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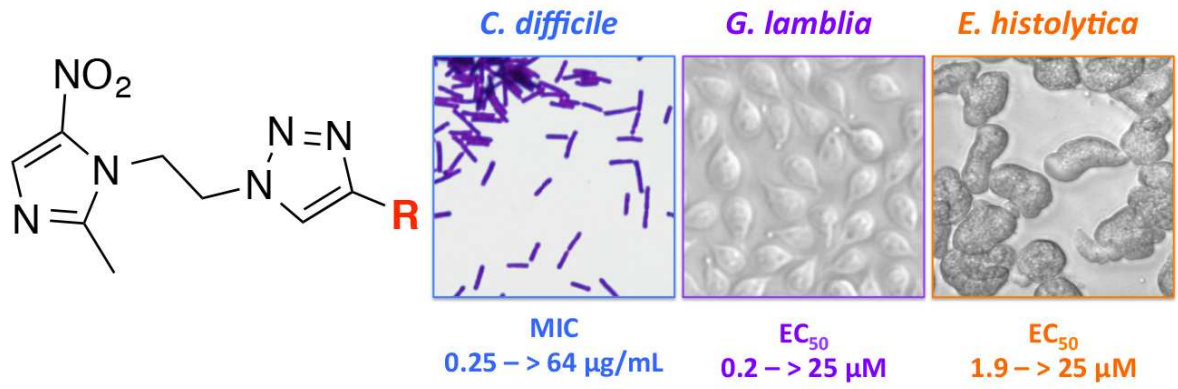

Metronidazole-triazole conjugates: activity against *Clostridium difficile* and parasites

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Abstract

Metronidazole has been used clinically for over 50 years as an antiparasitic and broad-spectrum antibacterial agent effective against anaerobic bacteria. However resistance to metronidazole in parasites and bacteria has been reported, and improved second-generation metronidazole analogues are needed. The copper catalysed Huigsen azide-alkyne 1,3-dipolar cycloaddition offers a way to efficiently assemble new libraries of metronidazole analogues. Several new metronidazole-triazole conjugates (Mtz-triazoles) have been identified with excellent broad spectrum antimicrobial and antiparasitic activity targeting *Clostridium difficile*, *Entamoeba histolytica* and *Giardia lamblia*. Cross resistance to metronidazole was observed against stable metronidazole resistant *C. difficile* and *G. lamblia* strains. However for the most potent Mtz-triazoles, the activity remained in a therapeutically relevant window.

Keywords

Nitroimidazole

Click Chemistry

Antibiotic

Anaerobe

1 Introduction

For over 50 years the 5-nitroimidazole antibiotic metronidazole **1** has been in clinical use as a broad-spectrum agent for treatment of Gram-positive and Gram-negative anaerobic bacterial infections as well as parasitic infections.[1] Reduction of the nitro group to the nitro radical anion by electron carriers in an anaerobic environment leads to decomposition to form toxic metabolites, which cause DNA damage and nonspecific macromolecular damage leading to cell death.[2] However, metronidazole resistance has been observed in both parasites[3,4] and anaerobic bacteria.[5,6] Over the last ten years metronidazole has also been extensively used to treat *Clostridium difficile* infection (CDI), an intestinal infection that causes life-threatening severe diarrhea, abdominal pain and fever.[7] The spores produced by *C. difficile* are resistant to heat and alcohol based disinfectants, complicating eradication and promoting hospital-based outbreaks. Metronidazole is one of three antibiotics routinely prescribed to treat the disease.[8] Metronidazole is effective against mild to moderate *C. difficile* infection, but, while not wide-spread, resistance has been observed in clinical isolates.[9] To this end, the development of next generation metronidazole analogues that can overcome resistance is therapeutically important.

Beena *et al.* previously reported the synthesis of metronidazole-triazole conjugates (Mtz-triazoles) with potent activity (reported as IC_{50} values 0.06-0.35 µg/mL) against the facultative anaerobic bacteria *Staphylococcus aureus*, S*taphylococcus epidermis*, *Escherichia coli* and *Pseudomonas aeruginosa*.[10] Metronidazole is generally not active against Gram-negative and Gram-positive facultative anaerobic bacteria, so the potent activities of Mtz-triazoles observed by Beena *et al.* are suggestive of a different mode of action to metronidazole. Given that the active 5-nitroimidazole core ("warhead") in metronidazole is maintained in Mtz-triazoles, and that the hydroxyl group of metronidazole is amenable to modification[11] (Figure 1), we reasoned that this class might also exhibit potent activity against the anaerobic bacteria and parasites for which metronidazole is used as a treatment. Therefore, we believed Mtz-triazoles warranted further investigation for both antimicrobial and antiparasitic activity under anaerobic conditions.

Independently, during the course of this work, Miyamoto *et al.*[12] reported a similar approach to Mtz-triazoles, prepared by reaction of six different 5-nitroimidazole azides with a library of alkynes. The compounds were prepared for testing by dilution of the crude reaction mixtures (>85% purity by LCMS)

with dimethyl sulfoxide. This methodology bypassed the bottleneck of compound purification and characterization and allowed for the rapid assessment of activity. The solutions of crude material were tested against the parasites *Giardia lamblia* and *Trichomonas vaginalis*, the microaerophilic bacteria *Helicobacter pylori*, the anaerobes *C. difficile* and *Bacteroides fragilis* and the facultative anaerobic bacteria *E. coli*. It is possible that the crude mixtures contained unreacted starting azide **3**, which we have shown in this study retains activity, and may influence the reported crude compound potency. A key finding by Miyamoto was that Mtz-triazoles were often active against metronidazole resistant (MtzR) strains of *T. vaginalis* (47% of compounds) and *G. lamblia* (100% of compounds), but not against *H. pylori frxA* and *rdxA* double mutant (1.4% of compounds).[12] From this result, we hypothesized that Mtz-triazoles may possess activity against metronidazole resistant *Clostridium difficile*. More recently Beena *et al.* described the activity of Mtz-triazoles against the anaerobic protozoan *Entamoeba histolytica*.[13] They tested a library of 10 Mtz-triazoles and found **4a**, **4h**, **4p** and **4q** to have potent activity (IC_{50} = 0.008- 2.36 µM) against *E. histolytica* HM1: IMSS.[13]

We now report an expansion and advancement of these approaches with the synthesis of a small library of purified Mtz-triazoles **4a-t** (>95 % purity, Scheme 1), including ten novel derivatives (**4b**, **4c**, **4f, 4g**, **4i**, **4j**, **4l**, **4o**, **4s** and **4t**). We also explore the antimicrobial and antiparasitic properties of the set of Mtz-triazoles and the parent azide **3** (>95% purity) and determine their activity against additional microbial targets that have not previously been examined. We evaluated the activity of Mtz-triazoles against the parasites *E. histolytica* and *G. lamblia*, multiple pathogenic strains of the anaerobe *C. difficile*, the microaerophile *H. pylori* and the facultative anaerobic bacterial ESKAPE pathogens *E. coli*, methicillin resistant *S. aureus* (MRSA), *Klebsiella pneumoniae*, *Acinetobacter baumanni* and *P. aeruginosa*. The antibacterial activity was measured against a stable MtzR *C. difficile* strain (CD26A54_R), the parent *C. difficile* strain with elevated metronidazole minimum inhibitory concentration (MIC) (CD26A54_S) and MtzR *H. pylori* clinical isolates, while cytotoxicity of the compounds was evaluated against mammalian liver (HepG2) and kidney (HEK293) cell lines.

2 Materials and Methods

2.1 Synthesis of Mtz-triazole library

Mtz-triazoles were synthesized from metronidazole via an azide intermediate by activation of the hydroxyl group with methanesulfonyl chloride (Scheme 1). Displacement of methanesulfonate **2** with sodium azide provided the desired azide **3** by nucleophilic substitution. The azide substituent was then reacted with a library of alkynes to give **4a-t** by copper-catalysed Huisgen 1,3-dipolar cycloaddition with copper sulfate and sodium ascorbate in methanol, with heating to 45 ºC and/or additional reagents added if monitoring indicated the reaction was incomplete. All alkynes utilized were commercially available with the exception of pyrazole alkyne **6**. Pyrazole alkyne **6** was prepared by reacting pyrazole **5** with propargyl bromide in the presence of potassium carbonate and *t*-butylammonium bromide as a phase transfer catalyst (Scheme 1).[14] All compounds were characterized by ^{1}H and ^{13}C NMR, LCMS and HRMS and detailed experimental procedures and characterization are provided in the supplementary information.

2.2 MIC assays

All compounds were tested for activity against a set of anaerobic *C. difficile* bacteria (630 ATCC BAA-1382, NAP1/027 M7404, NAP1/027 ATCC BAA-1803, VPI10463 ATCC 43255, CD26A54_S and CD26A54_R) and representative ESKAPE pathogens *S. aureus* (MRSA ATCC 43300), *E. coli* (ATCC 25922), *K. pneumoniae* (ATCC 700603), *A. baumannii* (ATCC 19606) and *P. aeruginosa* (ATCC 27853) using a standard broth microdilution assay. Selected compounds were tested for activity against *H. pylori* strains (26695, and clinical isolates MtzS 13/25, MtzR 98/285 and MtzR 13/61) using agar plate dilution, and for antimicrobial activity against twelve Gram-positive bacterial strains: *Enterococcus faecalis* (VanA clinical isolate), *Enterococcus faecium* (MDR VanA ATCC 51559), *Streptococcus pneumoniae* (MDR ATCC 700677), *S. aureus* (MRSA ATCC 43300, MRSA clinical isolate, MRSA/DRSA clinical isolate, GISA NARSA NRS 1, GISA NARSA NRS 17, VRSA NARSA VRS1, VRSA NARSA VRS4 and VRSA NARSA VRS10) using broth microdilution. *C. difficile* strains were grown at 37 °C in a COY anaerobic chamber (5 % H_2, 10 % CO_2, 85% N_2). *H. pylori* strains were grown at 37 °C under a microaerobic atmosphere (5 % O_2, 10 % CO_2). All facultative anaerobic bacteria were grown at 37 °C with normal atmospheric oxygen levels. All experiments were performed in duplicate with metronidazole, vancomycin, linezolid or colistin as

positive controls for relevant strains (see Table 1). Positive growth control of bacteria and DMSO as well as a negative control of only media were included for every plate. Full assay details are provided in the supplementary information.

2.3 Antiparasitic assays

Compounds were tested for antiparasitic activity against *E. histolytica* (HM1:1MSS strain) and *G. lamblia* (WB, BRIS/87/HEPU/713 (713),[15] BRIS/83/HEPU/106 (106)[16] and the metronidazole resistant syngeneic line 713-M3[15,16]) using an ATP-bioluminescence based screen for cell growth and survival.[17,18] Assay plates were inoculated with trophozoites ($5x10^3$ parasites/well) and incubated in the GasPak™ EZ Anaerobe Gas Generating Pouch Systems (VWR, West Chester, PA) to maintain anaerobic conditions throughout the incubation period. The assays were performed in triplicate using the CellTiter-Glo Luminescent Cell Viability Assay.[17] Metronidazole was used as a positive control.

2.4 Cytotoxicity

Compounds were tested for cytotoxicity against mammalian HepG2 and HEK293 cell lines, as detailed in the supplementary information.

3 Results and Discussion

3.1 Design of metronidazole-triazole conjugate library

The Mtz-triazole library was designed to contain a variety of structural groups with some compounds identical to those reported by Beena *et al*. (**4a**, **4h**, **4p** and **4q**).[10] Initial results against *C. difficile* showed that hydrophobic **4a** (R = phenyl) maintained activity relative to metronidazole **1** while more hydrophilic **4p** (R = hydroxyethyl) and **4q** (R = hydroxymethyl) lost activity. Therefore the subsequent library was biased towards exploring variance of the aromatic ring substitutions, extension of the position of the aromatic ring relative to the triazole core and replacement of the phenyl group with various heterocycles, with several of the latter selected due to their rating in terms of medicinal chemistry 'developability'.[19] Amine **4t**, and acids **4r** and **4s** were included to explore the structure activity relationships of non-aromatic ionisable groups. Compounds **4a**, **4d**, **4e**, **4h**, **4k**, **4m**, **4n**, **4q** and **4r** were reported by Miyamoto[12], but **4a**, **4h** and

4q were not tested against *C. difficile*. Compounds **4b**, **4c**, **4f, 4g**, **4i**, **4j**, **4l**, **4o**, **4s** and **4t** are described for the first time.

3.2 Antimicrobial and antiparasitic activity of metronidazole-triazole conjugates

The antimicrobial and antiparasitic spectrum of action of Mtz-triazoles was assessed against the anaerobic bacteria *C. difficile* and the anaerobic parasites *E. histolytica* and *G. lamblia* (Table 1). Variations to the phenyl group of **4a** were well tolerated and activity of **4b-e** was maintained against *C. difficile, E. histolytica* and *G. lamblia* (Table 1). Phenyl derivatives **4a-e** were several fold more active against *G. lamblia* than metronidazole. Pyridine **4h** and thiophene **4n** heterocycles also possessed broad-spectrum activity against *C. difficile, E. histolytica* and *G. lamblia*. However, we found that **4h** was not as potent compared to metronidazole in the ATP-bioluminescence parasite assay in contrast to the eosin-stain method used by Beena *et al.*[13] The pyrazole **4g** and pyrimidine **4i-j** maintained activity against *C. difficile* within one to two 2-fold dilutions of metronidazole. Compounds **4g** and **4i-j** were still active against *G. lamblia* compared to metronidazole, but were several fold less potent than the phenyl derivatives **4a-e**. The pyrazole **4g** and pyrimidines **4i-j** did not inhibit *E. histolytica* at 25 µM, showing that the R group can be used as a handle to tune the selectivity of this class towards different organisms. Compound **4k** (benzyl) and **4m** (CH_2NMe-benzyl) maintained broad-spectrum activity but **4l** (CHOH-phenyl) was inactive against *E. histolytica* at 25 µM.

Several compounds were inactive, or weakly active, against all three microorganisms at the highest concentration tested. These included the non-aromatic thiomorpholine dioxide **4o**, amine **4t** and the carboxylic acids **4r** and **4s**. The methyl hydroxyl **4q** and ethyl hydroxyl **4p** were inactive against *E. histolytica* and *G. lamblia* and weakly potent against *C. difficile*, continuing the trend of reduced activity with more polar substituents.

We also demonstrate that the parent azide **3** possesses potent activity against *C. difficile*, *E. histolytica* and *G. lamblia*. Therefore, when assessing combinatorial-like libraries of crude material for biological activity, the activity of any unreacted parent compounds is an important consideration, particularly when the compound warhead is maintained.

The MICs of **1**, **3** and **4a-t** were >32 µg/mL against the representative ESKAPE pathogens (MRSA, *E. coli*, *K. pneumoniae*, *A. baumannii* and *P. aeruginosa*) tested under aerobic conditions. In addition, the MIC values of a subset of compounds (metronidazole **1**, azide **3, 4a** (phenyl) and **4q** (CH_2OH)) were all >64 µg/mL against a panel of 8 additional drug resistant *S. aureus* strains, vancomycin resistant *E. faecalis*, vancomycin resistant *E. faecium* and multidrug resistant *S. pneumoniae*. This lack of activity is contrary to Beena's report, but consistent with the reported inactivity of 378 Mtz-triazoles against *E. coli* in Miyamoto's study, and the inactivity of metronidazole against facultative anaerobic bacteria.

3.3 Activity of metronidazole-triazole conjugates against C. difficile strain panel

The activity of the Mtz-triazoles did not vary significantly against multiple strains of *C. difficile*, including two major pathogenic strains of NAP1/027 and a VPI10463 strain associated with epidemics (Table 1). The MICs of individual compounds typically remained within one 2-fold dilution against the four strains of *C. difficile* tested. This is important, as viable drug candidates must possess appropriate broad strain coverage. A review of antibiotic drug candidates in development found that activity against multiple strains of *C. difficile* could vary substantially.[20] This can be explained since *C. difficile* has a highly mobile, mosaic genome and there is wide strain diversity between isolates.[21,22] Isolates can be divided across five main phylogenetic clades, multiple ribotypes and toxinotypes.[23] Recently, genomic epidemiology studies examining and tracking *C. difficile* outbreaks in Europe found that a high proportion of strains causing infection were not related to prior infectious strains.[22] Therefore, there is a large pool of genetically diverse strains in the community and selection and spread of intrinsically resistant strains could occur. However, the broad-strain activity of Mtz-triazoles against *C. difficile* in this study encourages further development of this class.

3.4 Activity of metronidazole-triazole conjugates against metronidazole resistant C. difficile

While metronidazole resistance has been reported in the clinic,[9,24] performing antimicrobial susceptibility testing against resistant *C. difficile* isolates is problematic since the resistance phenotype is unstable and often reported to be lost on freeze thaw cycles or on passaging of the isolates.[5] However, Lynch *et al.* have reported a stable metronidazole resistant (MtzR) *C. difficile* clone.[25] The activity of Mtz-triazoles was

assessed against this stable MtzR strain of *C. difficile* (CD26A54_R) and the parent metronidazole sensitive (MtzS) (CD26A54_S) strain, which lost the metronidazole resistance on freeze thawing, but still retained a slightly elevated MIC value to metronidazole compared to the control MtzS NAP1/027 strain. The MIC to metronidazole in BHIS broth at 48 hr was lower than the metronidazole MIC determined by E-test on BAKHS (4 µg/mL vs 48 µg/mL), similar to values reported by Chong *et al.* (8 µg/mL vs >32 µg/mL).[26]

The resistance to metronidazole became more apparent on incubation in broth for 48 hr (MIC = 2 µg/mL after 24 hr incubation vs MIC = 4 µg/mL after 48 hr) (Supplementary Table 1). In contrast, MICs against the control MtzS NAP1/027 strain ATCC 1803 did not change significantly between 24 hr and 48 hr (Supplementary Table 1). Although several compounds (**4a**, **4e** and **4n**) were active against MtzR *C. difficile* at 1 dilution lower than metronidazole (MIC = 2 µg/mL vs 4 µg/mL), none of the Mtz-triazoles were active against MtzR *C. difficile* at levels comparable to the activity of metronidazole against MtzS strains (MIC = 0.5 µg/mL). Reduced activity against the parent *C. difficile* strain CD26A54_S with intermediary metronidazole susceptibility was also observed.

3.5 Activity of metronidazole-triazole conjugates against additional G. lamblia strains including metronidazole resistant G. lamblia

Selected compounds (**4a-f**, **4h**, **4k-n**) were tested against two additional strains of MtzS *G. lamblia* (106 and 713) and one MtzR strain (713-M3) derived from the parent MtzS strain 713 (*Single column size:*

Table 2). The Mtz-triazoles displayed similar activity against the additional MtzS strains (106 and 713) compared to *G. lamblia* strain WB. Encouragingly, the Mtz-triazoles were all more potent than metronidazole against MtzR *G. lamblia* 713-M3. However, with the exception of **4d**, all of the Mtz-triazoles lost activity against the MtzR *G. lamblia* 713-M3 when compared to the parent MtzS strain 713, similar to the loss of activity against MtzR *C. difficile*. Mtz-triazoles of scaffold **4** were also all observed by Miyamoto *et al.* to lose activity against MtzR *G. lamblia* 713-M3 and 106-2ID10. However, while activity diminished against the MtzR 713M strain, the Mtz-triazoles were often still much more potent than metronidazole itself, meaning that the EC_{50} remained within a therapeutically meaningful window.

The activities of purified Mtz-triazoles (>95% purity) against *G. lamblia* strains 106, 713 and 713M were compared with the literature activities[12] determined with crude reaction mixtures (Supplementary Table 3). The activities were generally in close agreement, supporting the methodology used by Miyamoto,[12] although a 3.7 to 8 fold difference was observed for Mtz-triazoles **4d**, **4e** and **4m** against 1 strain of *G. lamblia*.

3.6 Activity of metronidazole-triazole conjugates against metronidazole resistant H. pylori

Selected compounds (4f-h, 4m and 4s) were tested against a panel of *H. pylori* strains using the CLSI agar dilution susceptibility method (
Single column size:

Table 3).[27] These strains included the reference strain 26695 and three clinical isolates 13/25 (metronidazole E-test MIC = 2 µg/mL), 98/285 (E-test MIC = 24 µg/mL) and 13/61 (E-test MIC = 256 µg/mL) with a range of resistance levels to metronidazole determined by the E-test. The susceptibilities of the strains to metronidazole determined by the agar dilution method differed from the susceptibilities obtained from the E-test as has been described previously.[28] The methyl ester **4f** was weakly active against *H. pylori*, while the carboxylic acid **4s** was inactive against all *H. pylori* strains as observed with *E. histolytica* and *G. lamblia* as well. Compounds **4g**, **4h** and **4m** were more active than metronidazole against the MtzS strains (26695 and clinical isolate 13/25) and 1-2 dilutions more active than metronidazole against the MtzR strains (clinical isolates 98/285 and 13/61) but they could not completely overcome metronidazole resistance, similar to the loss of activity against metronidazole resistant *C. difficile*.

3.7 Cytotoxicity

All compounds, including metronidazole **1**, azide **3,** and **4a-t** showed no cytotoxicity against HepG2 and HEK293 cells at concentrations up to 100 µM (CC_{50}). This is consistent with the findings by Miyamoto *et al.* which did not observe cytotoxicity against HeLa cells at 50 µM.[12]

3.8 Relationship of compound activity with compound properties

In order to understand the relationship between compound properties and activity, compound properties including logP (or logD at pH = 7.4), molecular weight, topological polar surface area (tPSA), and predicted solubility (logS) were examined for correlation with activity against MtzS strains of *C. difficile*, *E. histolytica* and *G. lamblia*, with the activity expressed as pMIC ($-\log_{10}$MIC) or pEC_{50} ($-\log_{10}EC_{50}$)) (Supplementary Table 4). Inactive compounds were included in the analysis at the highest concentration of compound tested. A correlation can be observed between the logD values and the activity against *G. lamblia* (with R^2 (linear regression) = 0.84, Supplementary Figure 1) (Figure 2). The logD of the most potent compounds (**4a-e**, **4h**, **4k** and **4n**) against *G. lamblia* is between 1.5 and 3.0, with activity decreasing with lower logD. In contrast, only a mild correlation could be detected between logD and the activity against *C. difficile* (R^2 = 0.68, Supplementary Figure 1) and no correlation for activity against *E. histolytica* (R^2 = 0.48, Supplementary Figure 1). This might explain why the compounds with aromatic phenyl, thiophene and pyridine groups were more potent against *G. lamblia* than *E. histolytica* and *C. difficile*. Increasing the hydrophobicity (logD) of compounds to improve potency is generally considered undesirable in drug development due to the tendency for increased metabolism[29] and increased promiscuity[30]. However, in this case the antibiotic metronidazole has a low logD to begin with, such that the relatively higher logD of the compounds explored in this study does not place the compounds in an undesirable chemical space. On the other hand, the site of infection of *G. lamblia* is the small intestine, where low (< 1) or high (> 3) logD values,[29] associated with reduced systemic uptake, might be beneficial for efficacy. However, metronidazole is 100 % oral bioavailable[31] and so the importance of bioavailability for *in vivo* efficacy against *G. lamblia* infections is unclear.

4 Conclusion

Mtz-triazoles were synthesized and assessed for activity against the anaerobe *C. difficile*, microaerophile *H. pylori*, the parasites *E. histolytica* and *G. lamblia* as well as facultative anaerobic Gram-positive and Gram-negative bacteria. While no activity was observed against the facultative anaerobic bacteria, nine compounds were identified with potent broad spectrum activity against anaerobic organisms, while having no cytotoxicity against mammalian cell lines. Hydrophobic R groups such as the benzyl **4k**, varyingly

substituted phenyl derivatives **4a-e** and heterocyclic R groups such as pyridine **4h** and thiophene **4n** were well tolerated and favored broad spectrum anaerobic activity. In contrast, polar R groups including pyrimidines **4i-j**, carboxylic acids **4p** and **4r** and methyl amine **4t** either resulted in loss of broad spectrum activity or were inactive against all organisms tested.

While active Mtz-triazoles displayed a narrow MIC range against multiple strains of *C. difficile* and *G. lamblia*, there was metronidazole cross resistance against the stable MtzR *C. difficile* strain CD26A54_R, MtzR *G. lamblia* strain 713M and clinical isolates of MtzR *H.pylori*. Mtz-triazoles **4a-t** generally lost activity against MtzR *C. difficile*, MtzR *G. lamblia* and MtzR *H. pylori*. However, since the Mtz-triazoles were often much more potent than metronidazole against *G. lamblia*, the EC_{50} remained within a therapeutically meaningful window. It is possible that surveying a more diverse chemical space of R group substituents or using alternative 5-nitroimidazole scaffolds could provide access to Mtz-triazoles that are even more potent and therefore active against MtzR strains of *C. difficile* and *G. lamblia*. Future work will focus on identifying such compounds and explore their *in vivo* efficacy against anaerobic pathogens.

5 Supporting Information

The following file is available free of charge.

SI_Mtztriazole_EuJMedChem: Detailed descriptions of experimental procedure for chemical synthesis, biological assays and compound activity analysis. Supplementary Tables 1-4, Supplementary Figure 1.

6 Abbreviations

MIC- minimum inhibition concentration

MtzS- metronidazole sensitive

MtzR- metronidazole resistant

Clin. Isol.- clinical isolate

7 Author Information

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8 Author Contribution

Planned experiments: AMJ, TK, AD, MAB, MAC

Performed experiments: AMJ, AD, CYT, JH, GK, AG, YM, SR, AK, JZ

Wrote paper: AMJ, TK, LE, MAB, MAC

9 Conflict of Interest

The authors declare no competing financial interest.

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12 Figures

Single column size:

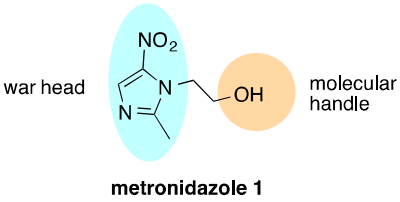

Figure 1. Metronidazole contains the 5-nitroimidazole warhead and a hydroxyl group amenable to modification

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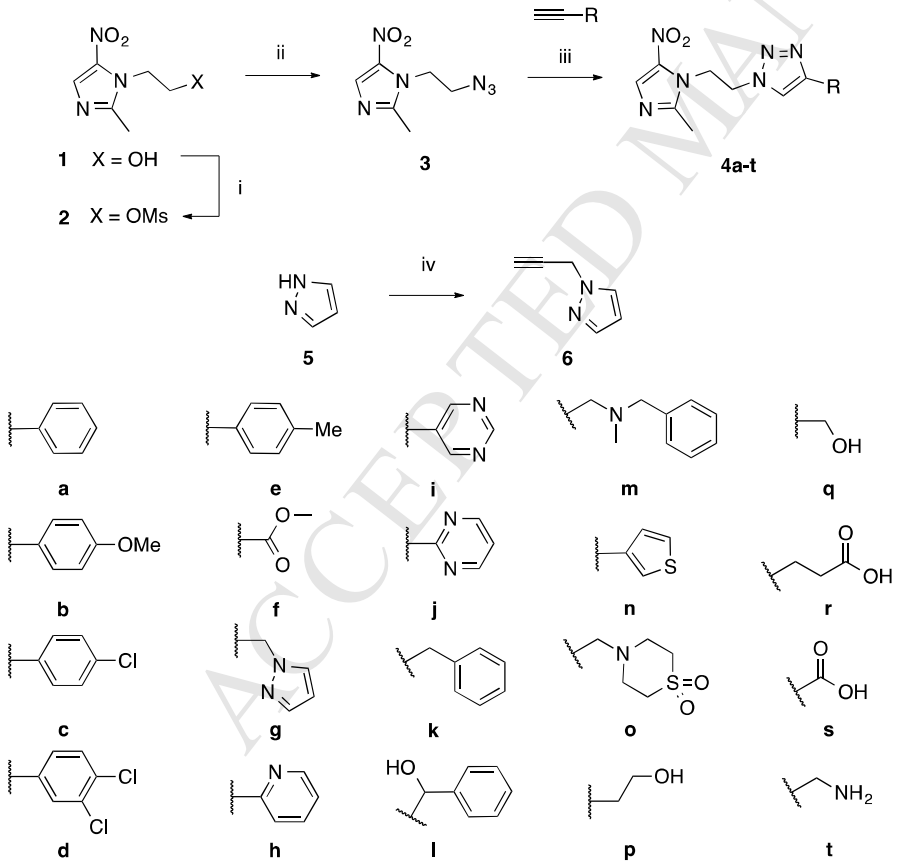


Scheme 1: Synthesis of metronidazole-triazole conjugate library. i) MsCl, TEA, DCM, 2 ºC to rt, 80 min, 85% yield ii) NaN_3, DMF, 50 ºC, 21 hr, quant. yield iii) $CuSO_4$, sodium ascorbate, MeOH, rt or 45 ºC, 20 min – 48 hr, 9 – 97% iv) propargyl bromide (80% w/v in toluene), K_2CO_3, TBAB, rt, 3 days, 16%.

Single column size:

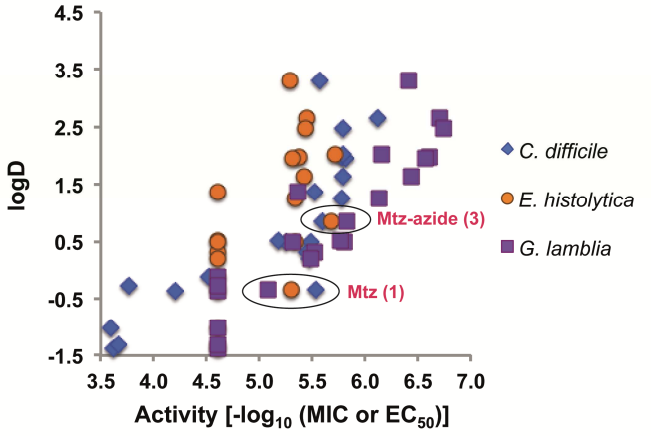


Figure 2: Correlation of activity against *G. lamblia*, *E. histolytica* and *C. difficile* with logD. Compounds with activity against parasites (EC_{50} < 25 µM) and *C . difficile* (MIC ≤ 8 µg/mL) have pEC50 or pMIC > 5.0.

13 Tables

Double column size:

Table 1: Activity of Mtz-triazoles against *C. difficile*, ESKAPE bacteria, *E. histolytica* and *G. lamblia*.

Compound		MIC (μg/mL)							EC_{50} (*μM*)	
		C. difficile						*ESKAPE bacteria*[h]	*E. histolytica*	*G. lamblia*
		630[b]	VPI 10463[e]	NAP1/ 027[c]	NAP1/ 027[d]	NAP1/ 027[f]	NAP1/ 027[g]		HM1:IMSS	WB
		MtzS	MtzS	MtzS	MtzS	MtzS	MtzR		MtzS	MtzS
1	metronidazole	0.5	0.5	0.5	0.5	1	4	>32	5	7.9
3	azide	1	0.5	0.5	0.5	2	8	>32	2.1	1.5
4a	phenyl	0.5	0.25-0.5	0.5	0.5	1	2	>32	4.2	0.25
4b	p-OMe-phenyl	0.5	0.25-0.5	0.5	0.5	1	4	>32	4.8	0.27
4c	p-Cl-phenyl	0.5	0.25	0.25	0.25	2	16	>32	3.6	0.20
4d	p,m-Cl_2-phenyl	0.5	0.5-1	1	1	1	8-16	>32	5.2	0.39
4e	p-Me-phenyl	0.5	0.25	0.5	0.5	1	2	>32	3.7	0.18
4f	methyl ester	1	1	1	1	2	4	>32	>25	3.0
4g	pyrazole	1	1	1	1	2	4	>32	>25	4.9
4h	pyridine	0.5	0.5	0.5	0.5	1	4	>32	4.6	0.74
4i	5-pyrimidine	1	1	0.5-1	1	1	4	>32	>25	3.3
4j	2-pyrimidine	2	2	2	2	2-4	4	>32	>25	1.7
4k	benzyl	0.5	0.25-0.5	0.5	0.5	1	4	>32	1.9	0.70
4l	CHOH-phenyl	1	0.5	1	0.5-1	2	4	>32	>25	4.4
4m	CH_2NMe-benzyl	2	1-2	1	2	2	8	>32	4.6	1.6
4n	thiophene	0.5	0.25-0.5	0.5	0.5	1	2	>32	3.8	0.37
4o	thiomorpholine	>64	>64	>64	>64	>64	>64	>32	>25	>25
4p	CH_2CH_2OH	8	8	16	8	8	16	>32	>25	>25
4q	CH_2OH	16	16	16-32	16	16-32	16	>32	>25	>25
4r	CH_2CH_2COOH	>64	>64	>64	>64	>64	>64	>32	>25	>25
4s	COOH	64	64	64	64	>64	>64	>32	>25	>25
4t	CH_2NH_2	>64	>64	>64	>64	>64	>64	>32	>25	>25

[a]MIC results determined against CD26A54_S and CD26A54_R after 48 hr growth, all other *C. difficile* strain MIC results determined at 24 hr. See Supplementary Table 1 for MIC at 24 hr for CD26A54_S and CD26A54_R and at 48 hr for ATCC BAA-1803. *C. difficile* MICs are the median of at least n = 4, except for CD26A54_R where n = 8. ESKAPE pathogen MICs were performed in a single concentration screen with n = 3. EC_{50} results n = 3.

[b]ATCC BAA-1382, [c]ATCC 43255, [d]M7404, [e]ATCC BAA-1803, [f]CD26A54_S, [g]CD26A54_R

[h]*S. aureus* MRSA (ATCC 43300), *E. coli* (ATCC 25922), *K. pneumoniae* (ATCC 700603), *A. baumannii* (ATCC 19606) and *P. aeruginosa* (ATCC 27853).

Single column size:

Table 2. EC_{50} of selected Mtz-triazoles against *G. lamblia* strains. The EC_{50} values for *G. lamblia* WB strain are shown from Table 1 for comparison.

Compound		EC_{50} (μM) *G. lamblia*			
		WB	106	713	713M
		MtzS	MtzS	MtzS	MtzR
1	metronidazole	7.9	2.8	2.3	17
4a	phenyl	0.25	0.28	0.16	2.3
4b	p-OMe-phenyl	0.27	0.64	0.64	1.0
4c	p-Cl-phenyl	0.20	0.89	1.2	3.5
4d	p,m-Cl_2-phenyl	0.39	1.1	2.5	1.2
4e	p-Me-phenyl	0.18	0.29	0.29	1.0
4f	methyl ester	3.0	2.0	1.8	3.1
4h	pyridine	0.74	0.38	0.28	0.95
4k	benzyl	0.70	0.51	0.32	1.1
4l	CHOH-phenyl	4.4	2.2	2.4	3.0
4m	CH_2NMe-benzyl	1.6	1.1	0.71	2.2
4n	thiophene	0.37	0.34	0.18	0.9

Single column size:

Table 3. MIC of selected Mtz-triazoles against *H. pylori* strains

Compound		MIC (μg/mL) *H. pylori*			
		26695	Clin. Isol. 13/25	Clin. Isol. 98/285	Clin. Isol. 13/61
		MtzS	MtzS	MtzR	MtzR
1	metronidazole	8	4-8	64	32-64
4f	methyl ester	32-64	32-64	64->64	64->64
4g	pyrazole	2-4	2	16-32	16-32
4h	pyridine	2	1	32	16
4m	CH_2NMe-benzyl	4	2	16->64	16
4s	COOH	>64	>64	>64	>64

Clin. Isol. = clinical isolate

Highlights:

Metronidazole-triazole conjugates: activity against *Clostridium difficile* and parasites

Angie M. Jarrad[a], Tomislav Karoli[a,e], Anjan Debnath[b], Chin Yen Tay[d], Johnny X. Huang[a], Geraldine Kaeslin[a], Alysha G. Elliott[a], Yukiko Miyamoto[c], Soumya Ramu[a], Angela M. Kavanagh[a], Lars Eckmann[c], Mark A.T Blaskovich[a] and Matthew A. Cooper[a,*]

- A series of 20 metronidazole (Mtz)-triazole conjugates were synthesised
- Several Mtz-triazoles had increased potency against pathogenic anaerobes
- The compounds were not cytotoxic at 100 µM against mammalian cell lines
- Cross-resistance was observed against Mtz-resistant bacteria and parasites
- Therapeutically relevant activity retained against Mtz-resistant *G. lamblia*

Supplementary Information

Metronidazole-triazole conjugates: activity against *Clostridium difficile* and parasites

Angie M. Jarrad, Tomislav Karoli, Anjan Debnath, Chin Y. Tay, Johnny X. Huang, Geraldine Kaeslin, Alysha G. Elliott, Yukiko Miyamoto, Soumya Ramu, Angela M. Kavanagh, J. Zuegg, Lars Eckmann, Mark A.T. Blaskovich and Matthew A. Cooper

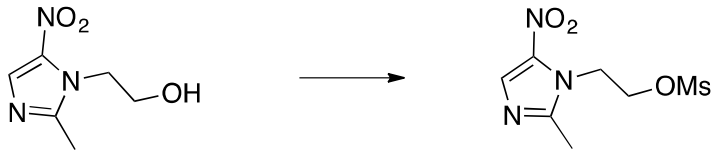
Table of Contents

1 General Methods

Reagents and anhydrous solvents (dimethylformamide, dichloromethane, and acetonitrile) were used as received. Reactions requiring anhydrous conditions were performed under an inert atmosphere of nitrogen. Reactions were monitored by thin layer chromatography (TLC) or LCMS. Analytical TLC was performed on Merck TLC aluminium sheets pre-coated with Silica Gel 60 F-254 and compounds were visualised using UV lamp and potassium permanganate stain. Melting points were determined using a Gallenkamp melting point apparatus and are corrected to a standard curve of the measured and literature melting points of vanillin, acetyl salicylic acid, 3-phenoxybenzoic acid and caffeine standards. Analytical LCMS was performed on Shimadzu LCMS using 0.05% formic acid in water as solvent A and 0.05% formic acid in acetonitrile as solvent B. LCMS conditions (solvent A = H_2O + 0.05% formic acid, solvent B = acetonitrile + 0.05% formic acid): Standard (unspecified): Column Zorbax Eclipse XDB-Phenyl, 3.0×100mm, 3.5 µ: Flow: 1 mL/min: Gradient timetable: 0.00 min, 5% B; 3.00 min, 100% B; 3.7 min, 100% B; 5.00 min, 5% B. Waters Column: Waters Atlantis T3, 2.1×50mm, 5 µ: Flow 1 mL/min, Gradient timetable: 0.00 min, 0% B; 3.30 min, 25% B; 3.50 min, 100% B; 4.00 min 100% B; 5.00 min, 0% B; 13.00 min, 5.0% B. Detection: UV at 254 nm, ELSD and electrospray MS. Compounds were purified using Biotage Isolera, Gilson or Grace Reveleris X2 chromatography systems. Commercially available cartridges were used for MPLC chromatography: Biotage SNAP cartridge HP-Silica 10, 25 or 50 g, Reveleris 4 g Silica (40 µm) cartridge or Reveleris C18 Reversed-Phase 12 g cartridge. All final products were obtained in >95% purity as determined by HPLC using UV at 254 nm, ESIMS and ELSD detection. NMR data were collected and calibrated in DMSO-d_6 or $CDCl_3$ at 298K on a Varian Unity 400 MHz or Bruker Avance-600 MHz spectrometers. Where appropriate, ^{1}H-coupling constants were examined using resolution enhancement with MestReNova software. Data are presented as follows: chemical shift (ppm), multiplicity (s = singlet, d = doublet, t= triplet, q = quartet, quin = quintet, m = multiplet, br = broad), coupling constant (Hz) and integration. High resolution mass spectrometry (HRMS) was performed on a Bruker MicroTOF mass spectrometer using (+)-ESI calibrated to HCOONa. For compounds purified by reverse phase chromatography, the exact concentration of the compounds was determined by the quantitative NMR integration 'PULCON' experiment.[1] These settings were used for all PULCON experiments: relaxation delay of 30 s, 8 scans, 2 dummy scans, 90° pulse and temperature at 298 K. The formula weight obtained from PULCON experiments was also used to calculate the percentage yields.

