SYNTHESIS OF FLUORESCENT ANTI-MALARIAL DRUG PROBES AND EVALUATION WITHIN PLASMODIUM FALCIPARUM

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INDEX

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"Common sense invents and constructs no less than its own field than science does in its domain. It is, however, in the nature of common sense not to be aware of this situation."

- *Albert Einstein.*

"Science moves with the spirit of an adventure characterized both by youthful arrogance and by the belief that the truth, once found, would be simple as well as pretty."

- *James Watson.*

I am deeply indebted to the Department of Chemistry, National University of Singapore for their funding support and to my mentor and a father figure ***Dr. Martin. J. Lear*** for imbibing into me common sense towards successful completion of my Masters Thesis work on *"Synthesis of Fluorescent Anti-Malarial drug probes and evaluation of pathway within Plasmodium Falciparum"*. His fighting spirit, endurance & perseverance shall forever remain as fond memories for my future endeavours.

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Thus I dedicate this work to all people who have touched my life and time spent in Singapore.

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACN – Acetonitrile.

Ar – Argon.

Ato – Atovaquone

AtoR – Atovaquone resistant.

Ato-PG – Atovaquone proguanil combination.

Ato-PGR – Atovaquone Proguanil combination resistance.

Art – Artemisinin.

Art-comb – Artemisinin combination.

$CHCl_3$ – Chloroform.

CH_2Cl_2 – Dichloromethane.

CQ – Chloroquine.

CQR – Chloroquine Resistance.

calc. – calculated.

CSP – Circumsporozoite surface protein.

DCC – Dicyclohexylcarbene.

DMF – Dimethyformamide.

DMP – Dess-Martin Periodinane.

DIPEA – Di-isopropylethylamine.

EtOAc – Ethyl acetate

Ret. Time – Retention Time.

Fmoc-OSu – N-(9-Fluorenylmethoxy

GI50 – 50% Growth inhibition

HATU – (2-(7-Aza-1H-benztriazole-1-yl)) carbonyloxy) succinimide -1,1,3,3,-tetramethylammonium hexafluorophosphate.

Hal – Halofanthrine

HalR – Halofanthrine resistant.

HOAt – 3H-[1,2,3]triazolo[4,5-b] pyridine-3-ol.

HOBt – N-Hydroxybenzotriazole.

HRMS – High resolution Mass spectroscopy

HPLC – High Performance Liquid Chromatography

K_2CO_3 – Potassium carbonate.

LCMS – Liquid Chromatography Mass Spectroscopy.

LD – LapDap (chlorproguanil

LDR – LapDap (chlorproguanil

dapsone combination).

dapsone combination resistance).

LC_{50} – 50% Lethal Concentration

Mef – Mefloquine.

MefR – Mefloquine resistance.

MeOH – Methanol.

MSP-1 – Merozoite surface protein

$NaHCO_3$ – Sodium bicarbonate

NaOH – Sodium Hydroxide.

Na_2SO_4 – Sodium Sulfate.

$NaBH(OAc)_3$ – Sodium triacetoxy borohydride.

obsvd. – observed.

PI – Propidium Iodide.

Pyr – Pyrimethamine.

PyrR – Pyrimethamine resistant

Pyr-SDX – Pyrimethamine Sulfadoxine

Pyr-SDX – Pyrimethamine Sulfadoxine Resistance.

TEA – Triethylamine.

TLC – Thin layer chromatography

TFA – Trifluoroacetic acid.

TGI 50 – Total growth inhibition

LIST OF SYMBOLS

α – alpha.

β – beta.

ö – expressed in ppm for NMR.

$ö_H$ – proton NMR.

$ö_C$ – carbon NMR.

µL – micro litre.

$[M]^+$ – Molecular ion.

mgs – milligrams.

mM – milli moles.

m/z – mass to charge ratio.

nM – nano-Molar.

