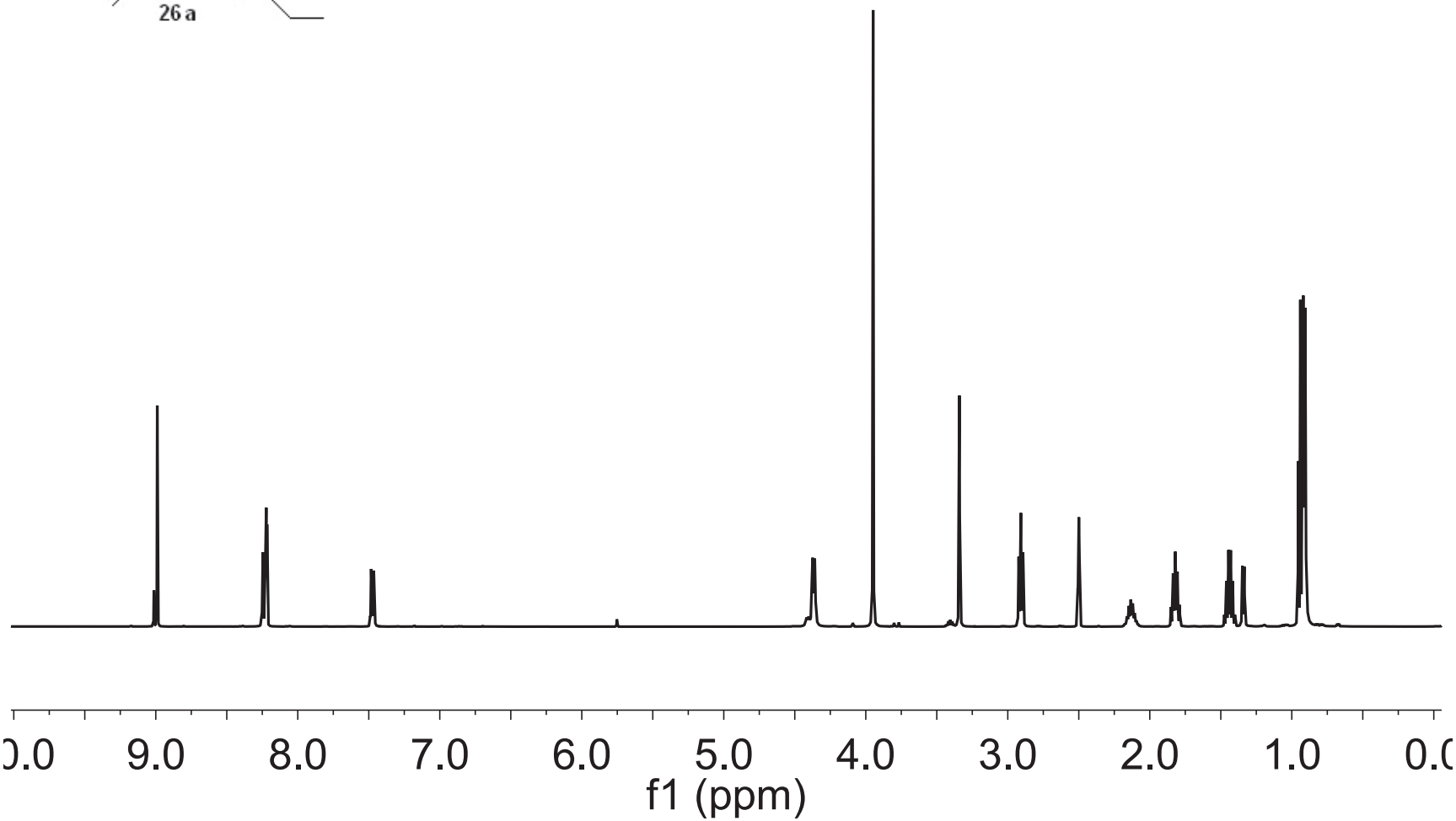


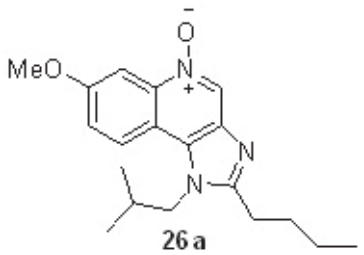
25f



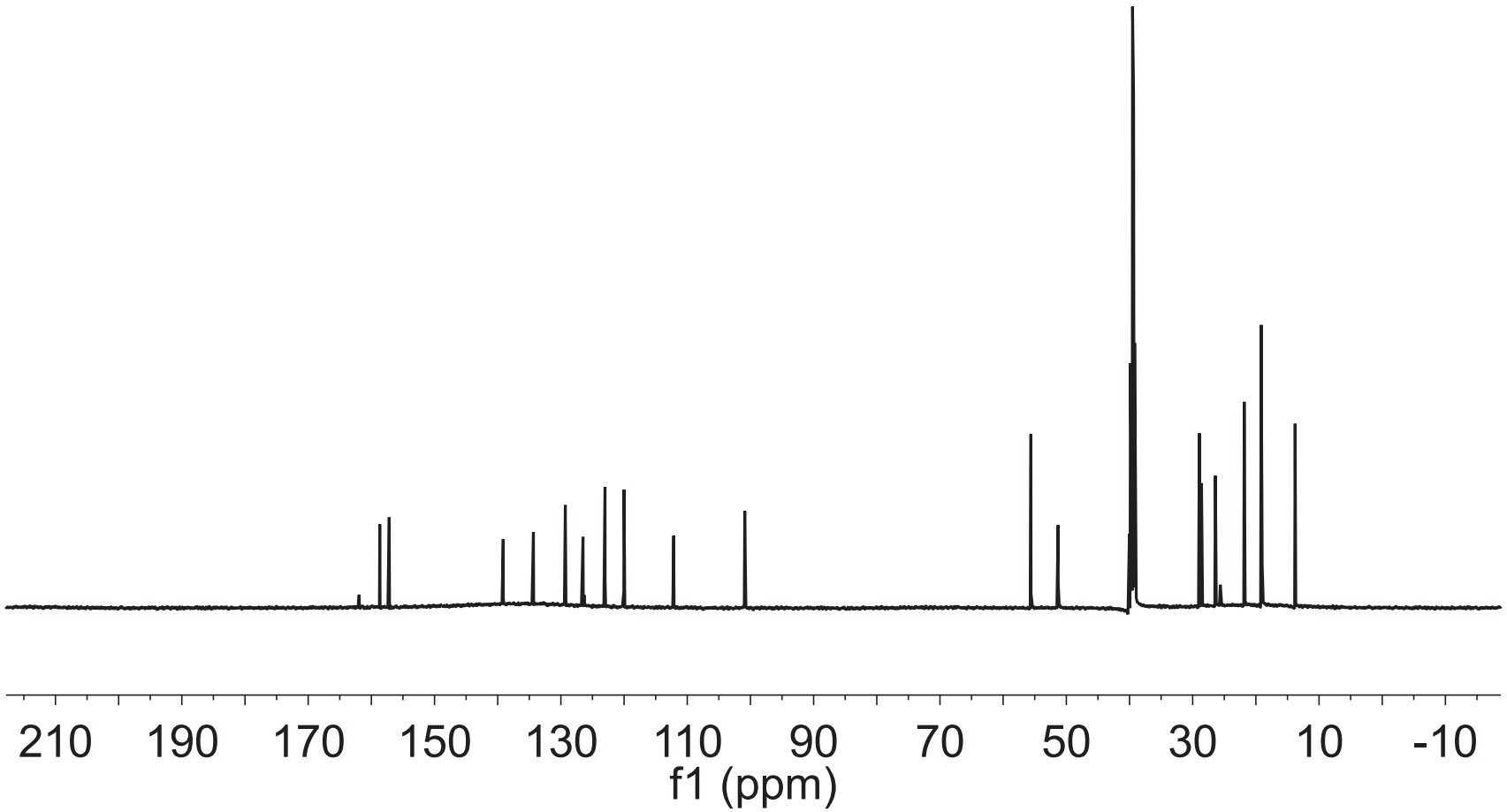


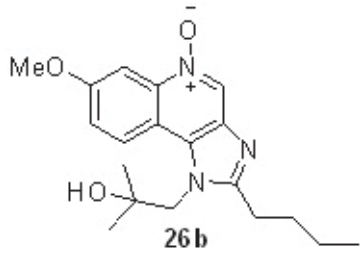
26 a





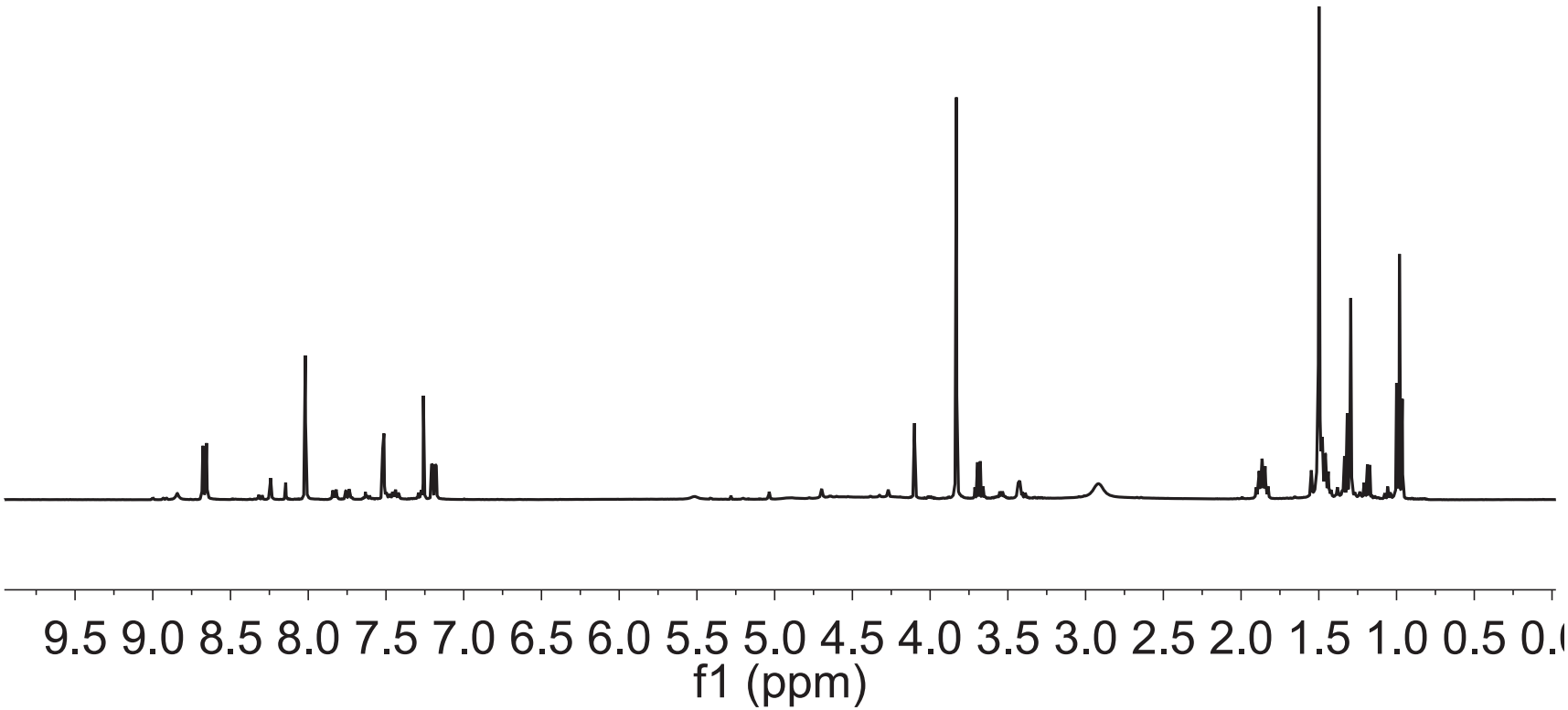
26 a

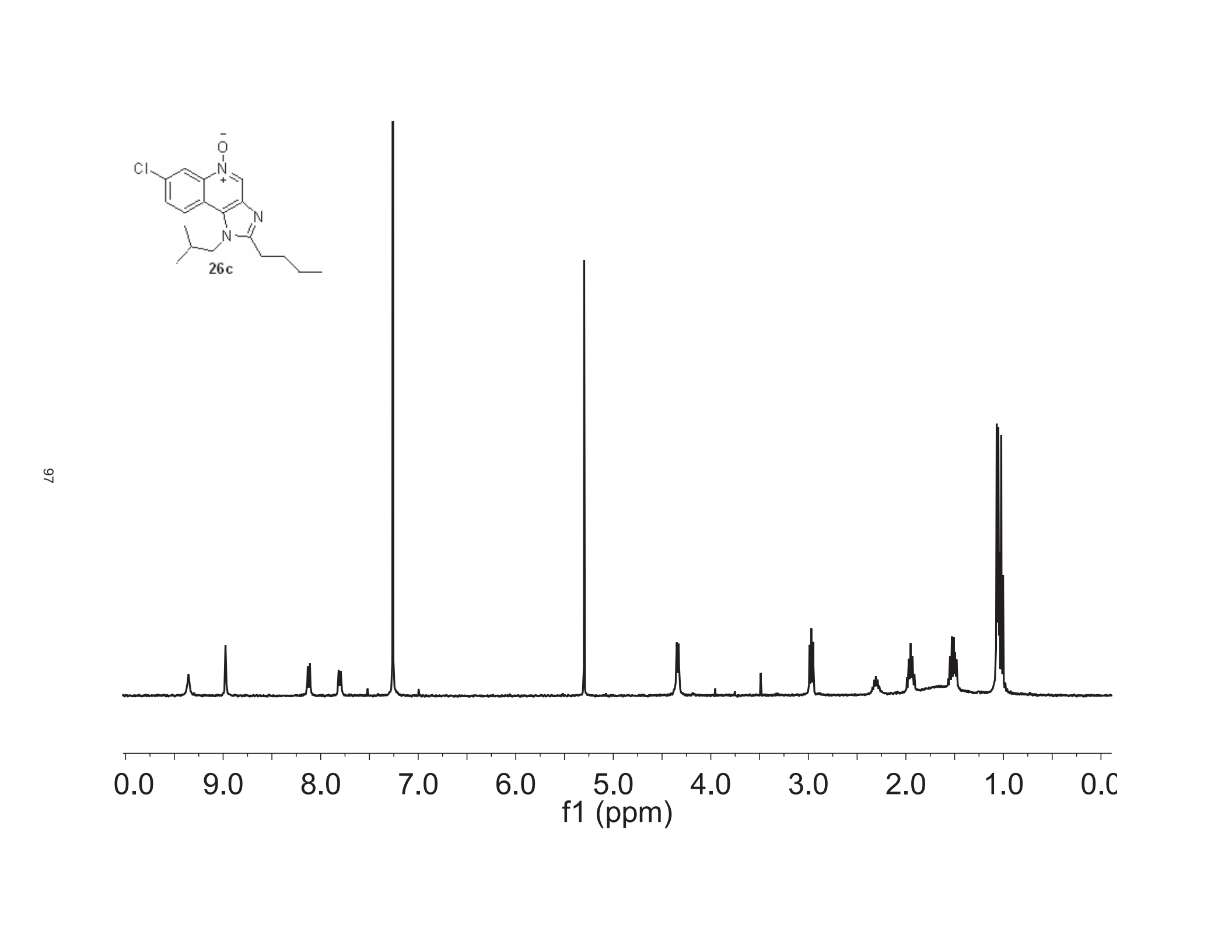


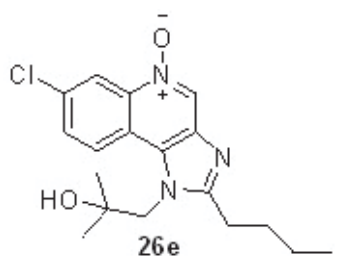

MeO
O
N
+
N
N
HO

26b

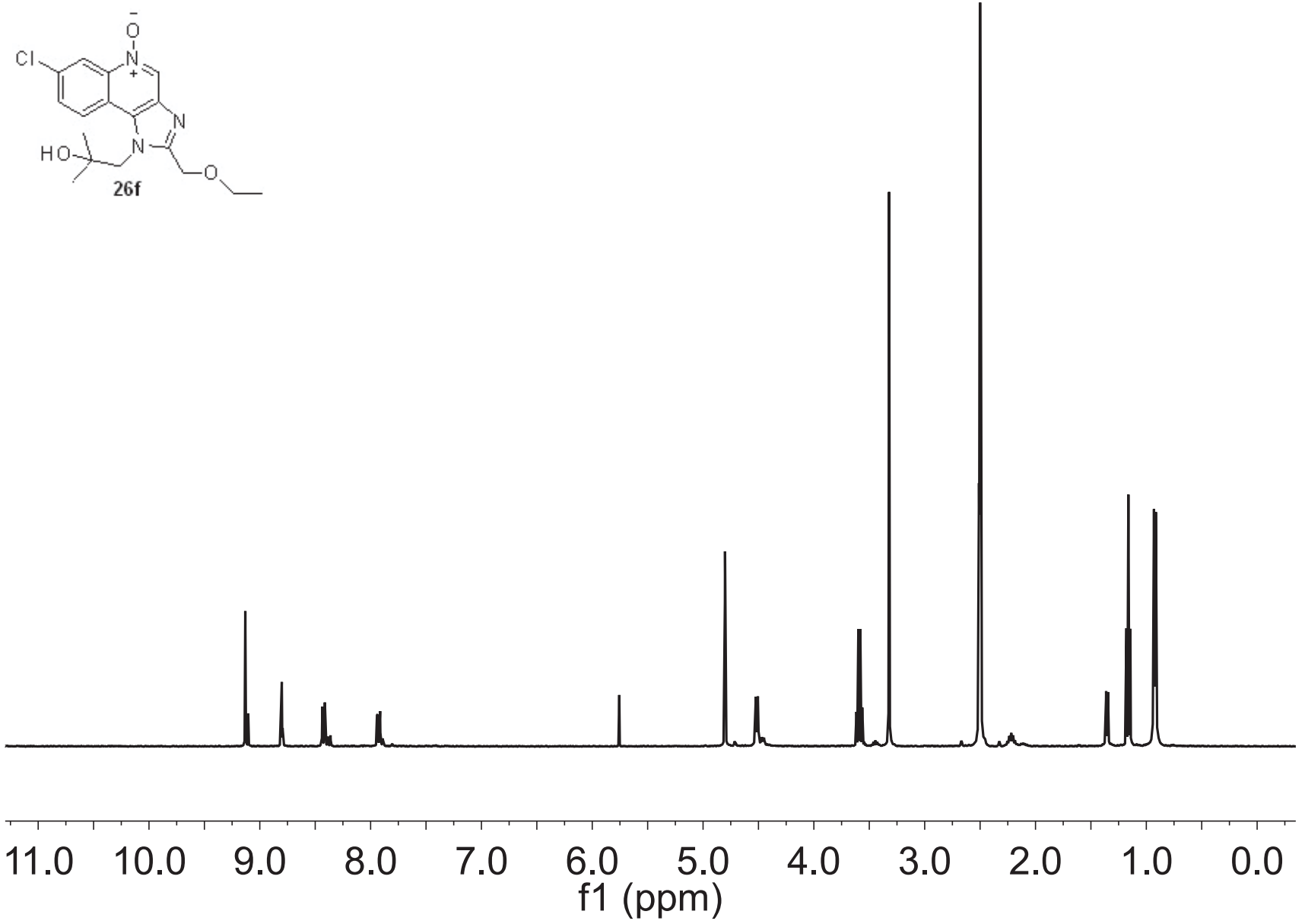