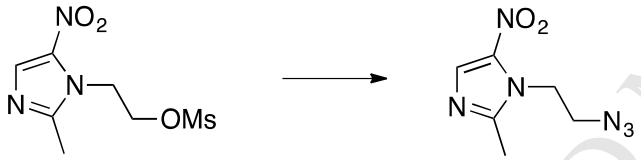
2 Synthesis

2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl methanesulfonate; ***2***



Methanesulfonyl chloride (2.7 mL, 35.0 mmol) was added dropwise to a stirring suspension of metronidazole (4.99 g, 29.2 mmol) and TEA (6.1 mL, 43.7 mmol) in anh. DCM (25 mL) cooled to 2 ℃. The reaction was warmed to room temperature and stirred for 1 hr 20 min. Volatiles were removed *in vacuo*. The solid was washed with water (40 mL), triturated with pet. spirits (20 mL) and washed with DCM (15 mL) and then dried under vacuum. An off-white solid was obtained (6.18 g, 85%). LCMS: R_t = 2.28 min, 99 A% @ 254 nm, $[M + H]^+$ = 249.8. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.06 (s, 1H), 4.65 (dd, *J* = 5.4, 4.5 Hz, 2H), 4.55 (dd, *J* = 5.4, 4.4 Hz, 2H), 3.15 (s, 3H), 2.46 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 150.7, 138.4, 133.1, 68.4, 45.1, 36.7, 14.0. HRMS calcd for $C_7H_{11}N_3NaO_5S$ $[M + Na]^+$, 272.0312; found, 272.0312.

1-(2-azidoethyl)-2-methyl-5-nitro-1H-imidazole; ***3***



To stirring suspension of mesylate (4.50 g, 18.1 mmol) in anh. DMF (45 mL) was added NaN_3 (1.48 g, 22.8 mmol). The reaction was stirred under N_2 at 50 ˚C overnight before pouring into H_2O (450 mL) and extracting with EtOAc (3 x 360 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and volatiles removed *in vacuo* to give an orange solid (3.60 g, 100%). LCMS: Rt = 2.50 min, 99 A% @ 254 nm, $[M + H]^+$ = 197.1. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.06 (s, 1H), 4.48 (dd, *J* = 6.1, 5.3 Hz, 2H), 3.79 (dd, *J* = 6.1, 5.3 Hz, 2H), 2.48 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 151.5, 138.3, 133.1, 50.0, 44.9, 14.0. HRMS calcd for $C_6H_8N_6NaO_2$ $[M + Na]^+$, 219.0601; found, 219.0590.

Triazole General Procedure A:

To a stirring solution of **3** (1 eq.) in MeOH (5 vol) was added alkyne (1 eq.) followed by 100 mM aq. $CuSO_4$ (5 mol %) and 100 mM aq. sodium ascorbate (10 mol %).

Triazole General Procedure B:

As for General procedure A, except that if the reaction was not proceeding when monitored by LCMS, the reaction was warmed to 45 ℃ and an addit ional portion of methanol (5 vol) was added.

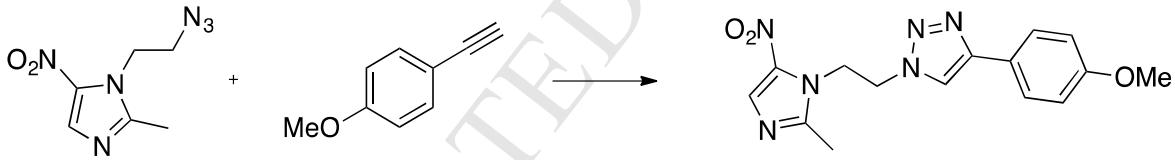
Triazole General Procedure C:

As for General procedure A, except that if the reaction was not proceeding when monitored by LCMS, further portions of 100 mM aq. $CuSO_4$ (5 mol %) and 100 mM aq. sodium ascorbate (10 mol %) were added.

1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-phenyl-1H-1,2,3-triazole; ***4a***

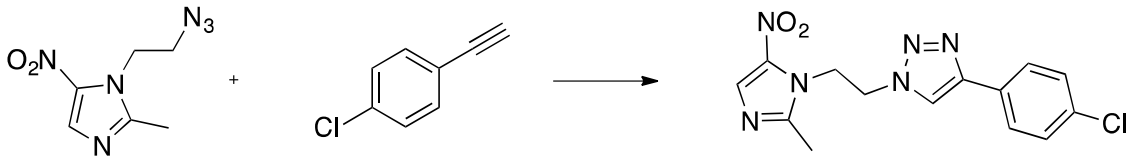
General procedure A. The reaction mixture was concentrated to dryness and purified by MPLC over silica gel (Biotage, 75- 100% EtOAc in pet. spirits then 0-10% MeOH in EtOAc) to give a colourless solid (21 mg, 68%). LCMS: R_t = 2.51 min, 99 A% @ 254 nm, $[M+ ACN + H]^+$ = 340.0. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.44 (s, 1H), 8.07 (s, 1H), 7.86 – 7.62 (m, 2H), 7.49 – 7.41 (m, 2H), 7.39 – 7.30 (m, 2H), 4.88 (dd, *J* = 7.1, 6.1 Hz, 2H), 4.77 (dd, *J* = 6.9, 5.8 Hz, 2H), 1.92 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 151.2, 146.5, 138.4, 133.3, 130.3, 128.9, 128.0, 125.1, 122.2, 48.9, 46.1, 12.9. HRMS calcd for $C_{14}H_{14}N_6NaO_2$ $[M + Na]^+$, 321.1070; found, 321.1063.

1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(4-methoxyphenyl)-1H-1,2,3-triazole; ***4b***



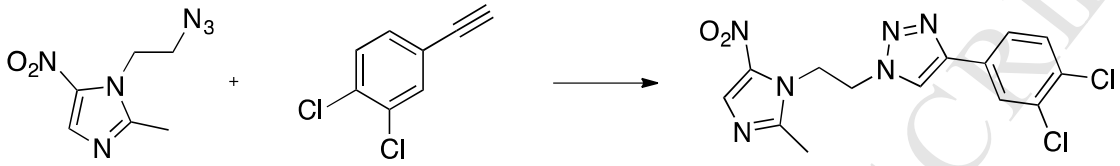
General procedure A. The reaction mixture was concentrated to dryness and purified by MPLC over silica gel (Gilson, 100% EtOAc to 30% MeOH) to give a colourless solid (71 mg, 42%). LCMS: R_t = 2.50 min, 99 A% @ 254 nm, $[M + H]^+$ = 329.3. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.31 (s, 1H), 8.06 (s, 1H), 7.71 – 7.65 (m, 2H), 7.04 – 6.98 (m, 2H), 4.85 (dd, *J* = 6.5, 4.5 Hz, 2H), 4.76 (dd, *J* = 6.6, 4.4 Hz, 2H), 3.78 (s, 3H), 1.91 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 159.1, 151.2, 146.5, 138.4, 133.3, 126.5, 122.9, 121.3, 114.3, 55.1, 48.8, 46.1, 12.9. HRMS calcd for $C_{15}H_{16}N_6NaO_3$ $[M + Na]^+$, 351.1176; found, 351.1178.

1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(4-chlorophenyl)-1H-1,2,3-triazole; ***4c***



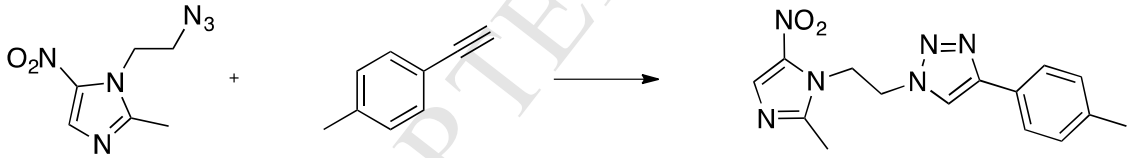
General procedure B. The reaction mixture was concentrated to dryness and purified by MPLC over silica gel (Biotage, 0- 10% MeOH in DCM) to obtain a colourless solid (82 mg, 48%). LCMS: R_t = 2.69 min, 99 A% @ 254 nm, $[M + H]^+$ = 332.9. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.49 (s, 1H), 8.06 (s, 1H), 7.81 – 7.75 (m, 2H), 7.54 – 7.49 (m, 2H), 4.88 (dd, *J* = 6.4, 4.7 Hz, 2H), 4.77 (dd, *J* = 6.6, 4.6 Hz, 2H), 1.94 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 151.2, 145.4, 138.3, 133.3, 132.4, 129.2, 129.0, 126.8, 122.6, 48.9, 46.0, 12.9. HRMS calcd for $C_{14}H_{13}ClN_6NaO_2$ $[M + Na]^+$, 355.0681; found, 355.0682.

1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(3,4-dichlorophenyl)-1H-1,2,3-triazole; ***4d***



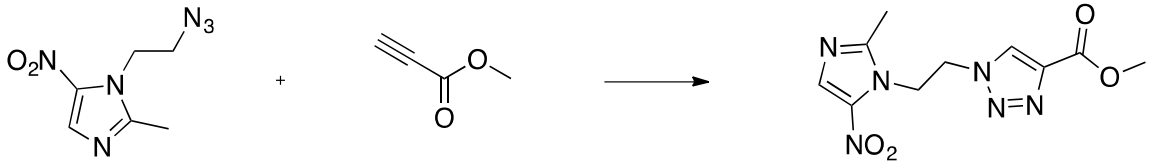
General procedure B. The reaction mixture was concentrated to dryness and purified by MPLC over silica gel (Biotage, 0-10% MeOH in DCM) to obtain a colourless pearlescent solid (129 mg, 69%). LCMS: R_t = 2.86 min, 99 A% @ 254 nm, $[M + H]^+$ = 366.9. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.59 (s, 1H), 8.06 (s, 1H), 8.02 (d, *J* = 2.0 Hz, 1H), 7.77 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.72 (d, *J* = 8.3 Hz, 1H), 4.89 (dd, *J* = 6.5, 4.7 Hz, 2H), 4.77 (dd, *J* = 6.5, 4.7 Hz, 2H), 1.96 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 151.2, 144.3, 138.3, 133.3, 131.7, 131.3, 131.0, 130.3, 126.7, 125.1, 123.3, 49.0, 45.9, 13.0. HRMS calcd for $C_{14}H_{12}Cl_2N_6NaO_2$ $[M + Na]^+$, 389.0291; found, 389.0292.

1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(p-tolyl)-1H-1,2,3-triazole; ***4e***



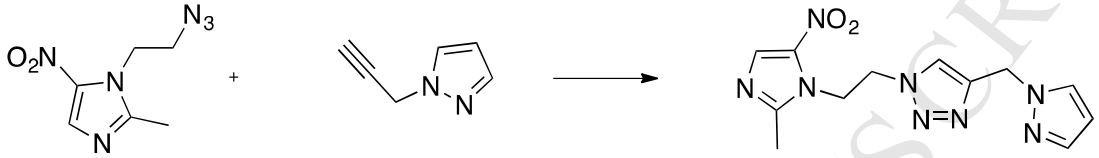
General procedure A. The reaction mixture was concentrated to dryness and purified by MPLC over silica gel (Biotage, 0- 10% MeOH in DCM gradient) to give a colourless solid (129 mg, 81%). LCMS: R_t = 2.62 min, 99 A% @ 254 nm, $[M + H]^+$ = 313.0. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.37 (s, 1H), 8.06 (s, 1H), 7.67 – 7.61 (m, 2H), 7.27 – 7.22 (m, 2H), 4.86 (dd, *J* = 6.5, 4.5 Hz, 2H), 4.76 (dd, *J* = 6.6, 4.5 Hz, 2H), 2.32 (s, 3H), 1.92 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 151.2, 146.6, 138.3, 137.3, 133.2, 129.4, 127.6, 125.0, 121.8, 48.8, 46.0, 20.8, 12.9. HRMS calcd for $C_{15}H_{16}N_6NaO_2$ $[M + Na]^+$, 335.1227; found, 335.1222.

1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(methoxycarbonyl)- 1H-1,2,3-triazole; ***4f***



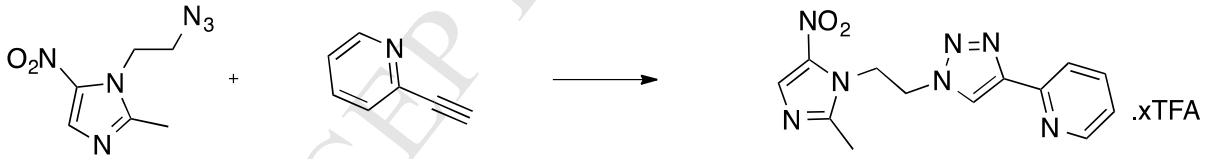
General procedure A. The reaction mixture was concentrated to dryness and purified by MPLC over silica gel (Biotage, 2-10% MeOH in DCM gradient and then Grace Reveleris X2, 2-10% MeOH in DCM gradient). The product crystallised from a fraction and was collected by filtration to give a colourless solid (43 mg, 30%). Mp = 197 - 198 ℃ (decomposed). LCMS: R_t = 2.02 min, 99 A% @ 254 nm, $[M + H]^+$ = 280.8. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.71 (s, 1H), 8.05 (s, 1H), 4.90 (dd, *J* = 6.5, 4.4 Hz, 2H), 4.77 (dd, *J* = 6.6, 4.5 Hz, 2H), 3.82 (s, 3H), 1.93 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.4, 151.0, 138.6, 138.4, 133.2, 130.0, 51.8, 49.1, 45.7, 12.9. HRMS calcd for $C_{10}H_{12}N_6NaO_4$ $[M + Na]^+$, 303.0812; found, 303.0814.

1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-((1H-pyrazol-1-yl)methyl)-1H-1,2,3-triazole; ***4g***



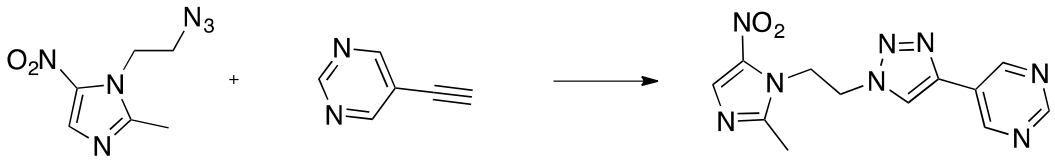
General procedure A. The reaction mixture was concentrated to dryness and the residue was purified by MPLC over silica gel (Biotage, 2- 15% MeOH in DCM gradient). The product was obtained as a colourless solid (57 mg, 55%). LCMS: R_t = 2.03 min, 99 A% @ 254 nm, $[M + H]^+$ = 302.9. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.03 (s, 1H), 7.95 (s, 1H), 7.71 (dd, *J* = 2.3, 0.7 Hz, 1H), 7.42 (dd, *J* = 1.9, 0.7 Hz, 1H), 6.24 (t, *J* = 2.1 Hz, 1H), 5.37 (s, 2H), 4.81 (dd, *J* = 6.5, 4.4 Hz, 2H), 4.69 (dd, *J* = 6.5, 4.5 Hz, 2H), 1.79 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 151.2, 143.3, 138.9, 138.3, 133.2, 129.6, 124.6, 105.4, 48.7, 46.2, 46.1, 12.7. HRMS calcd for $C_{12}H_{14}N_8NaO_2$ $[M + Na]^+$, 325.1132; found, 325.1129.

1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(2-pyridinyl)-1H-1,2,3-triazole.xTFA; ***4h***



General procedure A. The crude mixture was diluted with water and the organics were extracted with DCM (3 x 50 mL). The combined organics were washed with brine, dried over anh. $MgSO_4$ and filtered. Volatiles were removed *in vacuo* and the residue was purified by MPLC over C18 silica gel (Grace Reveleris X2, A: H_2O + 0.1% TFA, B: ACN + 0.1% TFA, 5-30% B). The eluent was removed by lyophilisation to give a yellowish solid (148 mg, 97%). LCMS: R_t = 2.08 min, 99 A% @ 254 nm, $[M + H]^+$ = 299.8. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.59 (ddd, *J* = 5.0, 1.8, 1.0 Hz, 1H), 8.55 (s, 1H), 8.06 (s, 1H), 8.03 (dt, *J* = 8.0, 1.1 Hz, 1H), 7.92 (td, *J* = 7.7, 1.8 Hz, 1H), 7.38 (ddd, *J* = 7.6, 4.9, 1.2 Hz, 1H), 4.92 (dd, *J* = 6.5, 4.4 Hz, 2H), 4.80 (dd, *J* = 6.5, 4.4 Hz, 2H), 1.93 (s, 3H). ^{13}C NMR (150 MHz, DMSO- d_6) δ 158.5, 158.3, 158.0, 157.8, 151.1, 149.3, 147.0, 138.4, 137.7, 133.1, 124.4, 123.2, 119.6, 49.0, 46.0, 12.9. HRMS calcd for $C_{13}H_{14}N_7O_2$ $[M + H]^+$, 300.1203; found, 300.1194.

1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(5-pyrimidinyl)-1H-1,2,3-triazole; ***4i***

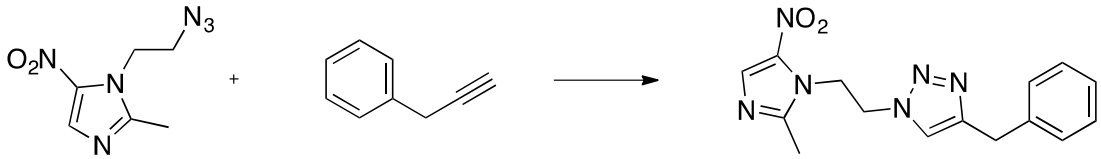


General procedure A. The reaction was diluted with DCM (10 mL). The tan ppt was then collected by filtration. The organic layer filtrate was evaporated to give a crude crystalline solid. The ppt and crystals were combined and purified by MPLC over C18 silica gel (Grace Reveleris X2, A: H_2O + 0.1% TFA, B: ACN + 0.1% TFA, 5-30% B). The eluent was removed by lyophilisation to give a colourless powder (86 mg, 56%). LCMS: R_t = 1.99 min, 99 A% @ 254 nm, $[M + H]^+$ = 300.8. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.19 (s, 2H), 9.18 (s, 1H), 8.70 (s, 1H), 8.07 (s, 1H), 4.94 (dd, *J* = 6.5, 4.7 Hz, 2H), 4.79 (dd, *J* = 6.5, 4.8 Hz, 2H), 2.00 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 157.7, 153.3, 151.3, 140.8, 138.4, 133.3, 124.7, 123.7, 49.1, 45.9, 13.0. HRMS calcd for $C_{12}H_{12}N_8NaO_2$ $[M + Na]^+$, 323.0975; found, 323.0966.

1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(2-pyrimidinyl)-1H-1,2,3-triazole; ***4j***

General procedure A. The reaction was poured into water (20 mL) and extracted with DCM (4 x 50 mL). The combined organics were washed with brine (50 mL), dried over anh. $MgSO_4$ and filtered. The volatiles were removed *in vacuo* and the crude was purified by MPLC over C18 silica gel (Grace Reveleris X2, A: H_2O + 0.1% TFA, B: ACN + 0.1% TFA, 5-25%) then repurified (Grace Reveleris X2, A: H_2O + 0.1% TFA, B: ACN + 0.1% TFA, 5-100% B) to give a cream powder (16 mg, 9%). LCMS: R_t = 2.21 min, 99 A% @ 254 nm, $[M + H]^+$ = 300.8. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.88 – 8.84 (m, 2H), 8.69 (s, 1H), 8.06 (s, 1H), 7.44 (t, *J* = 4.9 Hz, 1H), 4.93 (dd, *J* = 6.5, 4.4 Hz, 2H), 4.80 (dd, *J* = 6.6, 4.4 Hz, 2H), 1.93 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 158.3, 157.8, 151.1, 146.5, 138.4, 133.2, 127.3, 120.2, 48.9, 46.0, 12.9. HRMS calcd for $C_{12}H_{12}N_8NaO_2$ $[M + Na]^+$, 323.0975; found, 323.0976.

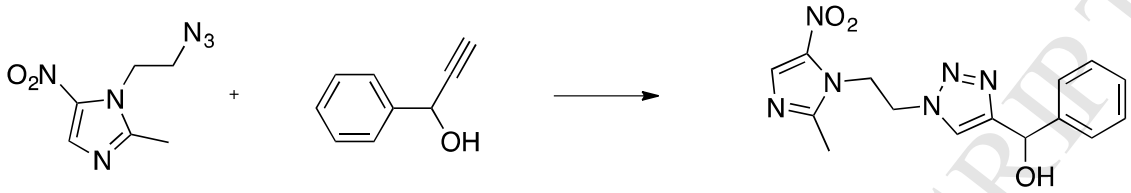
1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)- 4-benzyl-1H-1,2,3-triazole; ***4k***



General procedure A. The reaction mixture was concentrated to dryness and purified by MPLC over silica gel (Biotage, 0- 10% MeOH in DCM gradient) to give a colourless solid (34 mg, 21%).

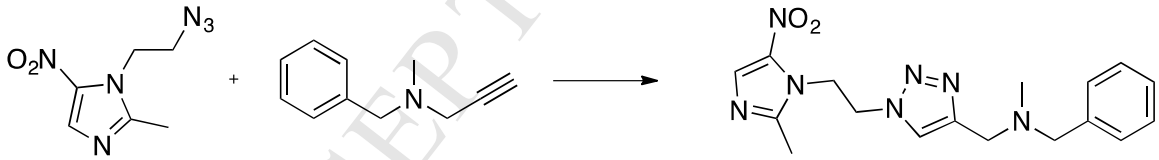
LCMS: R_t = 2.50 min, 99 A% @ 254 nm, $[M + H]^+$ = 313.0. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.03 (s, 1H), 7.69 (t, *J* = 0.7 Hz, 1H), 7.32 – 7.25 (m, 2H), 7.23 – 7.15 (m, 3H), 4.77 (dd, *J* = 6.5, 4.3 Hz, 2H), 4.68 (dd, *J* = 6.5, 4.4 Hz, 2H), 3.95 (s, 2H), 1.80 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 151.1, 146.4, 139.4, 138.3, 133.2, 128.4, 128.3, 126.1, 123.4, 48.5, 46.2, 31.0, 12.8. HRMS calcd for $C_{15}H_{16}N_6NaO_2$ $[M + Na]^+$, 335.1227; found, 335.1224.

1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(1-phenyl-1-hydroxylmethyl)- 1H-1,2,3-triazole; ***4l***



General procedure A. The reaction mixture was concentrated to dryness and purified by MPLC over silica gel (Biotage, 0- 10% MeOH in DCM gradient) to give an off-white solid which was recrystallised (EtOAc) to yield an off-white crystalline solid (75 mg, 45%). Mp = 136-137 ℃. LCMS: R_t = 2.19 min, 99 A% @ 254 nm, $[M + H]^+$ = 328.9. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.03 (s, 1H), 7.77 – 7.74 (m, 1H), 7.35 – 7.28 (m, 4H), 7.28 – 7.21 (m, 1H), 5.96 (d, *J* = 4.7 Hz, 1H), 5.77 (d, *J* = 4.7 Hz, 1H), 4.83 – 4.73 (m, 2H), 4.73 – 4.61 (m, 2H), 1.75 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 151.9, 151.2, 143.9, 138.3, 133.2, 128.0, 127.0, 126.3, 122.9, 67.8, 48.5, 46.2, 12.7. HRMS calcd for $C_{15}H_{16}N_6NaO_3$ $[M + Na]^+$, 351.1176; found, 351.1173.

1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(N-benzyl, N-methyl aminomethyl)- 1H-1,2,3-triazole; ***4m***

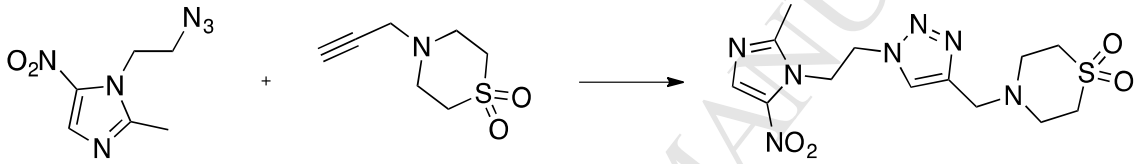


General procedure A. The reaction mixture was concentrated to dryness and purified by MPLC over silica gel (Biotage, 0- 20% MeOH in DCM gradient, recolumned 0- 15% MeOH in DCM) to give an orange waxy solid (77 mg, 42%). LCMS: R_t = 1.88 min, 99 A% @ 254 nm, $[M + H]^+$ = 356.1. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.03 (s, 1H), 7.89 (s, 1H), 7.35 – 7.27 (m, 4H), 7.27 – 7.22 (m, 1H), 4.85 – 4.80 (m, 2H), 4.76 – 4.71 (m, 2H), 3.57 (s, 2H), 3.42 (s, 2H), 2.05 (s, 3H), 1.87 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 151.1, 143.7, 138.7, 138.3, 133.2, 128.6, 128.1, 126.8, 124.6, 60.0, 51.0, 48.6, 46.2, 41.2, 12.9. HRMS calcd for $C_{17}H_{22}N_7O_2$ $[M + H]^+$, 356.1829; found, 356.1824.

1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(thiophen-3-yl)-1H-1,2,3-triazole; ***4n***

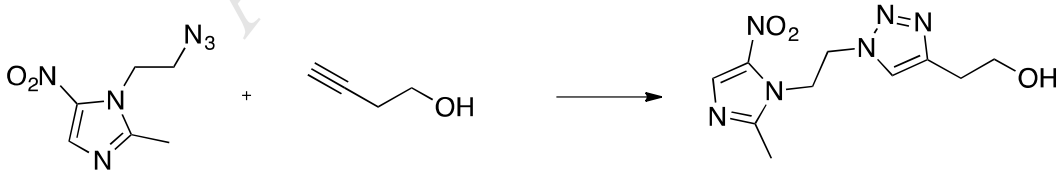
General procedure A. The reaction mixture was concentrated to dryness and purified by MPLC over C18 silica gel (Grace Reveleris X2, A: H_2O + 0.1% TFA, B: ACN + 0.1% TFA, 20-50% B). The eluent was removed by lyophilisation to give a colourless powdery solid (22.4 mg, 14%). LCMS: R_t = 2.43 min, 99 A% @ 254 nm, $[M + H]^+$ = 305.1. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.30 (s, 1H), 8.06 (s, 1H), 7.80 (dd, *J* = 3.0, 1.3 Hz, 1H), 7.64 (dd, *J* = 5.0, 2.9 Hz, 1H), 7.43 (dd, *J* = 5.0, 1.3 Hz, 1H), 4.86 (dd, *J* = 6.6, 4.5 Hz, 2H), 4.76 (dd, *J* = 6.6, 4.5 Hz, 2H), 1.93 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 151.2, 143.0, 138.3, 133.3, 131.6, 127.2, 125.6, 121.9, 121.0, 48.8, 46.0, 12.9. HRMS calcd for $C_{12}H_{12}N_6NaO_2S$ $[M + Na]^+$, 327.0635; found, 327.0627.

1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-((1,1-dioxothiomorpholin-4-yl)methyl)- 1H-1,2,3-triazole; ***4o***



General procedure C. The reaction was evaporated directly onto C18 silica and purified by MPLC over C18 silica gel (Grace Reveleris X2, A: H_2O + 0.1% TFA, B: ACN + 0.1% TFA, 5-10%). The eluent was removed by lyophilisation to give an aqua coloured powder. The solid was dissolved in H_2O (5 mL), adjusted to pH 5 with 2M aq. NaOH and passed over Chelex 100 resin, 200-400 mesh. The eluent was removed by lyophilisation to give a tan solid (93 mg, 43%). LCMS: R_t = 1.85 min, 99 A% @ 254 nm, $[M + H]^+$ = 370.0. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.05 (s, 1H), 7.88 (s, 1H), 4.82 (dd, *J* = 6.6, 4.3 Hz, 2H), 4.72 (dd, *J* = 6.6, 4.3 Hz, 2H), 3.72 (s, 2H), 3.10 – 3.05 (m, 4H), 2.86 – 2.80 (m, 4H), 1.87 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 151.2, 143.2, 138.3, 133.2, 124.8, 50.5, 50.5, 49.7, 48.7, 46.1, 12.9. HRMS calcd for $C_{13}H_{19}N_7NaO_4S$ $[M + Na]^+$, 392.1111; found, 392.1110.

1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(2-hydroxyethyl)-1H-1,2,3-triazole; ***4p***



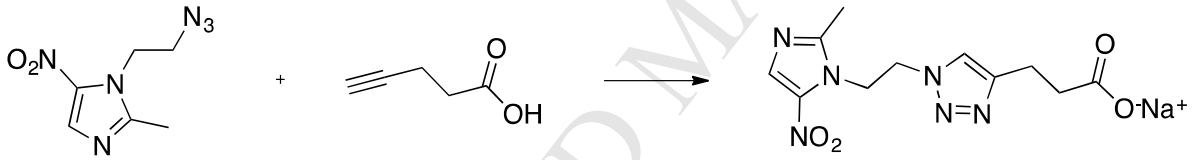
General procedure B and C. An additional portion of acetylene (1 eq.) was added. The crude reaction mixture was concentrated to dryness and purified by MPLC over silica gel (Biotage, 15-25% MeOH in DCM) to give a colourless solid (49 mg, 68%). LCMS: R_t = 1.73 min, 99 A% @ 254 nm, $[M + H]^+$ = 267.1. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.04 (s, 1H), 7.74 (s, 1H), 4.80 – 4.75 (m,

2H), 4.71 – 4.65 (m, 3H), 3.58 – 3.52 (m, 2H), 2.75 – 2.69 (m, 2H), 1.84 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 151.2, 144.7, 138.3, 133.2, 123.3, 60.4, 48.5, 46.2, 29.0, 12.7. HRMS calcd for $C_{10}H_{14}N_6NaO_3$ $[M + Na]^+$, 289.1020; found, 289.1013.

1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(hydroxymethyl)- 1H-1,2,3-triazole; ***4q***

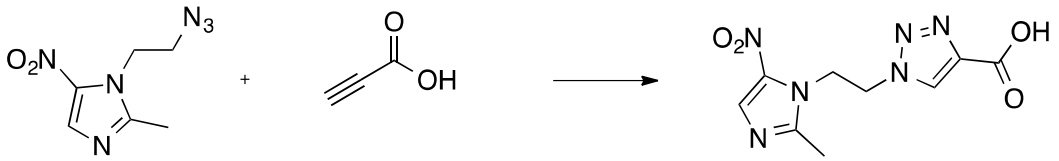
General procedure B and C. An additional portion of acetylene (1 eq.) was added. The crude reaction mixture was concentrated to dryness and purified by MPLC over silica gel (Biotage, 2-15% MeOH in DCM) to give a colourless solid (34 mg, 51%). LCMS: R_t = 1.67 min, 99 A% @ 254 nm, $[M + H]^+$ = 253.0. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.05 (s, 1H), 7.87 (s, 1H), 5.20 (t, *J* = 5.6 Hz, 1H), 4.81 (dd, *J* = 6.5, 4.4 Hz, 2H), 4.71 (dd, *J* = 6.5, 4.4 Hz, 2H), 4.49 – 4.45 (m, 2H), 1.85 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 151.2, 148.3, 138.3, 133.2, 123.6, 54.8, 48.5, 46.2, 12.8. HRMS calcd for $C_9H_{12}N_6NaO_3$ $[M + Na]^+$, 275.0863; found, 275.0865.

sodium 1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(carboxyethyl)- 1H-1,2,3-triazole; ***4r***



General procedure C. The reaction was evaporated directly onto C18 silica and purified by MPLC over C18 silica gel (Grace Reveleris X2, A: H_2O + 0.1% TFA, B: ACN + 0.1% TFA, 5-14% B). The eluent was removed by lyophilisation to give an aqua coloured powder. The solid was dissolved in ACN: H_2O (30:70, 20 mL), adjusted to pH 5 with 2M aq. NaOH and passed over Chelex 100 resin, 200-400 mesh. The eluent was removed by lyophilisation to give a tan powder (109 mg, 73%). LCMS: R_t = 1.82 min, 99 A% @ 254 nm, $[M + H]^+$ = 294.7. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.04 (s, 1H), 7.70 (s, 1H), 4.78 – 4.73 (m, 2H), 4.70 – 4.65 (m, 2H), 2.76 (br s, 2H), 2.36 (br s, 2H), 1.82 (br s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 175.0*, 151.2, 147.0, 138.3, 133.2, 122.7, 48.27, 46.21, 35.8*, 21.46, 12.8. *Carbon peaks were confirmed by HSQC and HMBC correlations. HRMS calcd for $C_{11}H_{14}N_6NaO_4$ $[M + Na]^+$, 317.0969; found, 317.0958.

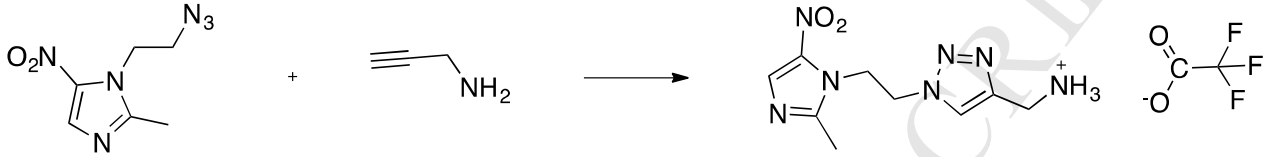
1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(carboxy)- 1H-1,2,3-triazole; ***4s***



General procedure A. The reaction suspension was filtered. The solid was washed with water (5 x 0.5 mL) and EtOAc (3 x 0.5 mL). The solid was purified by MPLC over C18 silica gel (Grace

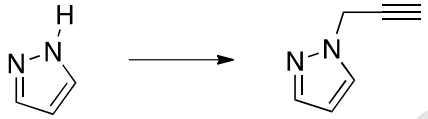
Reveleris X2, A: H_2O + 0.1% TFA, B: ACN + 0.1% TFA, 5-15%). The eluent was removed by lyophilisation to give a colourless solid (49 mg, 36%). LCMS: R_t = 1.72 min, 99 A% @ 254 nm, $[M + H]^+$ = 266.8. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.59 (s, 1H), 8.05 (s, 1H), 4.89 (dd, *J* = 6.5, 4.4 Hz, 2H), 4.76 (dd, *J* = 6.6, 4.4 Hz, 2H), 1.93 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 161.4, 151.0, 139.7, 138.4, 133.2, 129.8, 49.0, 45.8, 12.9. HRMS calcd for $C_9H_{10}N_6NaO_4$ $[M + Na]^+$, 289.0656; found, 289.0655.

1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(aminomethyl)- 1H-1,2,3-triazole trifluoroacetate salt; ***4t***



General procedure A. The reaction was evaporated directly onto C18 silica and purified by MPLC over C18 silica gel (Grace Reveleris X2, A: H_2O + 0.1% TFA, B: ACN + 0.1% TFA, 5-10% B). The eluent was removed by lyophilisation to give a yellow oil (34 mg, 17%). LCMS: R_t = 1.26 min, 99 A% @ 254 nm, $[M^+H]^+$ = 251.8. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.24 (br s, 3H), 8.072 (s, 1H), 8.068 (s, 1H), 4.89 (dd, *J* = 6.5, 4.5 Hz, 2H), 4.74 (dd, *J* = 6.6, 4.6 Hz, 2H), 4.11 (q, *J* = 5.8 Hz, 2H), 1.92 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 151.3, 140.3, 138.4, 133.2, 125.1, 48.8, 46.0, 33.7, 13.0. HRMS calcd for $C_9H_{14}N_7O_2$ $[M + H]^+$, 252.1203; found, 252.1204.