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SUMMARY

On World Malaria Day April 2010, impetus has been towards reducing Malaria burden in 2010 to half as compared to the year 2000 levels and to achieve eradication of malaria by 2015 through progressive elimination methods[1]. These methods rely heavily upon effective and efficient diagnosis of the parasite making it a crucial step towards early identification, control and subsequent elimination of the disease. The gold standard for malaria diagnosis still continues to be optical microscopy, although it has severe limitations due to its ease of availability, labor intensive process and need for highly skilled technicians. The emergence of chloroquine resistant strains in 1957and the further discovery of multi-drug resistant strains (MDRSs) and recent Artemisinin resistant strains[3] in 2009 along the Thai-Cambodian border, has been a cause of grave concern. The current diagnostic techniques do not address the above need for differentiating sensitive vs resistant strains of the parasite, which would be an important factor in determining the clinical administration of the effective drug. My current thesis involving *"Synthesis of fluorescent anti-malarial drug probes and evaluation within plasmodium falciparum"* addresses the above requirement for a robust, fast, sensitive, & portable diagnostic technique for determination of drug resistant *Plasmodium falciparum* strains within patient blood samples. The probes designed would help in reliable data collection and administration of the appropriate drug dosage. The thesis discusses the drug design rationale, synthesis and results of the application of the probes in (1) malaria diagnosis (in collaboration with Dr. Kevin Tan), (2) cancer studies (in collaboration with National Cancer Institute, USA) and (3) bio-imaging studies on macrophages (studies done by myself in collaboration with Dr. Kevin Tan). The probes are mainly designed on chloroquine and artemisinin analogues, which are the preliminary drugs administered for the treatment of malaria. The probes tested on *Plasmodium falciparum* & mammalian cell lines established their lysosomotropic nature thus providing potential insight into the pathway within the parasite and macrophages. The future lies in utilizing the concept of drug probes or *"Medicinal Probes"* towards evaluation and bio-imaging studies on various diseases.

INTRODUCTION

A deadly mosquito borne disease, *"Malaria"* was the cause of 7% of global deaths in children in 2008. According to WHO estimates last year malaria accounted for 250 million cases which lead to 850,000 deaths worldwide in the developing countries, especially Africa. Global Malaria commitment and funding has increased 10-fold to about US$1.8 billion accounting for external funding sources and other donors like GFATM (The Global Fund to Fight Aids, Tuberculosis and Malaria), UNITAID, US-PMI. On World Malaria Day April 2010, impetus has been towards reducing Malaria burden in 2010 to half compared to 2000 levels and to achieve eradication of malaria by 2015 through progressive elimination methods[1] as described in Fig. 1

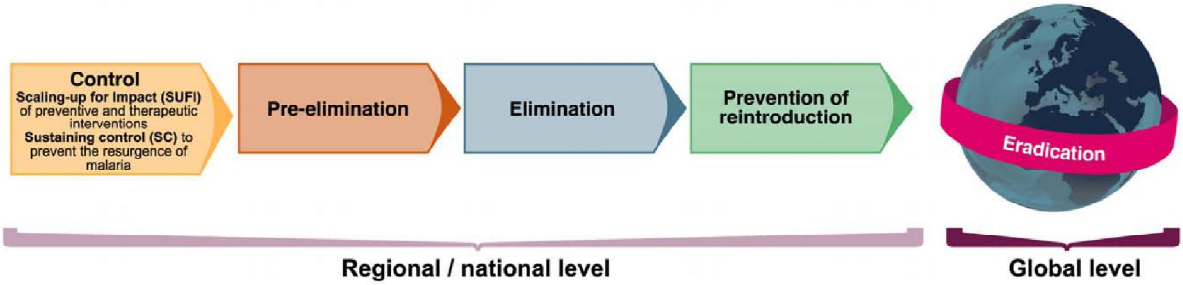


Fig.1 – WHO Roll back Malaria goals[3]

The intervention methods coupled with better diagnostic techniques have shown success in the past 6 years (2000-06) by reduction in malaria burden by 50%[2]. The challenges for achieving WHOs goal of control, elimination and subsequent eradication of malaria lies in making improvements in the following tools[3]:

a) SERCaP (Single Encounter radical cure and prophylaxis).