96

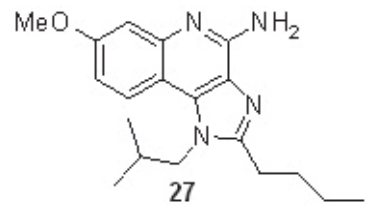

9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.
f1 (ppm)


Cl
O
N
+
N
N
26c
0.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f1 (ppm)

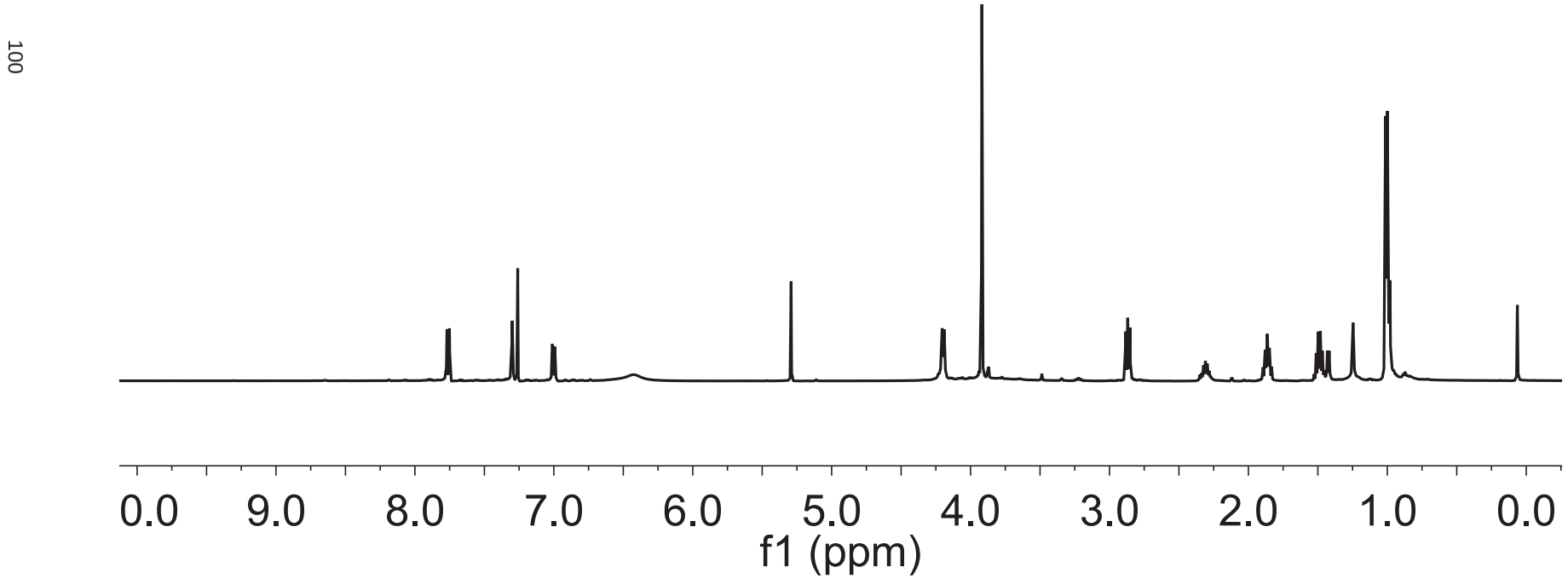


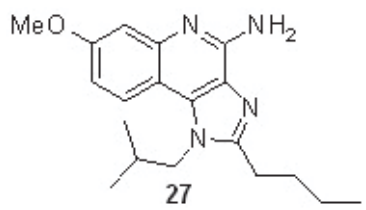
26e


Cl
O
N
+
N
HO
N
O
26f
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f1 (ppm)