1-(prop-2-yn-1-yl)-1H-pyrazole; ***6***

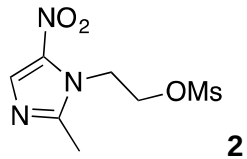


To a solid mixture of 1H-pyrazole (1.00 g, 14.7 mmol), K_2CO_3 (3.04 g, 22.0 mmol) and tetrabutylammonium bromide (237 mg, 0.73 mmol) was added propargyl bromide (80% weight in toluene) (2.5 mL, 22.0 mmol).[2] The reaction was stirred vigorously at rt for 3 days before pouring into H_2O (50 mL) and extracting with EtOAc (50 mL x 3). The combined organics were washed with brine (50 mL), dried with anh. $MgSO_4$ and filtered. Volatiles removed *in vacuo* to give a crude brown oil. The crude material was purified by MPLC over silica gel (Biotage, 5- 40% EtOAc in pet. spirits gradient) to give a pale yellow oil (245 mg, 16%). LCMS: R_t = 1.82 min, A% @ 200 nm, $[M + H]^+$ = not detected by ESI/API. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.62 – 7.58 (m, 1H), 7.56 – 7.52 (m, 1H), 6.30 (m, 1H), 4.96 (d, *J* = 2.6 Hz, 2H), 2.50 (t, *J* = 2.6 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 140.0, 128.7, 106.2, 76.8, 74.5, 41.5. Spectra were indistinguishable from Mohr et al.[3]

3 Spectra

2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl methanesulfonate

^{1}H NMR (600 MHz DMSO-d_6)



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3	Owner	chemistry
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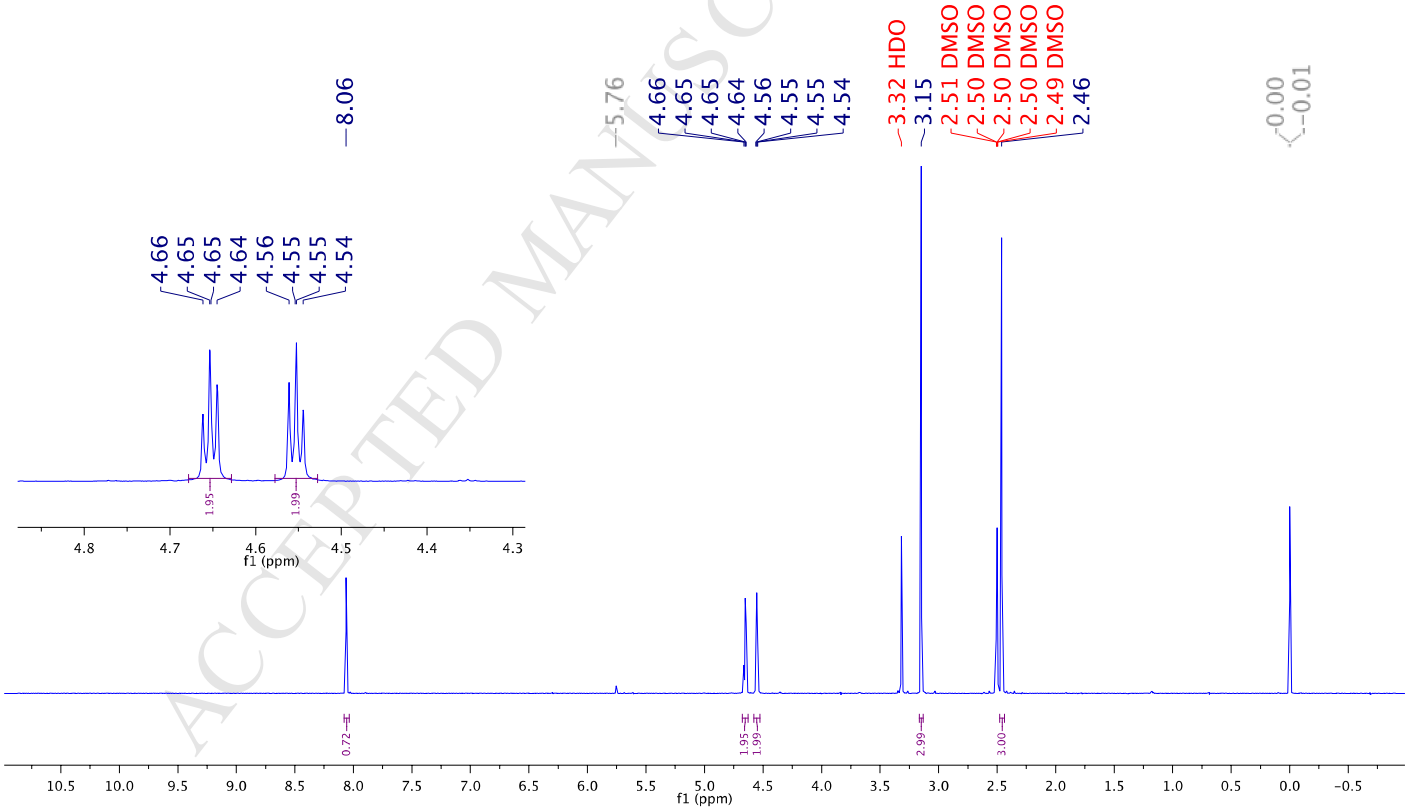
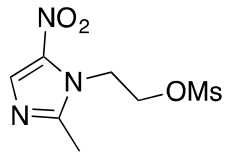
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	zg
8	Experiment	1D
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	8
11	Receiver Gain	32
12	Relaxation Delay	10.0000
13	Pulse Width	7.8000
14	Presaturation Frequency	
15	Acquisition Time	2.2808
16	Acquisition Date	2014-03-04T04:29:00
17	Modification Date	2014-03-04T04:29:10
18	Spectrometer Frequency	600.13
19	Spectral Width	7183.9
20	Lowest Frequency	-591.6
21	Nucleus	1H
22	Acquired Size	16384
23	Spectral Size	65536



2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl methanesulfonate

^{13}C JMOD (150 MHz, DMSO-d_6)



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4	Spectrometer	spect
5	Solvent	DMSO
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7	Pulse Sequence	jmod
8	Experiment	JMOD
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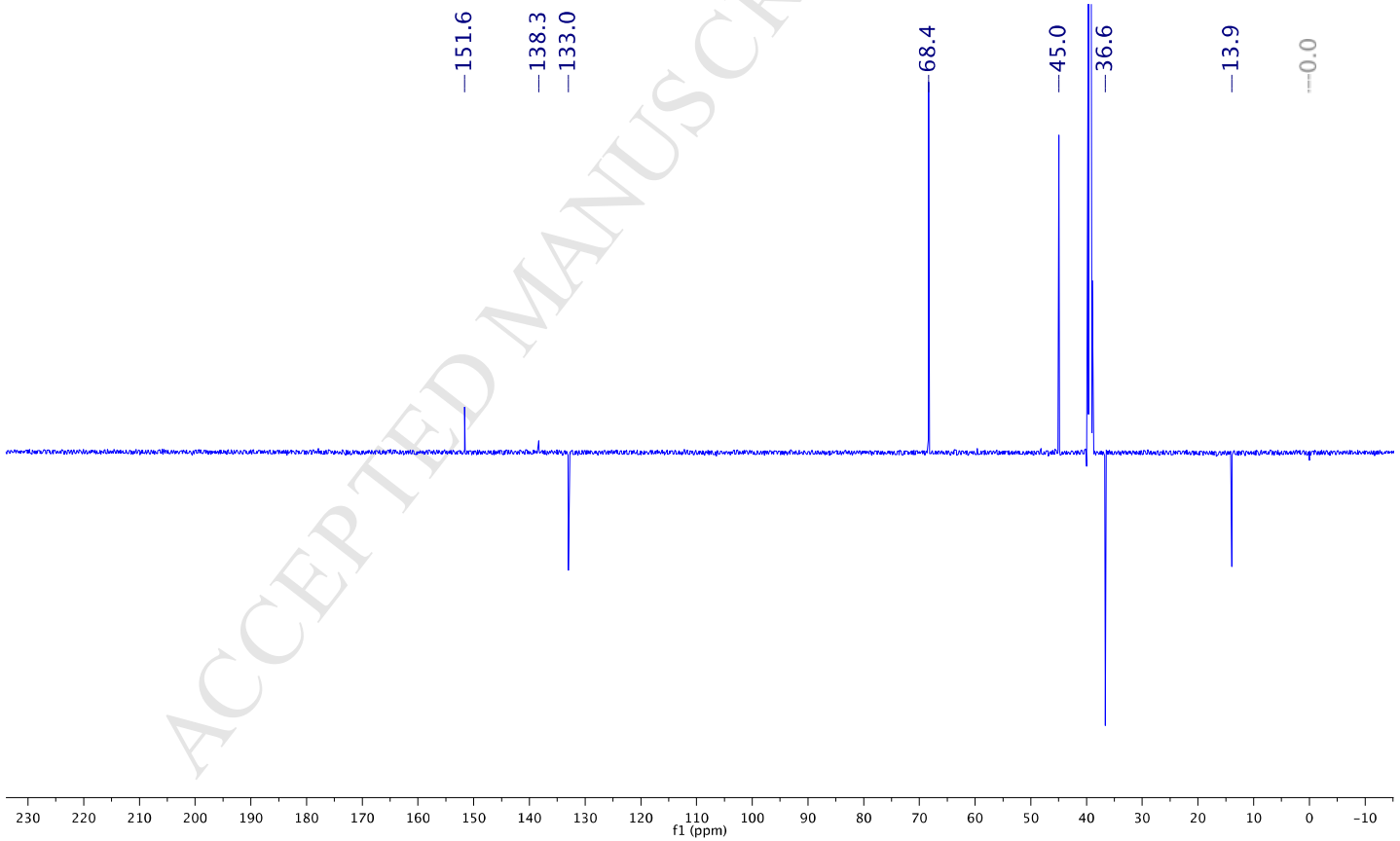
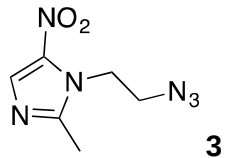
10	Number of Scans	2048
11	Receiver Gain	32768
12	Relaxation Delay	1.0000
13	Pulse Width	12.0000
14	Presaturation Frequency	
15	Acquisition Time	0.8717
16	Acquisition Date	2014-03-04T05:37:00
17	Modification Date	2014-03-04T05:37:10
18	Spectrometer Frequency	150.92
19	Spectral Width	37594.0
20	Lowest Frequency	-2273.6
21	Nucleus	13C
22	Acquired Size	32768
23	Spectral Size	65536



1-(2-azidoethyl)-2-methyl-5-nitro-1H-imidazole

^{1}H NMR (600 MHz, DMSO-d_6)



3

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3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	zg
8	Experiment	1D
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	8
11	Receiver Gain	32
12	Relaxation Delay	10.0000
13	Pulse Width	7.8000
14	Presaturation Frequency	
15	Acquisition Time	2.2808
16	Acquisition Date	2014-03-04T05:41:00
17	Modification Date	2014-03-04T05:41:52
18	Spectrometer Frequency	600.13
19	Spectral Width	7183.9
20	Lowest Frequency	-591.2
21	Nucleus	1H
22	Acquired Size	16384
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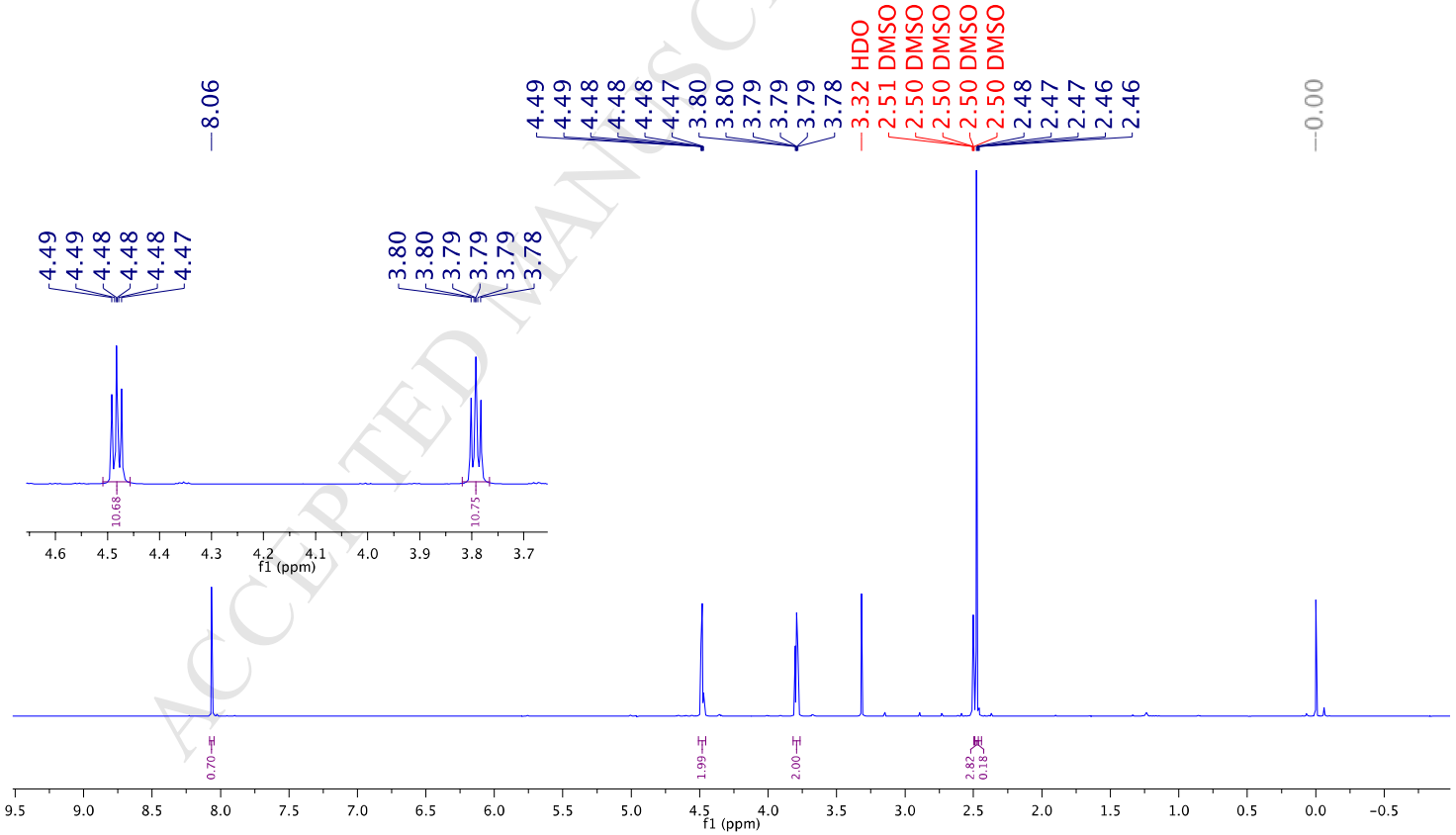
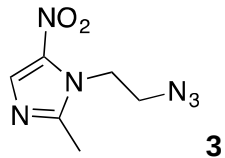


1-(2-azidoethyl)-2-methyl-5-nitro-1H-imidazole

^{13}C JMOD (150 MHz, DMSO-d_6)



3

	Parameter	Value
1	Title	AMJ6941_016_crudeppt_FP.2.fid
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3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	jmod
8	Experiment	JMOD
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10	Number of Scans	2048
11	Receiver Gain	41285
12	Relaxation Delay	1.0000
13	Pulse Width	12.0000
14	Presaturation Frequency	
15	Acquisition Time	0.8717
16	Acquisition Date	2014-03-04T06:49:00
17	Modification Date	2014-03-04T06:49:48
18	Spectrometer Frequency	150.92
19	Spectral Width	37594.0
20	Lowest Frequency	-2273.6
21	Nucleus	13C
22	Acquired Size	32768
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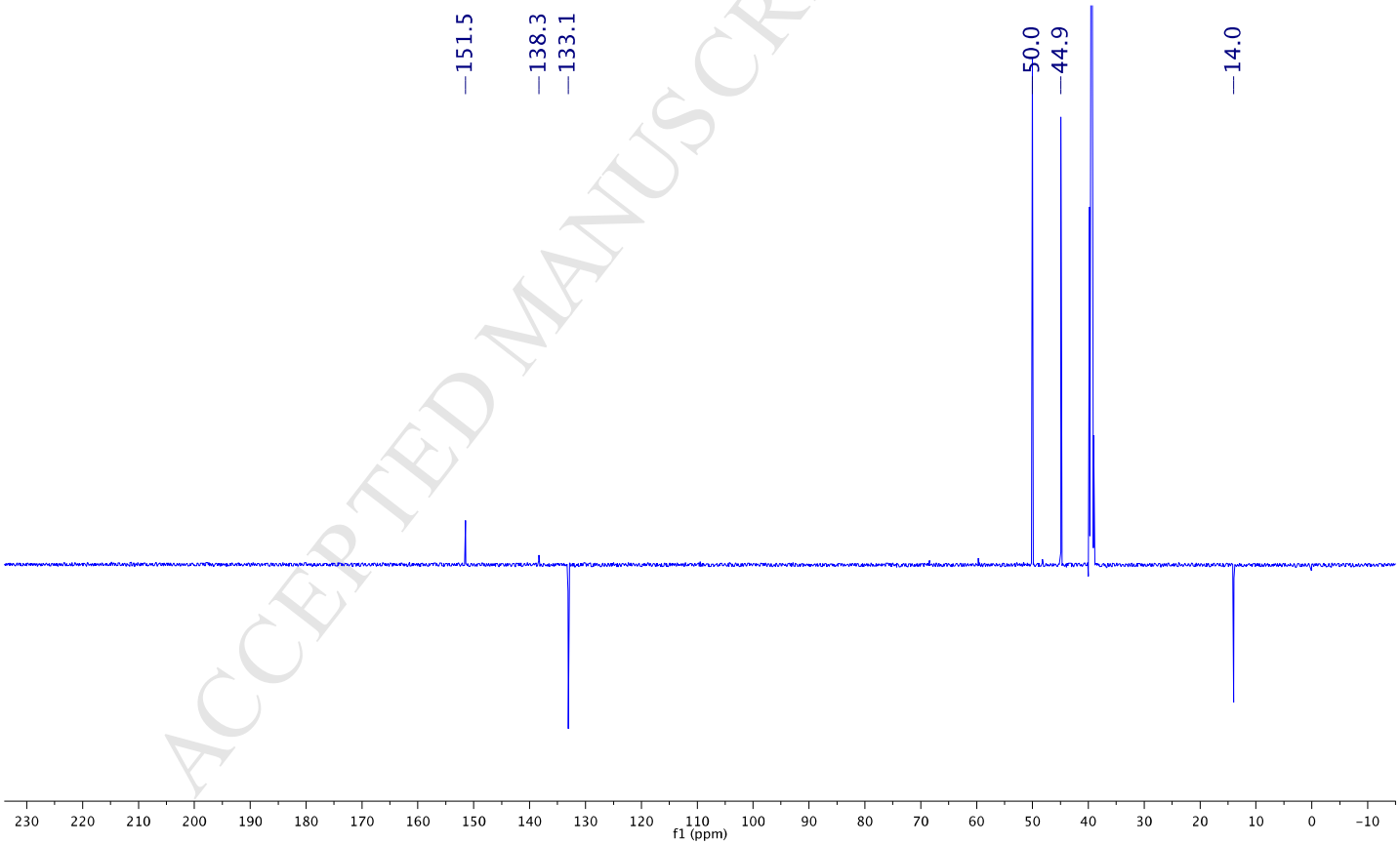
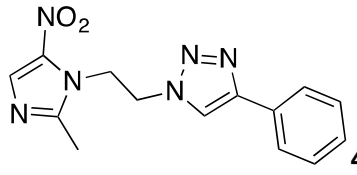


1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-phenyl-1H-1,2,3-triazole

^{1}H NMR (600 MHz, DMSO-d_6)



4a

	Parameter	Value
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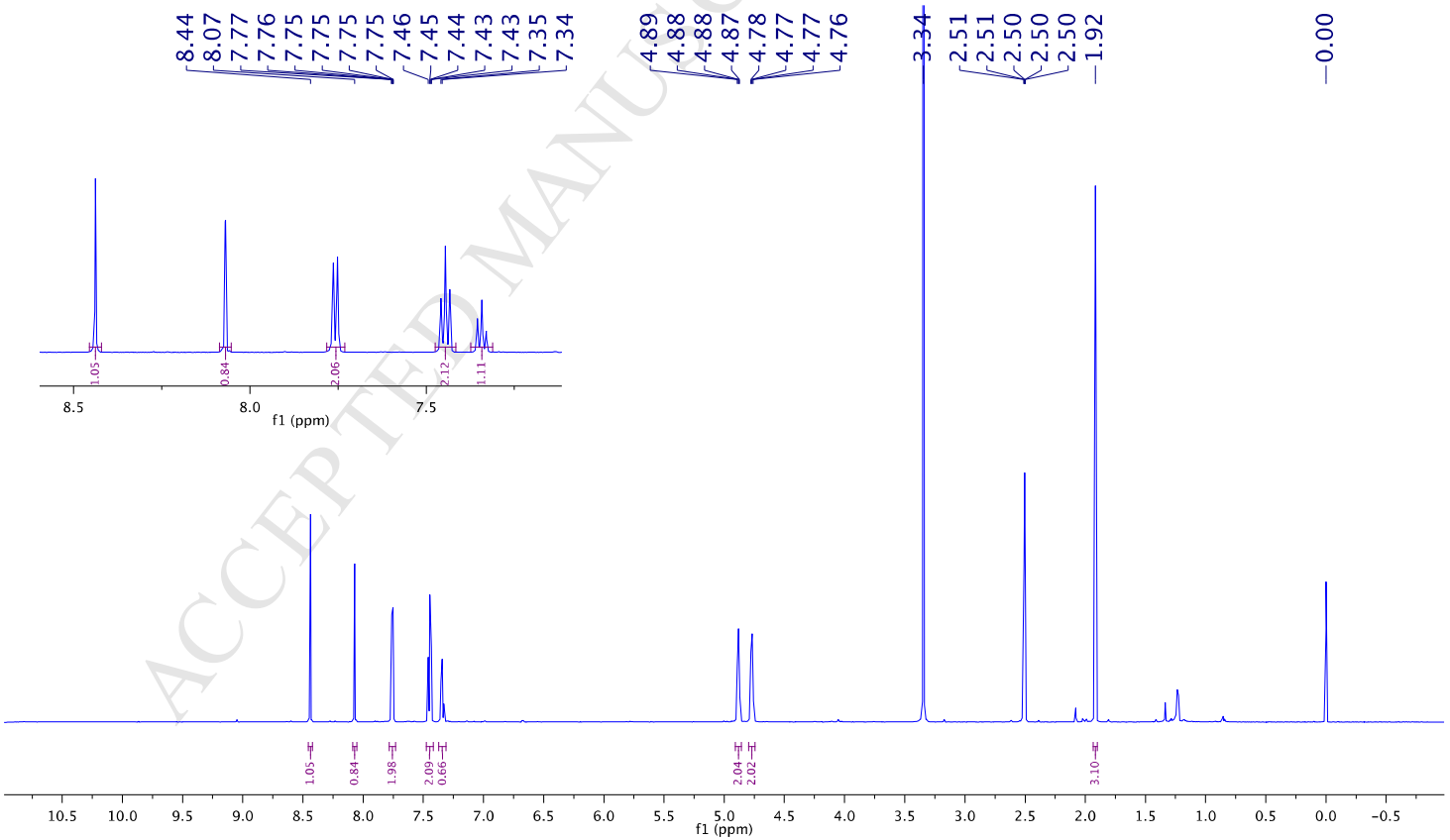
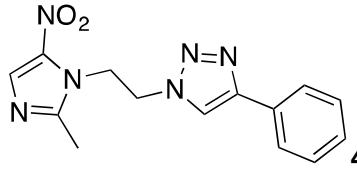
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	zg
8	Experiment	1D
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	8
11	Receiver Gain	23
12	Relaxation Delay	10.0000
13	Pulse Width	7.8000
14	Presaturation Frequency	
15	Acquisition Time	2.2808
16	Acquisition Date	2013-05-16T18:10:00
17	Modification Date	2013-05-16T18:11:06
18	Spectrometer Frequency	600.13
19	Spectral Width	7183.9
20	Lowest Frequency	-591.5
21	Nucleus	1H
22	Acquired Size	16384
23	Spectral Size	65536



1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-phenyl-1H-1,2,3-triazole

^{13}C JMOD (150 MHz, DMSO-d_6)



4a

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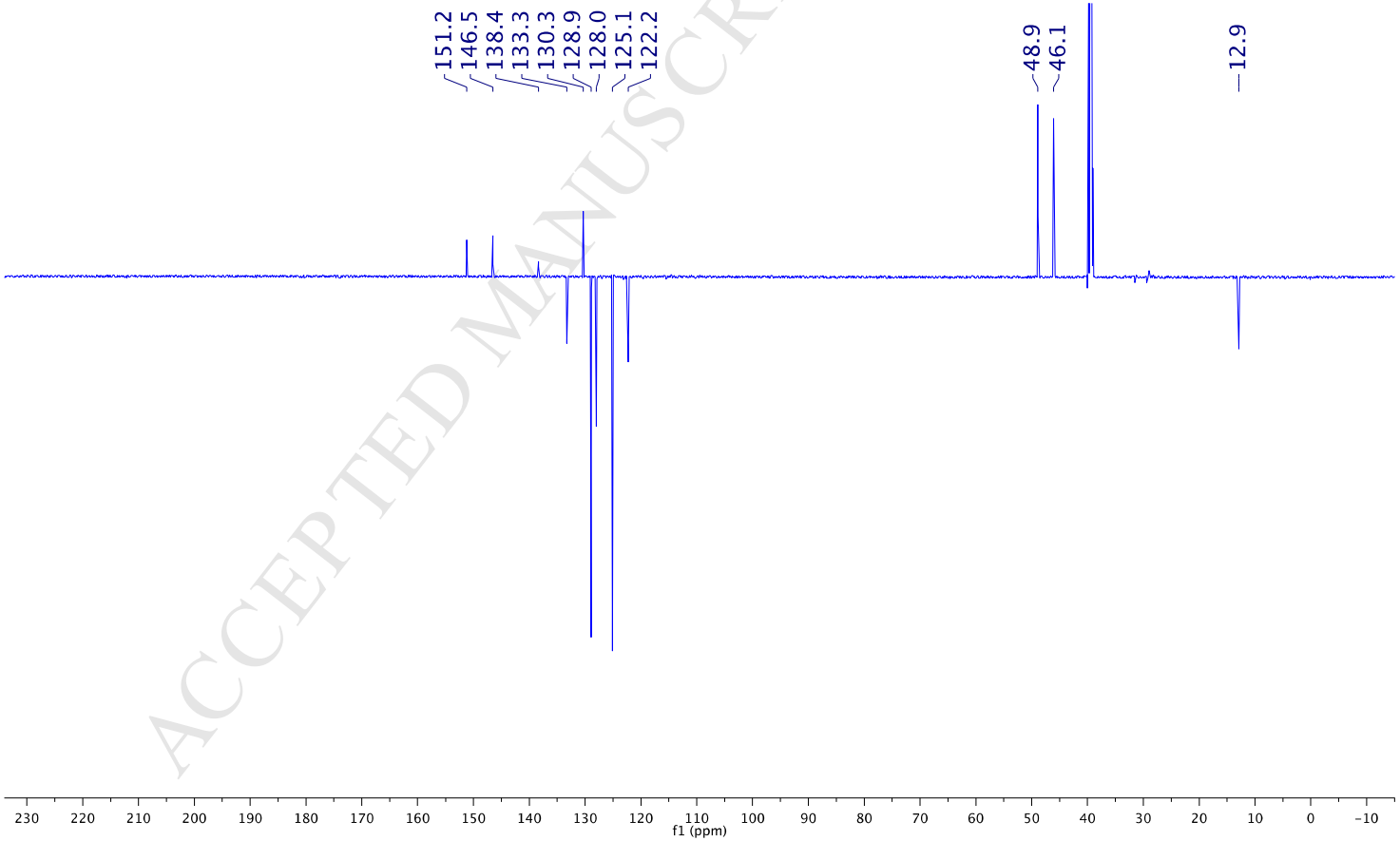
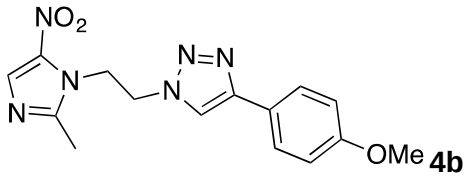
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	jmod
8	Experiment	JMOD
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	2048
11	Receiver Gain	46341
12	Relaxation Delay	1.0000
13	Pulse Width	12.0000
14	Presaturation Frequency	
15	Acquisition Time	0.8717
16	Acquisition Date	2013-05-16T19:18:00
17	Modification Date	2013-05-16T19:18:50
18	Spectrometer Frequency	150.92
19	Spectral Width	37594.0
20	Lowest Frequency	-2273.6
21	Nucleus	13C
22	Acquired Size	32768
23	Spectral Size	65536



1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(4-methoxyphenyl)-1H-1,2,3-triazole

^{1}H NMR (600 MHz, DMSO-d_6)



4b

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3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	zg
8	Experiment	1D
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	8
11	Receiver Gain	32
12	Relaxation Delay	10.0000
13	Pulse Width	7.8000
14	Presaturation Frequency	
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16	Acquisition Date	2013-08-02T10:25:00
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18	Spectrometer Frequency	600.13
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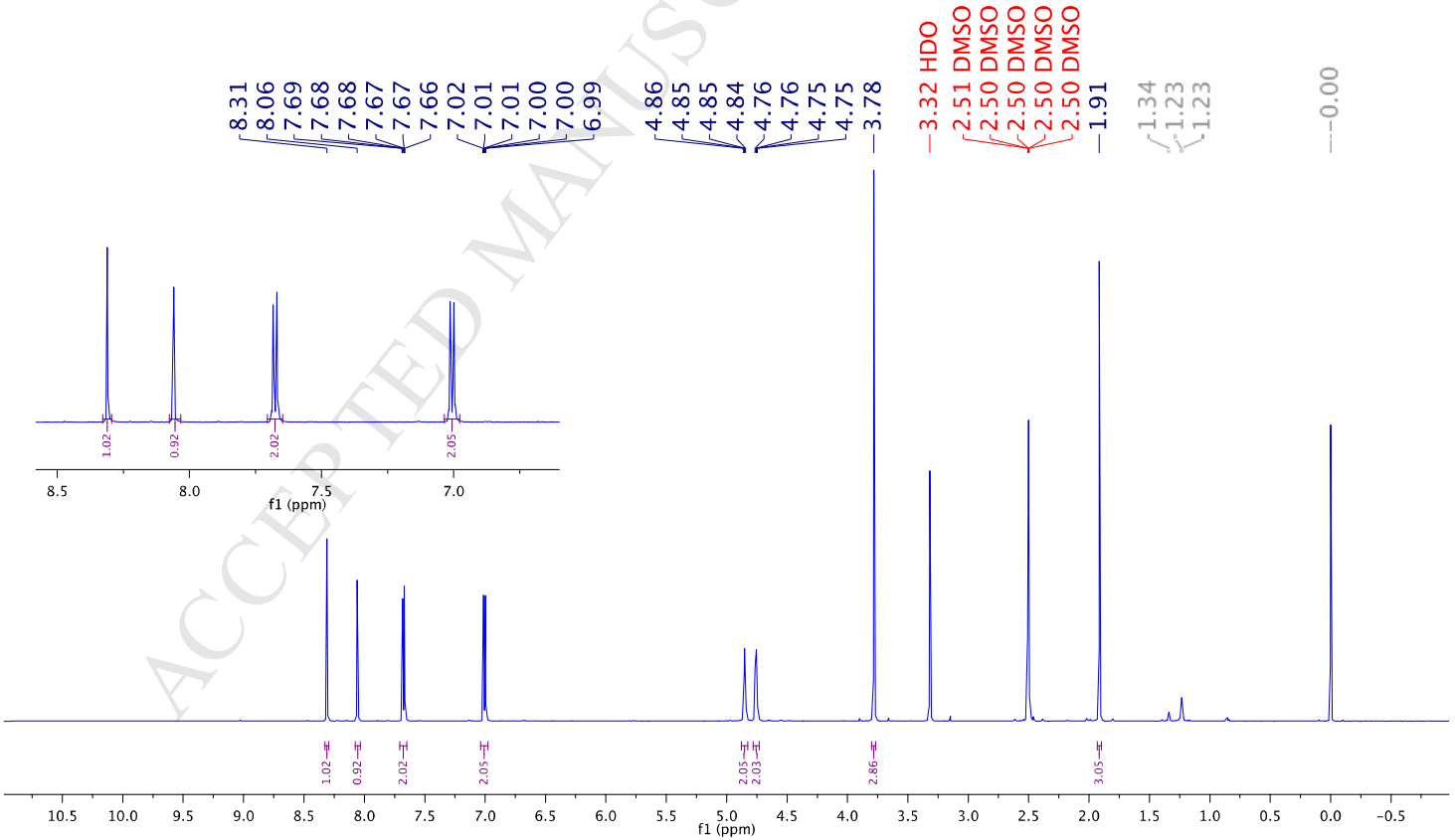
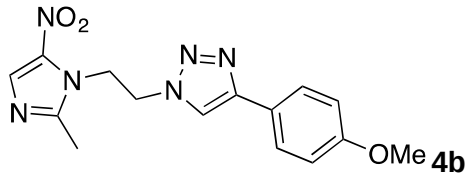
20	Lowest Frequency	-591.1
21	Nucleus	1H
22	Acquired Size	16384
23	Spectral Size	65536



1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(4-methoxyphenyl)-1H-1,2,3-triazole

^{13}C JMOD NMR (150 MHz, DMSO-d_6)



4b

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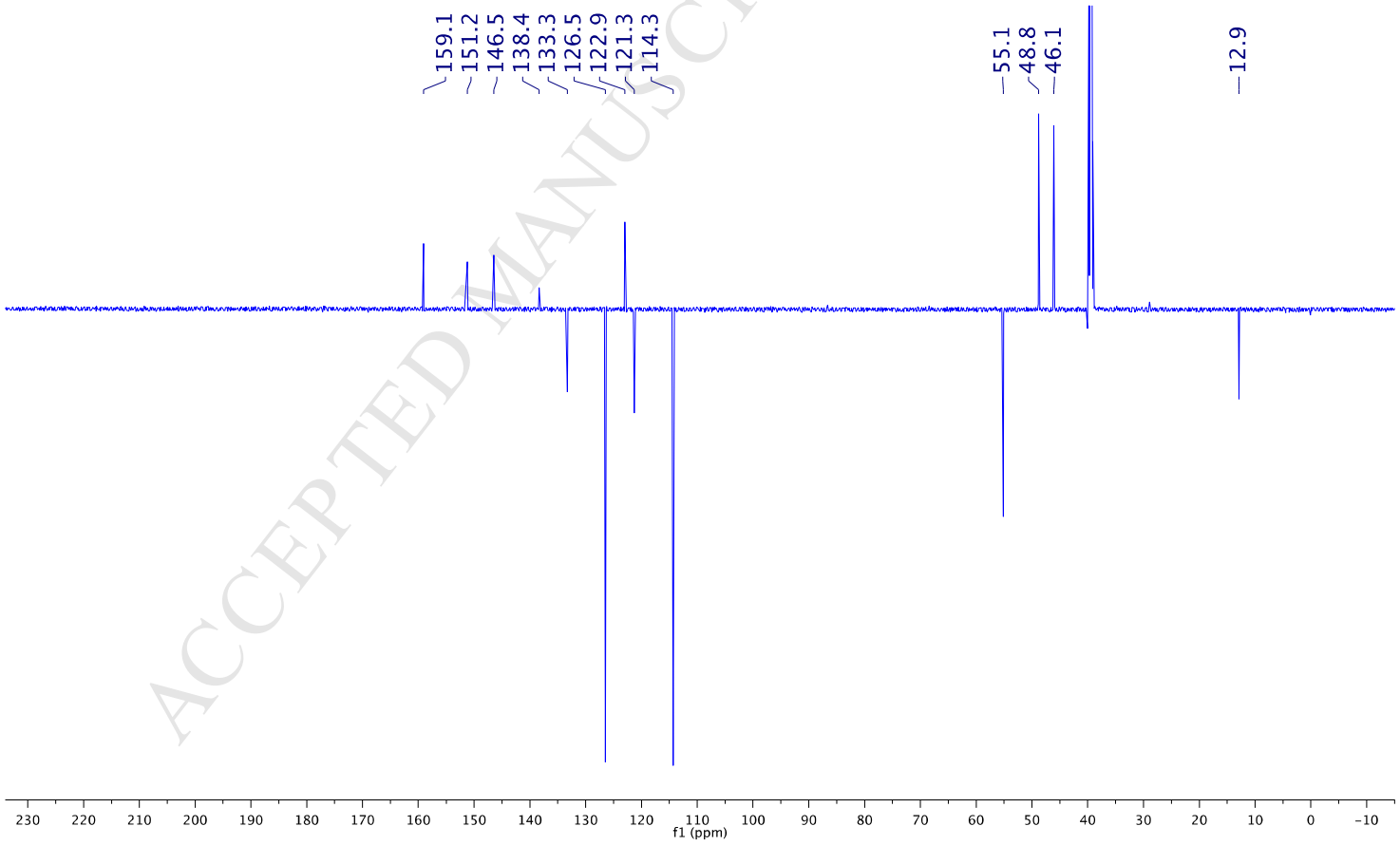
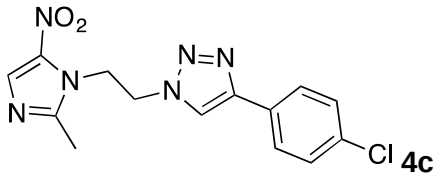
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	jmod
8	Experiment	JMOD
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	2048
11	Receiver Gain	41285
12	Relaxation Delay	1.0000
13	Pulse Width	12.0000
14	Presaturation Frequency	
15	Acquisition Time	0.8717
16	Acquisition Date	2013-08-02T14:52:00
17	Modification Date	2013-08-02T14:52:59
18	Spectrometer Frequency	150.92
19	Spectral Width	37594.0
20	Lowest Frequency	-2273.6
21	Nucleus	13C
22	Acquired Size	32768
23	Spectral Size	65536



1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(4-chlorophenyl)-1H-1,2,3-triazole

^{1}H NMR (600 MHz, DMSO-d_6)



4c

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4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	zg
8	Experiment	1D
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	8
11	Receiver Gain	32
12	Relaxation Delay	10.0000
13	Pulse Width	7.8000
14	Presaturation Frequency	
15	Acquisition Time	2.2808
16	Acquisition Date	2013-08-31T17:08:00
17	Modification Date	2013-08-31T17:08:56
18	Spectrometer Frequency	600.13
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21	Nucleus	1H
22	Acquired Size	16384
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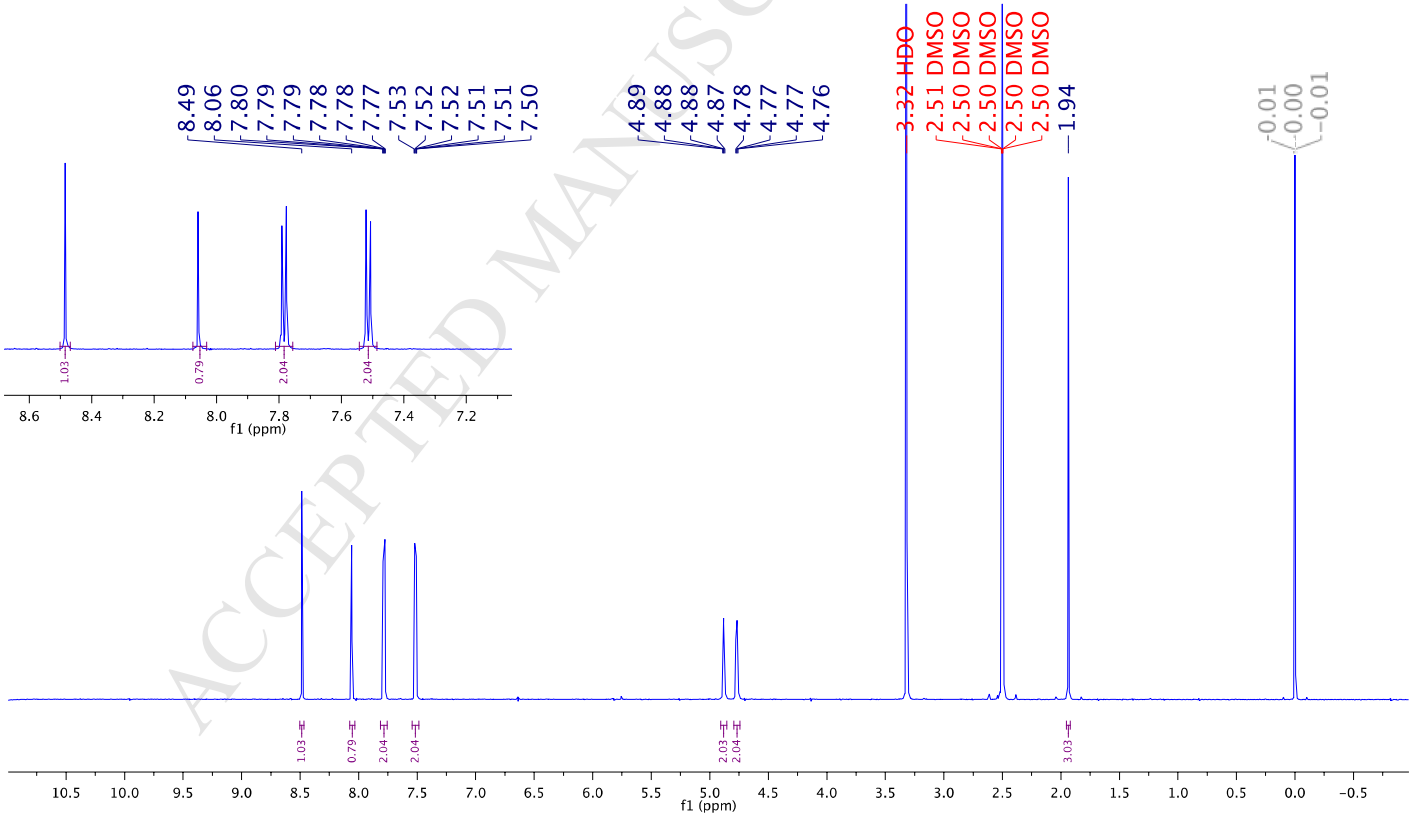
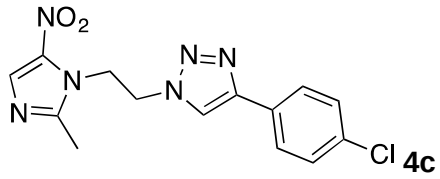