b) VIMT (Vaccines that interrupt malaria transmission).

c) Vector Control techniques.

d) Improved diagnostics and surveillance.

a) SERCaP – The objective of SERCaP type of drug would be to provide radical cure and prophylaxis for a period of at least *1month* outlasting the typical development period of *P.falciparum* parasites. Chloroquine and derivatives, quinine and artemisinin were the first line of defence against malaria due to their clinical effectiveness and low-cost. Fig 2 highlights the year of introduction of anti-malarial drugs administration and the subsequent clinical observations of emergence of resistant strain[4] denoted by the suffix "R" after every drug., e.g. – "*CQ-Chloroquine"* was introduced as the drug of choice for administration to malaria patients in 1945 and subsequently in the year 1955-1960 "CQR – Chloroquine resistance" due to emergence of chloroquine resistant strains of parasites was observed. (Abbreviations of other drugs are enclosed in List of Abbreviations IV-V)

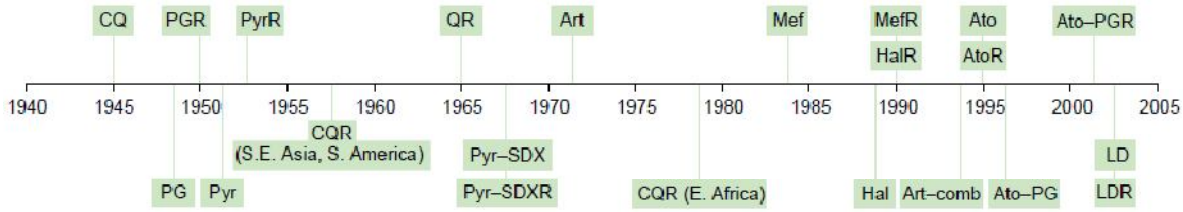


Fig.2 – Anti-Malarial drug introduction and emergence of resistance[4]

Fig. 3 highlights the mode of action of various anti-malarial drugs within the parasitic cellular components[5]. It is interesting to note that respite the varied mode of action of the above mentioned drugs, the parasite was still successful to genetically modify its cellular components to give rise to the drug specific or even multi-drug resistant strain. The emergences of multiple drug resistant strains (MDRSs) have been attributed to the single dose therapies or improper dosages. These have led to recrudescence i.e. generation of mutant plasmodium strain[4].