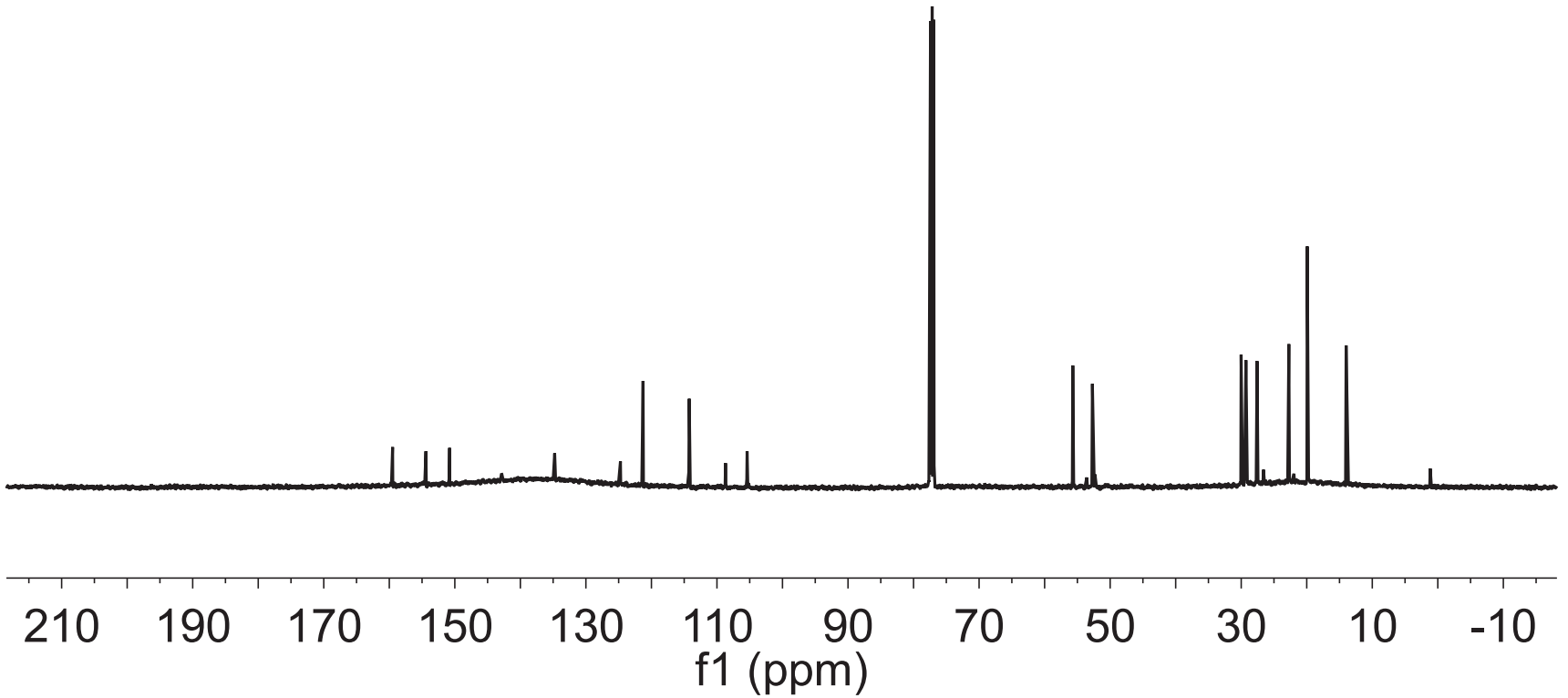


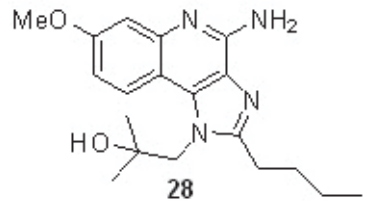
27



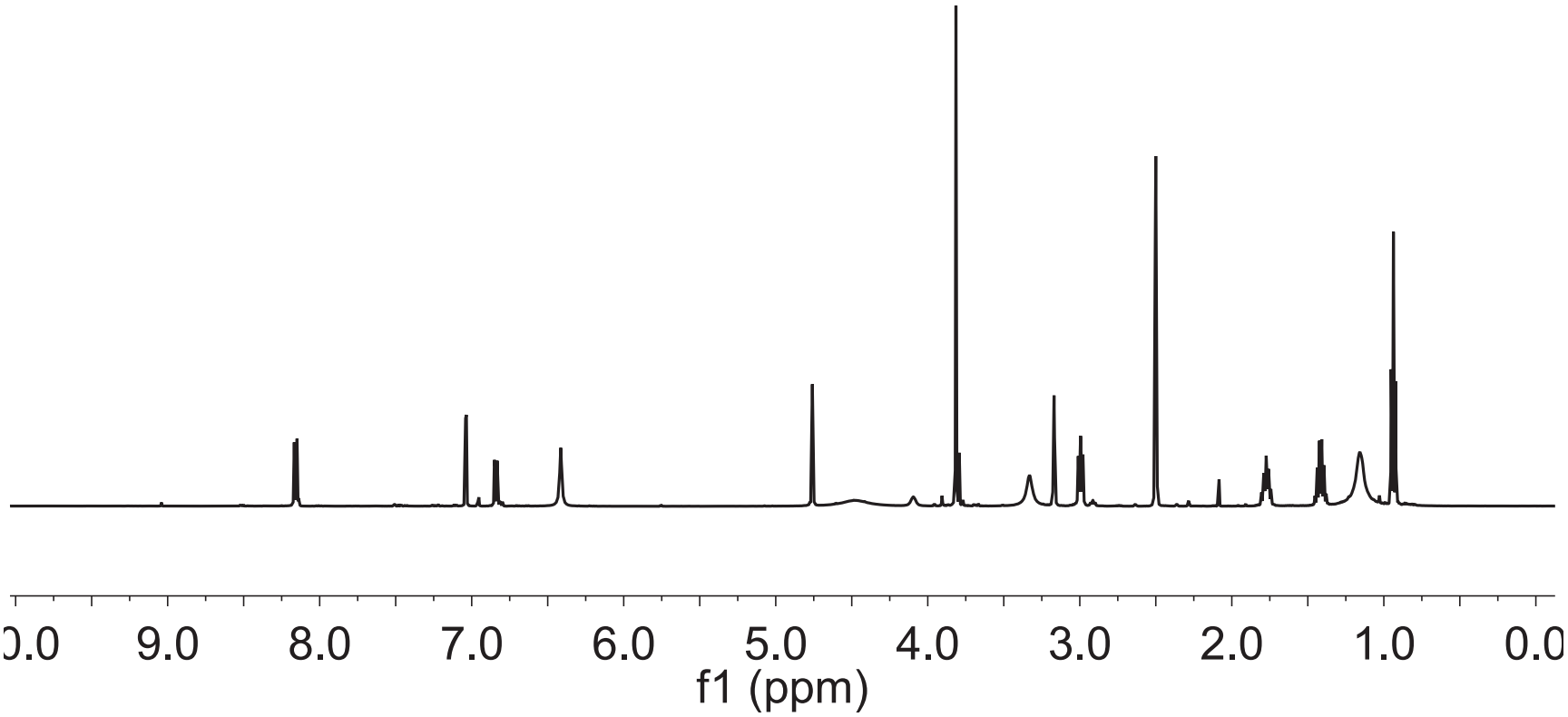


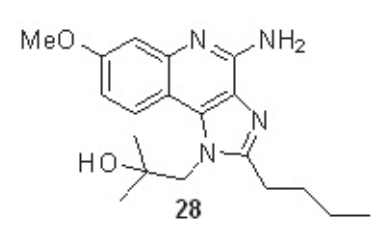
27



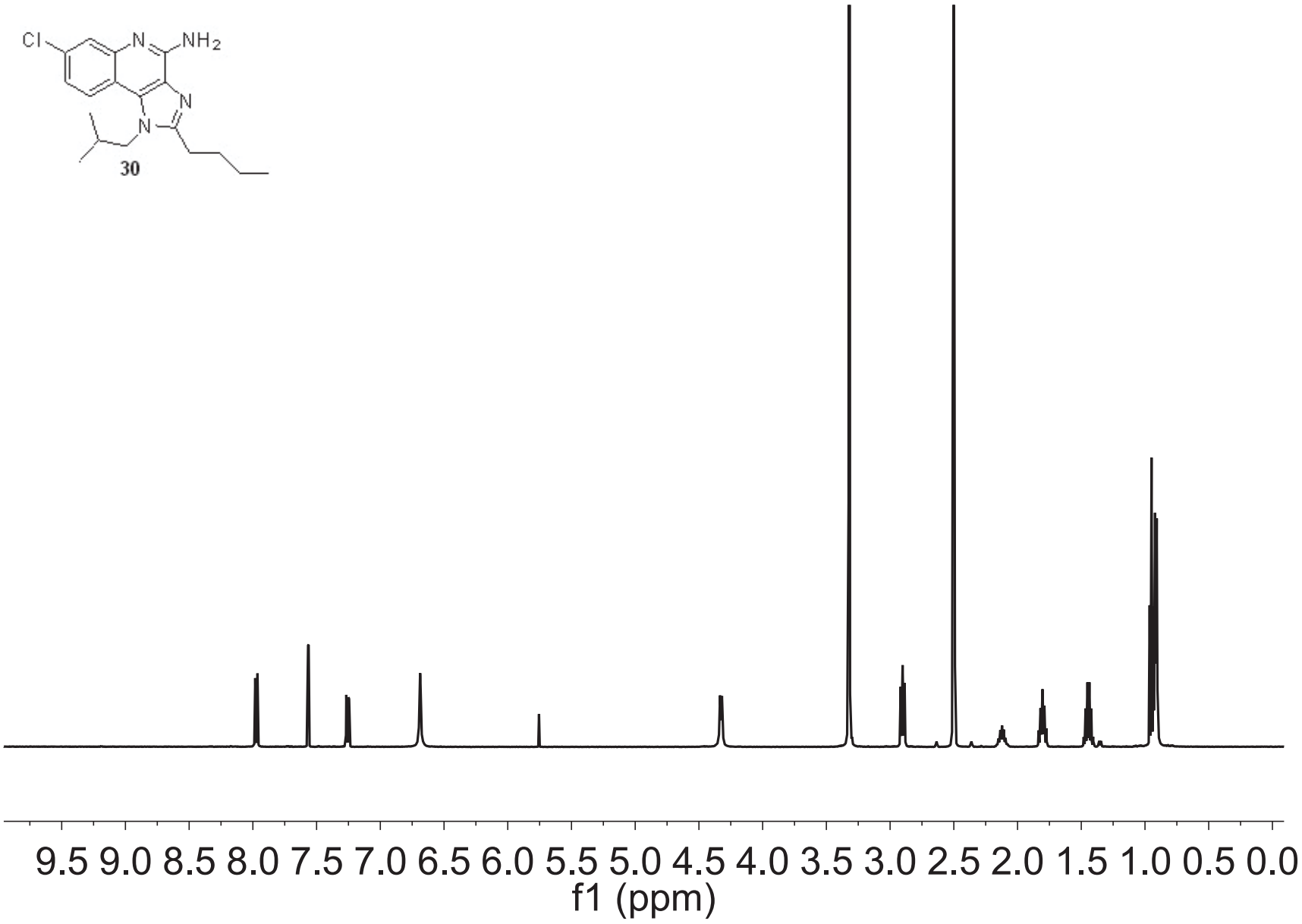


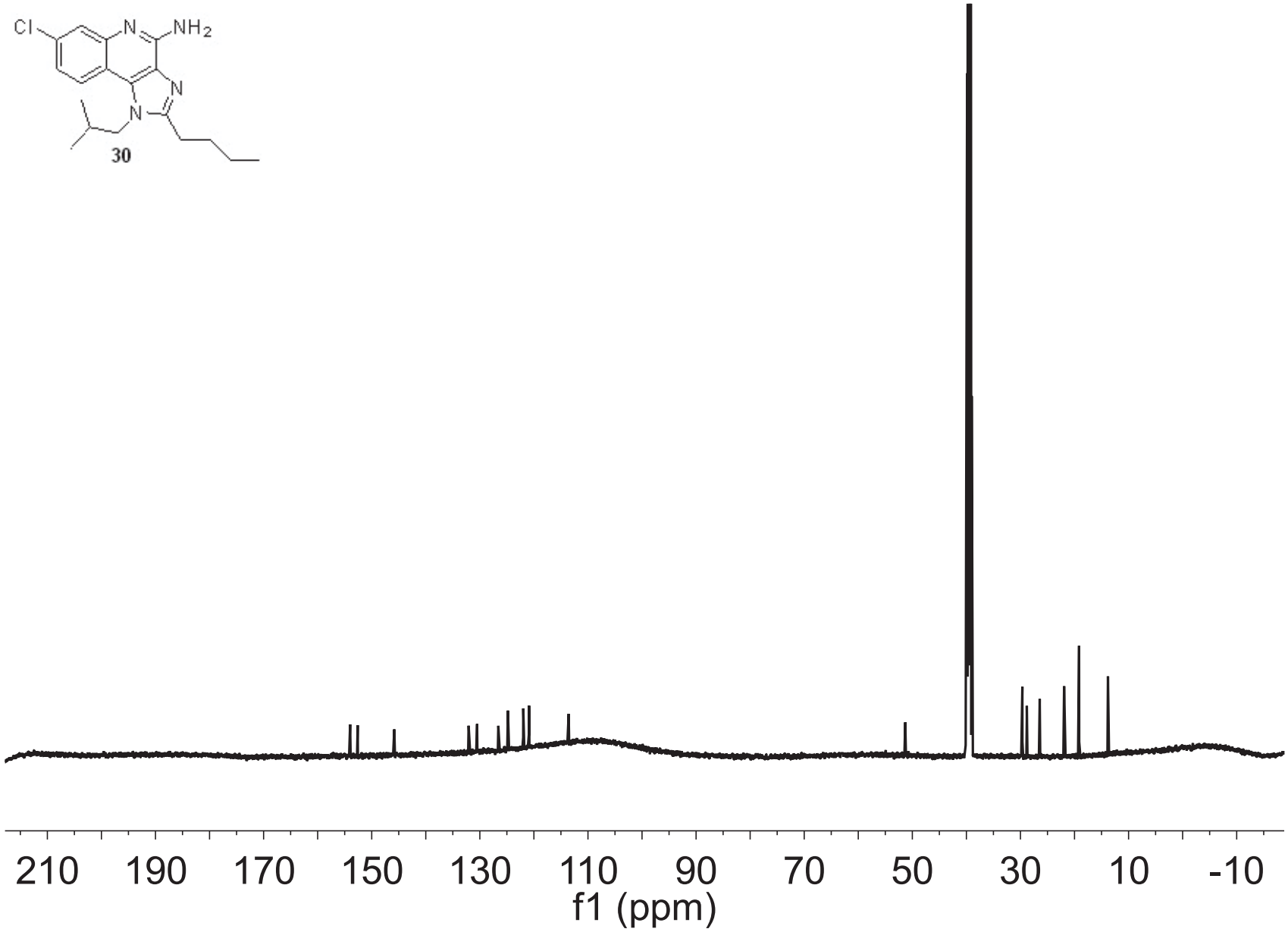