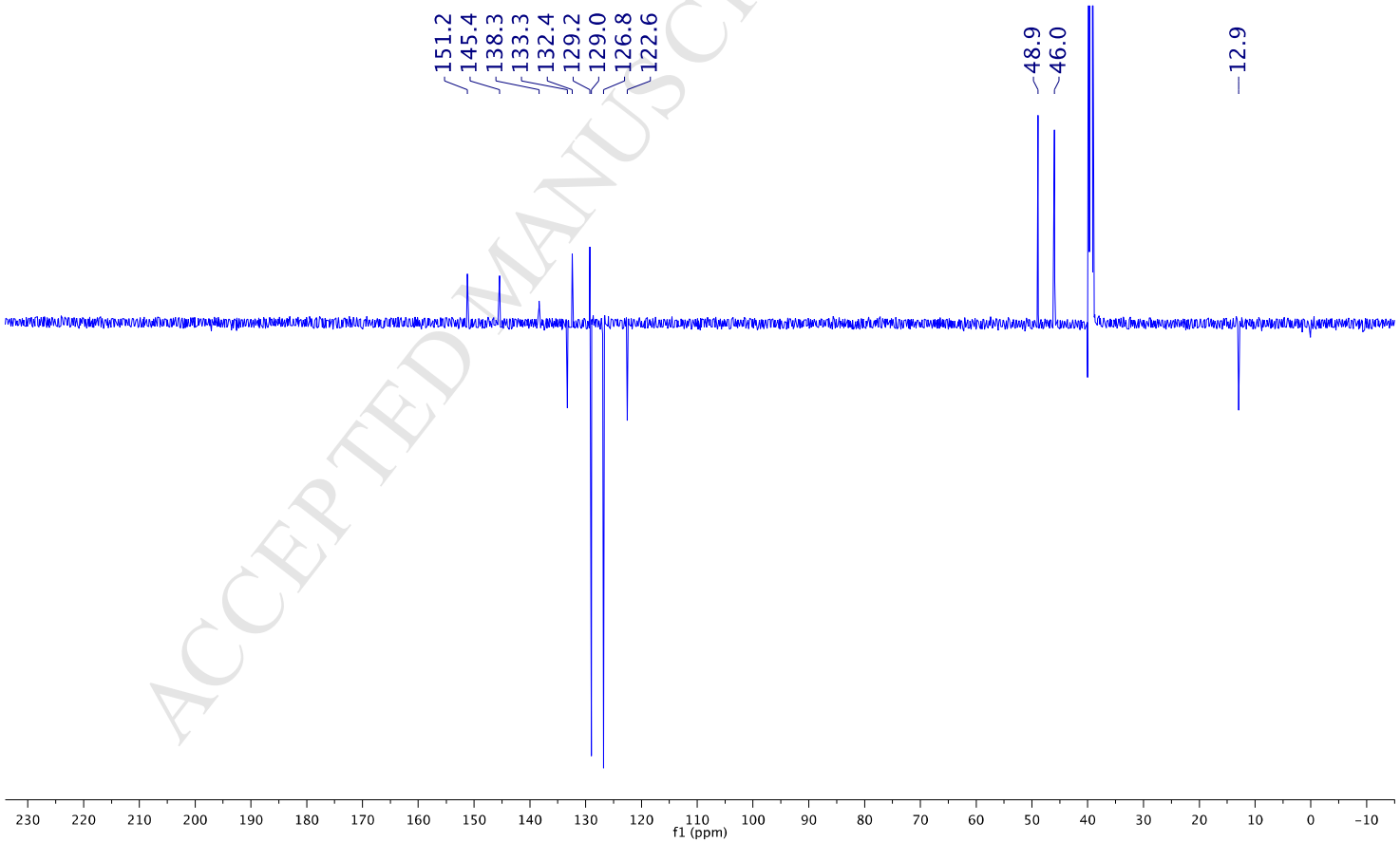
1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(4-chlorophenyl)-1H-1,2,3-triazole

^{13}C JMOD NMR (150 MHz, DMSO-d_6)



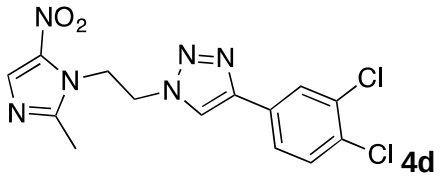
4c

	Parameter	Value
1	Title	AMJ6768_004A_f8.2.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	jmod
8	Experiment	JMOD
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	2048
11	Receiver Gain	46341
12	Relaxation Delay	1.0000
13	Pulse Width	12.0000
14	Presaturation Frequency	
15	Acquisition Time	0.8717
16	Acquisition Date	2013-08-31T18:16:00
17	Modification Date	2013-08-31T18:16:11
18	Spectrometer Frequency	150.92
19	Spectral Width	37594.0
20	Lowest Frequency	-2273.6
21	Nucleus	13C
22	Acquired Size	32768
23	Spectral Size	65536



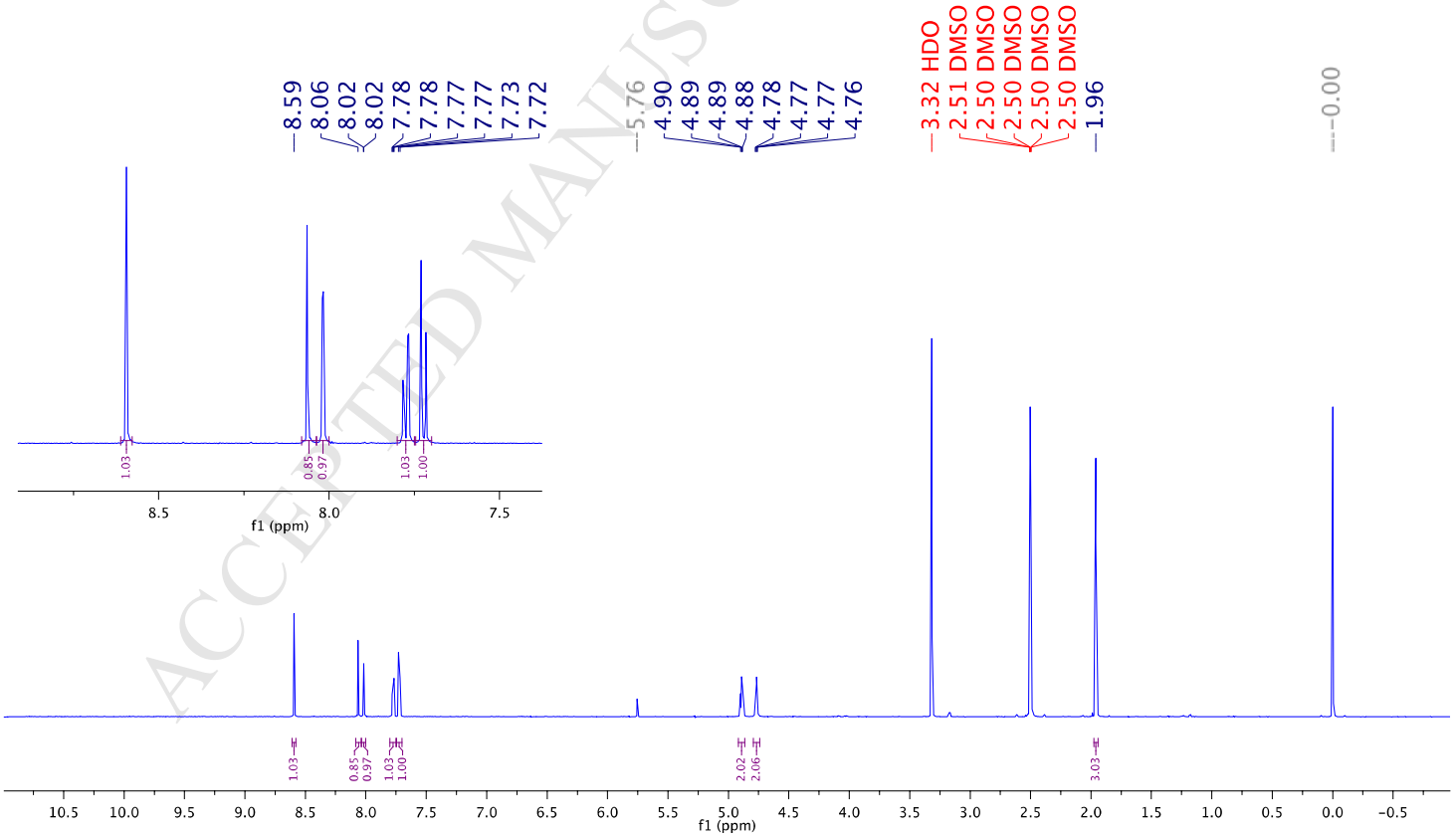
1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(3,4-dichlorophenyl)-1H-1,2,3-triazole

^{1}H NMR (600 MHz, DMSO-d_6)



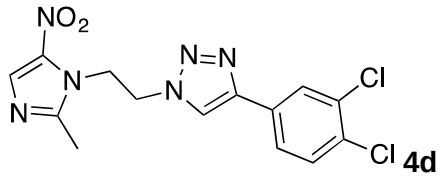
4d

	Parameter	Value
1	Title	AMJ6768_004B_f9_recrys EtOac_redissolved_higvac .1.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	zg
8	Experiment	1D
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	8
11	Receiver Gain	32
12	Relaxation Delay	10.0000
13	Pulse Width	7.8000
14	Presaturation Frequency	
15	Acquisition Time	2.2808
16	Acquisition Date	2013-09-15T22:02:00
17	Modification Date	2013-09-15T22:02:21
18	Spectrometer Frequency	600.13
19	Spectral Width	7183.9
20	Lowest Frequency	-585.2
21	Nucleus	1H
22	Acquired Size	16384
23	Spectral Size	65536



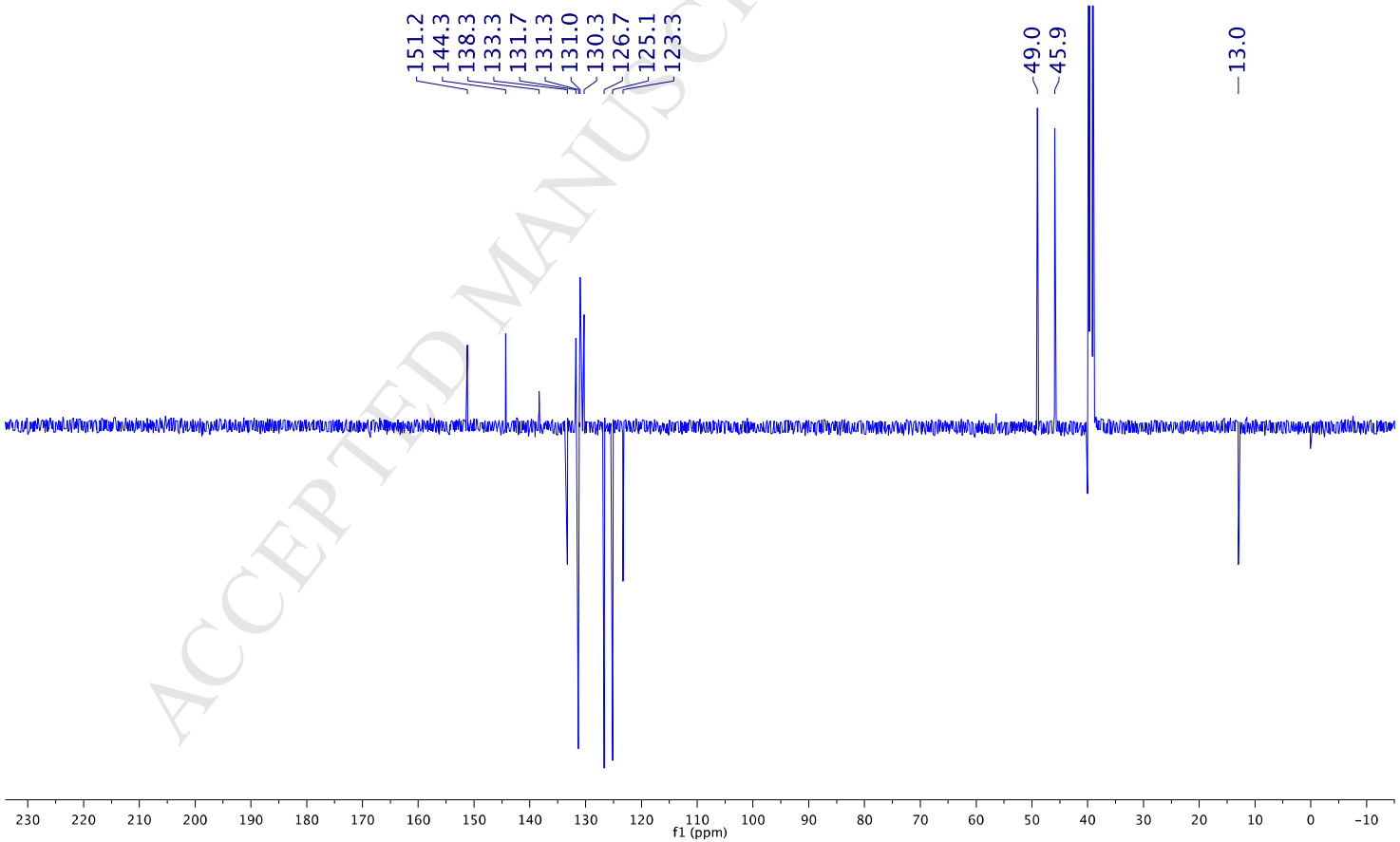
1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(3,4-dichlorophenyl)-1H-1,2,3-triazole

^{13}C JMOD NMR (150 MHz, DMSO-d_6)



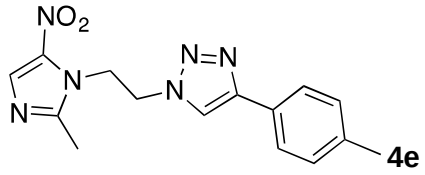
4d

	Parameter	Value
1	Title	AMJ6768_004B_f9_recrys EtOac_redissolved_higvac .2.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	jmod
8	Experiment	JMOD
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	2048
11	Receiver Gain	41285
12	Relaxation Delay	1.0000
13	Pulse Width	12.0000
14	Presaturation Frequency	
15	Acquisition Time	0.8717
16	Acquisition Date	2013-09-15T23:09:00
17	Modification Date	2013-09-15T23:09:47
18	Spectrometer Frequency	150.92
19	Spectral Width	37594.0
20	Lowest Frequency	-2273.6
21	Nucleus	13C
22	Acquired Size	32768
23	Spectral Size	65536



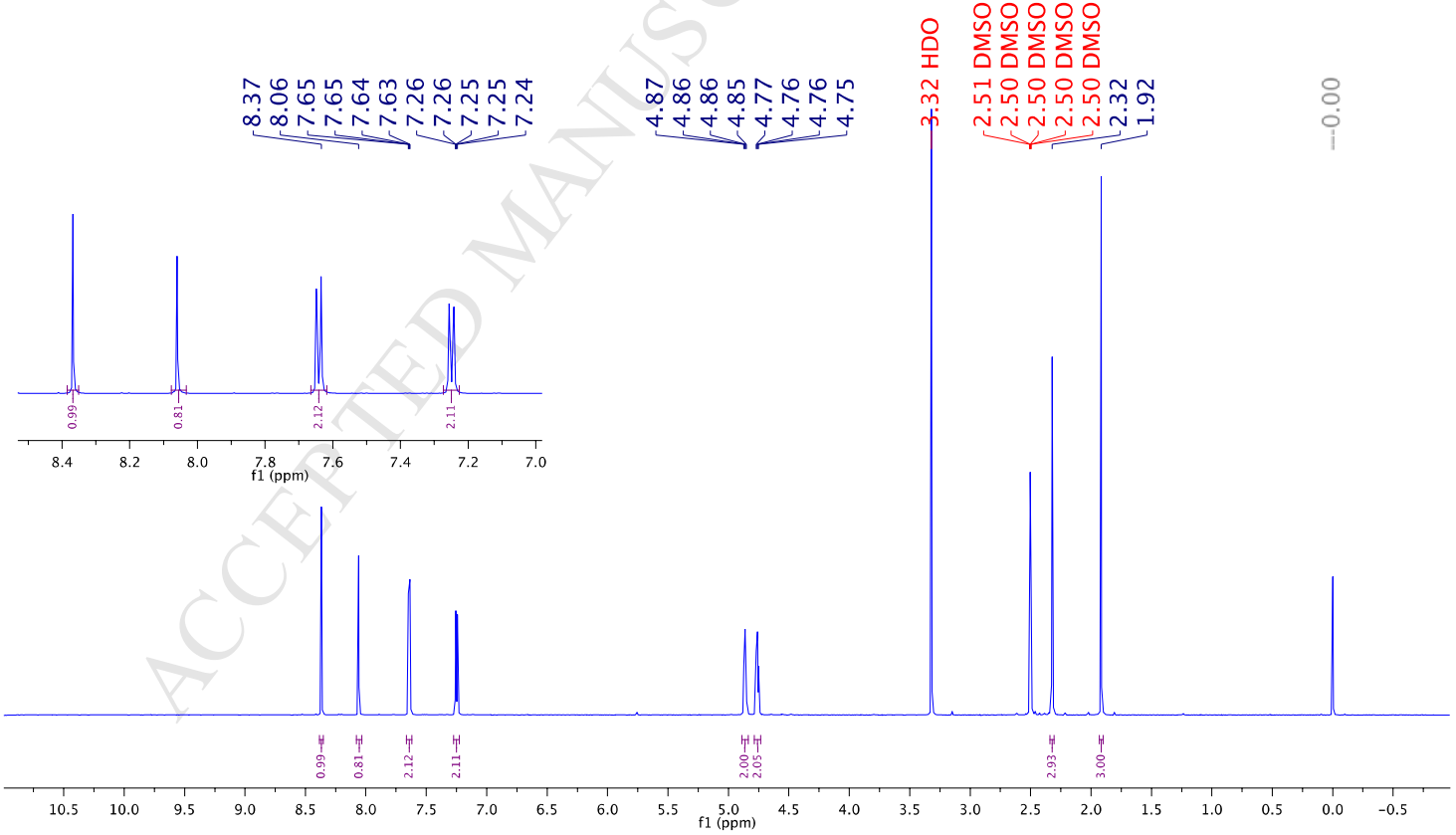
1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(p-tolyl)-1H-1,2,3-triazole

^{1}H NMR (600 MHz, DMSO-d_6)



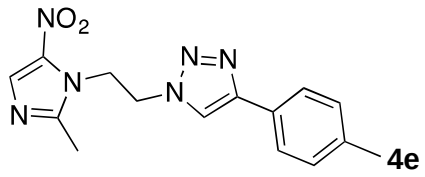
4e

	Parameter	Value
1	Title	AMJ6768_020A_f9.1.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	zg
8	Experiment	1D
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	8
11	Receiver Gain	32
12	Relaxation Delay	10.0000
13	Pulse Width	7.8000
14	Presaturation Frequency	
15	Acquisition Time	2.2808
16	Acquisition Date	2013-08-31T13:42:00
17	Modification Date	2013-08-31T13:42:29
18	Spectrometer Frequency	600.13
19	Spectral Width	7183.9
20	Lowest Frequency	-585.0
21	Nucleus	1H
22	Acquired Size	16384
23	Spectral Size	65536



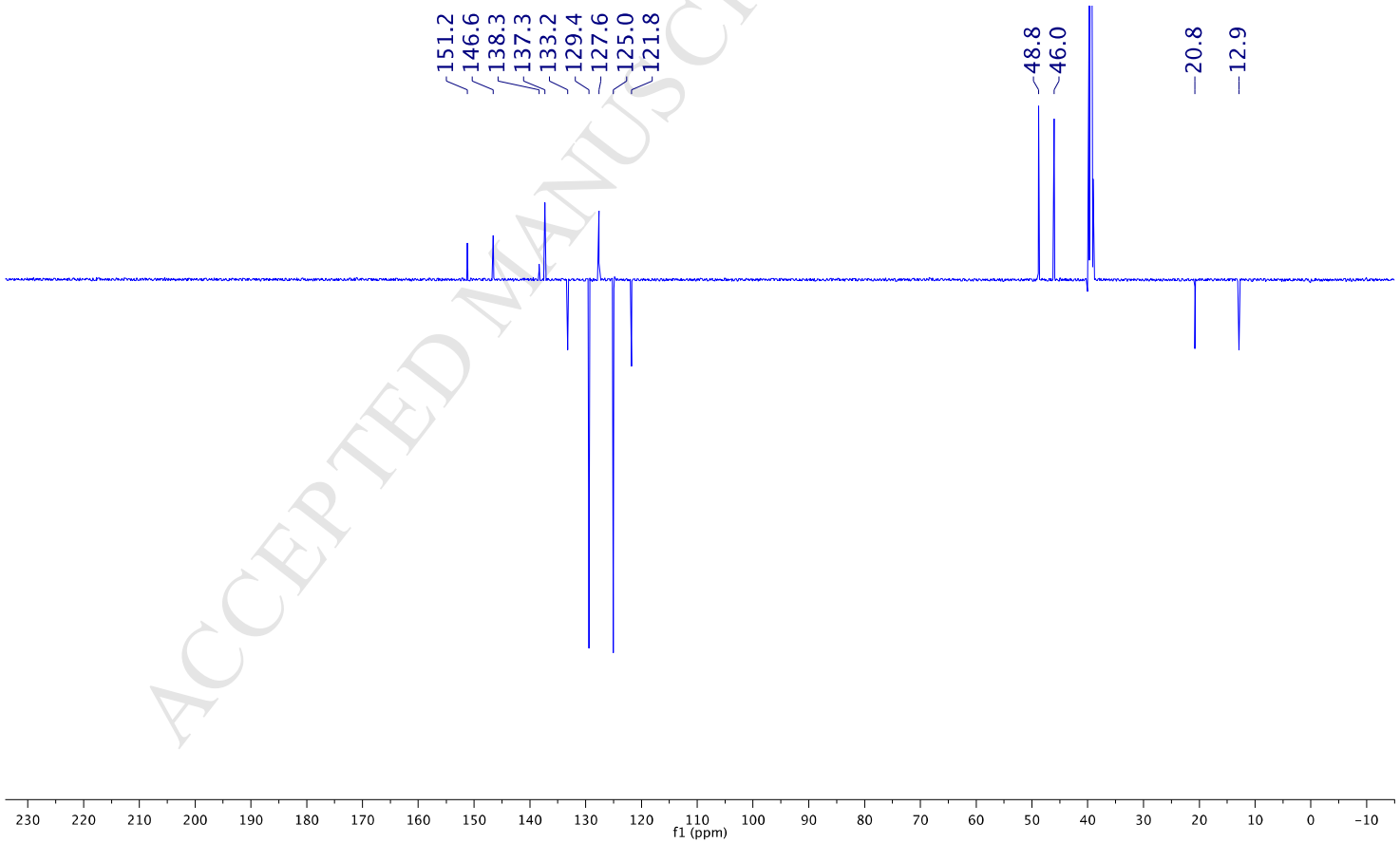
1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(p-tolyl)-1H-1,2,3-triazole

^{13}C JMOD NMR (150 MHz, DMSO-d_6)



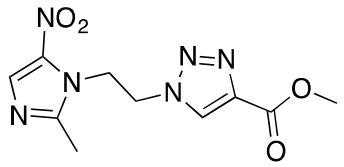
4e

	Parameter	Value
1	Title	AMJ6768_020A_f9.2.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	jmod
8	Experiment	JMOD
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	2048
11	Receiver Gain	29193
12	Relaxation Delay	1.0000
13	Pulse Width	12.0000
14	Presaturation Frequency	
15	Acquisition Time	0.8717
16	Acquisition Date	2013-08-31T14:49:00
17	Modification Date	2013-08-31T14:49:45
18	Spectrometer Frequency	150.92
19	Spectral Width	37594.0
20	Lowest Frequency	-2273.6
21	Nucleus	13C
22	Acquired Size	32768
23	Spectral Size	65536



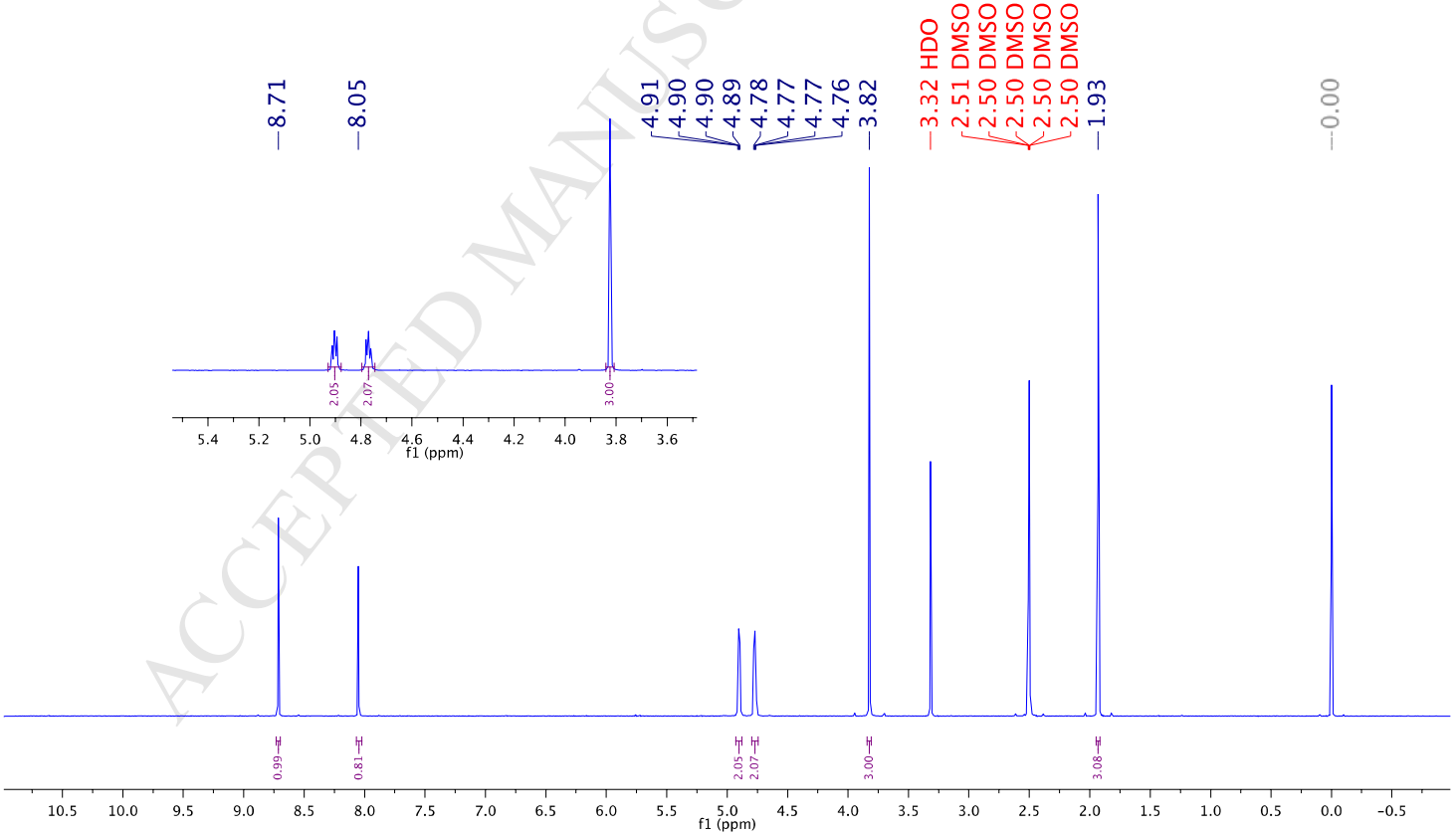
1-[2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl]-4-(methoxycarbonyl)- 1H-1,2,3-triazole

^{1}H NMR (600 MHz, DMSO-d_6)



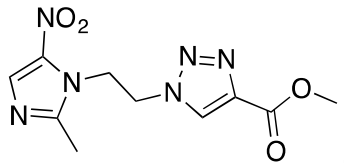
4f

	Parameter	Value
1	Title	AMJ6768_068E_f7_ppt.1.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	zg
8	Experiment	1D
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	8
11	Receiver Gain	32
12	Relaxation Delay	10.0000
13	Pulse Width	7.8000
14	Presaturation Frequency	
15	Acquisition Time	2.2808
16	Acquisition Date	2013-10-18T16:18:00
17	Modification Date	2013-10-18T16:18:30
18	Spectrometer Frequency	600.13
19	Spectral Width	7183.9
20	Lowest Frequency	-591.3
21	Nucleus	1H
22	Acquired Size	16384
23	Spectral Size	65536



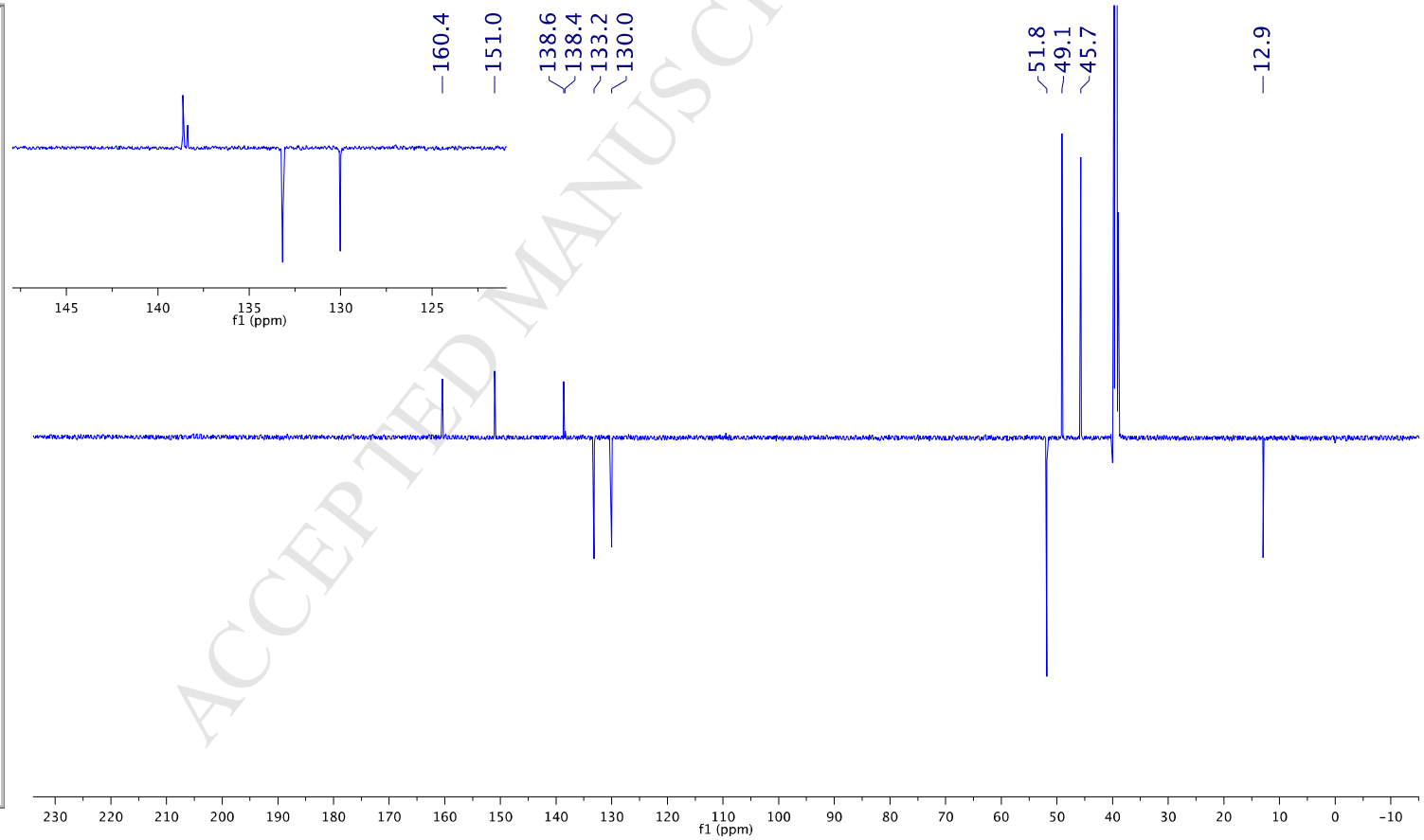
1-[2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl]-4-(methoxycarbonyl)- 1H-1,2,3-triazole

^{13}C JMOD NMR (150 MHz, DMSO-d_6).



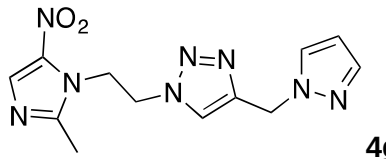
4f

	Parameter	Value
1	Title	AMJ6768_068E_f7_filteredppt.2.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	jmod
8	Experiment	JMOD
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	3584
11	Receiver Gain	41285
12	Relaxation Delay	1.0000
13	Pulse Width	12.0000
14	Presaturation Frequency	
15	Acquisition Time	0.8717
16	Acquisition Date	2014-04-07T17:06:00
17	Modification Date	2014-04-07T17:07:07
18	Spectrometer Frequency	150.92
19	Spectral Width	37594.0
20	Lowest Frequency	-2273.6
21	Nucleus	13C
22	Acquired Size	32768
23	Spectral Size	65536



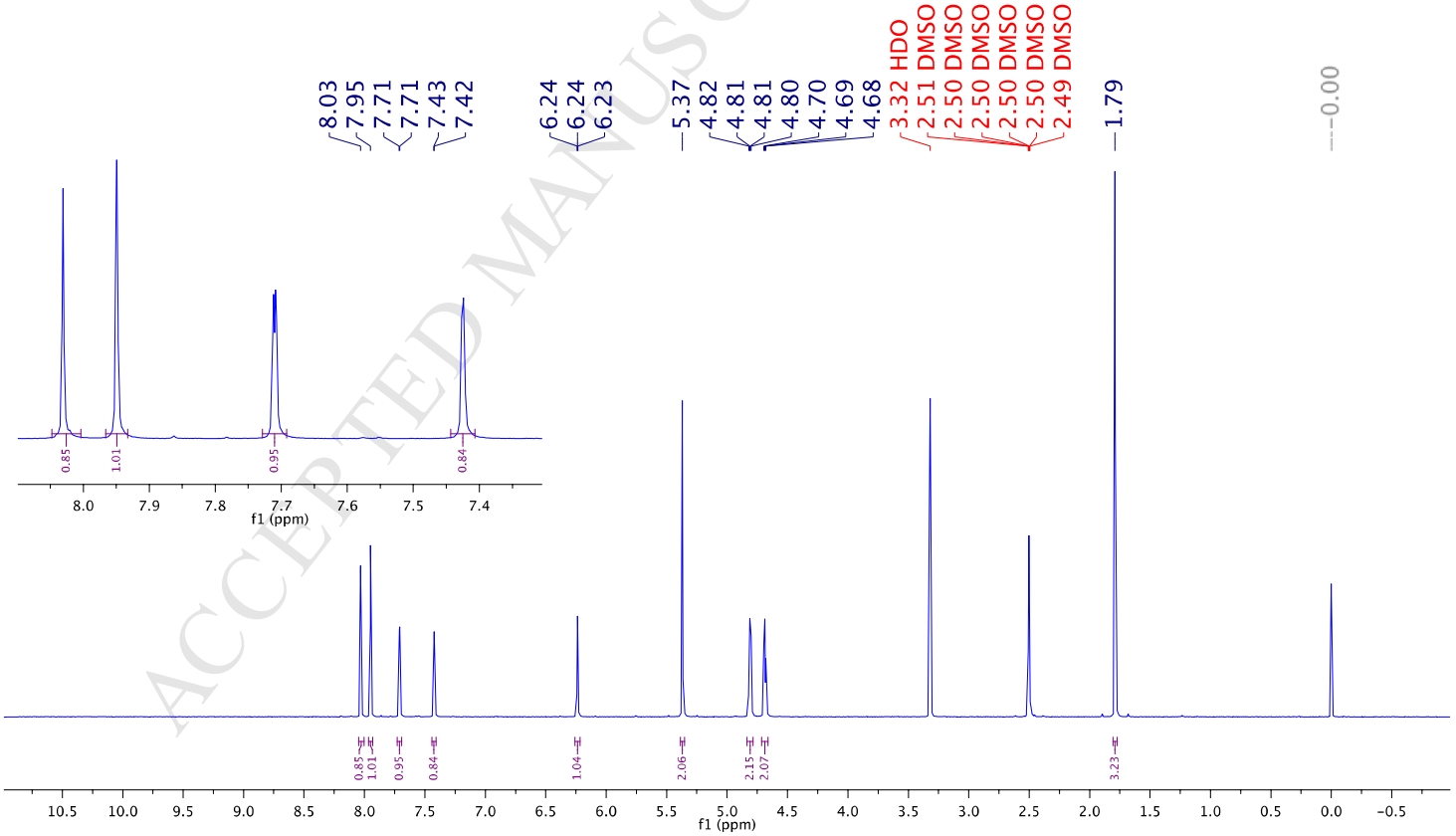
1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-((1H-pyrazol-1-yl)methyl)-1H-1,2,3-triazole

^{1}H NMR (600 MHz, DMSO-d_6)



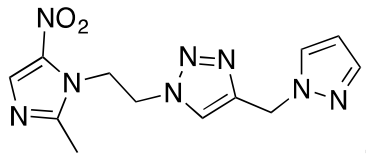
4g

	Parameter	Value
1	Title	AMJ6768_068A_conc_f8-9.1.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	zg
8	Experiment	1D
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	8
11	Receiver Gain	32
12	Relaxation Delay	10.0000
13	Pulse Width	7.8000
14	Presaturation Frequency	
15	Acquisition Time	2.2808
16	Acquisition Date	2013-11-07T17:51:00
17	Modification Date	2013-11-07T17:51:59
18	Spectrometer Frequency	600.13
19	Spectral Width	7183.9
20	Lowest Frequency	-591.9
21	Nucleus	1H
22	Acquired Size	16384
23	Spectral Size	65536



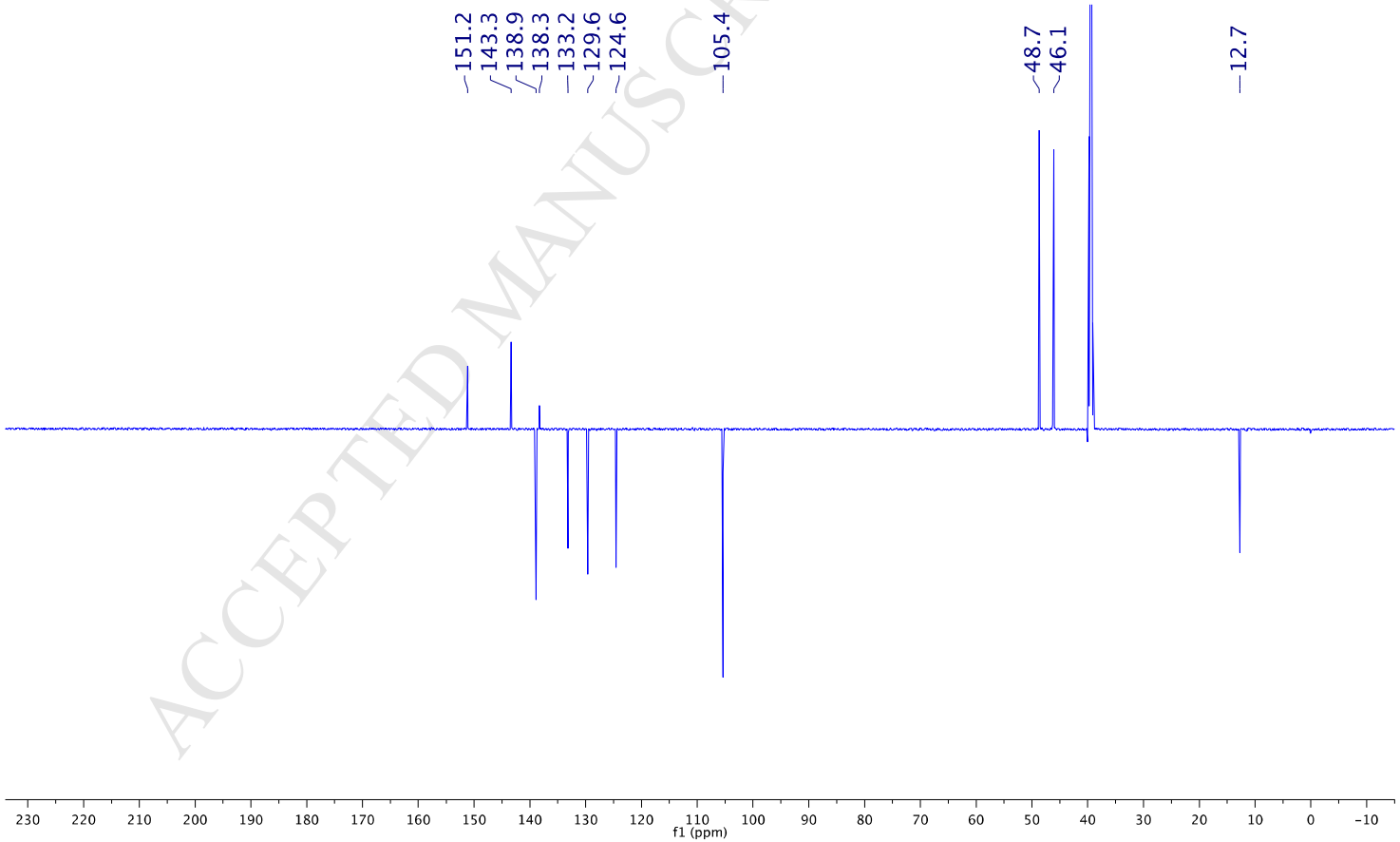
1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-((1H-pyrazol-1-yl)methyl)-1H-1,2,3-triazole