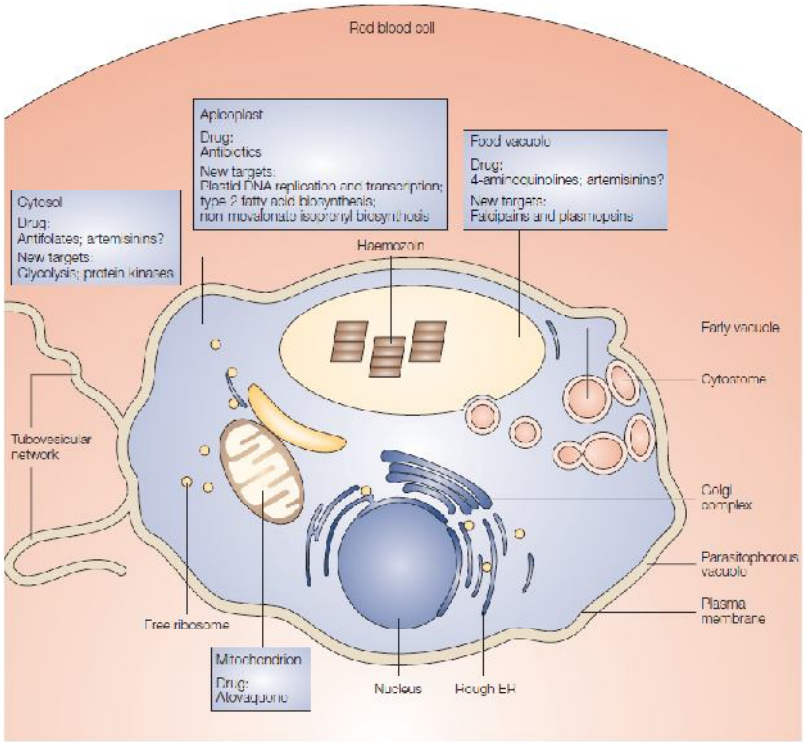
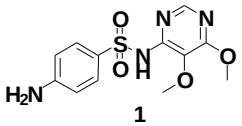
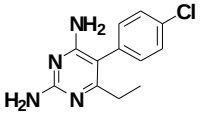
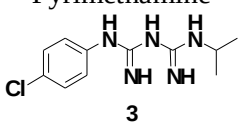
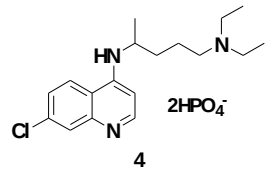
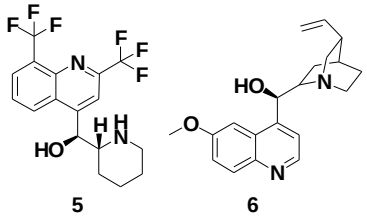
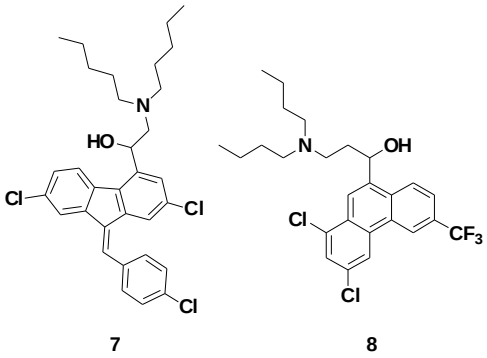
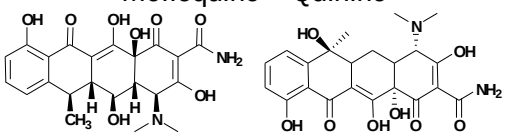
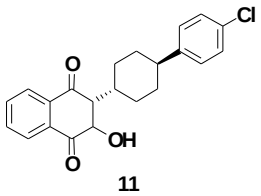
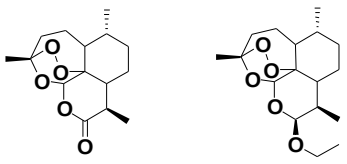
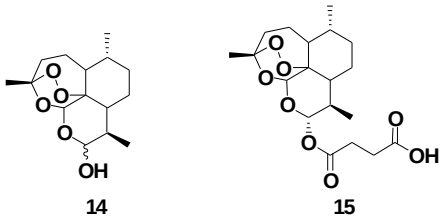


Fig.3 – Intra-erythrocytic *P.falciparum* trophozoite and anti-malarial drug targets[5]

The amino acid mutations in the cell components of the *P.falciparum* parasites from the field have been characterized and summarized below (Table 1)[4].

Drug	Gene encoding target	Principal amino acid associated with resistance levels in the field
 1 Sulfadoxine	Dihydropteroate synthase (dhps)	S436A/F, A437G, K540E.
 2 Pyrimethamine	Dihydrofolate Reductase (dhfr)	N51l, C59R, S108N.
 3 Chlorproguanil	Dihydrofolate reductase (dhfr)	A16V, S108T, C59R.

 4 Chloroquine diphosphate	chloroquine resistance (crt) transporter, multi-drug resistance1 (mdr1)	C72S, M74I, N86Y, Y184F.
 5 6 Mefloquine Quinine	multi-drug resistance1 (mdr1)	Copy number > 1; wild-type N86
 7 8 Mefloquine Quinine	multi-drug resistance1 (mdr1)	Copy number > 1; wild-type N86
 9 10 Doxycycline Tetracycline	mt protein synthesis	Not yet characterized
 11 Atovaquone	Cytochrome b	Y268S/N
 12 13 Artemisinin Artemether	ATPase, mdr1	Clinical resistance recently observed in 2009 but the mutation cannot be confirmed.