28

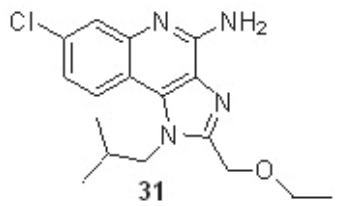




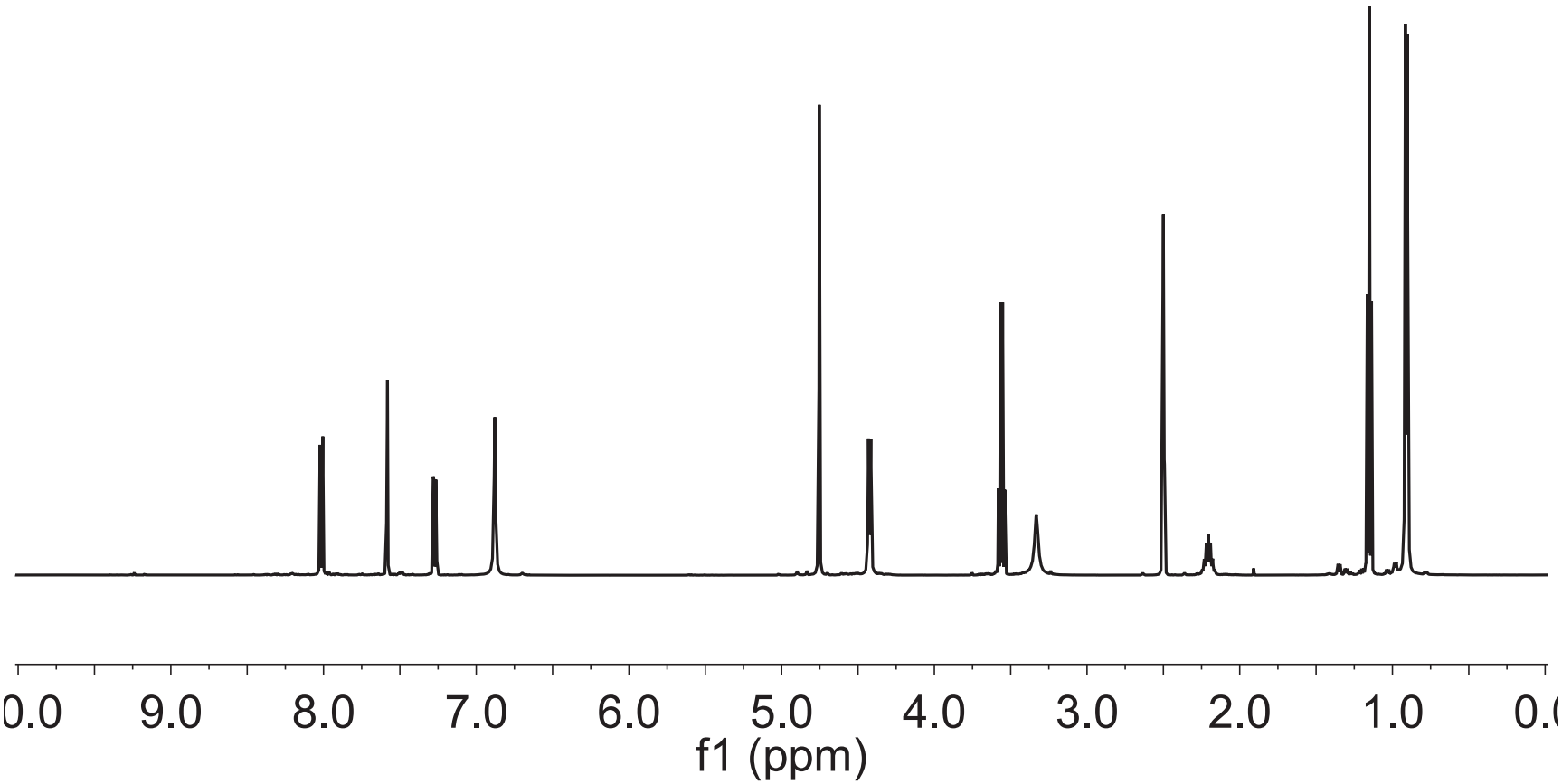
28

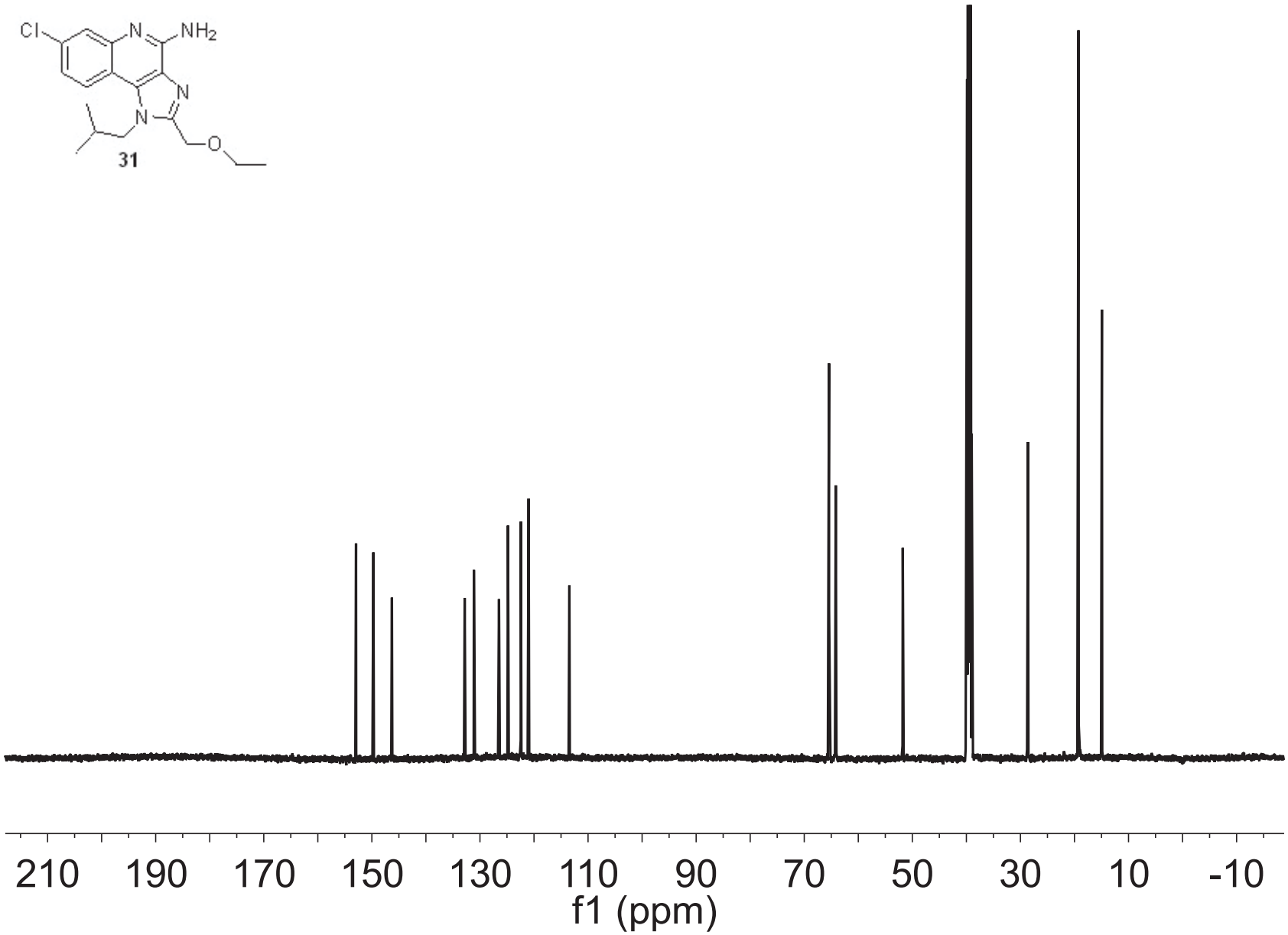

Cl
N
NH2
N
N
30
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

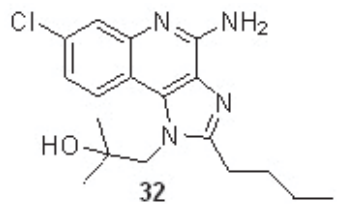