^{13}C JMOD NMR (150 MHz, DMSO-d_6)



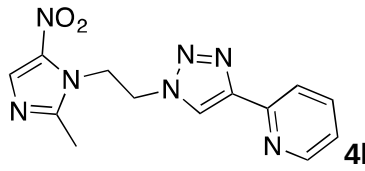
4g

	Parameter	Value
1	Title	AMJ6768_068A_conc_f8-9.2.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	jmod
8	Experiment	JMOD
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	4096
11	Receiver Gain	29193
12	Relaxation Delay	1.0000
13	Pulse Width	12.0000
14	Presaturation Frequency	
15	Acquisition Time	0.8717
16	Acquisition Date	2013-11-07T20:04:00
17	Modification Date	2013-11-07T20:04:41
18	Spectrometer Frequency	150.92
19	Spectral Width	37594.0
20	Lowest Frequency	-2273.6
21	Nucleus	13C
22	Acquired Size	32768
23	Spectral Size	65536



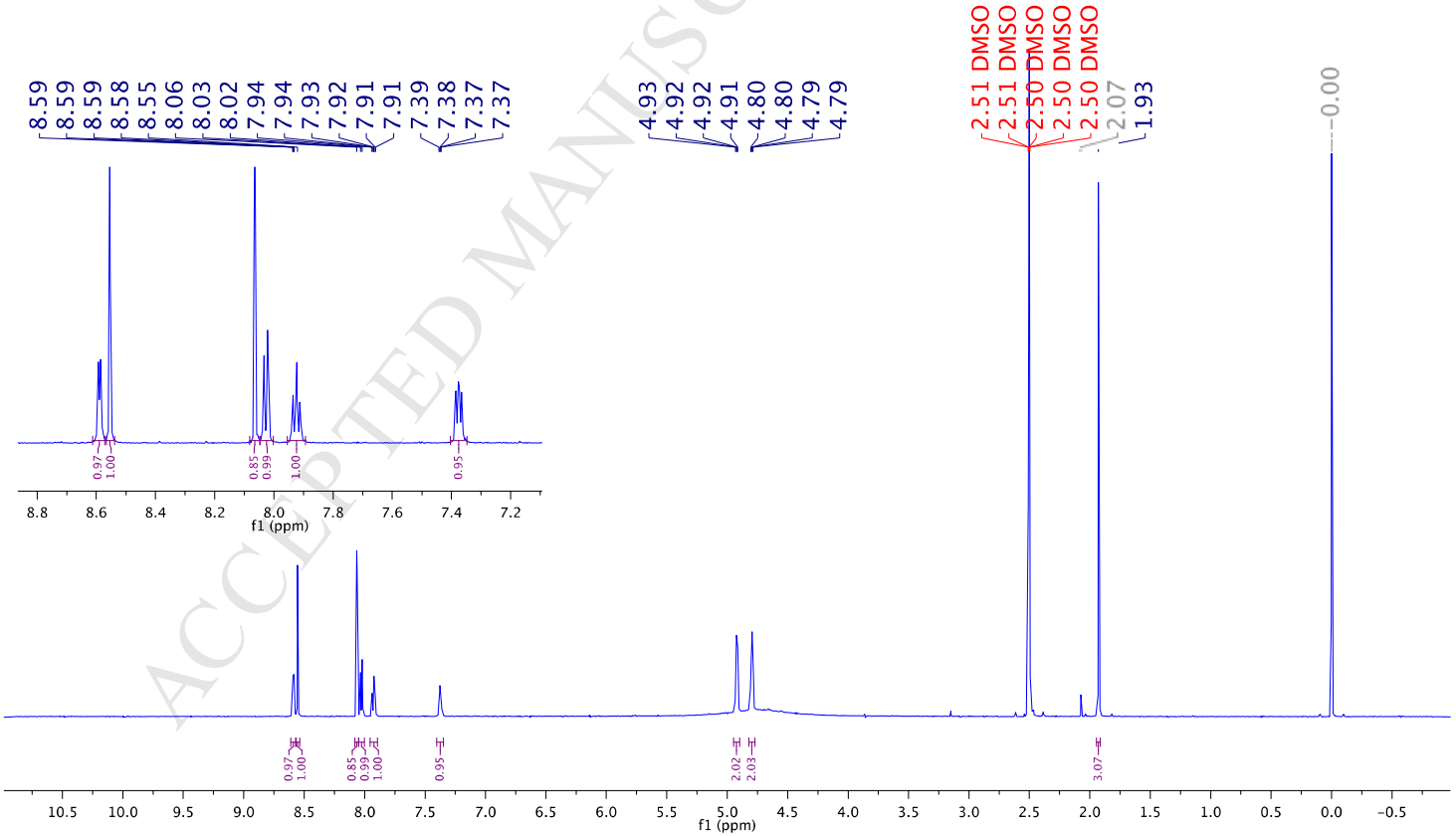
1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(2-pyridinyl)-1H-1,2,3-triazole.xTFA

^{1}H NMR (600 MHz, DMSO-d_6)



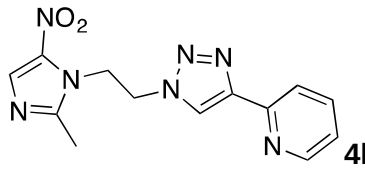
4h

	Parameter	Value
1	Title	AMJ6768_030B_c18_f7-10.1.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	zg
8	Experiment	1D
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	8
11	Receiver Gain	32
12	Relaxation Delay	10.0000
13	Pulse Width	7.8000
14	Presaturation Frequency	
15	Acquisition Time	2.2808
16	Acquisition Date	2013-10-19T14:11:00
17	Modification Date	2013-10-19T14:11:24
18	Spectrometer Frequency	600.13
19	Spectral Width	7183.9
20	Lowest Frequency	-591.0
21	Nucleus	1H
22	Acquired Size	16384
23	Spectral Size	65536



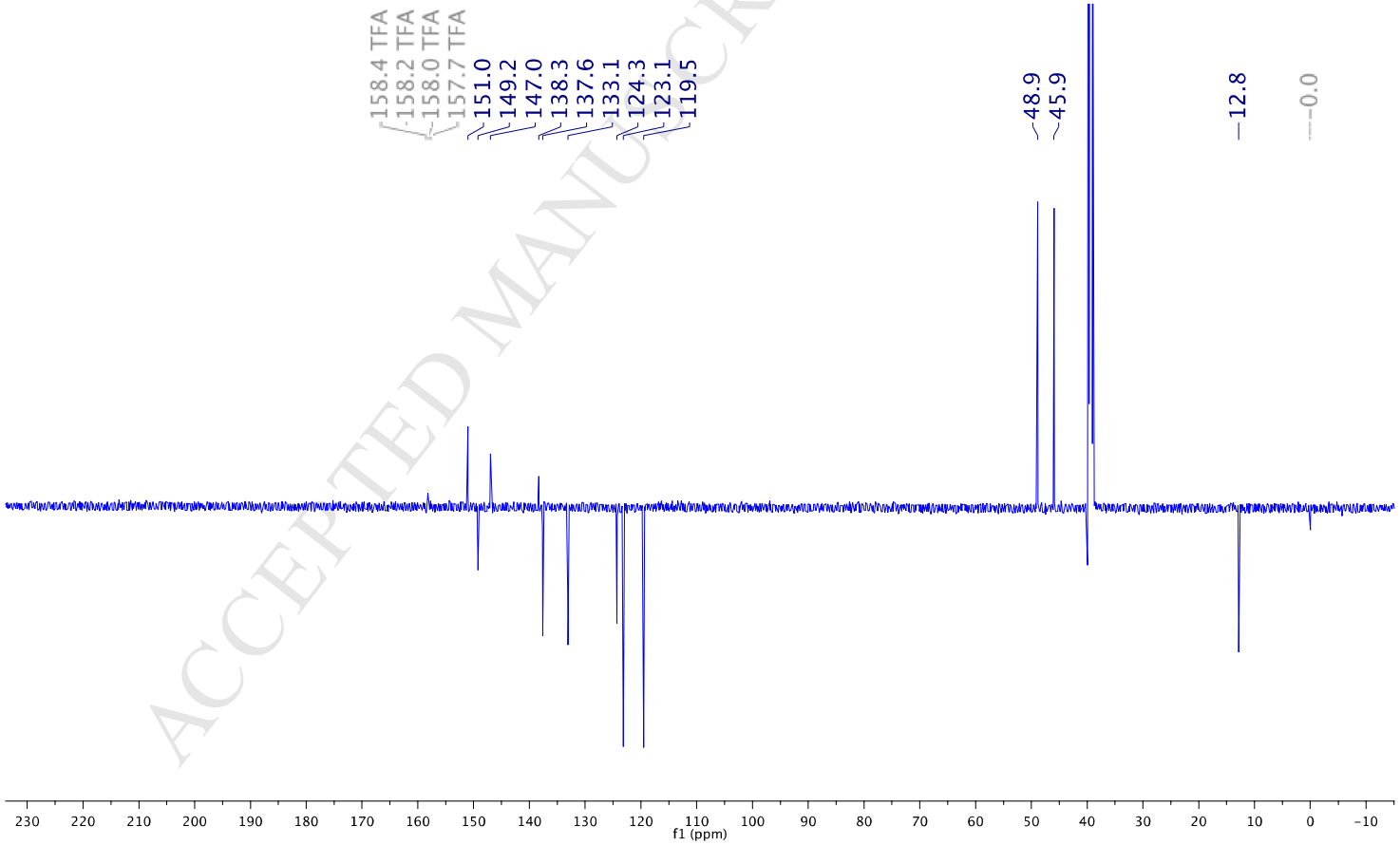
1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(2-pyridinyl)-1H-1,2,3-triazole.xTFA

^{13}C JMOD NMR (150 MHz, DMSO-d_6)



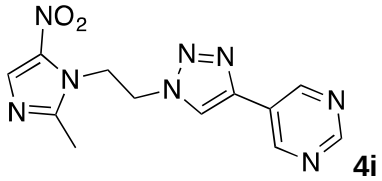
4h

	Parameter	Value
1	Title	AMJ6768_030B_c18_f7-10.2.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	jmod
8	Experiment	JMOD
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	4096
11	Receiver Gain	41285
12	Relaxation Delay	1.0000
13	Pulse Width	12.0000
14	Presaturation Frequency	
15	Acquisition Time	0.8717
16	Acquisition Date	2013-10-19T16:24:00
17	Modification Date	2013-10-19T16:24:09
18	Spectrometer Frequency	150.92
19	Spectral Width	37594.0
20	Lowest Frequency	-2273.6
21	Nucleus	13C
22	Acquired Size	32768
23	Spectral Size	65536



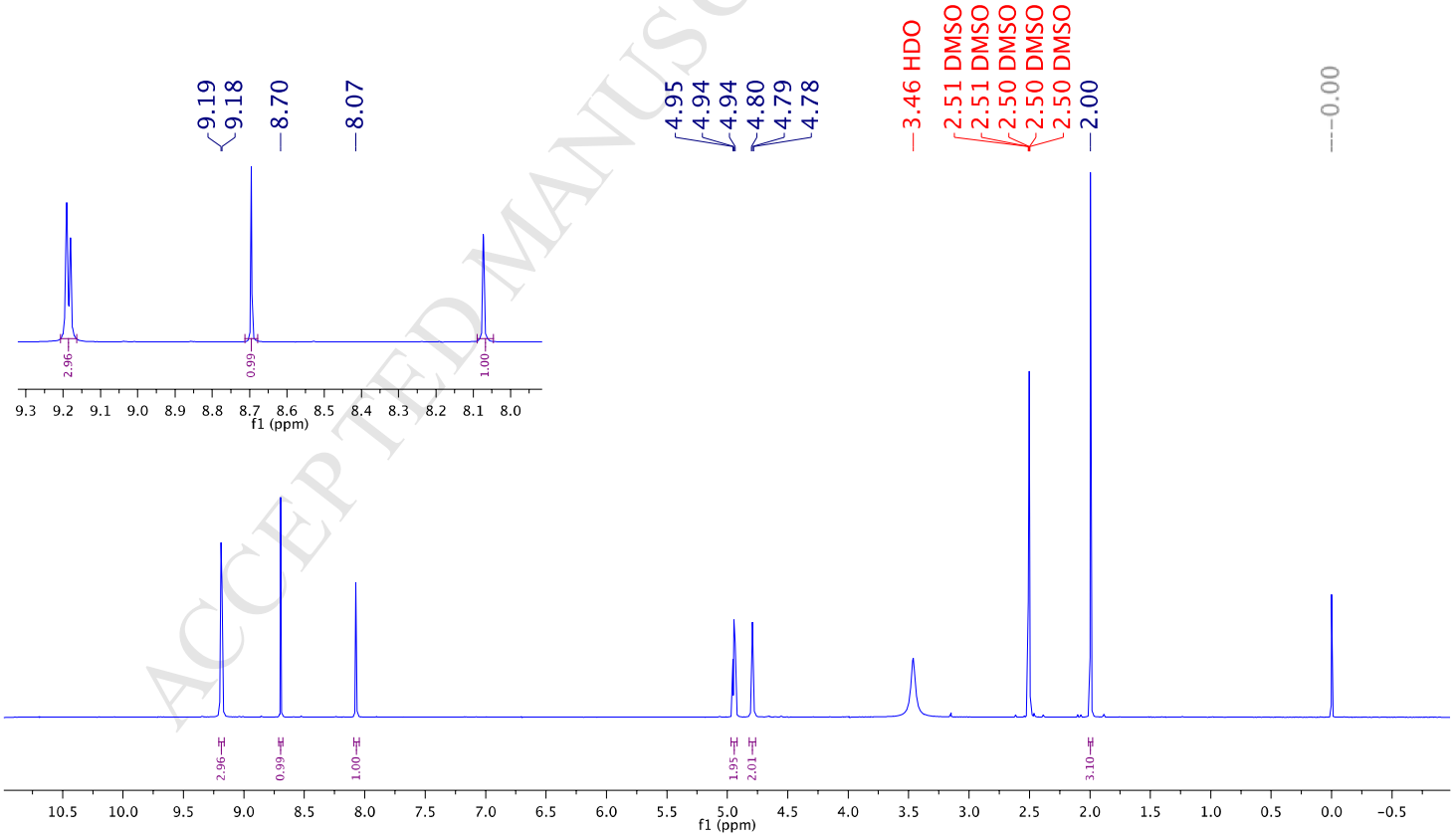
1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(5-pyrimidinyl)-1H-1,2,3-triazole

^{1}H NMR (600 MHz, DMSO-d_6)



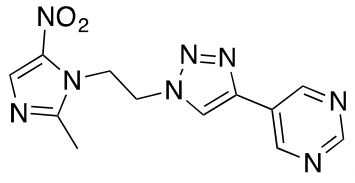
4i

	Parameter	Value
1	Title	AMJ6768_030D_c18_f10-12.1.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.1
7	Pulse Sequence	zg
8	Experiment	1D
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	8
11	Receiver Gain	32
12	Relaxation Delay	10.0000
13	Pulse Width	7.8000
14	Presaturation Frequency	
15	Acquisition Time	2.2808
16	Acquisition Date	2013-10-18T16:36:00
17	Modification Date	2013-10-18T16:36:36
18	Spectrometer Frequency	600.13
19	Spectral Width	7183.9
20	Lowest Frequency	-590.4
21	Nucleus	1H
22	Acquired Size	16384
23	Spectral Size	65536



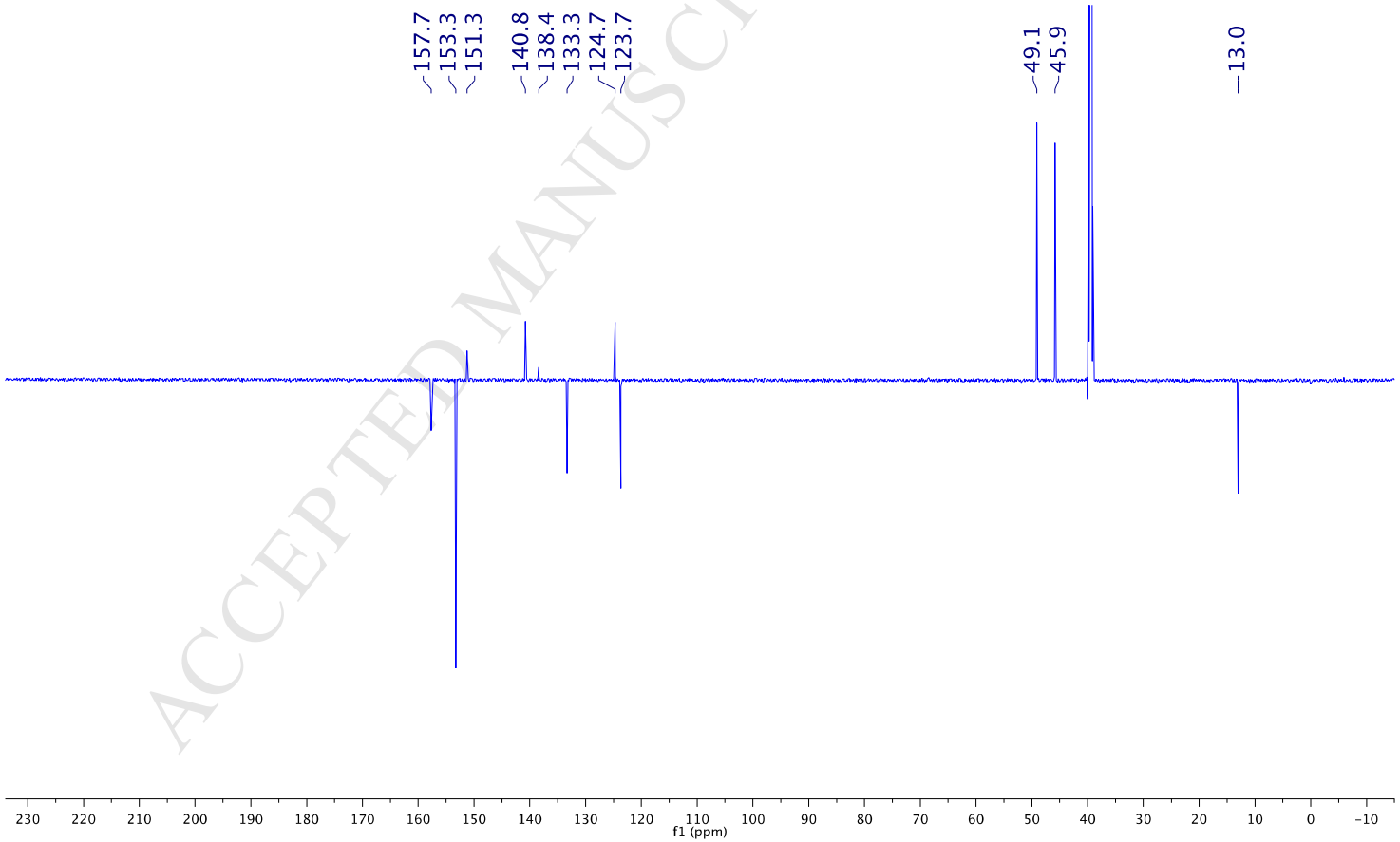
1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(5-pyrimidinyl)-1H-1,2,3-triazole

^{13}C JMOD NMR (150 MHz, DMSO-d_6)



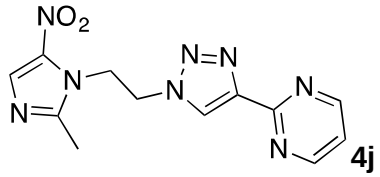
4i

	Parameter	Value
1	Title	AMJ6768_030D_c18_f10-12.2.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	jmod
8	Experiment	JMOD
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	4096
11	Receiver Gain	41285
12	Relaxation Delay	1.0000
13	Pulse Width	12.0000
14	Presaturation Frequency	
15	Acquisition Time	0.8717
16	Acquisition Date	2013-10-18T18:49:00
17	Modification Date	2013-10-18T18:49:20
18	Spectrometer Frequency	150.92
19	Spectral Width	37594.0
20	Lowest Frequency	-2273.6
21	Nucleus	13C
22	Acquired Size	32768
23	Spectral Size	65536



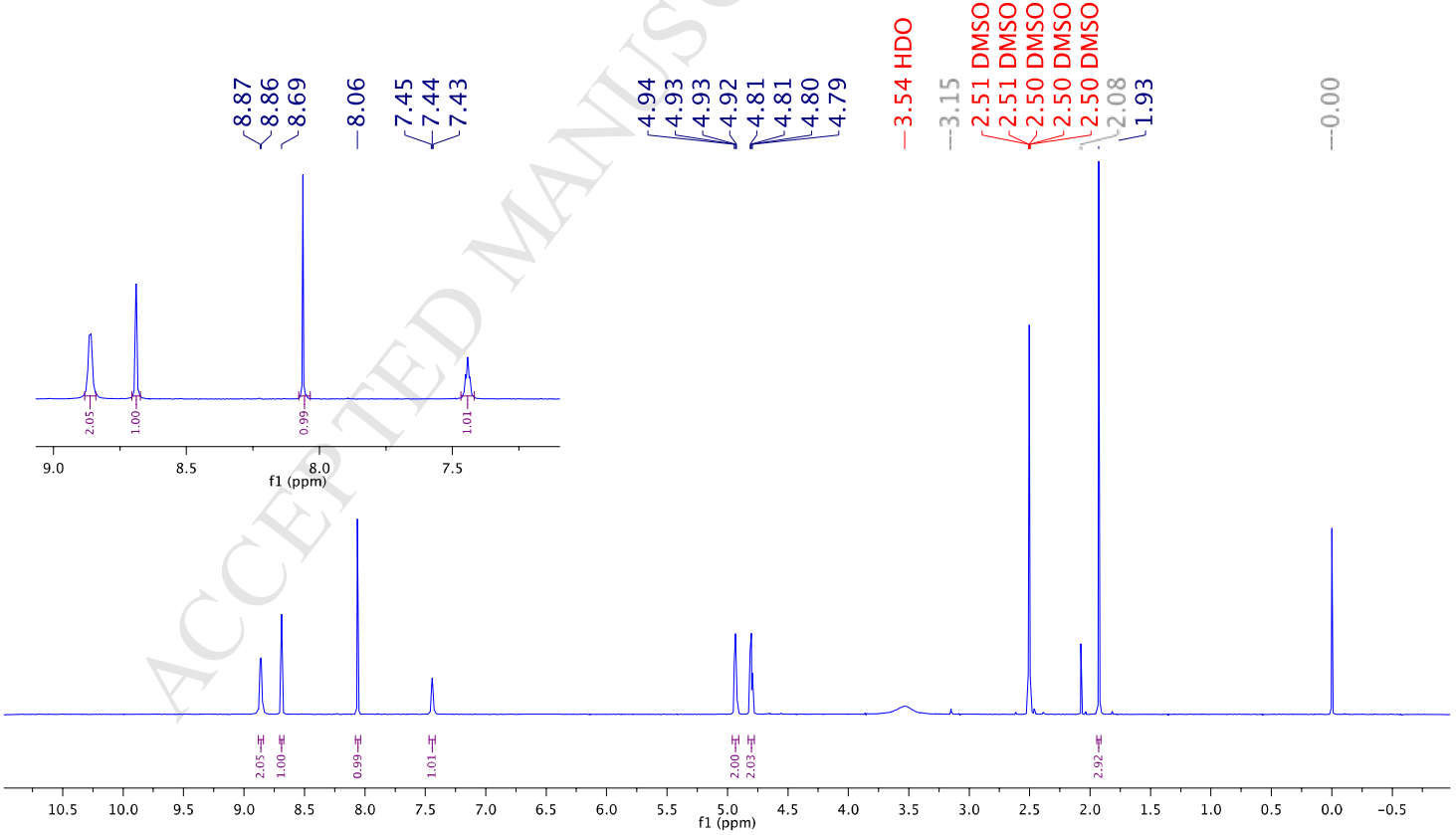
1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(2-pyrimidinyl)-1H-1,2,3-triazole

^{1}H NMR (600 MHz, DMSO-d_6)



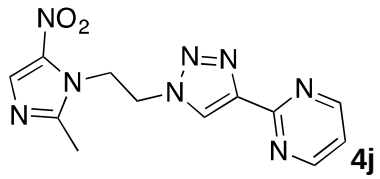
4j

	Parameter	Value
1	Title	AMJ6768_030C_col2_f27-29.1.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	zg
8	Experiment	1D
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	8
11	Receiver Gain	32
12	Relaxation Delay	30.0000
13	Pulse Width	7.3565
14	Presaturation Frequency	
15	Acquisition Time	2.2808
16	Acquisition Date	2013-12-11T11:55:00
17	Modification Date	2013-12-11T11:55:52
18	Spectrometer Frequency	600.13
19	Spectral Width	7183.9
20	Lowest Frequency	-589.6
21	Nucleus	1H
22	Acquired Size	16384
23	Spectral Size	65536



1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(2-pyrimidinyl)-1H-1,2,3-triazole

^{13}C JMOD NMR (150 MHz, DMSO-d_6)



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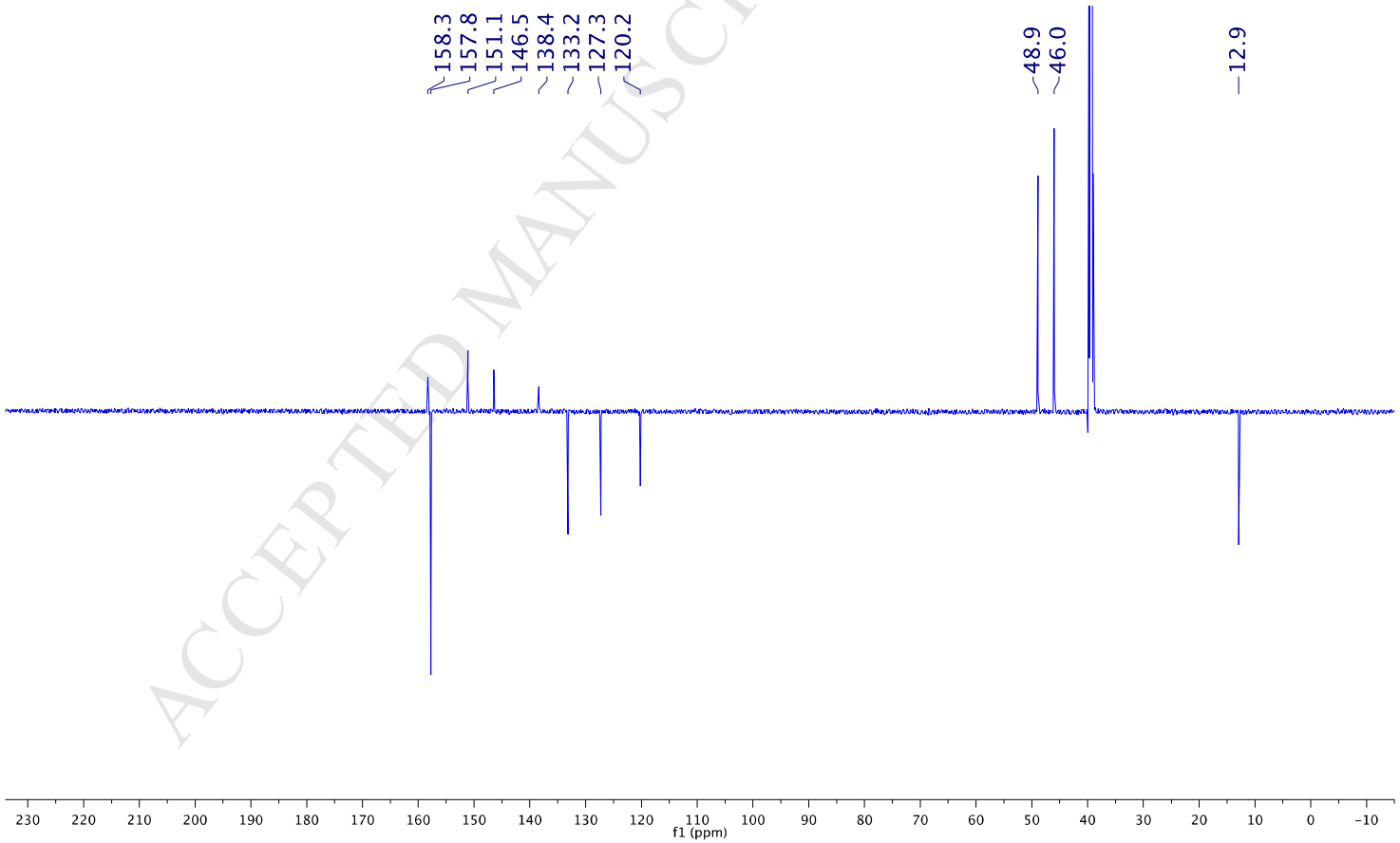
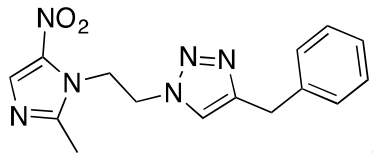
	Parameter	Value
1	Title	AMJ6768_030C_c18_col2_f27-29.2.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	jmod
8	Experiment	JMOD
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	3584
11	Receiver Gain	41285
12	Relaxation Delay	1.0000
13	Pulse Width	12.0000
14	Presaturation Frequency	
15	Acquisition Time	0.8717
16	Acquisition Date	2014-04-07T15:05:00
17	Modification Date	2014-04-07T15:05:55
18	Spectrometer Frequency	150.92
19	Spectral Width	37594.0
20	Lowest Frequency	-2273.6
21	Nucleus	13C
22	Acquired Size	32768
23	Spectral Size	65536



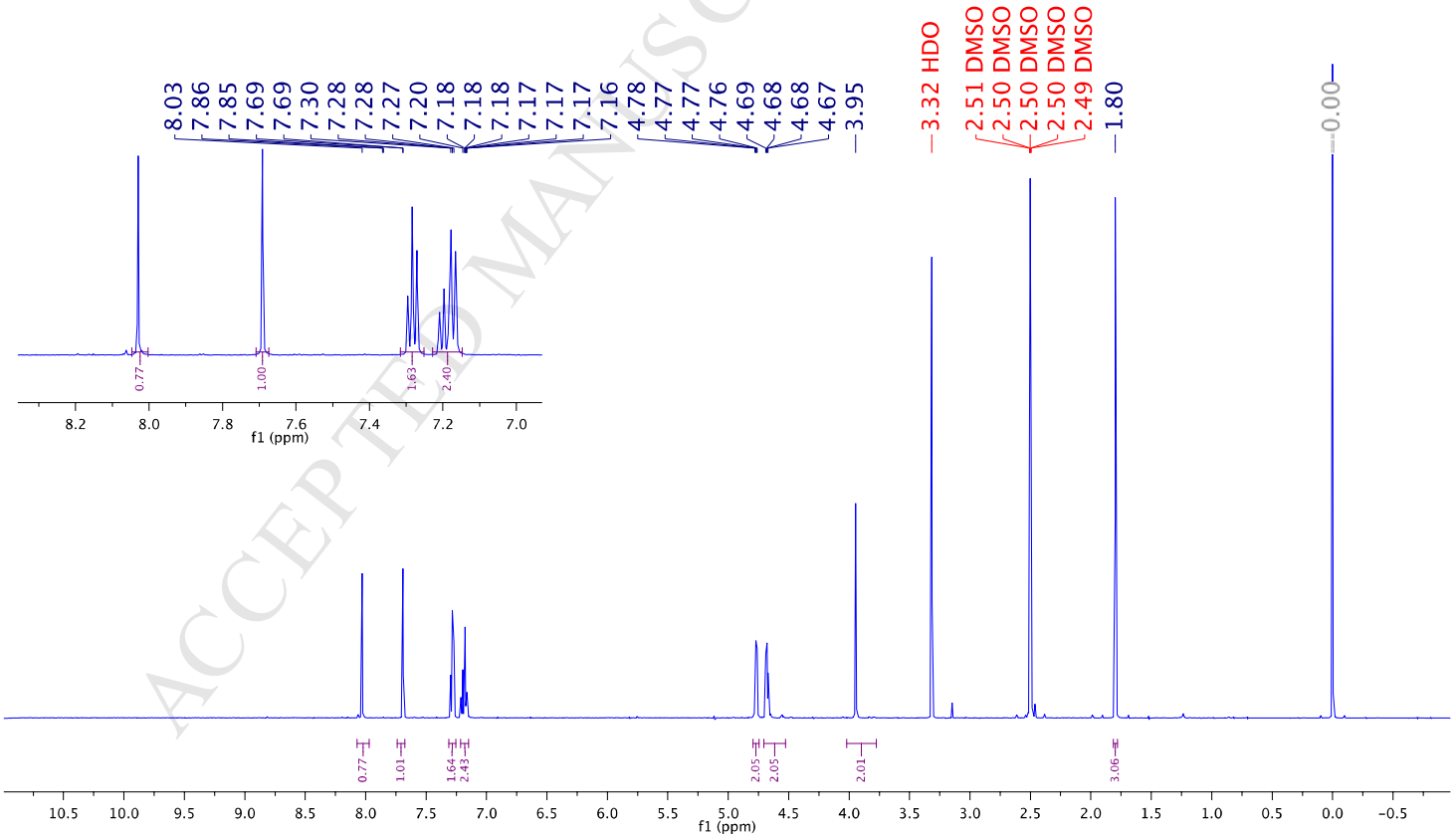
1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)- 4-benzyl-1H-1,2,3-triazole

^{1}H NMR (600 MHz, DMSO-d_6)



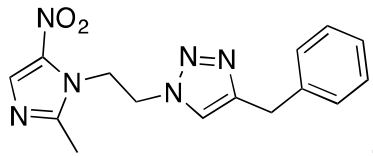
4k

	Parameter	Value
1	Title	AMJ6768_020B_f9-10_col2_f8_col3_f7_highvac.1.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	zg
8	Experiment	1D
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	8
11	Receiver Gain	32
12	Relaxation Delay	10.0000
13	Pulse Width	7.8000
14	Presaturation Frequency	
15	Acquisition Time	2.2808
16	Acquisition Date	2013-09-16T01:28:00
17	Modification Date	2013-09-16T01:29:04
18	Spectrometer Frequency	600.13
19	Spectral Width	7183.9
20	Lowest Frequency	-585.8
21	Nucleus	1H
22	Acquired Size	16384
23	Spectral Size	65536



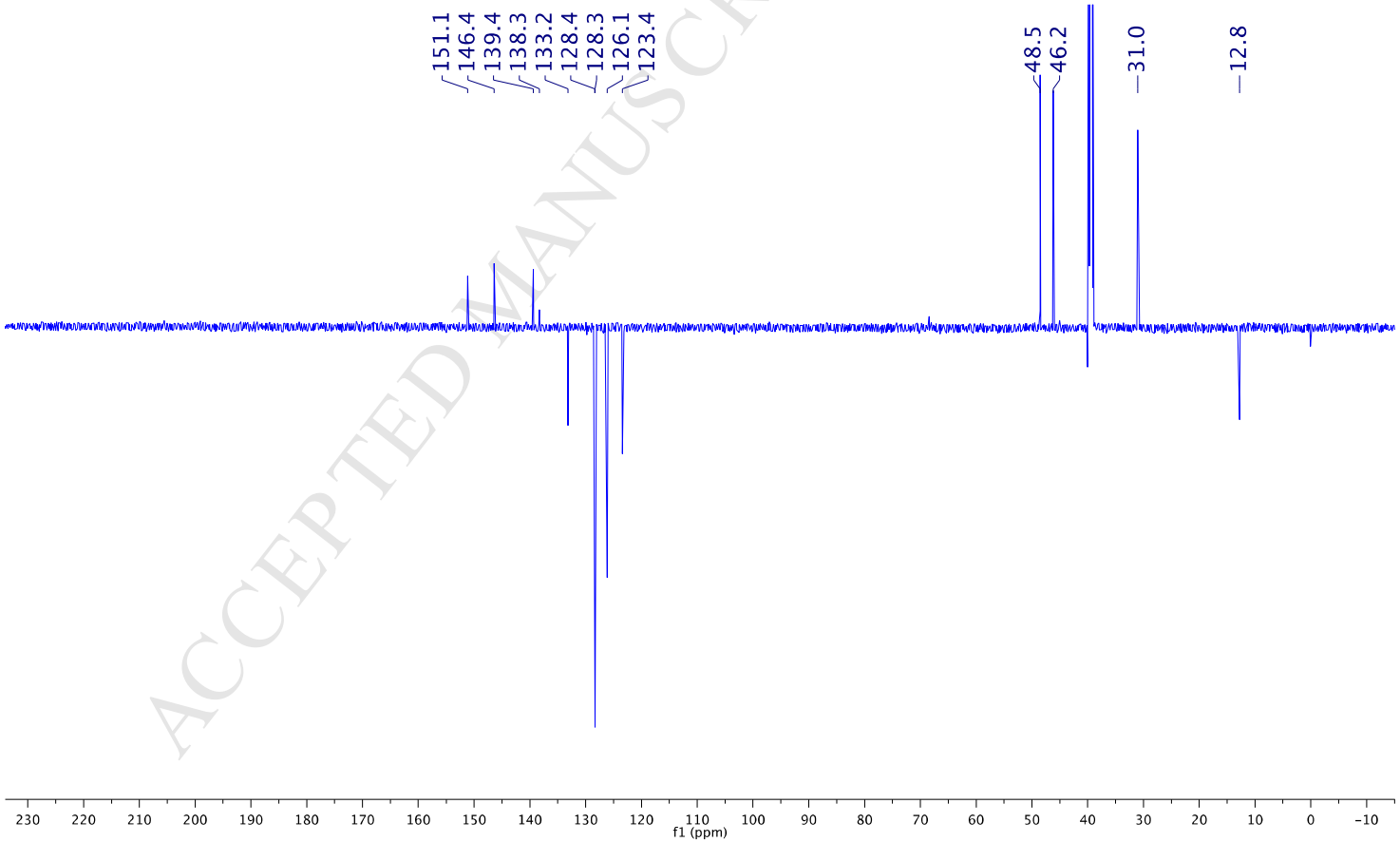
4-benzyl-1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-1H-1,2,3-triazole

^{13}C JMOD NMR (150 MHz, DMSO-d_6)