14 15

Dihydroartemisinin α-Artesunate

Table 1: Genetic changes in *P.falciparum* associated with resistance to current drugs.

Reports from 1995-2010 extensively highlight research contributions into new drug development[6-11], typically by utilizing active components from medicinal plants and marine natural products, which have been recommended as replacements for existing anti-malarial therapeutics. The molecules cover wide range of structures like alkaloids **(16)**, peptides **(17)**, flavonoids **(18)**, limonoids **(19)**, quinones **(20)**, terpenes **(21)**, trioxolanes **(22)**, poly-ether type SF2487 **(23)**[6-11].

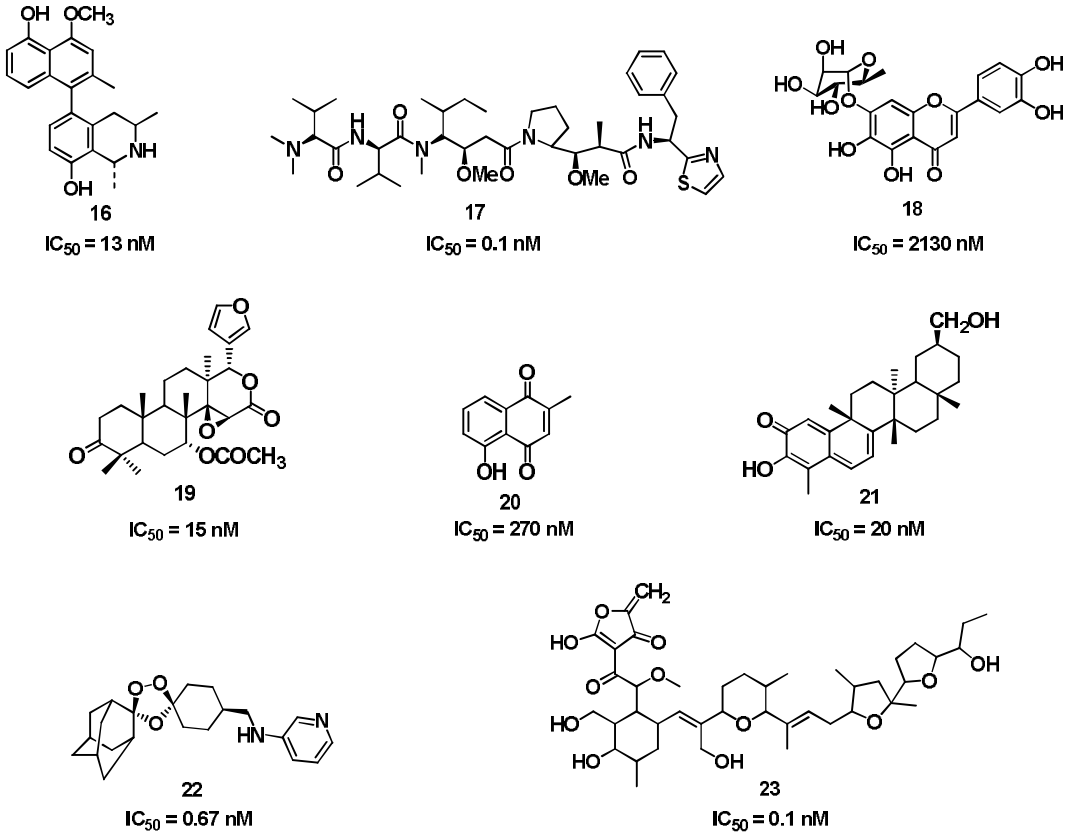


Fig. 4 – Structures of anti-malarial drugs derived from natural or marine sources.