Cl
N
NH2
N
N
30
210
190
170
150
130
110
90
70
50
30
10
-10
f1 (ppm)



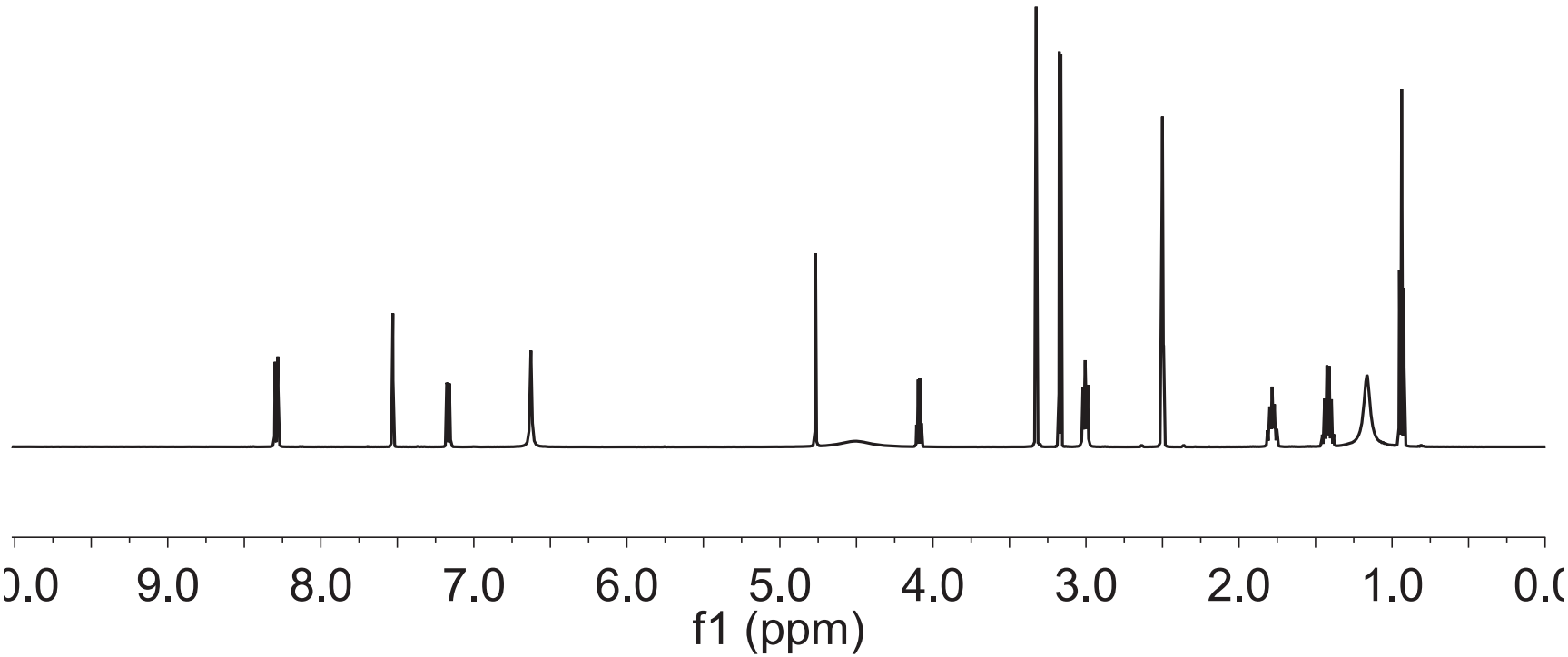
31

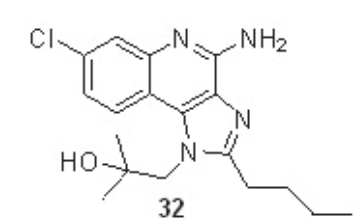



Cl
N
NH2
N
N
O
31
210
190
170
150
130
110
90
70
50
30
10
-10
f1 (ppm)