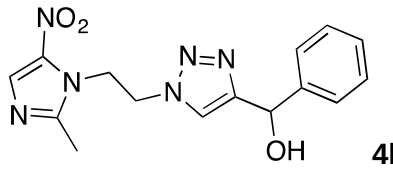
4k

	Parameter	Value
1	Title	AMJ6768_020B_f9-10_col2_f8_col3_f7_highvac.2.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	jmod
8	Experiment	JMOD
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	2048
11	Receiver Gain	46341
12	Relaxation Delay	1.0000
13	Pulse Width	12.0000
14	Presaturation Frequency	
15	Acquisition Time	0.8717
16	Acquisition Date	2013-09-16T02:36:00
17	Modification Date	2013-09-16T02:36:18
18	Spectrometer Frequency	150.92
19	Spectral Width	37594.0
20	Lowest Frequency	-2273.6
21	Nucleus	13C
22	Acquired Size	32768
23	Spectral Size	65536



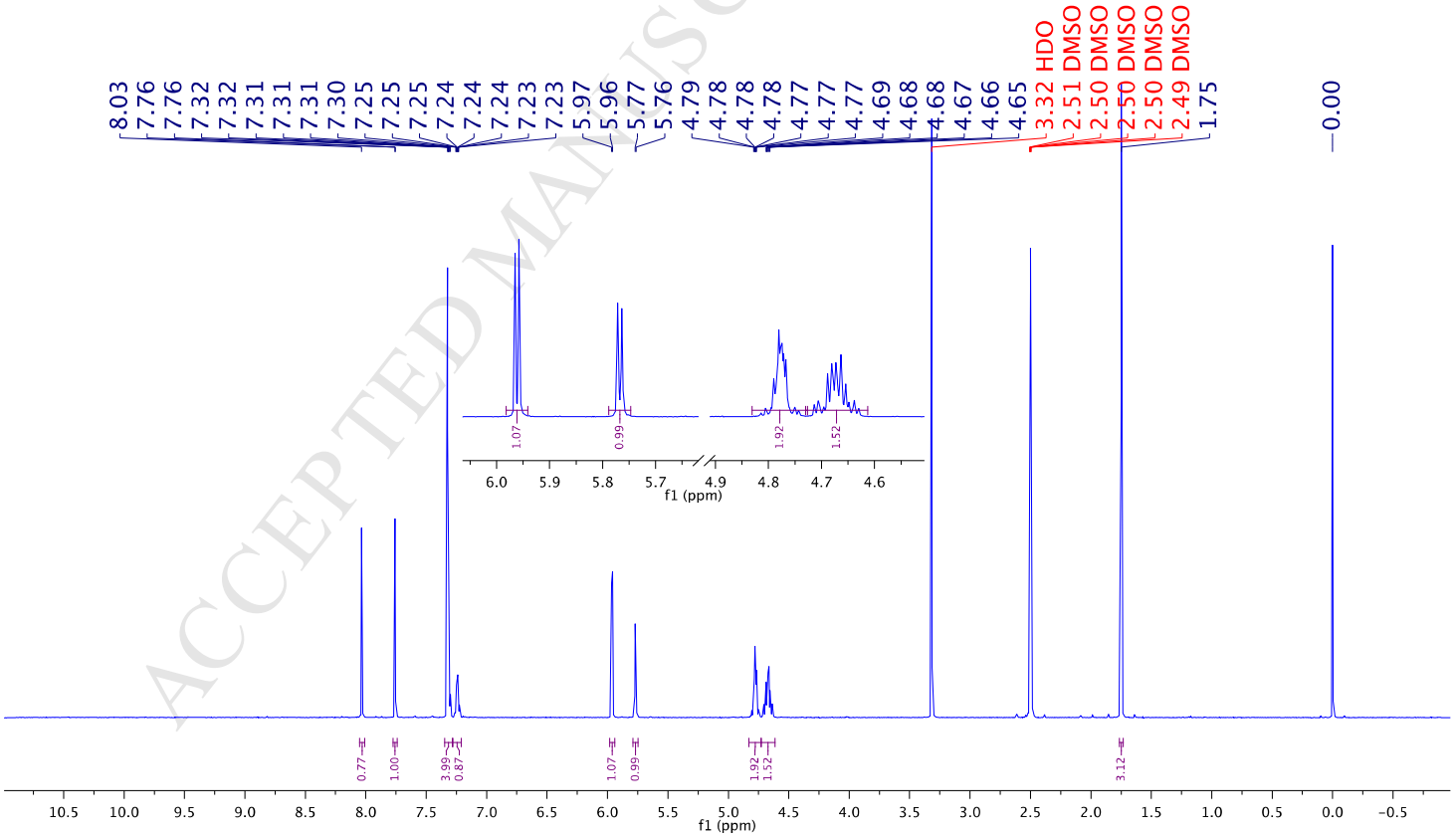
1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(1-phenyl-1-hydroxylmethyl)- 1H-1,2,3-triazole

^{1}H NMR (600 MHz, DMSO-d_6)



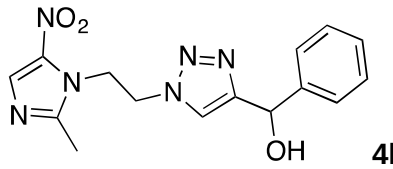
4l

	Parameter	Value
1	Title	AMJ6768_020C_f7_recrys EtOAc.1.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	zg
8	Experiment	1D
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	8
11	Receiver Gain	32
12	Relaxation Delay	10.0000
13	Pulse Width	7.8000
14	Presaturation Frequency	
15	Acquisition Time	2.2808
16	Acquisition Date	2013-08-31T05:47:00
17	Modification Date	2013-08-31T05:47:25
18	Spectrometer Frequency	600.13
19	Spectral Width	7183.9
20	Lowest Frequency	-585.9
21	Nucleus	1H
22	Acquired Size	16384
23	Spectral Size	65536



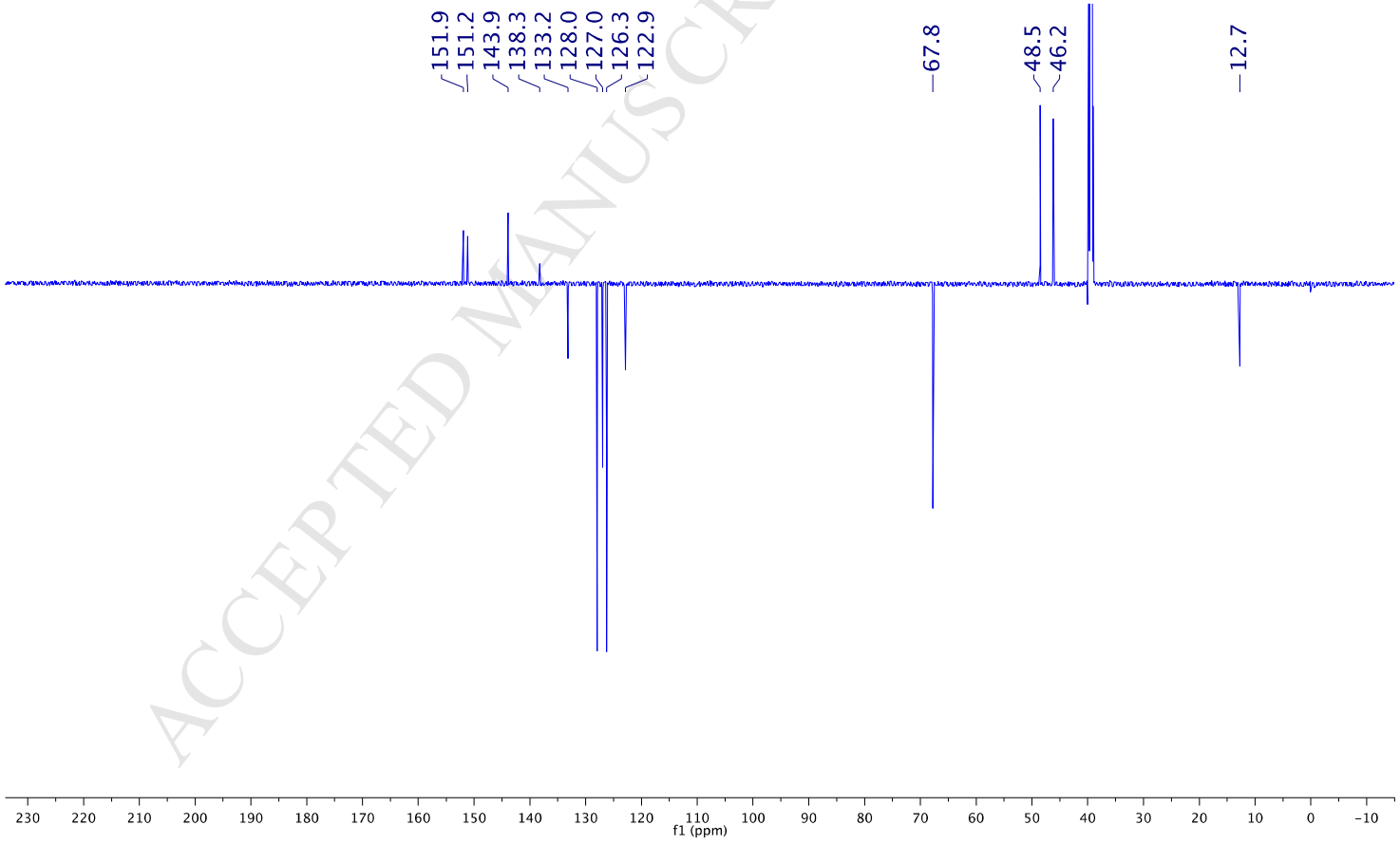
1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(1-phenyl-1-hydroxylmethyl)- 1H-1,2,3-triazole

^{13}C JMOD NMR (150 MHz, DMSO-d_6)



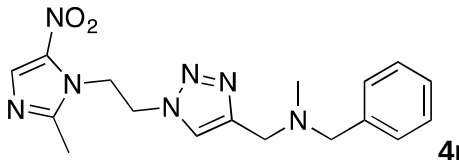
4l

	Parameter	Value
1	Title	AMJ6768_020C_f7_recrysEtOAc.2.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	jmod
8	Experiment	JMOD
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	2048
11	Receiver Gain	46341
12	Relaxation Delay	1.0000
13	Pulse Width	12.0000
14	Presaturation Frequency	
15	Acquisition Time	0.8717
16	Acquisition Date	2013-08-31T06:54:00
17	Modification Date	2013-08-31T06:54:40
18	Spectrometer Frequency	150.92
19	Spectral Width	37594.0
20	Lowest Frequency	-2273.6
21	Nucleus	13C
22	Acquired Size	32768
23	Spectral Size	65536



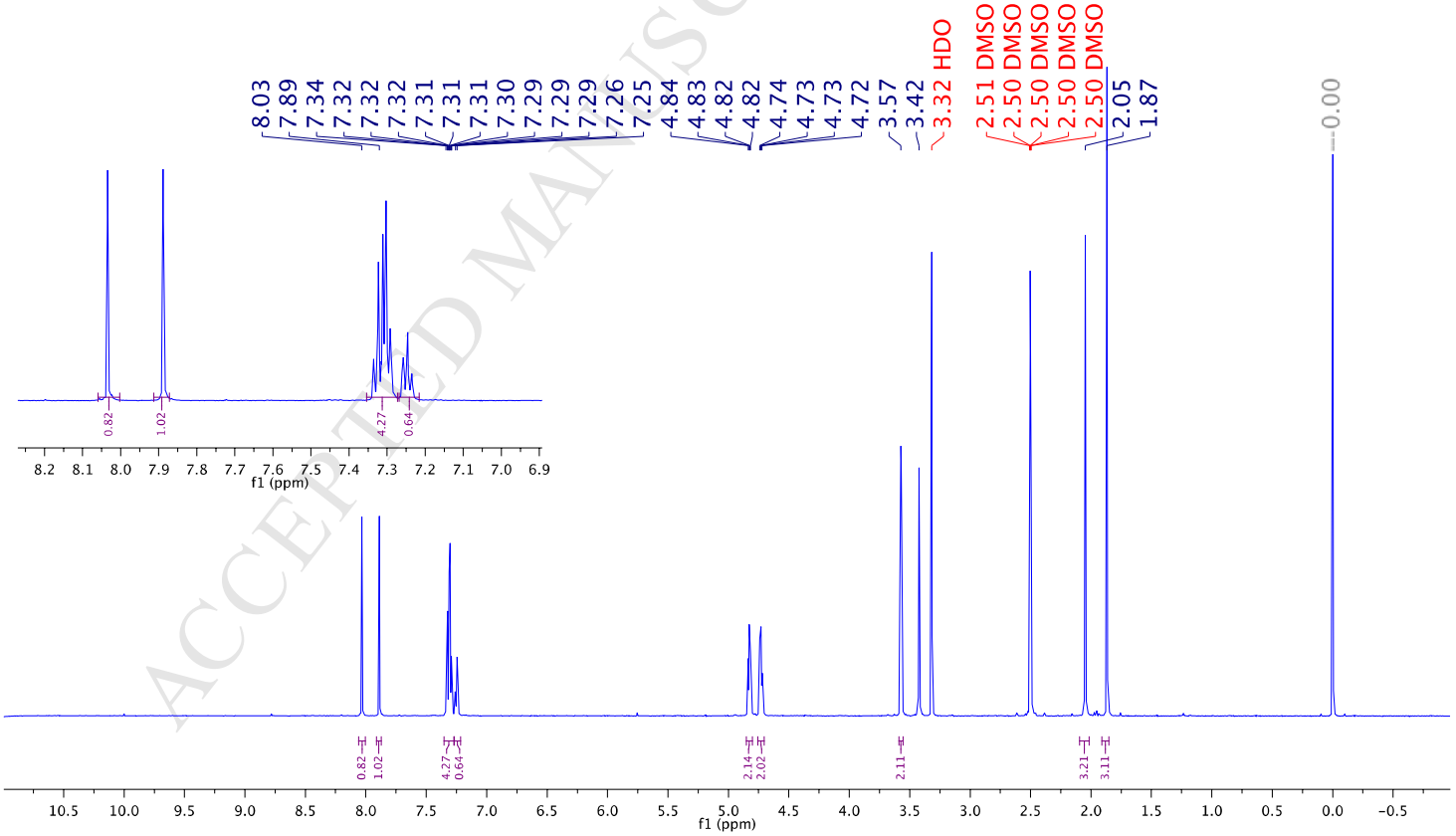
1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(N-benzyl, N-methyl aminomethyl)- 1H-1,2,3-triazole

^{1}H NMR (600 MHz, DMSO-d_6)



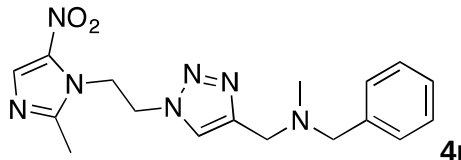
4m

	Parameter	Value
1	Title	AMJ6768_020D_f11_col2 _f10_11_highvac.1.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	zg
8	Experiment	1D
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	8
11	Receiver Gain	32
12	Relaxation Delay	10.0000
13	Pulse Width	7.8000
14	Presaturation Frequency	
15	Acquisition Time	2.2808
16	Acquisition Date	2013-09-15T17:12:00
17	Modification Date	2013-09-15T17:12:17
18	Spectrometer Frequency	600.13
19	Spectral Width	7183.9
20	Lowest Frequency	-585.2
21	Nucleus	1H
22	Acquired Size	16384
23	Spectral Size	65536



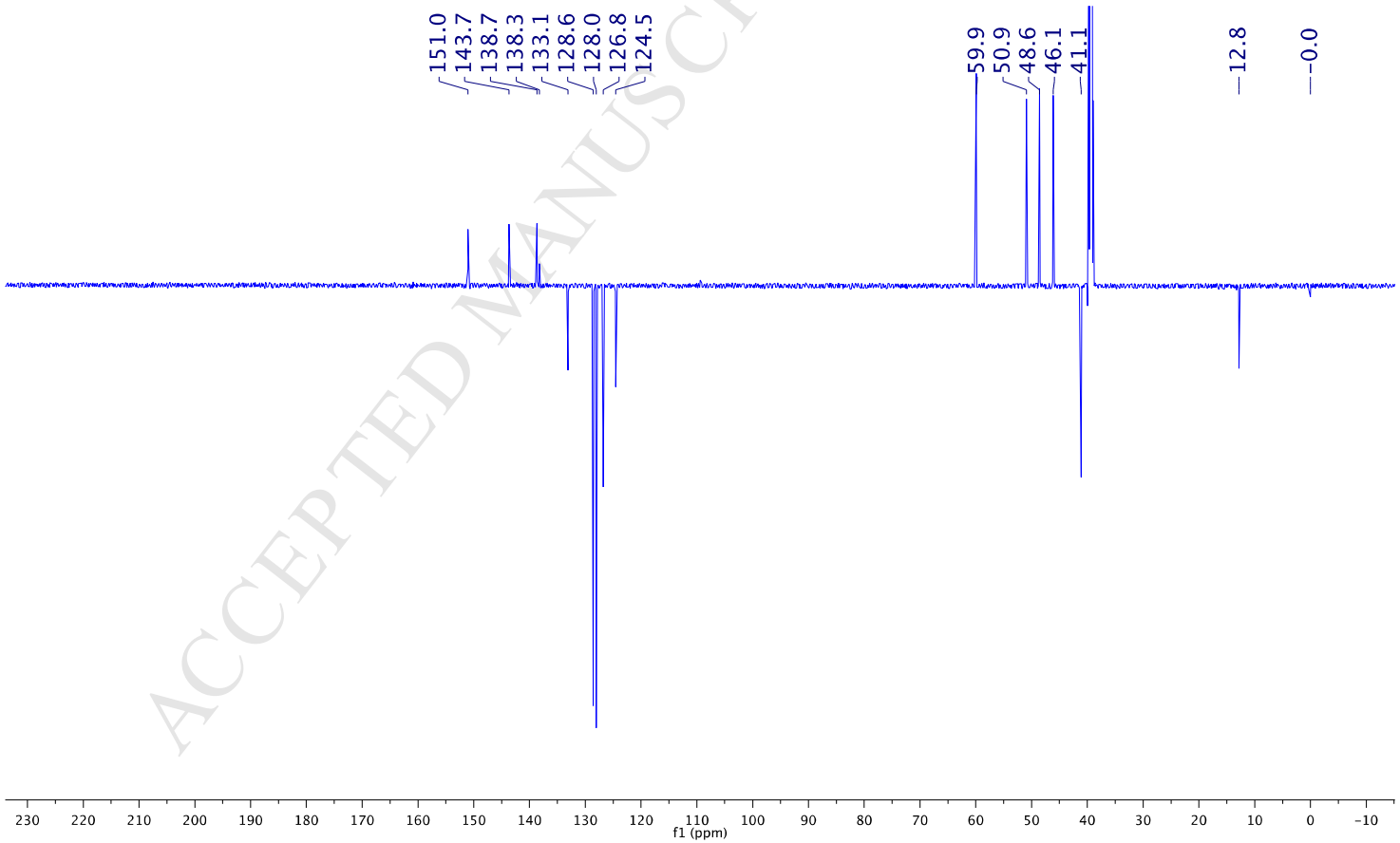
1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(N-benzyl, N-methyl aminomethyl)- 1H-1,2,3-triazole

^{13}C JMOD NMR (150 MHz, DMSO-d_6)



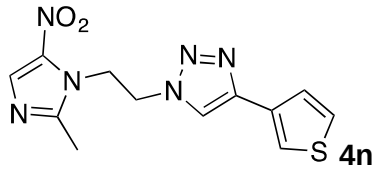
4m

	Parameter	Value
1	Title	AMJ6768_020D_f11_col2 _f10_11_highvac.2.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	jmod
8	Experiment	JMOD
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	2048
11	Receiver Gain	41285
12	Relaxation Delay	1.0000
13	Pulse Width	12.0000
14	Presaturation Frequency	
15	Acquisition Time	0.8717
16	Acquisition Date	2013-09-15T18:19:00
17	Modification Date	2013-09-15T18:19:32
18	Spectrometer Frequency	150.92
19	Spectral Width	37594.0
20	Lowest Frequency	-2273.6
21	Nucleus	13C
22	Acquired Size	32768
23	Spectral Size	65536



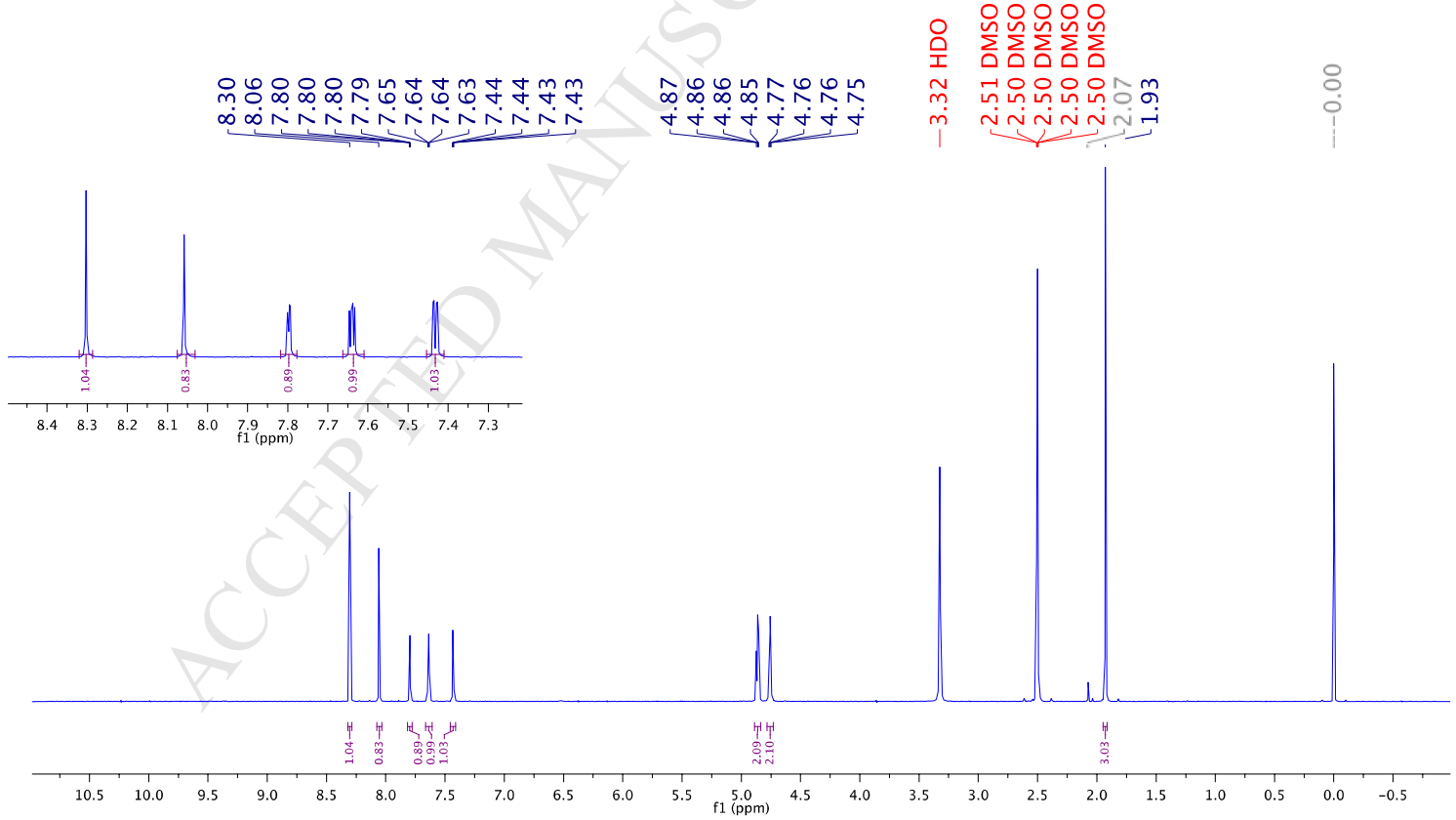
1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(thiophen-3-yl)-1H-1,2,3-triazole

^{1}H NMR (600 MHz, DMSO-d_6)



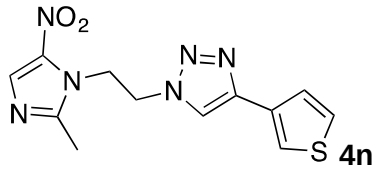
4n

	Parameter	Value
1	Title	AMJ6768_030A_c18_f29-30.1.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	zg
8	Experiment	1D
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	8
11	Receiver Gain	32
12	Relaxation Delay	10.0000
13	Pulse Width	7.8000
14	Presaturation Frequency	
15	Acquisition Time	2.2808
16	Acquisition Date	2013-10-19T10:49:00
17	Modification Date	2013-10-19T10:50:00
18	Spectrometer Frequency	600.13
19	Spectral Width	7183.9
20	Lowest Frequency	-591.5
21	Nucleus	1H
22	Acquired Size	16384
23	Spectral Size	65536



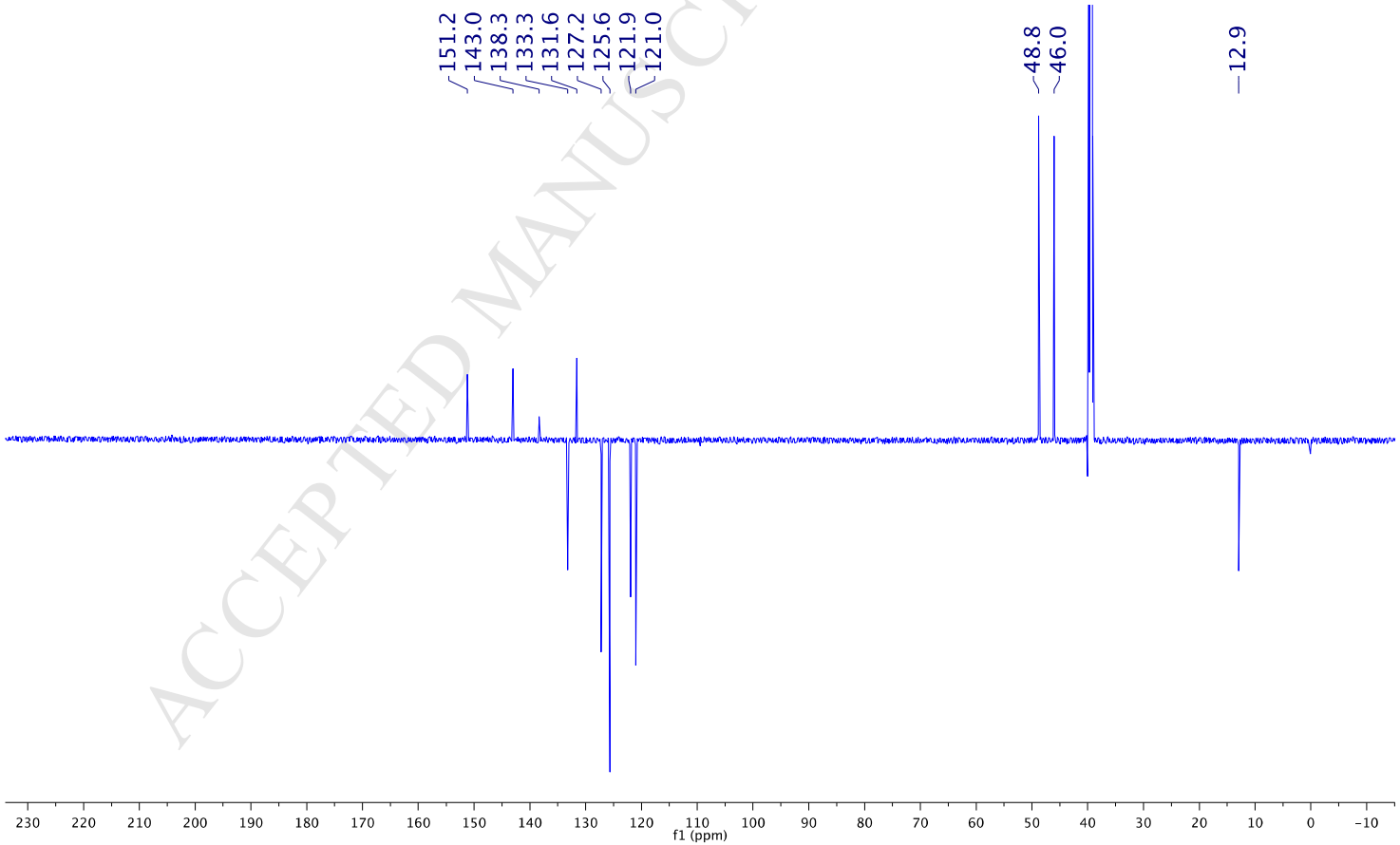
1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(thiophen-3-yl)-1H-1,2,3-triazole

^{13}C JMOD NMR (150 MHz, DMSO-d_6)



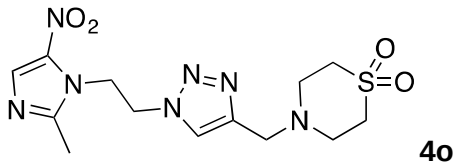
4n

	Parameter	Value
1	Title	AMJ6768_030A_c18_f29-30.2.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	jmod
8	Experiment	JMOD
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	4096
11	Receiver Gain	41285
12	Relaxation Delay	1.0000
13	Pulse Width	12.0000
14	Presaturation Frequency	
15	Acquisition Time	0.8717
16	Acquisition Date	2013-10-19T13:02:00
17	Modification Date	2013-10-19T13:02:46
18	Spectrometer Frequency	150.92
19	Spectral Width	37594.0
20	Lowest Frequency	-2273.6
21	Nucleus	13C
22	Acquired Size	32768
23	Spectral Size	65536



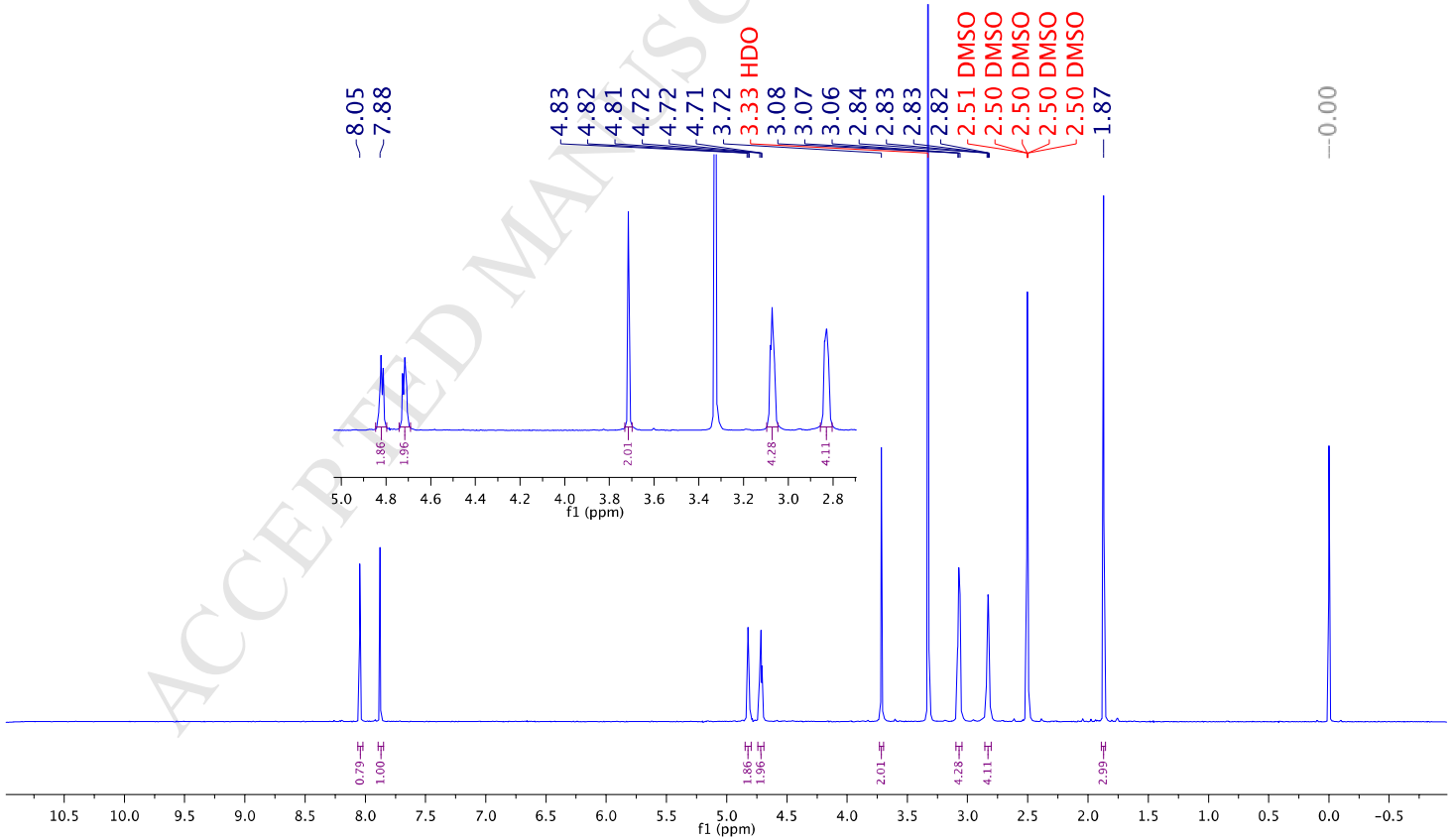
1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-((1,1-dioxothiomorpholin-4-yl)methyl)- 1H-1,2,3-triazole

^{1}H NMR (600 MHz, DMSO-d_6)



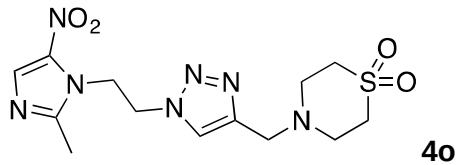
4o

	Parameter	Value
1	Title	AMJ6768_068B_f22-26_chelex.1.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	zg
8	Experiment	1D
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	8
11	Receiver Gain	32
12	Relaxation Delay	10.0000
13	Pulse Width	7.8000
14	Presaturation Frequency	
15	Acquisition Time	2.2808
16	Acquisition Date	2013-11-16T05:39:00
17	Modification Date	2013-11-16T05:40:06
18	Spectrometer Frequency	600.13
19	Spectral Width	7183.9
20	Lowest Frequency	-591.2
21	Nucleus	1H
22	Acquired Size	16384
23	Spectral Size	65536



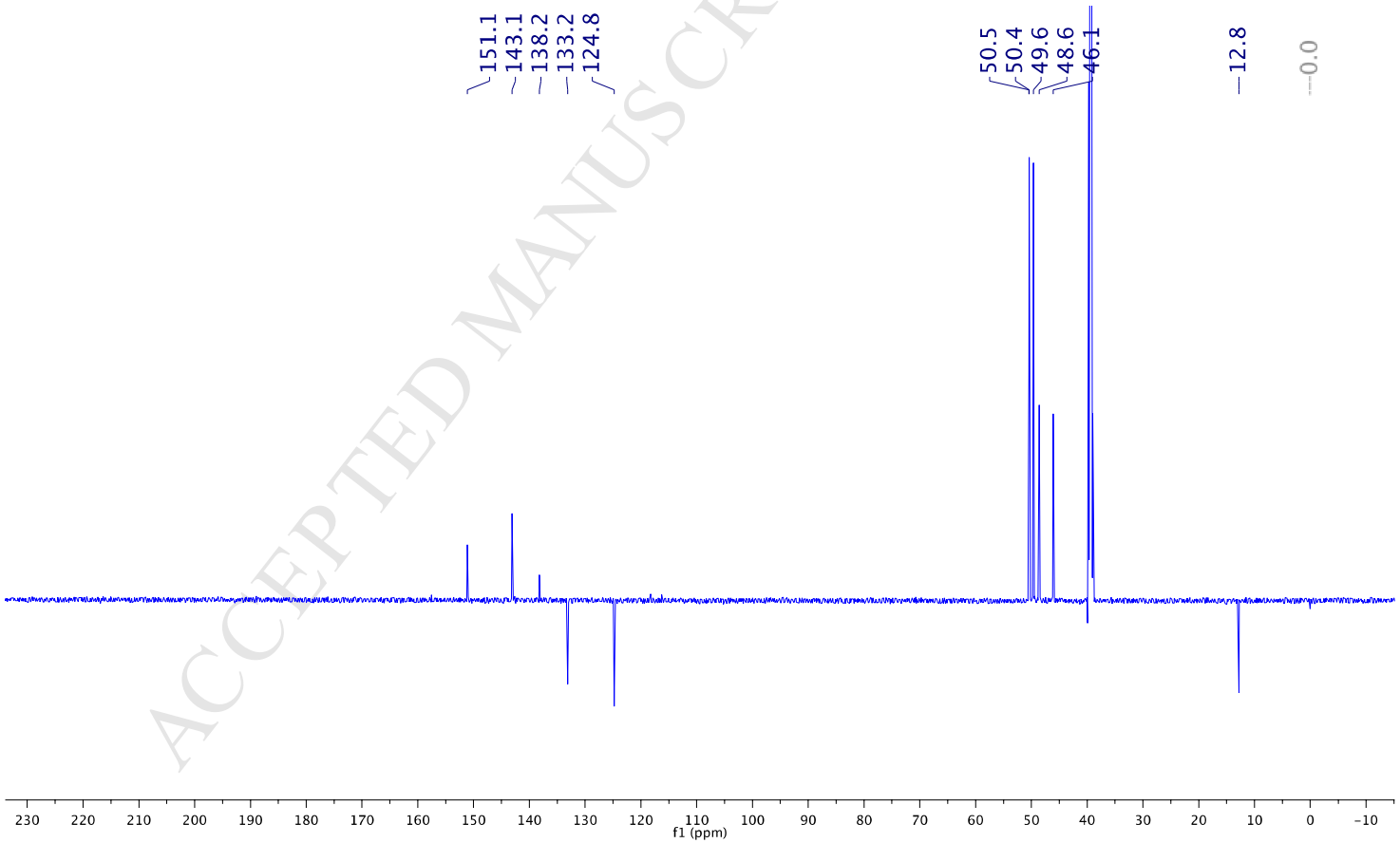
1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-((1,1-dioxothiomorpholin-4-yl)methyl)- 1H-1,2,3-triazole

^{13}C JMOD NMR (150 MHz, DMSO-d_6)



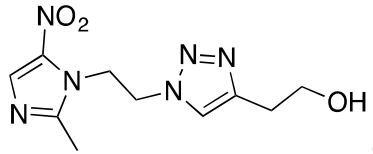
4o

	Parameter	Value
1	Title	AMJ6768_068B_f22-26_chelex.2.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	jmod
8	Experiment	JMOD
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	2048
11	Receiver Gain	41285
12	Relaxation Delay	1.0000
13	Pulse Width	12.0000
14	Presaturation Frequency	
15	Acquisition Time	0.8717
16	Acquisition Date	2013-11-16T06:47:00
17	Modification Date	2013-11-16T06:47:22
18	Spectrometer Frequency	150.92
19	Spectral Width	37594.0
20	Lowest Frequency	-2273.6
21	Nucleus	13C
22	Acquired Size	32768
23	Spectral Size	65536