Since malaria remains confined to developing or third-world nations, cost effectiveness, ready availability and clinical suitability of the above highly efficacious anti-malarial agents are the most important factors for successful implementation.

Thus WHO has recommended use of artemisinin combination therapy (ACT) to contain the emergence of resistant strain.

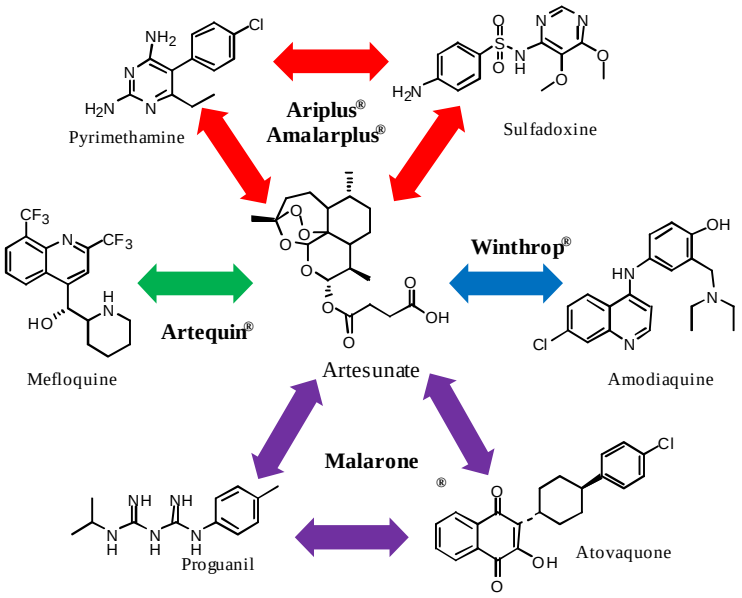


Fig. 5 – Artemisinin combination therapy (ACT).

Fig. 5 above shows combination therapies of artesunate with various anti-malarial drugs (as depicted by arrows) recommended by WHO.

Artemisinin multiple mode of action was expected to discourage the emergence of artemisinin resistant strains. Unfortunately, the discovery of existence of artemisinin resistant strains in 2009 and 2010 along the Thai-Cambodian border has been a cause of grave concern, as the cause for resistance has not been elucidated[13-17].

b) VIMT (Vaccines that interrupt malaria transmission) – The existing vaccines in clinical development have the objective of reducing morbidity and mortality in young children in highly endemic countries. However future vaccines are expected to function as VIMT's with the ultimate of purpose of complete eradication. Vaccine development[18-20] has taken a great leap with certain vaccines already reaching Phase III trials of testing. These vaccines are expected to create an immunological response to two specific parasite surface proteins namely MSP-1 (merozoite surface protein) and CSP (circumsporozoite protein). The vaccine RTS/S (from GlaxoSmith Kline based on CSP) has shown 65% efficacy and has currently progressed to Phase III clinical trials. First yet unsurpassed success in inducing complete and permanent protective immunity responses against malaria was achieved with irradiated sporozoites in human studies. However mass production of these sporozoites still remains a challenge. Other vaccination techniques are summarized below (Table 2).

c) Vector Control techniques – These techniques rely upon interventions like indoor residual insecticide spraying and insecticide treated bed-nets to reduce vector daily survival rates. The challenge lies in developing broader ranges of insecticides that can circumvent emerging resistance to existing insecticides. The other challenge lies in the development of interventions for vectors that do not lie or feed indoors[3].

d) Improved diagnostics and surveillance – Current methods for measuring transmission are time consuming, expensive and have low sensitivity for use in conditions of low and non-uniform infection. The main challenge for achieving eradication lies in creating a robust, sensitive and specific standardized method for the assessment of transmission intensity in the intervening period of low and non-random

levels of transmission[3]. The diagnostic methods are effective, but do not provide fast diagnosis and have to rely upon highly skilled technicians. The current gold standard