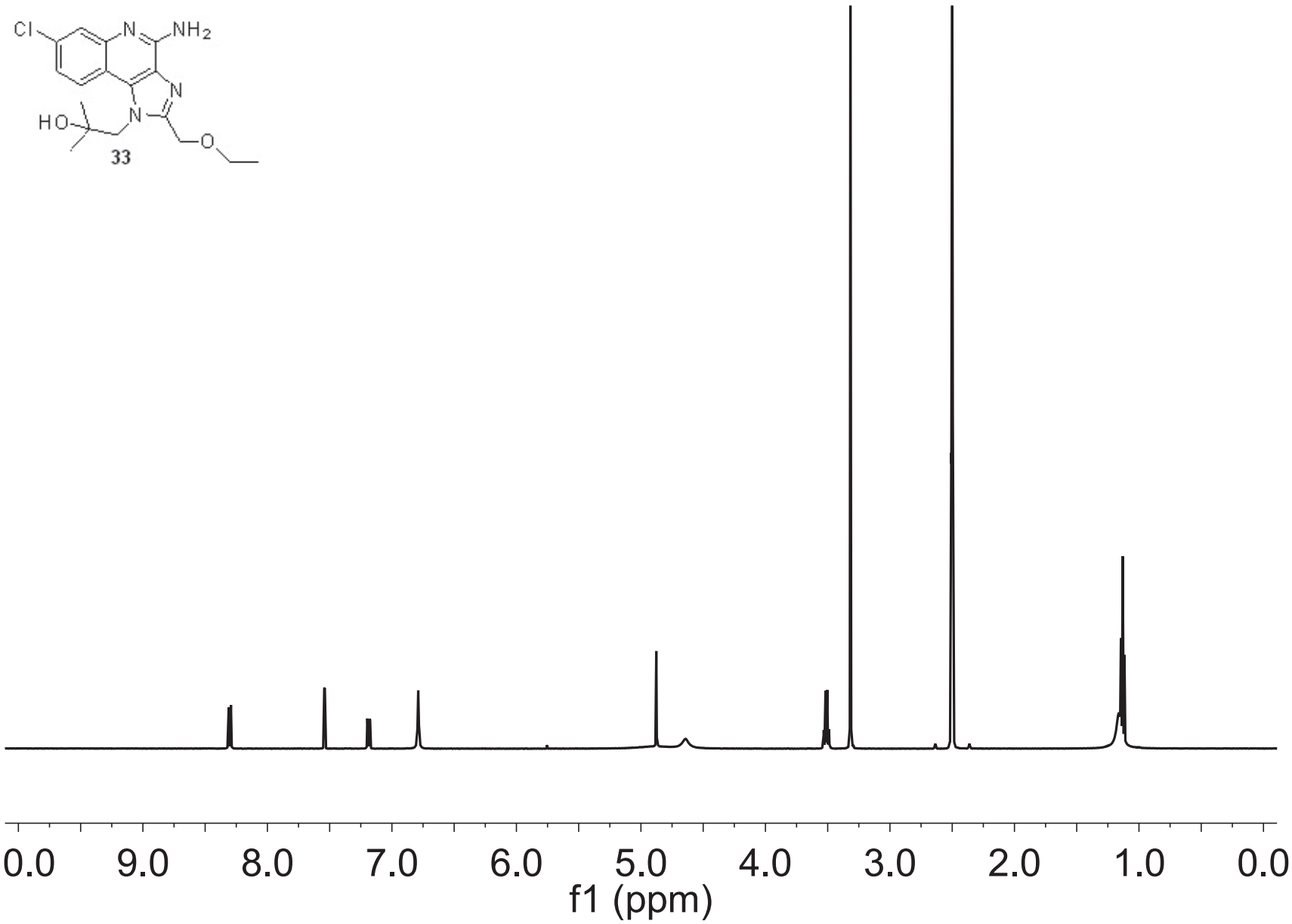


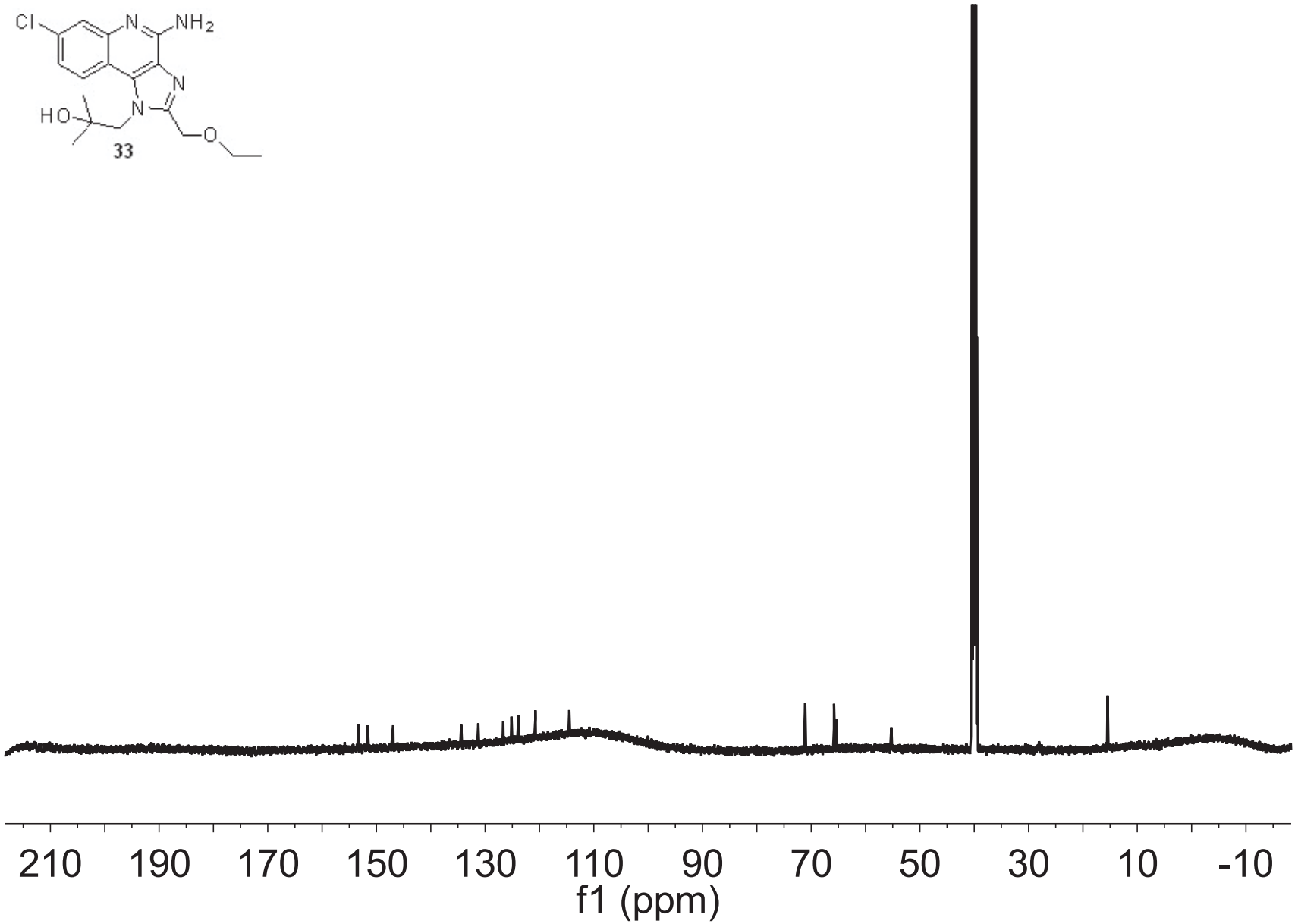
32

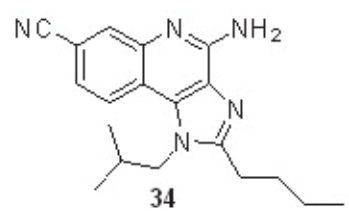




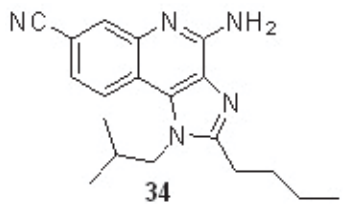
32


Cl
N
NH2
N
N
HO
O
33
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f1 (ppm)

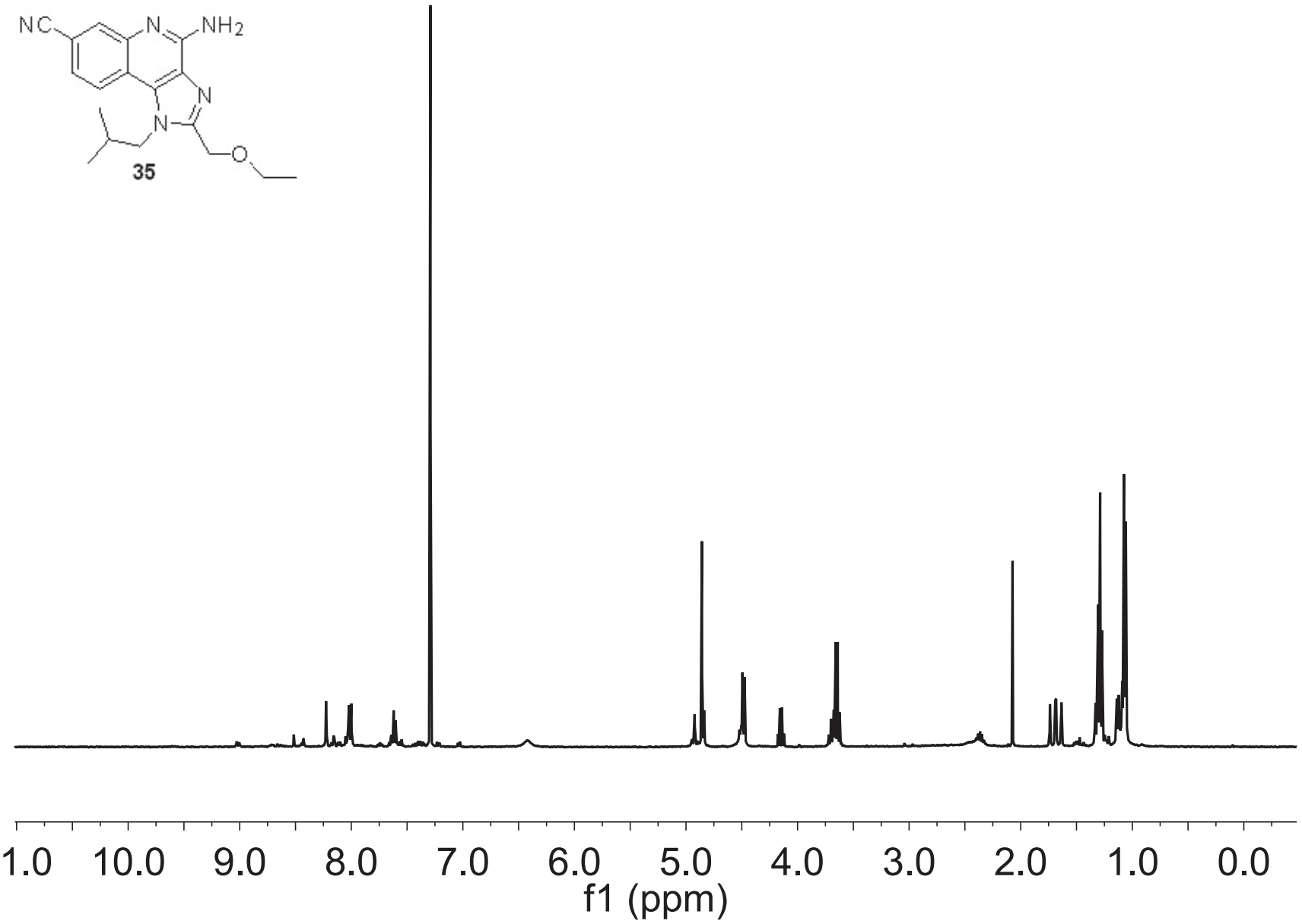

Cl
N
NH2
N
HO
N
O
33
210
190
170
150
130
110
90
70
50
30
10
-10
f1 (ppm)