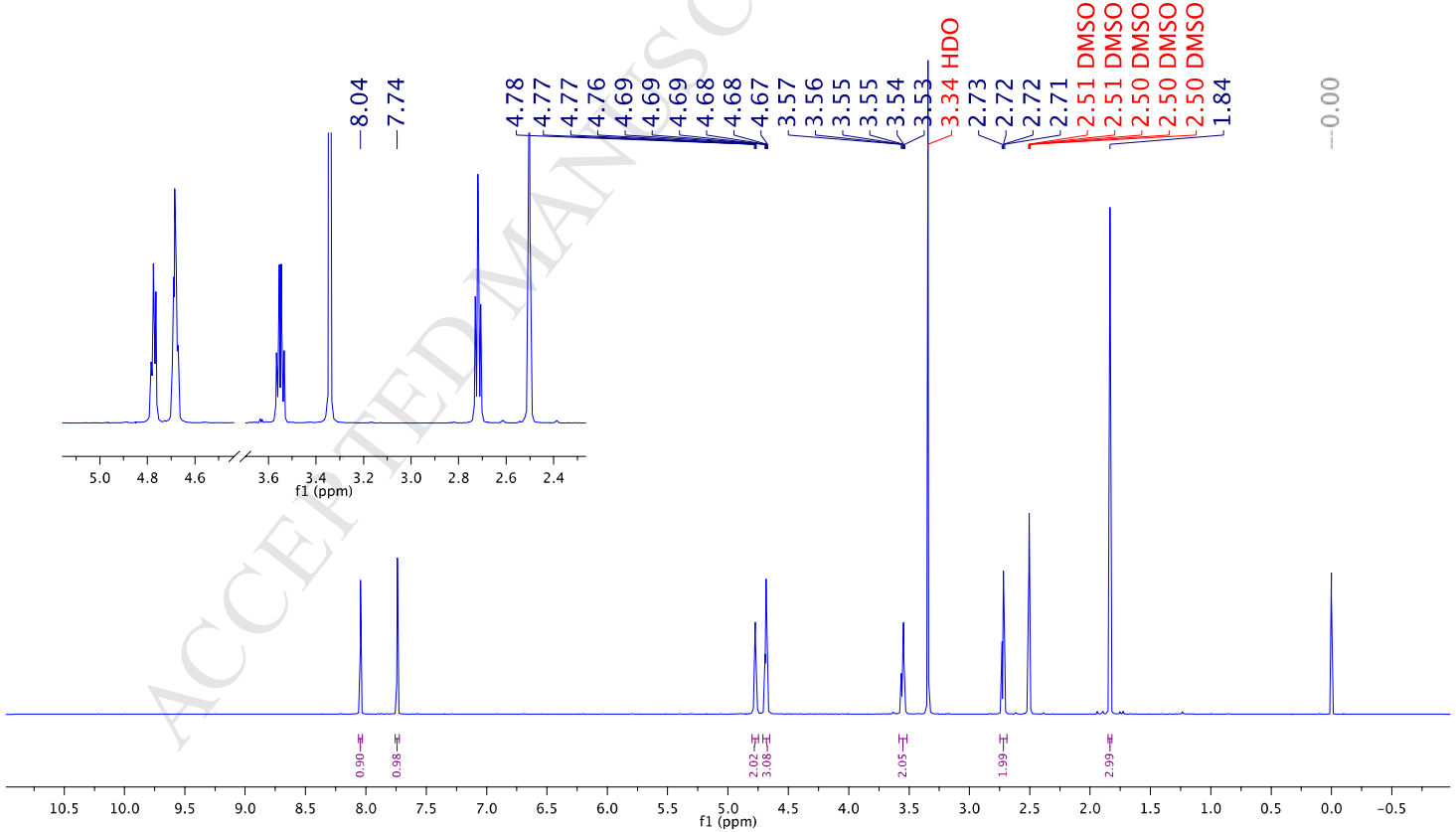
1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(2-hydroxyethyl)-1H-1,2,3-triazole

^{1}H NMR (600 MHz, DMSO-d_6)



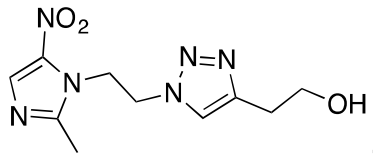
4p

	Parameter	Value
1	Title	AMJ6417_060D_elute2_f 10_13.10.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	zg
8	Experiment	1D
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	8
11	Receiver Gain	32
12	Relaxation Delay	10.0000
13	Pulse Width	7.8000
14	Presaturation Frequency	
15	Acquisition Time	2.2808
16	Acquisition Date	2013-05-16T21:33:00
17	Modification Date	2013-05-16T21:33:46
18	Spectrometer Frequency	600.13
19	Spectral Width	7183.9
20	Lowest Frequency	-592.0
21	Nucleus	1H
22	Acquired Size	16384
23	Spectral Size	65536



1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(2-hydroxyethyl)-1H-1,2,3-triazole

^{13}C JMOD NMR (150 MHz, DMSO-d_6)



4p

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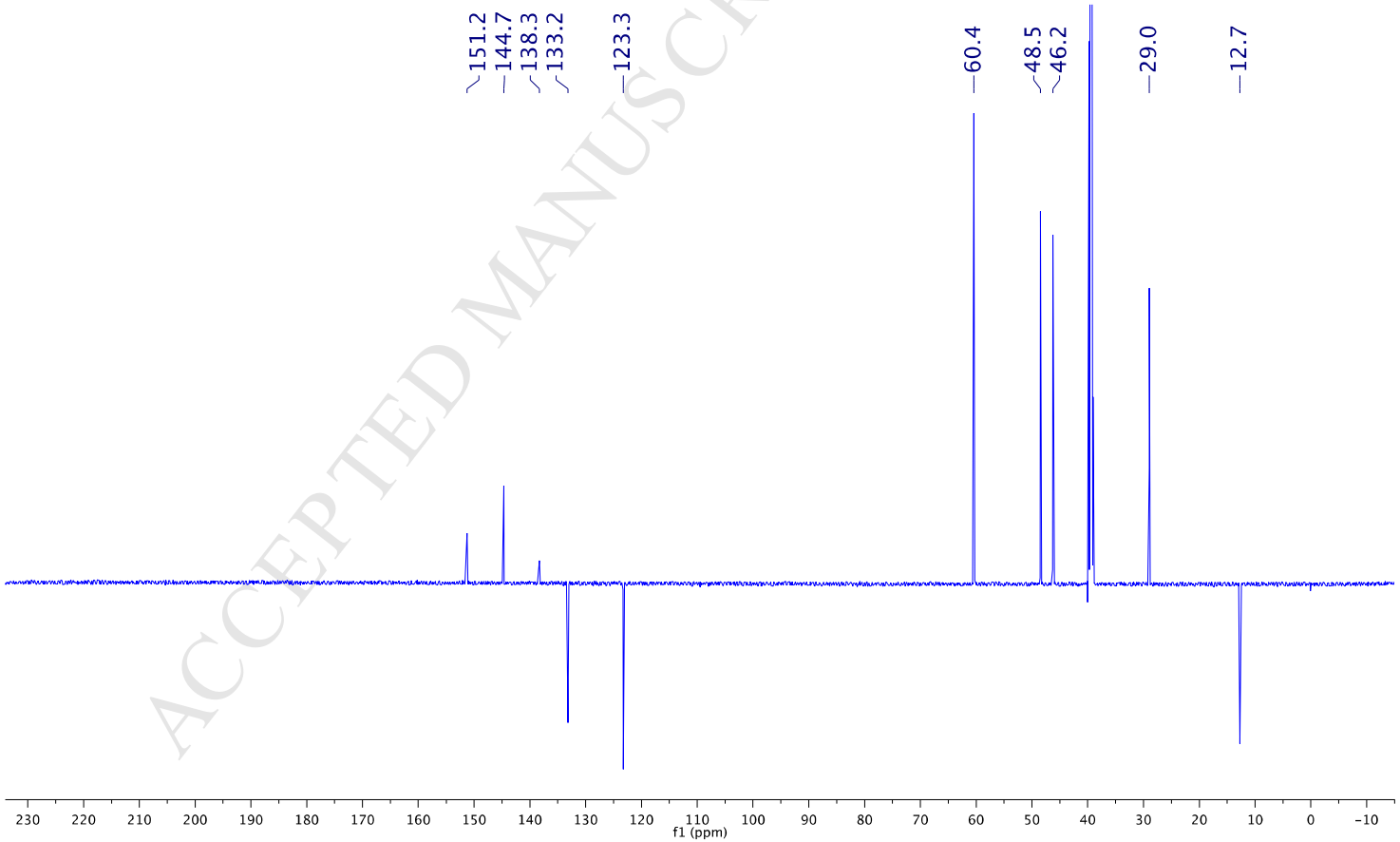
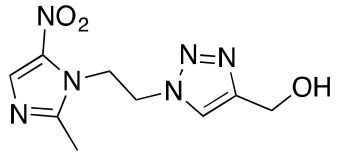
1	Title	AMJ6417_060D_elute2_f1 0_13.12.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	jmod
8	Experiment	JMOD
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	2048
11	Receiver Gain	32768
12	Relaxation Delay	1.0000
13	Pulse Width	12.0000
14	Presaturation Frequency	
15	Acquisition Time	0.8717
16	Acquisition Date	2013-05-16T22:40:00
17	Modification Date	2013-05-16T22:40:14
18	Spectrometer Frequency	150.92
19	Spectral Width	37594.0
20	Lowest Frequency	-2273.6
21	Nucleus	13C
22	Acquired Size	32768
23	Spectral Size	65536



1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(hydroxymethyl)- 1H-1,2,3-triazole

^{1}H NMR (600 MHz, DMSO-d_6)



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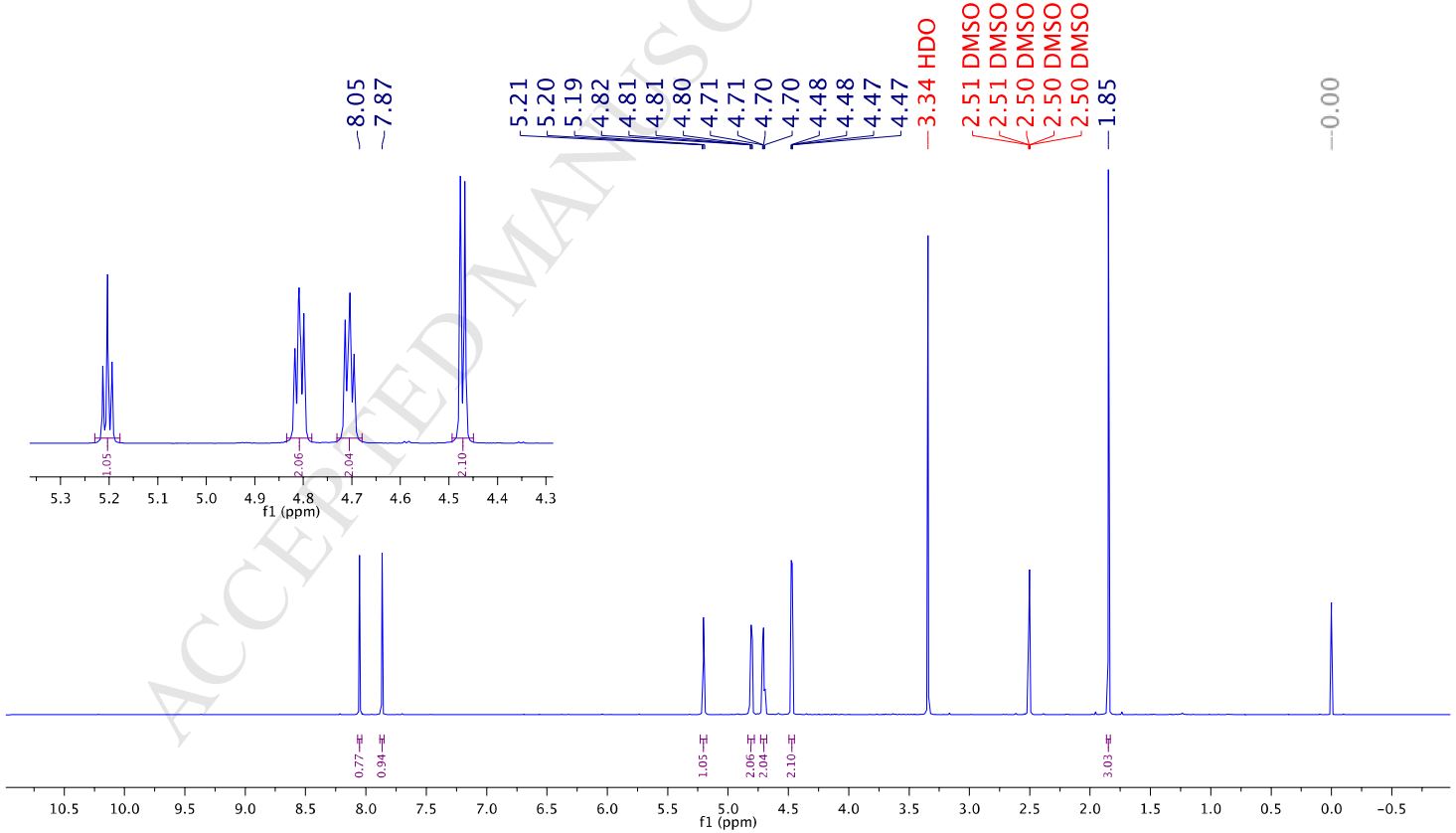
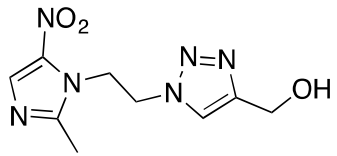
1	Title	AMJ6417_060E_f12_14.10.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	zg
8	Experiment	1D
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	8
11	Receiver Gain	23
12	Relaxation Delay	10.0000
13	Pulse Width	7.8000
14	Presaturation Frequency	
15	Acquisition Time	2.2808
16	Acquisition Date	2013-05-17T00:51:00
17	Modification Date	2013-05-17T00:51:46
18	Spectrometer Frequency	600.13
19	Spectral Width	7183.9
20	Lowest Frequency	-592.0
21	Nucleus	1H
22	Acquired Size	16384
23	Spectral Size	65536



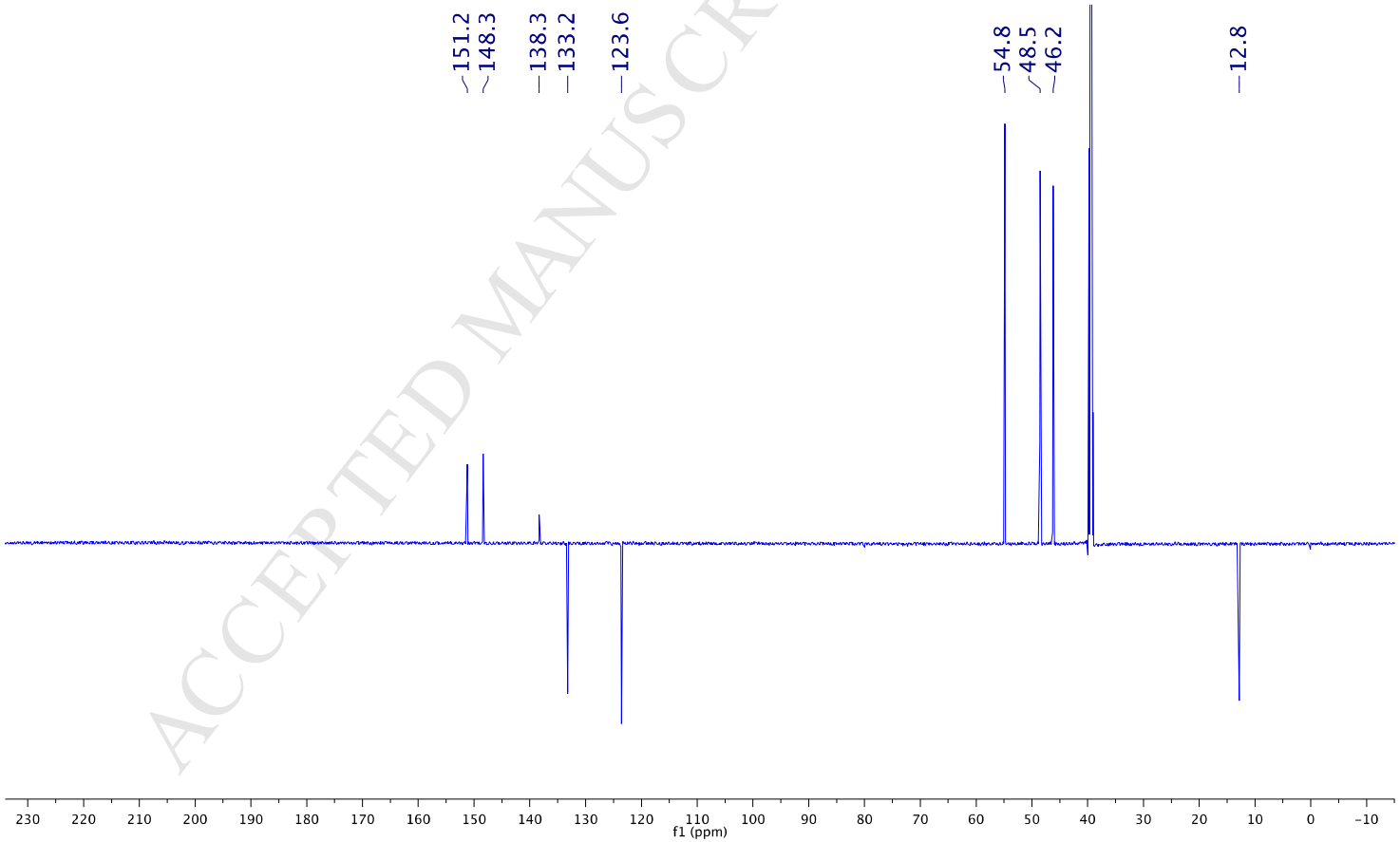
1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(hydroxymethyl)- 1H-1,2,3-triazole

^{13}C JMOD NMR (150 MHz, DMSO-d_6)



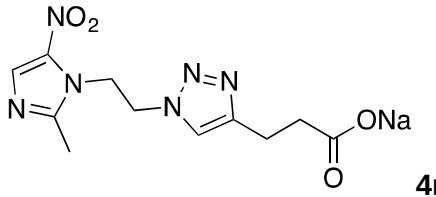
4q

	Parameter	Value
1	Title	AMJ6417_060E_f12_14.12.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	jmod
8	Experiment	JMOD
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	2048
11	Receiver Gain	41285
12	Relaxation Delay	1.0000
13	Pulse Width	12.0000
14	Presaturation Frequency	
15	Acquisition Time	0.8717
16	Acquisition Date	2013-05-17T01:58:00
17	Modification Date	2013-05-17T01:58:15
18	Spectrometer Frequency	150.92
19	Spectral Width	37594.0
20	Lowest Frequency	-2273.6
21	Nucleus	13C
22	Acquired Size	32768
23	Spectral Size	65536



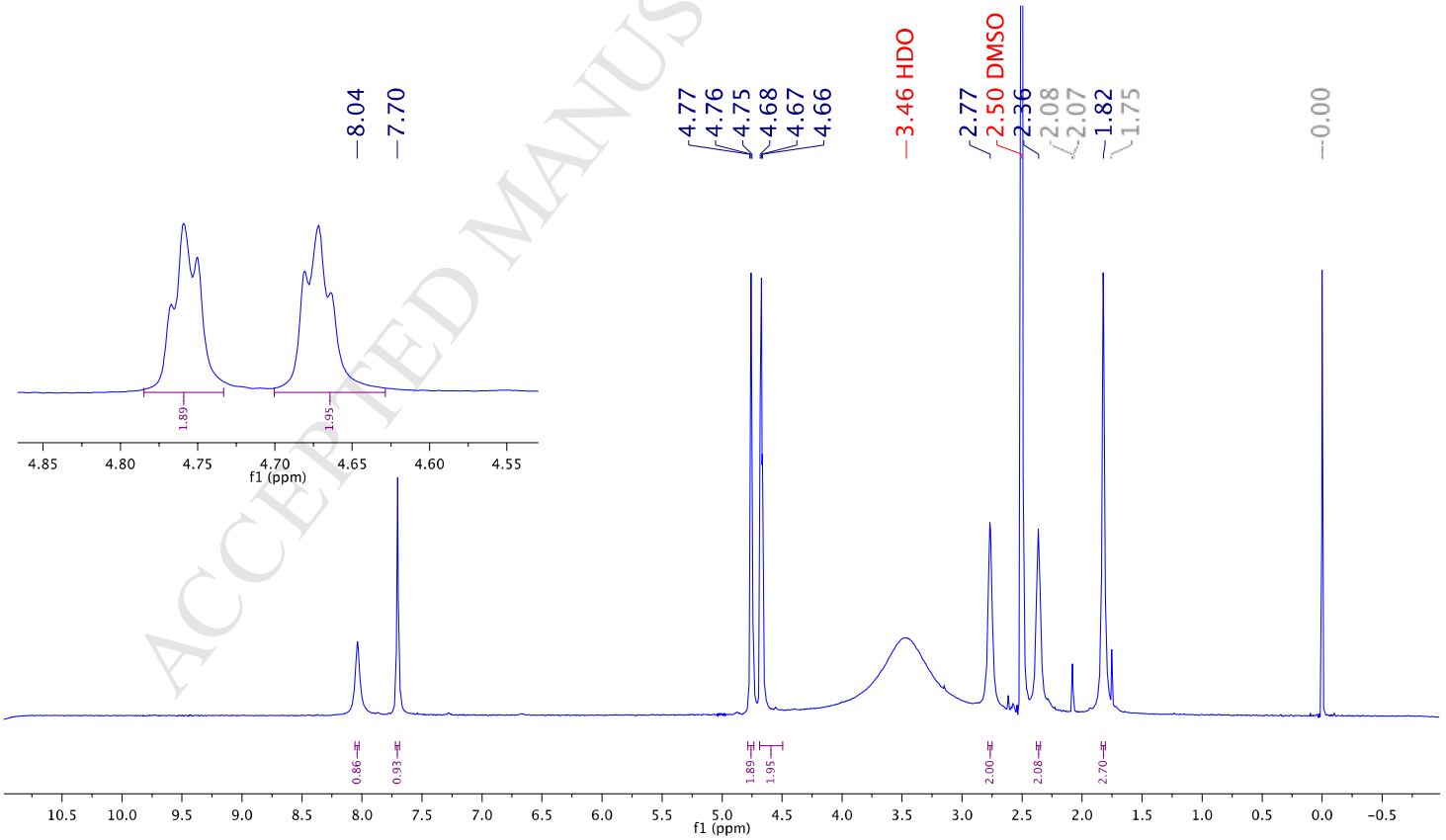
sodium 1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(carboxyethyl)- 1H-1,2,3-triazole

^{1}H NMR (600 MHz, DMSO-d_6)



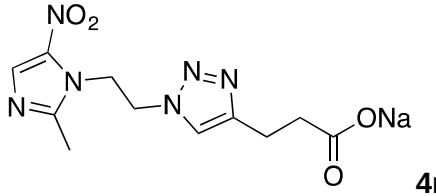
4r

	Parameter	Value
1	Title	AMJ6768_068D_f58-60_concsample.1.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	zg
8	Experiment	1D
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	8
11	Receiver Gain	32
12	Relaxation Delay	10.0000
13	Pulse Width	7.8000
14	Presaturation Frequency	
15	Acquisition Time	2.2808
16	Acquisition Date	2013-11-17T17:41:00
17	Modification Date	2013-11-17T17:41:42
18	Spectrometer Frequency	600.13
19	Spectral Width	7183.9
20	Lowest Frequency	-590.9
21	Nucleus	1H
22	Acquired Size	16384
23	Spectral Size	65536



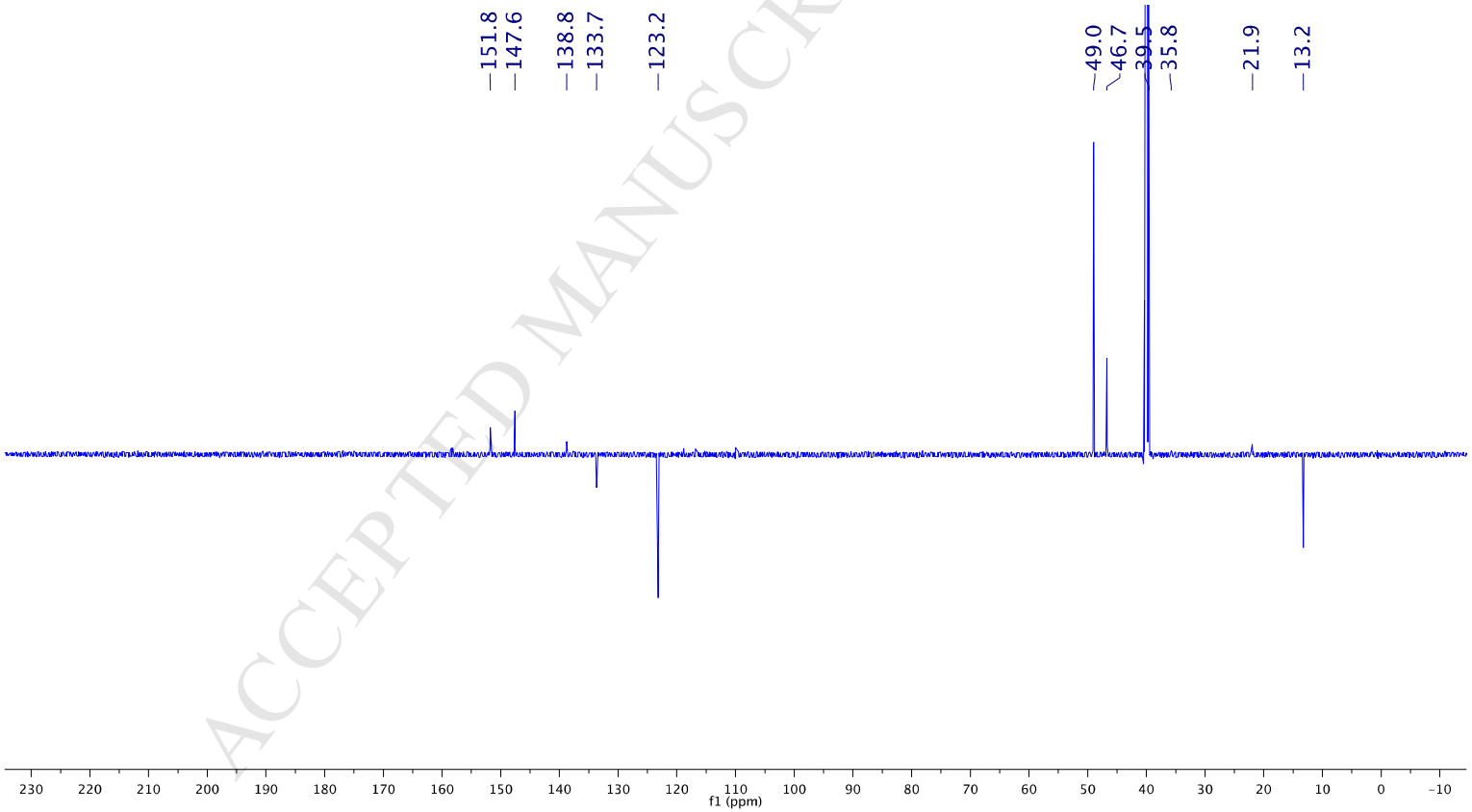
sodium 1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(carboxyethyl)- 1H-1,2,3-triazole

^{13}C JMOD NMR (150 MHz, DMSO-d_6). Missing carbon resonances at 35.8 ppm and 175.0 ppm were assigned with gHSQC and gHMBC correlations.



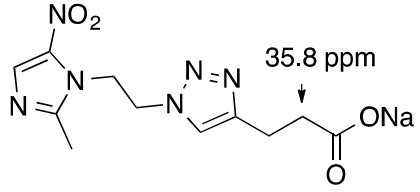
4r

	Parameter	Value
1	Title	AMJ6768_068D_f58-60_chelex_9mg/2
2	Origin	Bruker BioSpin GmbH
3	Owner	labadmin
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	jmod
8	Experiment	JMOD
9	Probe	5 mm CPTCI 1H/19F-13C/15N/D Z-GRD Z129649/0009
10	Number of Scans	4096
11	Receiver Gain	46341
12	Relaxation Delay	1.0000
13	Pulse Width	12.0000
14	Presaturation Frequency	
15	Acquisition Time	0.8716
16	Acquisition Date	2014-12-07T15:56:06
17	Modification Date	2014-12-07T17:50:56
18	Spectrometer Frequency	150.92
19	Spectral Width	37594.0
20	Lowest Frequency	-2198.2
21	Nucleus	13C
22	Acquired Size	32768
23	Spectral Size	65536



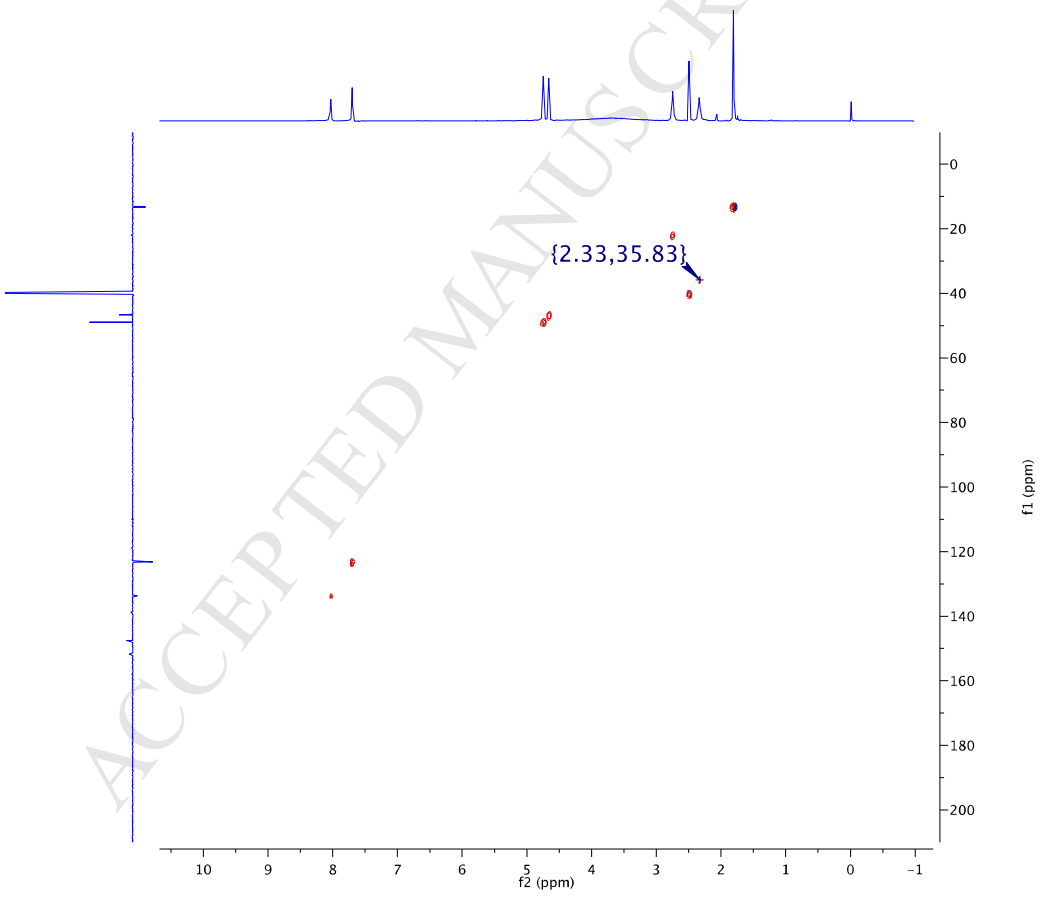
sodium 1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(carboxyethyl)- 1H-1,2,3-triazole

gHSQC NMR (150 MHz, DMSO-d_6).



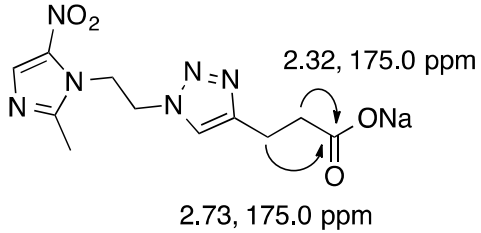
4r

	Parameter	Value
1	Title	AMJ6768_068D_f58-60_chelex_9mg/11
2	Origin	Bruker BioSpin GmbH
3	Owner	labadmin
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	hsqcetgpsisp2.2
8	Experiment	HSQC
9	Probe	5 mm CPTCI 1H/19F-13C/15N/D Z-GRD Z129649/0009
10	Number of Scans	8
11	Receiver Gain	29193
12	Relaxation Delay	2.0000
13	Pulse Width	7.8000
14	Presaturation Frequency	
15	Acquisition Time	0.1425
16	Acquisition Date	2014-12-07T22:30:15
17	Modification Date	2014-12-07T23:44:06
18	Spectrometer Frequency	(600.13, 150.92)
19	Spectral Width	(7183.9, 33201.6)
20	Lowest Frequency	(-775.7, -1511.0)
21	Nucleus	(1H, 13C)
22	Acquired Size	(1024, 256)
23	Spectral Size	(1024, 1024)



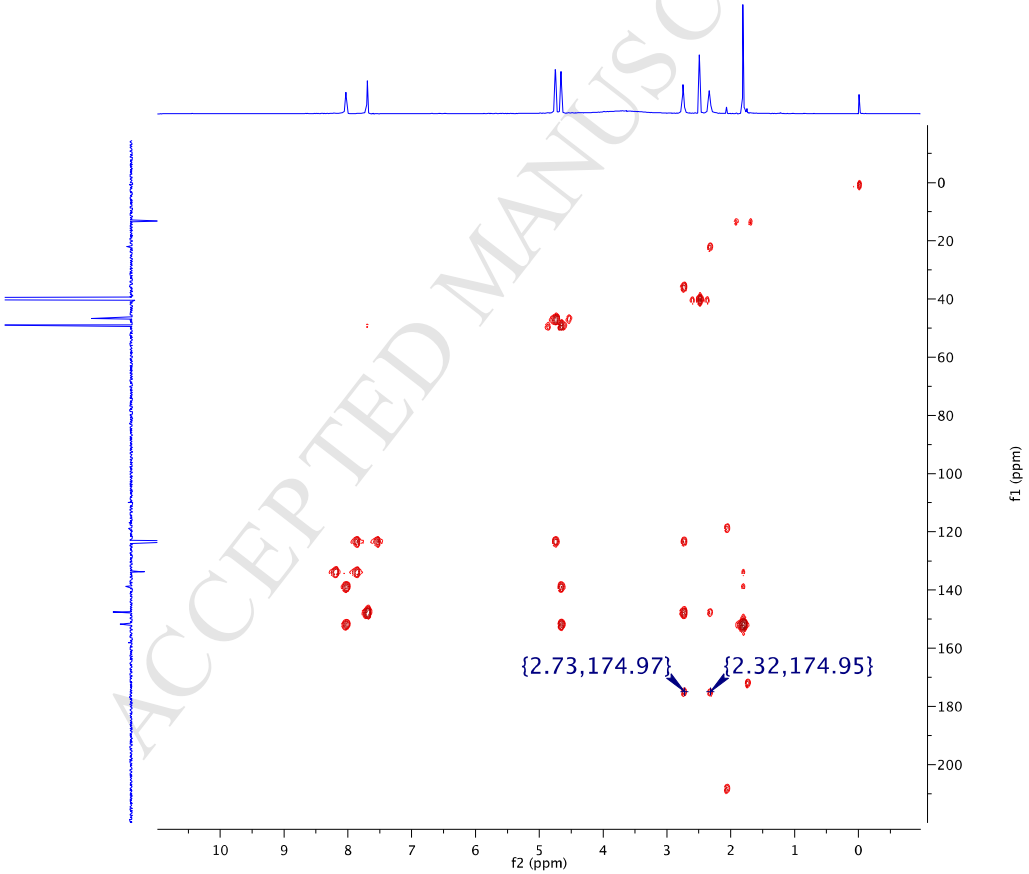
sodium 1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(carboxyethyl)- 1H-1,2,3-triazole

gHMBC NMR (150 MHz, DMSO-d_6).