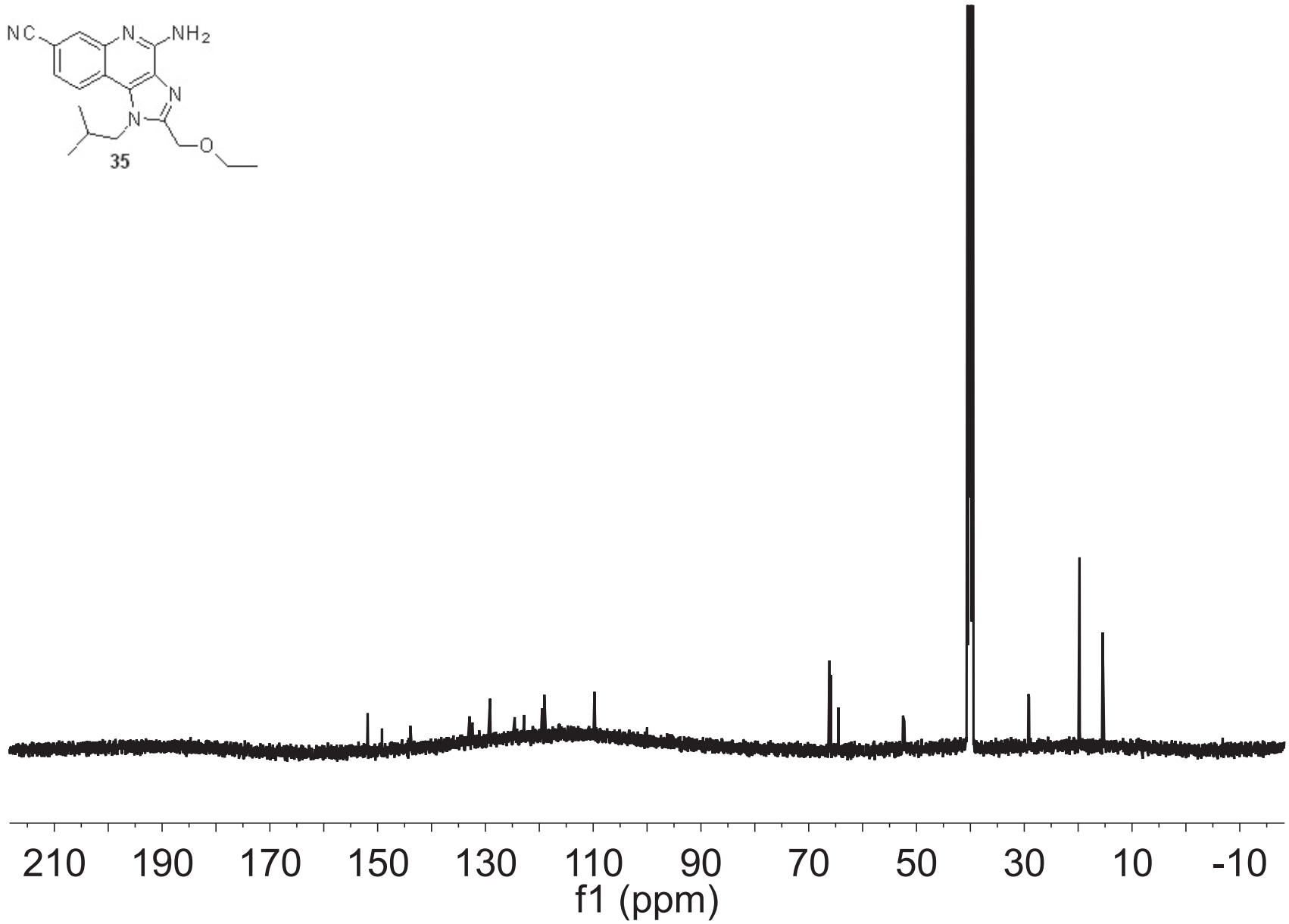


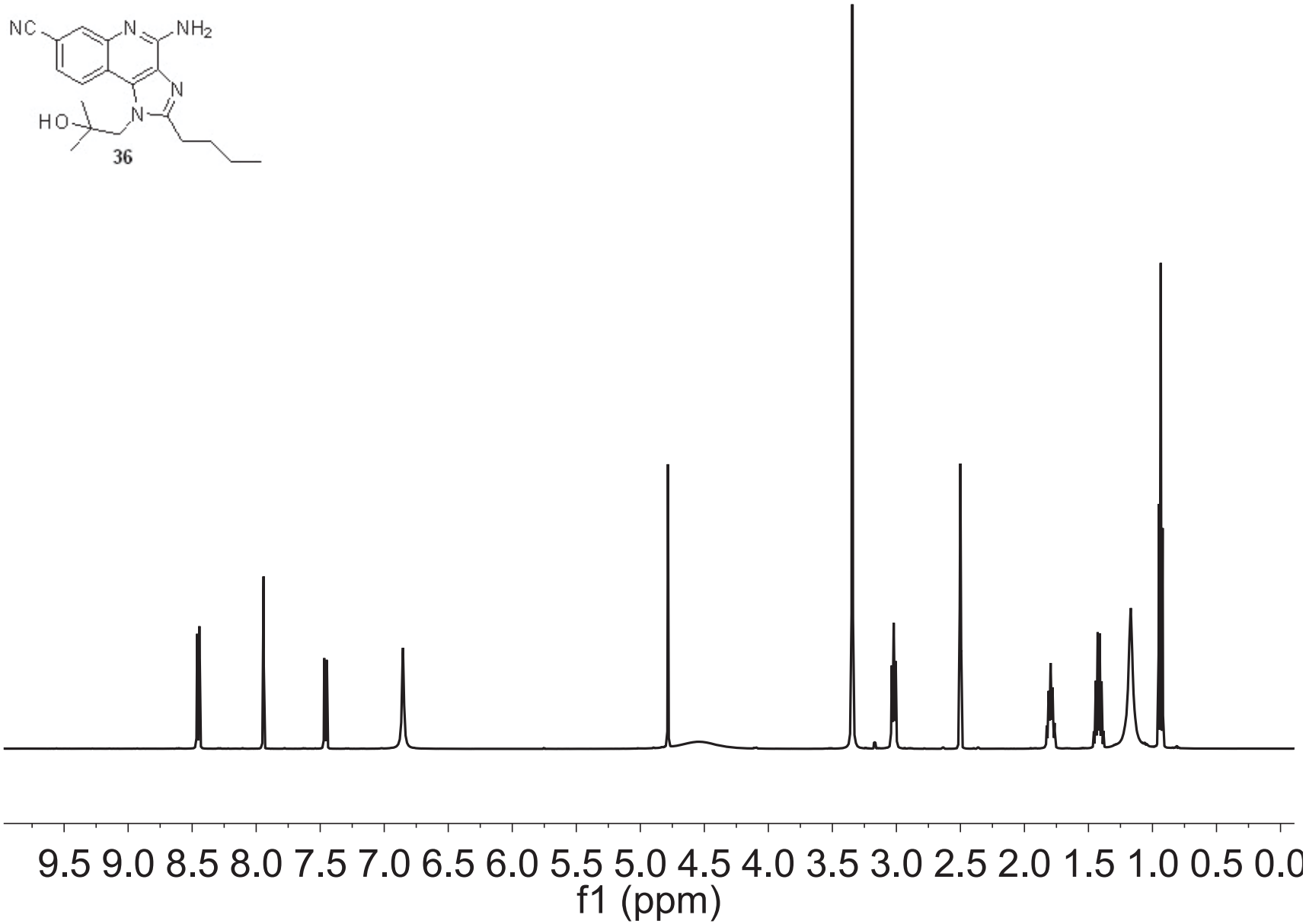
34

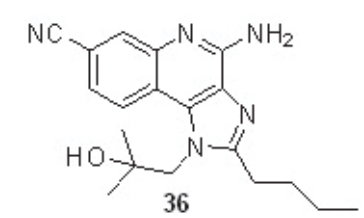


34

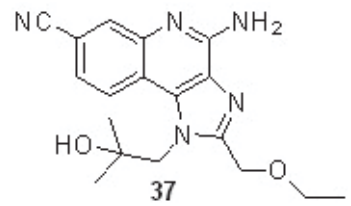

NC
N
NH2
N
N
O
35
1.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f1 (ppm)


NC
N
NH2
N
N
O
35
210
190
170
150
130
110
90
70
50
30
10
-10
f1 (ppm)

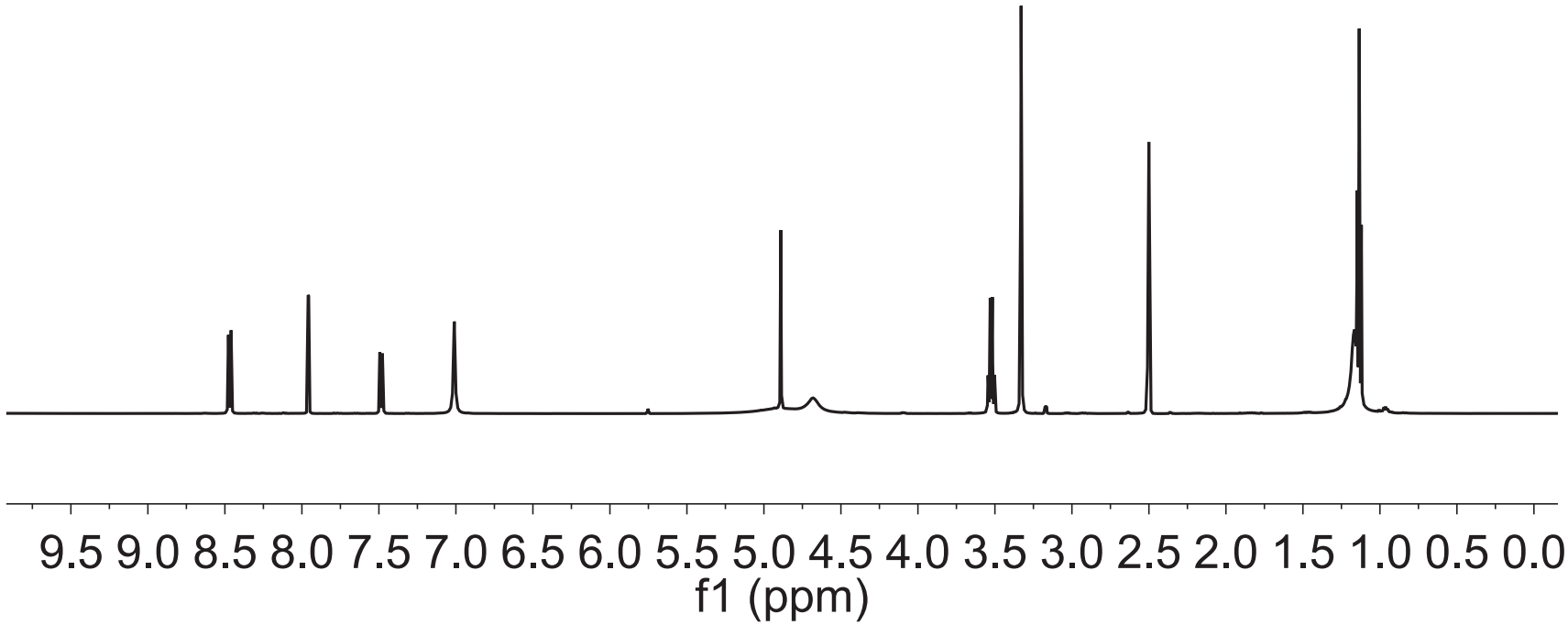

NC
N
NH2
N
N
HO
36
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

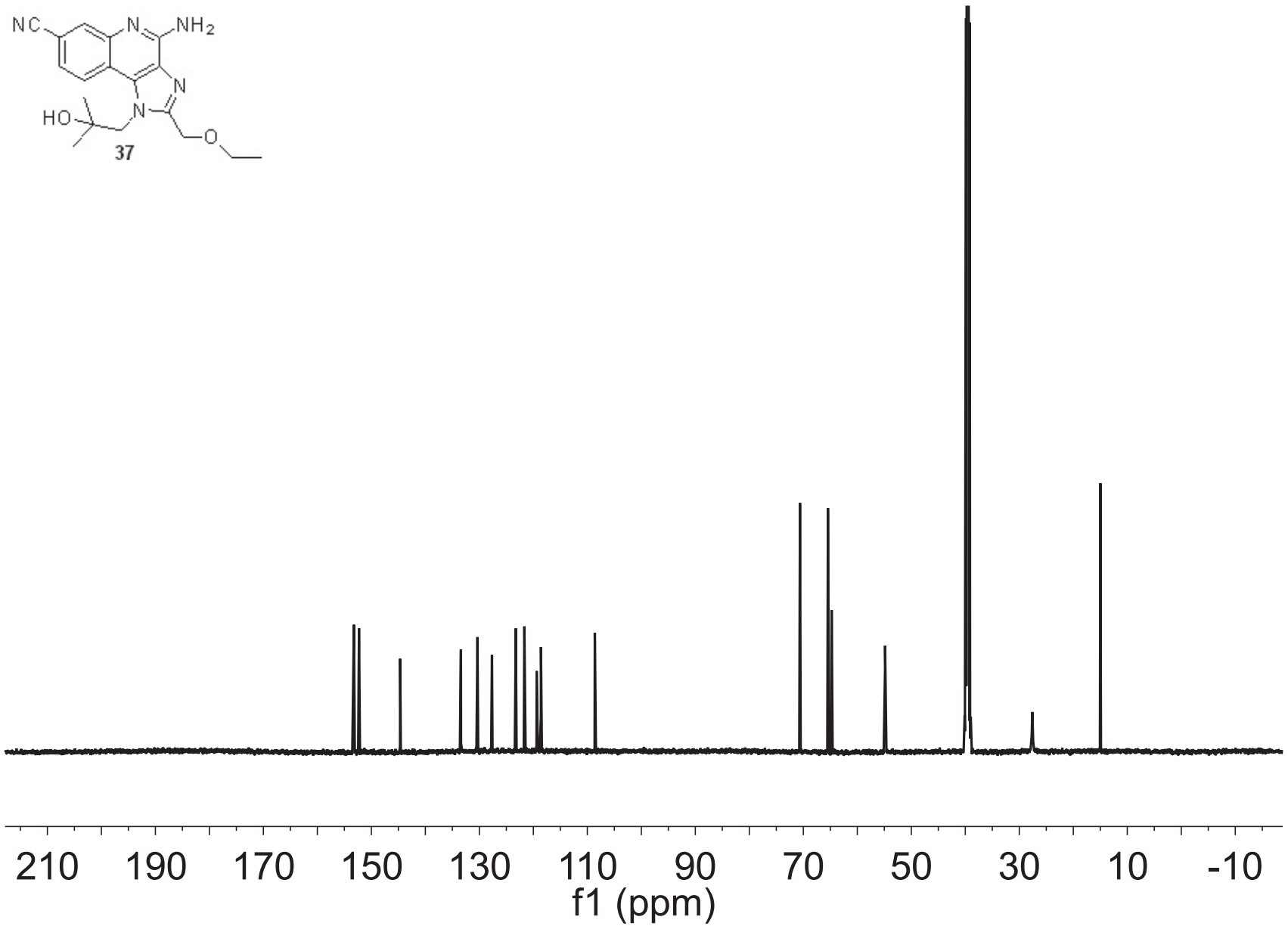


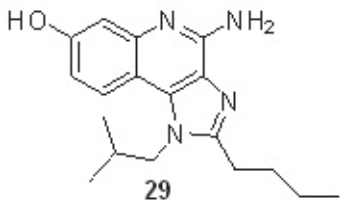
36


NC
N
NH2
N
N
HO
O

37


9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


NC
N
NH2
N
N
HO
O
37
210
190
170
150
130
110
90
70
50
30
10
-10
f1 (ppm)



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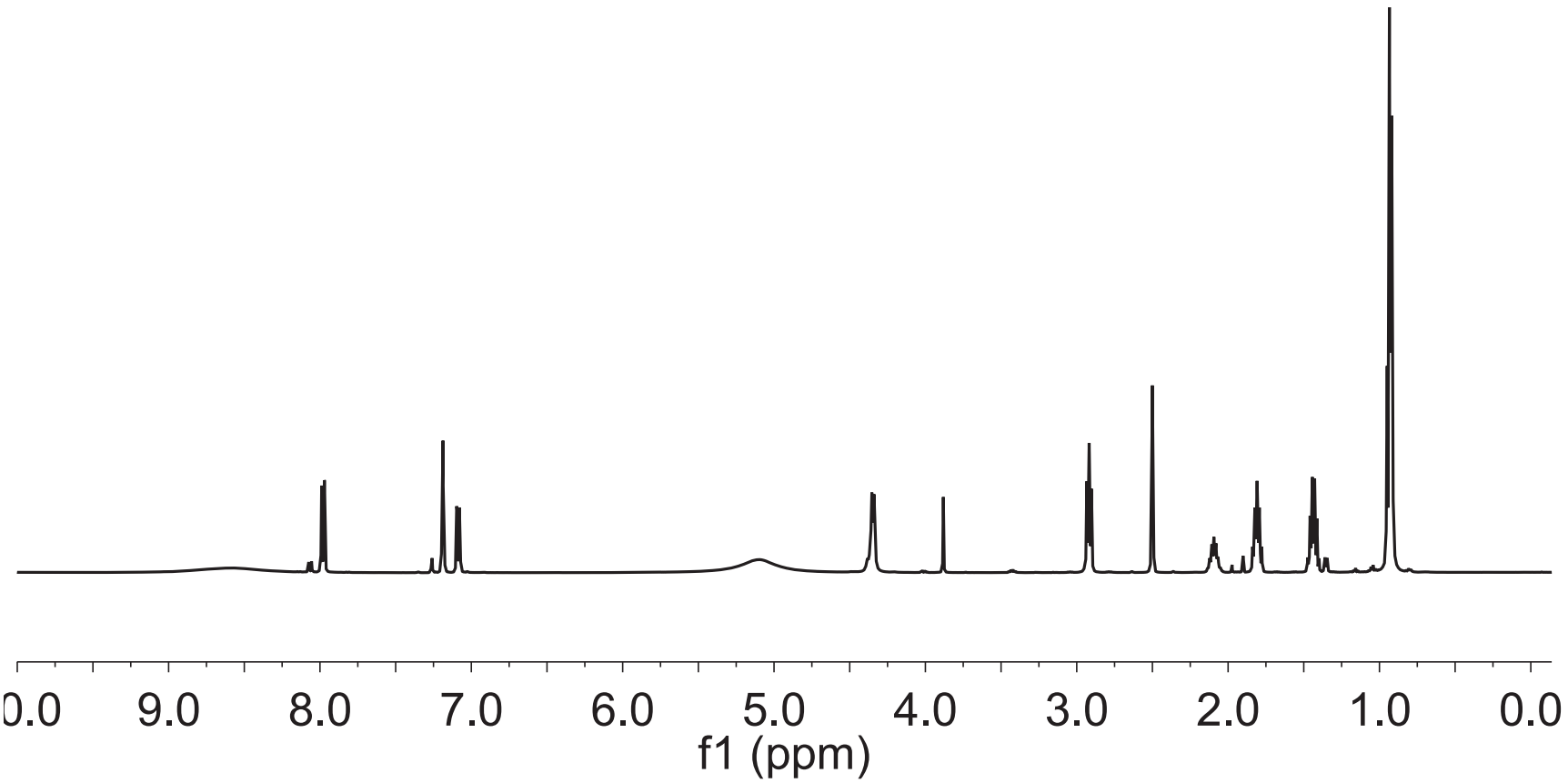

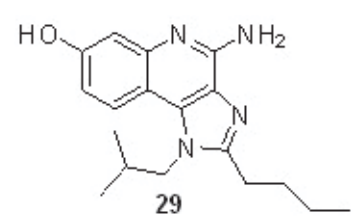

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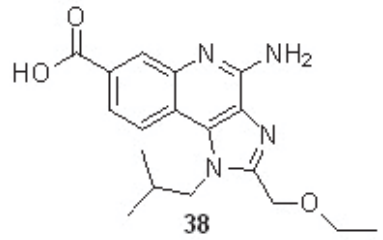

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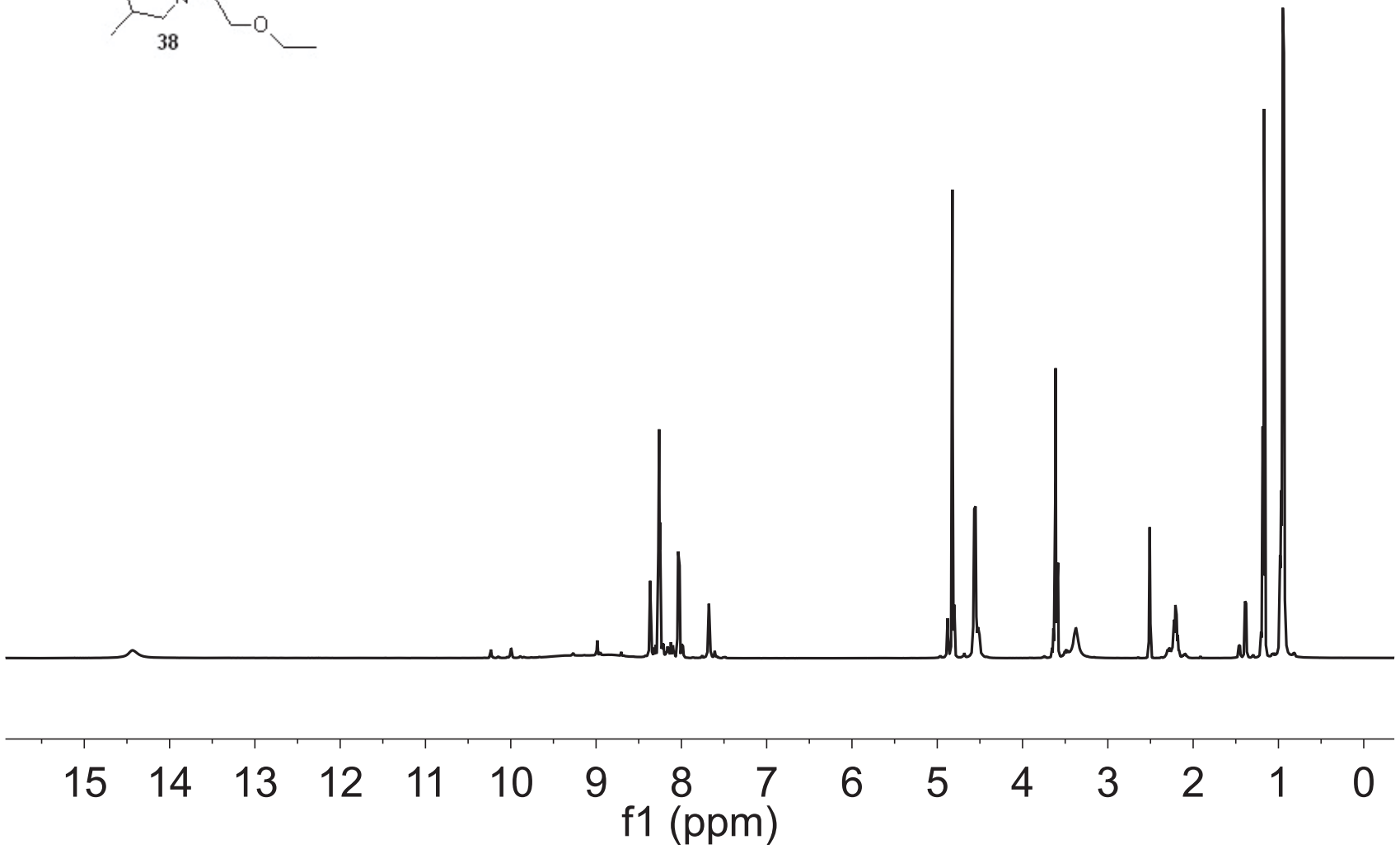

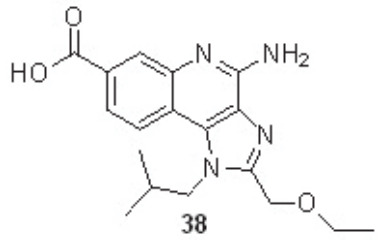

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