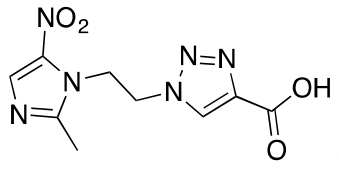
4r

	Parameter	Value
1	Title	AMJ6768_068D_f58-60_chelex_9mg/12
2	Origin	Bruker BioSpin GmbH
3	Owner	labadmin
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	hmbcgplpndqf
8	Experiment	HMBC
9	Probe	5 mm CPTCI 1H/19F-13C/15N/D Z-GRD Z129649/0009
10	Number of Scans	8
11	Receiver Gain	46341
12	Relaxation Delay	2.0000
13	Pulse Width	7.8000
14	Presaturation Frequency	
15	Acquisition Time	0.2851
16	Acquisition Date	2014-12-07T23:47:38
17	Modification Date	2014-12-08T01:08:08
18	Spectrometer Frequency	(600.13, 150.92)
19	Spectral Width	(7183.9, 36219.9)
20	Lowest Frequency	(-591.3, -3020.2)
21	Nucleus	(1H, 13C)
22	Acquired Size	(2048, 256)
23	Spectral Size	(2048, 1024)



1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(carboxy)- 1H-1,2,3-triazole

^{1}H NMR (600 MHz, DMSO-d_6)



4s

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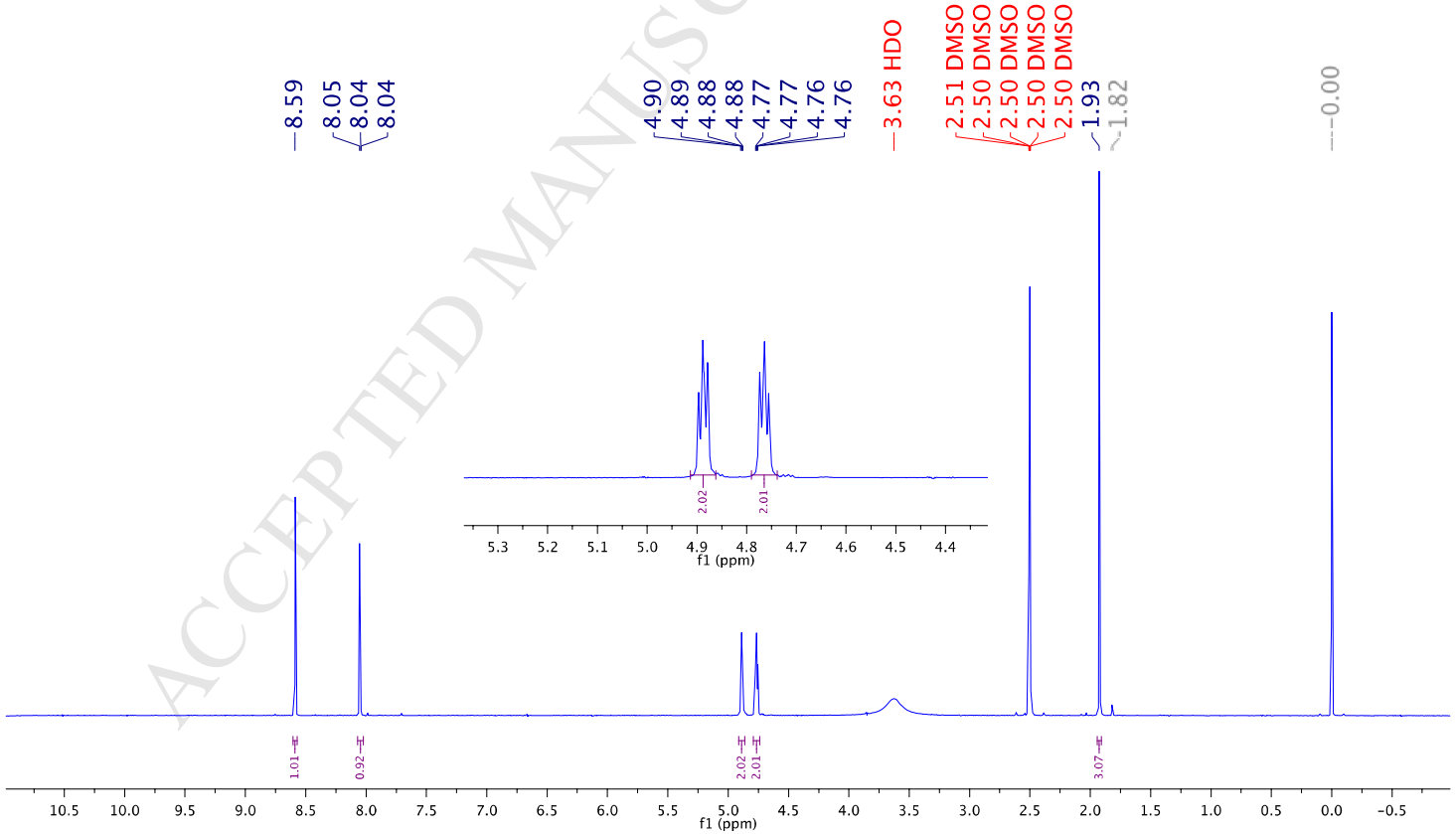
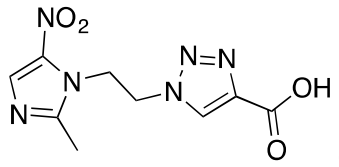
1	Title	AMJ6417_105_c18_f10-13.1.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	zg
8	Experiment	1D
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	8
11	Receiver Gain	32
12	Relaxation Delay	10.0000
13	Pulse Width	7.8000
14	Presaturation Frequency	
15	Acquisition Time	2.2808
16	Acquisition Date	2013-10-19T07:28:00
17	Modification Date	2013-10-19T07:28:35
18	Spectrometer Frequency	600.13
19	Spectral Width	7183.9
20	Lowest Frequency	-591.1
21	Nucleus	1H
22	Acquired Size	16384
23	Spectral Size	65536



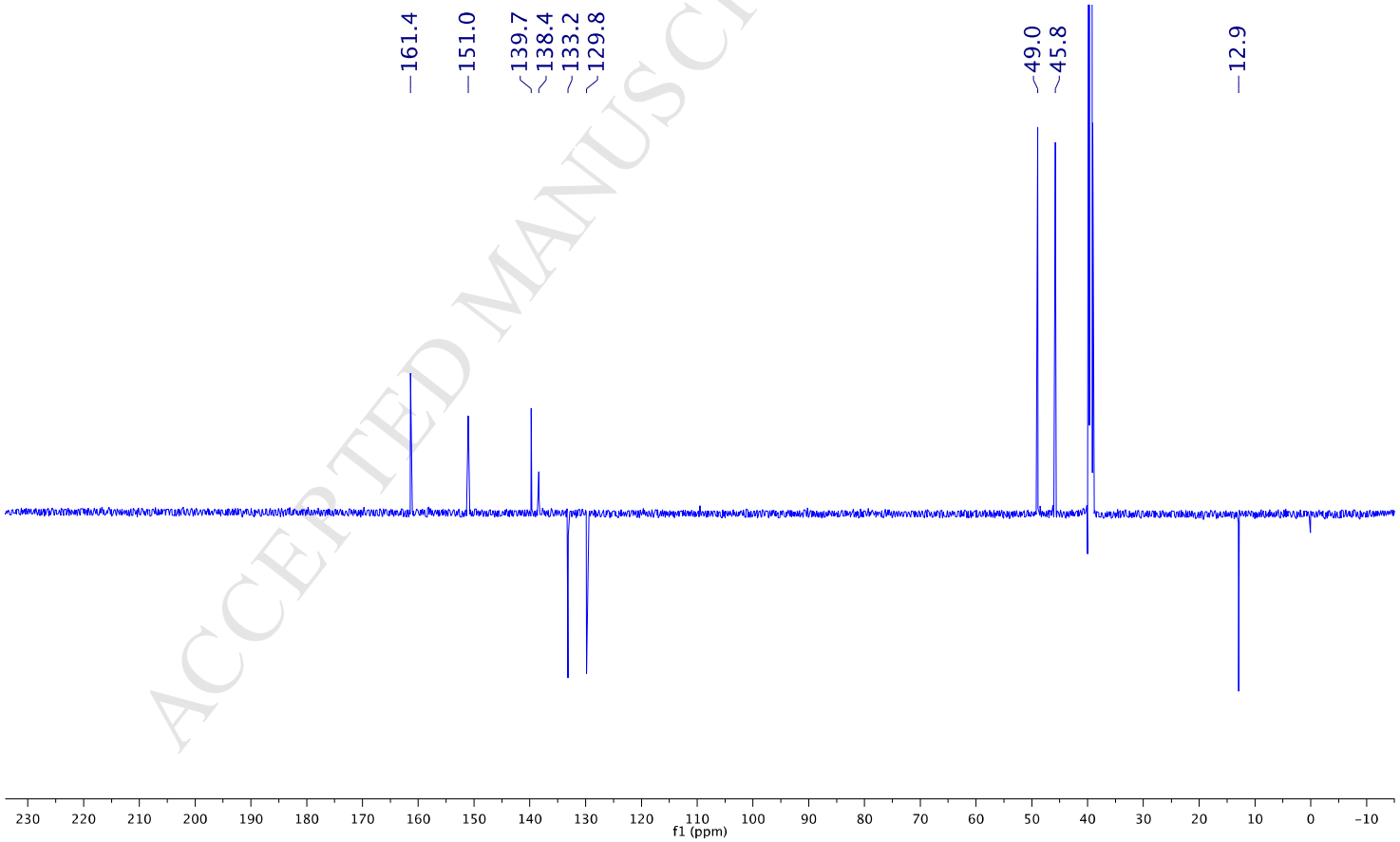
1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(carboxy)- 1H-1,2,3-triazole

^{13}C JMOD NMR (150 MHz, DMSO-d_6)



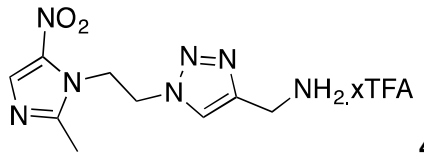
4s

	Parameter	Value
1	Title	AMJ6417_105_c18_f10-13.2.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	jmod
8	Experiment	JMOD
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	4096
11	Receiver Gain	41285
12	Relaxation Delay	1.0000
13	Pulse Width	12.0000
14	Presaturation Frequency	
15	Acquisition Time	0.8717
16	Acquisition Date	2013-10-19T09:41:00
17	Modification Date	2013-10-19T09:41:21
18	Spectrometer Frequency	150.92
19	Spectral Width	37594.0
20	Lowest Frequency	-2273.6
21	Nucleus	13C
22	Acquired Size	32768
23	Spectral Size	65536



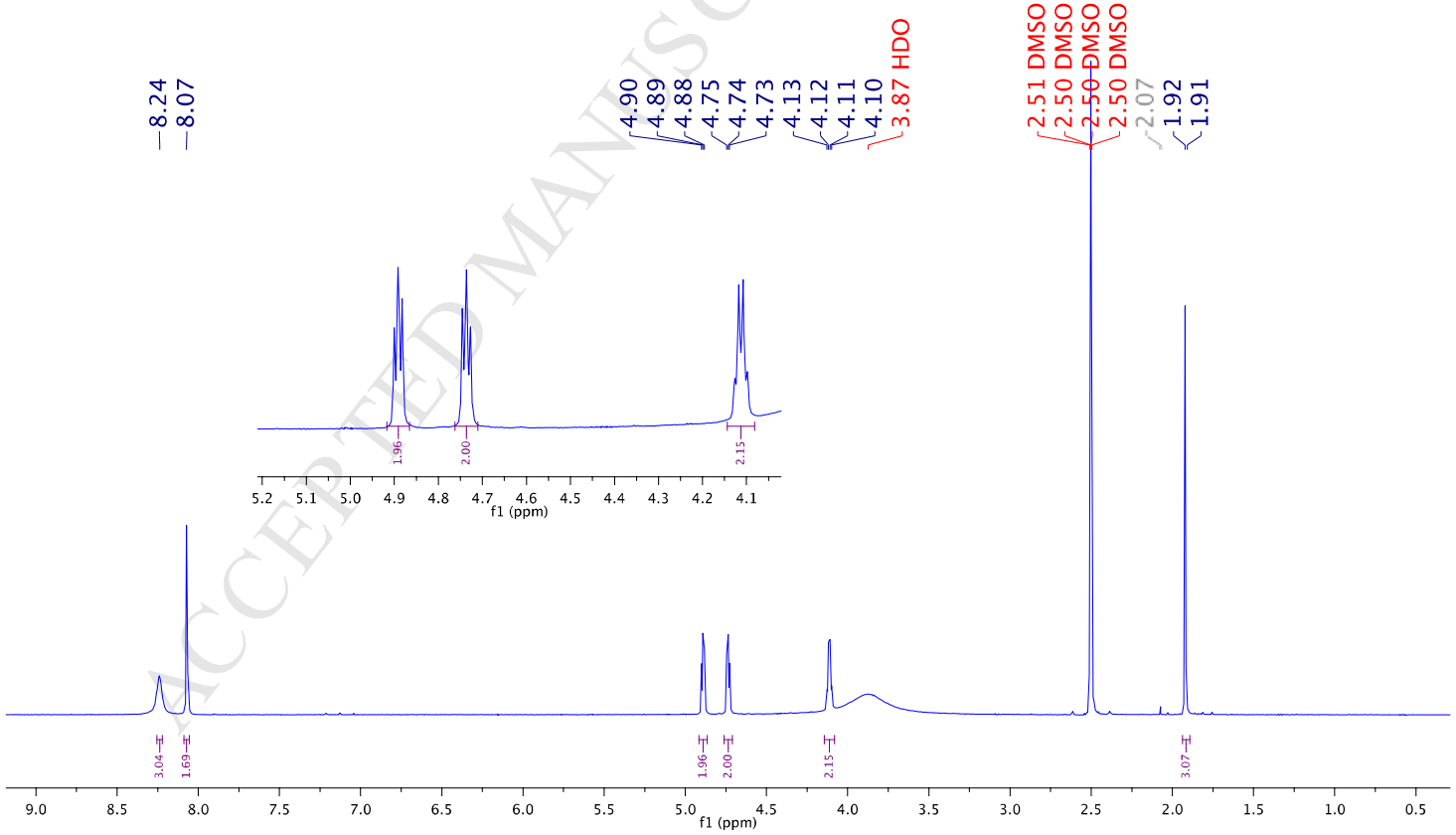
1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(aminomethyl)- 1H-1,2,3-triazole trifluoroacetate salt

^{1}H NMR (600 MHz, DMSO-d_6)



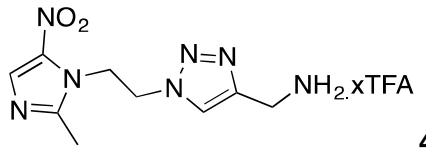
4t

	Parameter	Value
1	Title	AMJ6768_068C_f38_PULCON.1.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	zg
8	Experiment	1D
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	8
11	Receiver Gain	32
12	Relaxation Delay	30.0000
13	Pulse Width	7.4310
14	Presaturation Frequency	
15	Acquisition Time	2.2808
16	Acquisition Date	2013-11-21T11:44:00
17	Modification Date	2013-11-21T11:44:53
18	Spectrometer Frequency	600.13
19	Spectral Width	7183.9
20	Lowest Frequency	-591.0
21	Nucleus	1H
22	Acquired Size	16384
23	Spectral Size	65536



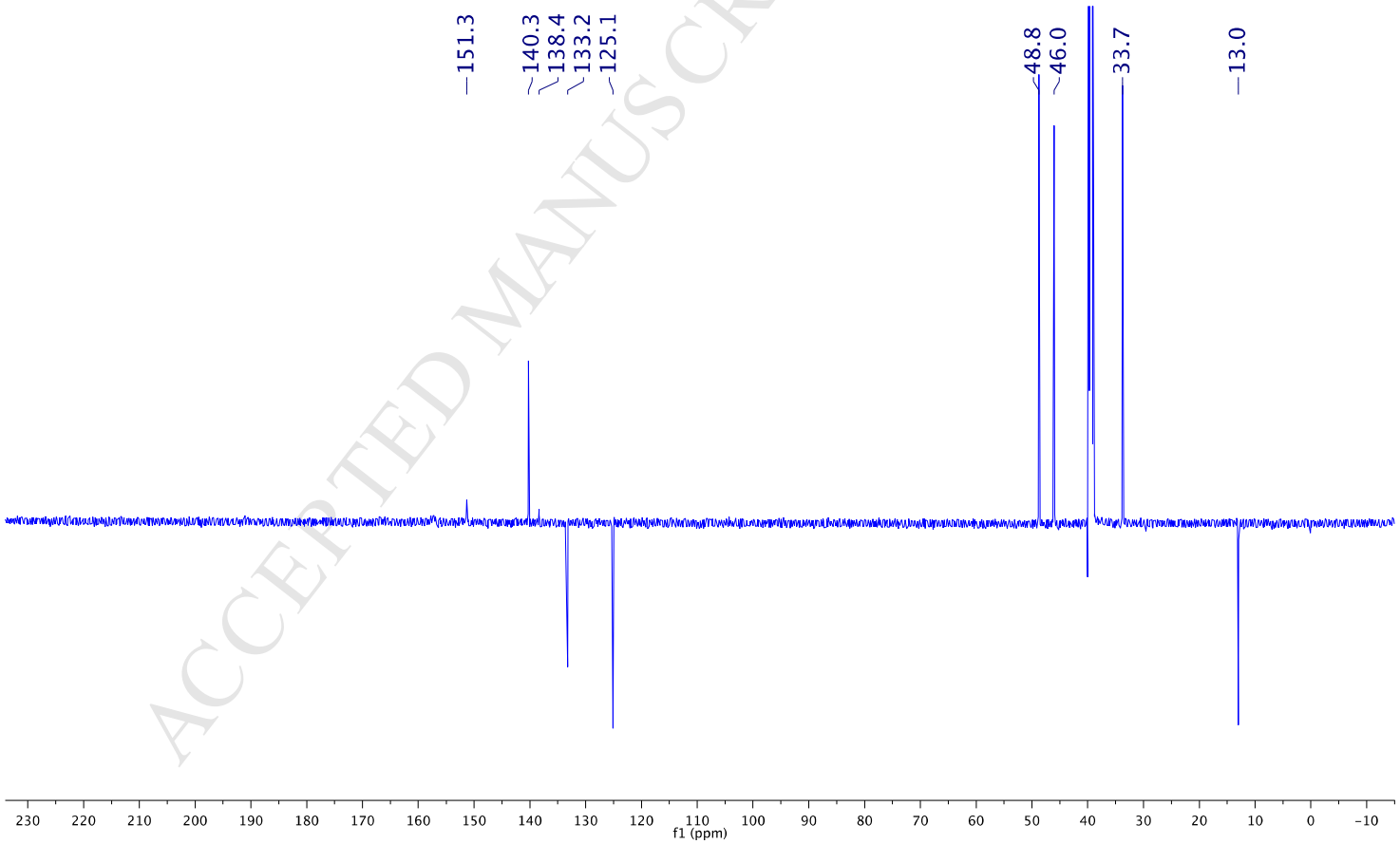
1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-(aminomethyl)- 1H-1,2,3-triazole trifluoroacetate salt

^{13}C JMOD NMR (150 MHz, DMSO-d_6)



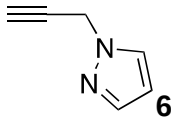
4t

	Parameter	Value
1	Title	AMJ6768_068C_f38_freezedriedinvial.2.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	DMSO
6	Temperature	298.0
7	Pulse Sequence	jmod
8	Experiment	JMOD
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	4096
11	Receiver Gain	36781
12	Relaxation Delay	1.0000
13	Pulse Width	12.0000
14	Presaturation Frequency	
15	Acquisition Time	0.8717
16	Acquisition Date	2013-11-17T16:36:00
17	Modification Date	2013-11-17T16:36:42
18	Spectrometer Frequency	150.92
19	Spectral Width	37594.0
20	Lowest Frequency	-2273.6
21	Nucleus	13C
22	Acquired Size	32768
23	Spectral Size	65536

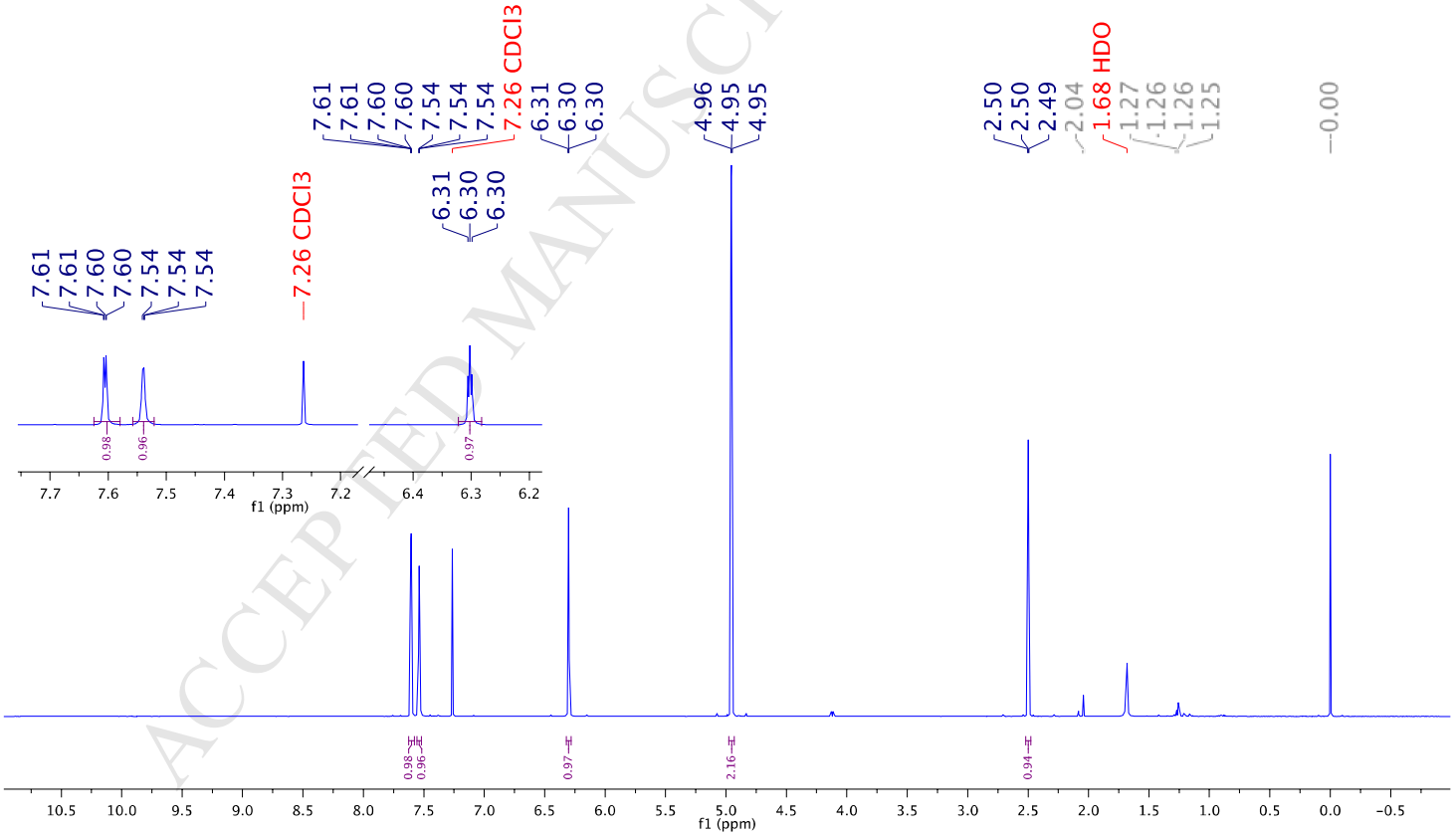


1-(prop-2-yn-1-yl)-1H-pyrazole

^{1}H NMR (600 MHz, $CDCl_3$)

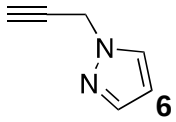


	Parameter	Value
1	Title	AMJ6768_013_f18_25_highvac1min.1.fid
2	Origin	Bruker BioSpin GmbH
3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	CDCl3
6	Temperature	298.0
7	Pulse Sequence	zg
8	Experiment	1D
9	Probe	5 mm CPTCI 1H/ 19F-13C/ 15N/ D Z-GRD Z129649/ 0009
10	Number of Scans	8
11	Receiver Gain	32
12	Relaxation Delay	10.0000
13	Pulse Width	7.8000
14	Presaturation Frequency	
15	Acquisition Time	2.2808
16	Acquisition Date	2013-08-31T20:36:00
17	Modification Date	2013-08-31T20:36:39
18	Spectrometer Frequency	600.13
19	Spectral Width	7183.9
20	Lowest Frequency	-597.2
21	Nucleus	1H
22	Acquired Size	16384
23	Spectral Size	65536



1-(prop-2-yn-1-yl)-1H-pyrazole

^{13}C JMOD (150 MHz, $CDCl_3$)



	Parameter	Value
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3	Owner	chemistry
4	Spectrometer	spect
5	Solvent	CDCl3
6	Temperature	298.0
7	Pulse Sequence	jmod
8	Experiment	JMOD
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12	Relaxation Delay	1.0000
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14	Presaturation Frequency	
15	Acquisition Time	0.8717
16	Acquisition Date	2013-08-31T21:43:00
17	Modification Date	2013-08-31T21:43:51
18	Spectrometer Frequency	150.92
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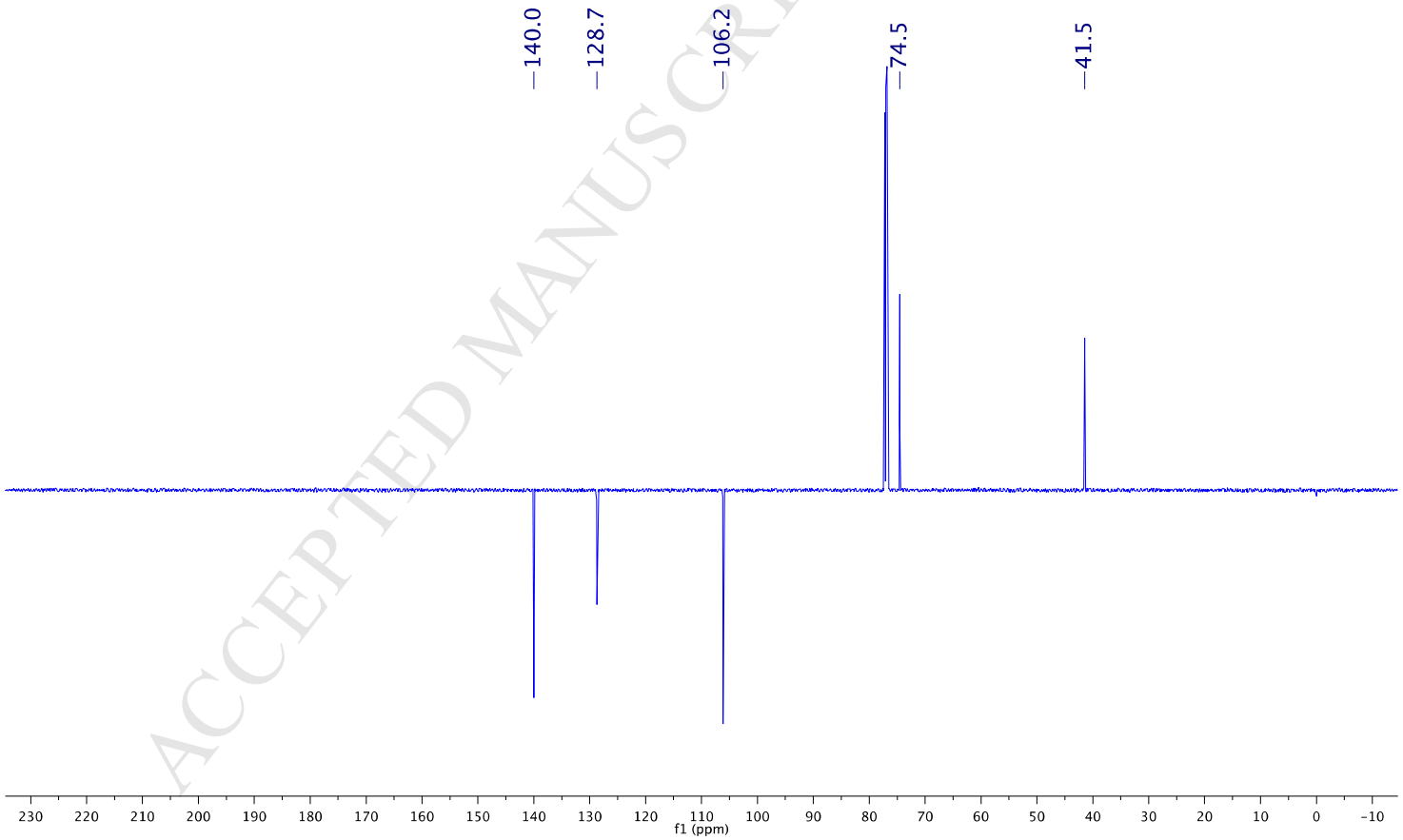

4 Biological Methods

4.1 Determination of Minimum Inhibition Concentration (MIC) against facultative anaerobic bacteria

MICs were determined by a two-fold serial broth micro dilution according to the recommendation of CLSI standards with an inoculum of 5×10^5 CFU/mL[4] The compounds along with standard antibiotics were serially diluted twofold across the wells of 96-well non-binding surface plates (NBS, Corning). Standards ranged from 64 to 0.03 µg/mL, and the compounds from 128 to 0.06 µg/mL with final volumes of 50 µL per well. Gram-positive and Gram-negative bacteria were cultured in Mueller Hinton broth (MHB) (Bacto laboratories, Cat. no. 211443) at 37 ℃ overnight. A sample of each culture was then diluted 40-fold in fresh MHB broth and incubated at 37 ℃ for 2-3 h. The resultant mid-log phase cultures were diluted to the final concentration of 5×10^5 CFU/mL, then 50 µL was added to each well of the compound containing 96-well plates. All the plates were covered and incubated at 37 ℃ for 24 h. MICs were the lowest concentration that showed no visible growth.

4.2 Maintenance of *C. difficile*[5]

Heat shocked spore stocks in H_2O

C. difficile was inoculated onto agar plates (TS + 5% sheep blood or BHIS agar) and incubated anaerobically at 37 ℃ for 3 to 7 days. The plates were removed from the anaerobic chamber and sterile PBS (1 mL) was added onto each agar plate. The colonies were suspended by scraping with a disposable inoculating loop. The cell suspension was transferred to an Eppendorf tube and this process was repeated with a further 0.5 mL of sterile PBS added to the agar plate. The cells were then heat shocked at 65 ℃ for 25 minutes to k ill vegetative cells. The cells were pelleted by centrifuging at 14000 RPM for 1 minute, the supernatant was discarded and the cell pellet was resuspended in sterile PBS (1 mL). This sequence was repeated 3 times before finally suspending the spores in sterile H_2O (1 mL) and storing at 4 ℃ until required.

Revival of C. difficile vegetative cells from spore stocks

C. difficile ATCC BAA-1382, ATCC BAA-1803, ATCC 43255 and M7404 heat shocked spore stocks (10 µL) were transferred into the anaerobic chamber and 10 µL streaked onto pre-reduced (at least 2.5 hr in anaerobic chamber) BHIS agar plates supplemented with 0.1% w/v sodium taurocholate (BHIS(TA)). Plates were incubated for 24 hours prior to assay to give colonies of size 1-3mm diameter with characteristic uneven borders.

Revival of C. difficile from Microbank beads stored at -80 ℃.

C. difficile CD26A54_S and *C. difficile* CD26A54_R on Microbank beads were transferred into the anaerobic chamber in a -20 °C Eppendorf IsoTherm cooler system. A single bead was used to inoculate individual prereduced BAKHS agar plates (brucella agar supplemented with 10 µg/mL vitamin K_1, 5 µg/mL hemin and 5% laked sheep blood).[6] Inoculated agar plates were incubated anaerobically for 48 hr before subculture onto BAKHS and finally onto BHIS(TA) prior to assay. As a control inoculated agar plates were incubated aerobically and no colonies grew.

4.3 Determination of Minimum Inhibition Concentration (MIC) against *C. difficile*

The minimum inhibition concentration was determined according to the CLSI Methods with modifications in broth and inoculum for *C. difficile* according to Babakhani *et al.*[7,8] Briefly, compounds were prepared to 20X final concentration in 100% water, 60% DMSO and 40% water or 60% DMSO and 40% media. The compounds along with standard antibiotics were serially diluted two-fold across the wells of 96-well non-binding surface plates (Non binding surface, Corning). The plates were placed in the anaerobic chamber overnight to reduce. *C. difficile* bacteria from BHIS(TA) agar plates were cultured anaerobically in BHIS at 37 °C overnight. A sample of culture was then diluted 40-fold in BHIS broth and incubated at 37 °C for approximately 4.5 hrs. The resultant mid-log phase culture (OD_{600}= 0.5-0.6) was diluted to the final concentration of ~1×10^6 CFU/mL, then 50 µL was added to each well of the compound-containing 96-well plates. This gave final cell concentration of 5×10^5 CFU/mL, final volume 100 µL and 3% maximum DMSO concentration. Compound concentration was typically from 64 to 0.03 µg/mL. An antibiotic standard was included on each 96 well plate as well as a positive growth control (no compound) and sterility control (no bacteria). All the plates were covered and incubated at 37 °C for 24 h. MICs were the lowest concentration showing **no visible** growth.

4.4 Determination of Minimum Inhibition Concentration (MIC) by E-test against *C. difficile*

Mid-log phase (OD_{600} =0.5- 0.6) culture was diluted to a 0.5 McFarland standard (OD_{600}~ 0.1) in prereduced broth. The diluted culture was inoculated onto duplicate BAKHS agar plate using a cotton tip applicator. The inoculum was allowed to dry before the Metronidazole E-test strip (Metronidazole MZH 256-S30, bioMérieux Australia) was placed in duplicate on the surface of the agar. The agar plate was inverted and incubated for up to 96 hr. The MIC was read as the point at which the elliptical zone of inhibition intercepted the E-test strip at 24, 48 and 96 hr.

4.5 Determination of Minimum Inhibition Concentration (MIC) against *H. pylori*

Helicobacter pylori strains, with a range of resistance levels to metronidazole (256 µg/ml, 64 µg/ml, 24 µg/ml, 2 µg/ml, and 0.04 µg/ml), were selected and obtained from Helicobacter Research

Laboratory, University of Western Australia. The resistance strength to metronidazole was recorded according to eTest (bioMérieux). *H. pylori* strain 26695 was used as the control. *H. pylori* was maintained on the non-selective Columbia blood agar plates (CBA) (Columbia agar base Oxoid) with 5% horse blood and incubated at 37 ℃ and 10% CO_2 for 48 hrs. The MIC of each strain for each compound was obtained using the CLSI agar dilution method, with final concentration of compound 0.0125 - 64 µg/ml.[9] A control plate that contain 3% DMSO and one without any antibiotics were included in this study. Each of the *H. pylori* strains were harvested, OD_{600} calibrated to 0.3, and spotted in triplicate on CBA pre-mixed with the compound. The lowest concentration of antibiotic showing no growth was read as the MIC. The experiment was performed in duplicate.

4.6 Maintenance of *E. histolytica* and *G. lamblia*

Trophozoites of *E. histolytica* strain HM1:IMSS and *G. lamblia* strains WB, BRIS/87/HEPU/713 (713),[10] BRIS/83/HEPU/106 (106)[11] and the metronidazole resistant syngenic line 713-M3[10,11] were axenically maintained in TYI-S-33 medium supplemented with penicillin (100 U/ml), streptomycin (100 µg/ml).[12] *E. histolytica* and *G. lamblia* trophozoites were counted using a particle counter (Beckman Coulter, Fullerton, CA). All experiments were performed using trophozoites harvested during the logarithmic phase of growth.

4.7 Determination of EC_{50} against *E. histolytica* and *G. lamblia* trophozoites

The compounds were screened for activity against *E. histolytica* and *G. lamblia* using an ATP-bioluminescence based assay for cell growth and survival.[13,14] Briefly, 2.5 µL of 5 mM stock compounds were diluted with 17.5 µL sterile water to yield 625 µM working concentration of compounds. A three-fold serial dilution was then performed yielding a concentration range 0.25-625 µM. From this dilution plate, 4 µL were transferred into the 96-well screen plates followed by addition of 96 µL of trophozoites (5,000 parasites) to yield a final 8-point concentration range spanning 0.01- 25 µM. Assay plates were incubated for 48 h at 37 ℃ in the GasPak™ EZ Anaerobe Gas Generating Pouch Systems (VWR, West Chester, PA) to maintain anaerobic condition throughout the incubation period. The assays were performed in triplicate using the CellTiter-Glo Luminescent Cell Viability Assay.[13]

4.8 Cytotoxicity

HEK293 and HepG2 cells were seeded as 3000 and 5000 cells per well in 384-well plates, respectively. Cells were cultured in DMEM with 1% FBS for 24 hours at 37 ℃, 5% CO_2. Then a dilution series of compounds was added into each well, with the highest concentration of 100 µM. The final concentration of DMSO in culture media was adjusted to 0.5%, which showed no effect on cell growth. After 24 hours incubation with the compounds, 5 µM resazurin were added into each well and incubated at 37 ℃ for 2 hours. As a negative control, 1% Triton X-100 was added

into the culture media to lyse all the cells. The fluorescence intensity was read using Polarstar Omega with excitation/emission 560/590. The data was analysed by GraphPad Prism software. Results are presented as the average percentage of control ± SD using the following equation: Percentage of Growth = (FI_{TEST} – $FI_{Negative}$/$FI_{UNTREATED}$ –$FI_{Negative}$)*100.

4.9 Correlation analysis of compound activity and properties

A correlation matrix between compound activity and properties was calculated using Excel correlation analysis (Table S4). The compound properties, logP, MW, logD, logS and tPSA, were calculated from the 2D structure of the compounds, using Pipeline Pilot (Accelrys, Version 8.5.0.200). The activity was expressed as $-\log_{10}$ values of MIC or EC_{50}, using average MIC (mol L^{-1}) of MtzS *C. difficile* ATCC BAA-1382, ATCC 43255, M7404, ATCC BAA-1803 strains and EC_{50} (mol L^{-1}) against *G. lamblia* WB strain and *E. histolytica* HM1:1MSS strain. For correlation between logD and individual activities the linear regression analysis in Excel was used, extracting the linear regression coefficients (R^2) (Figure S1).

5 Supplementary Results: Tables S1-3, Figure S1

5.1 MIC results of *Clostridium difficile* at 24 and 48 hr

Table S1: *Clostridium difficile* MIC values measured after 24 hr and 48 hr incubation for the metronidazole sensitive control strain NAP1/027 ATCC BAA-1803, the metronidazole resistant CD26A54_R strain and the parent sensitive CD26A54_S strain with intermediary resistance to metronidazole.

Compound		MIC (μg/mL)					
		C. difficile NAP1/027					
		ATCC BAA-1803		CD26A54_S		CD26A54_R	
		24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
1	metronidazole	0.5	0.5	1	1	2	4
3	azide	0.5	1	2	2	4	8
4a	phenyl	0.5	0.5	1	1	2	2
4b	p-OMe phenyl	0.5	0.5	1	1	4	4
4c	p-Cl phenyl	0.25	0.5	1-2	2	8	16
4d	p,m-Cl_2phenyl	1	0.5-8	1	1	8-16	8-16
4e	p-Me phenyl	0.5	0.5	1	1	2	2
4f	methyl ester	1	1	1	2	4	4
4g	pyrazole	1	1-2	2	2	4	4
4h	pyridine	0.5	0.5-1	1	1	2	4
4i	5-pyrimidine	1	1	1	1	2	4
4j	2-pyrimidine	2	2	2	2-4	4	4
4k	benzyl	0.5	0.5	1	1	2	4
4l	CHOH-phenyl	0.5-1	1	2	2	4	4
4m	CH_2NMe-benzyl	2	1-2	2	2	8	8
4n	thiophene	0.5	0.5	1	1	2	2
4o	thiomorpholine	>64	>64	>64	>64	>64	>64
4p	CH_2CH_2OH	8	8-16	8	8	8-16	16
4q	CH_2OH	16	16-32	16	16-32	16	16
4r	CH_2CH_2COOH	>64	>64	>64	>64	>64	>64
4s	COOH	64	64	>64	>64	>64	>64
4t	CH_2NH_2	>64	>64	>64	>64	>64	>64

5.2 Metronidazole E-test

Table S2: Metronidazole E-test MIC (µg/mL) determined on BAKHS.

Time (hrs)	Metronidazole E-test MIC (µg/mL)		
	C. difficile NAP1/027		
	ATCC BAA-1803	CD26A54_S	CD26A54_R
24	2	6	32
48	2	8	48
96	2	8	48

5.3 Comparison of purified and crude Mtz-triazole activity against *G. lamblia* strains

Table S3: Activity (EC_{50}) of selected purified Mtz-triazoles (>95% purify) against *G. lamblia* strains compared to literature values[15] for crude Mtz-triazoles (>85% purity).

	Compound	EC_{50} (µM) *G. lamblia*					
		106		713		713M	
		MtzS		MtzS		MtzR	
		This study	Lit.[15]	This study	Lit.[15]	This study	Lit.[15]
1	metronidazole	2.8	3.6	2.3	2.0	17	50
4a	phenyl	0.28	0.26	0.16	0.16	2.3	5.3
4d	p,m-Cl_2-phenyl	1.1	1.4	2.5	0.31	1.2	0.57
4e	p-Me-phenyl	0.29	2.1	0.29	0.53	1.0	8.1
4h	pyridine	0.38	0.56	0.28	0.35	0.95	14
4k	benzyl	0.51	0.71	0.32	0.39	1.1	5.2
4m	CH_2NMe-benzyl	1.1	0.30	0.71	0.91	2.2	9.3
4n	thiophene	0.34	0.34	0.18	0.23	0.9	6.9

5.4 Correlation matrix of compound activity and properties

Table S4: Correlation analysis of compound activity and properties.

	C. difficile	*E. histolytica*	*G. lamblia*	*logP*	*MW*	*logD*	*logS*	*tPSA*
C. difficile	1.00							
E. histolytica	0.68	1.00						
G. lamblia	0.86	0.77	1.00					
logP	0.70	0.72	0.89	1.00				
MW	0.14	0.01	0.36	0.56	1.00			
logD	0.82	0.69	0.91	0.92	0.47	1.00		
logS	-0.58	-0.46	-0.78	-0.91	-0.77	-0.83	1.00	
tPSA	-0.70	-0.71	-0.63	-0.61	0.04	-0.64	0.46	1.00

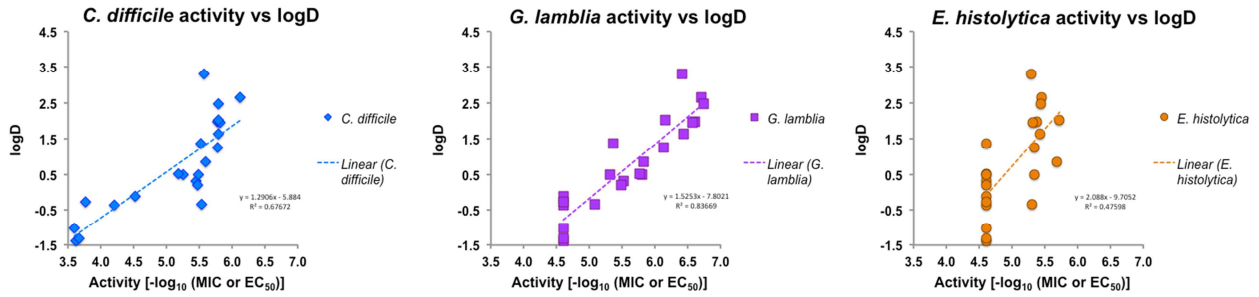

Figure S1: Activity vs logD for individual organisms with linear regression analysis.

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