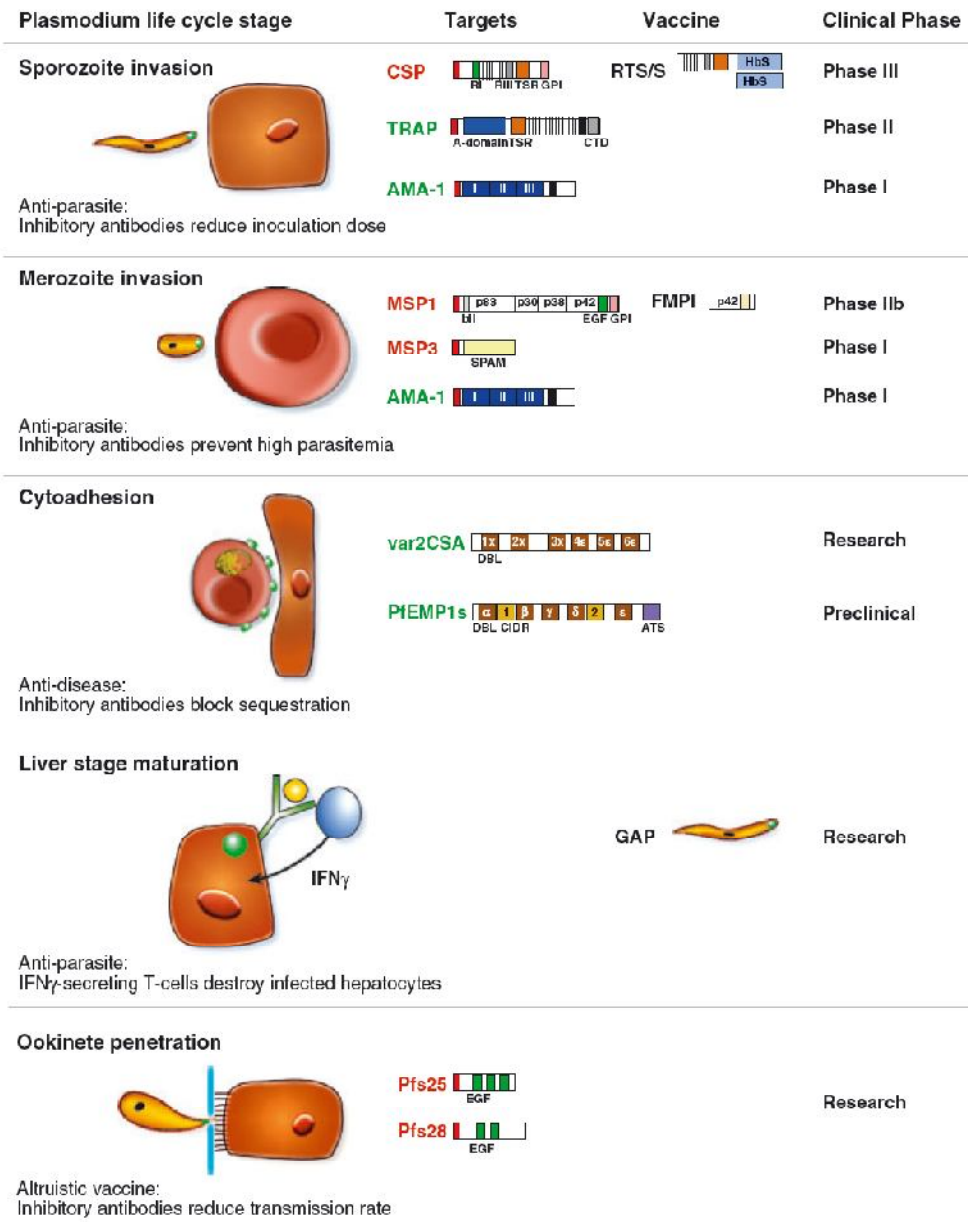


Table 2: Vaccination techniques and parasite targets[20].

for malaria diagnosis has been optical microscopy, but this has limitations due to its ease of availability, labor intensive process and need for a highly skilled technician. The WHO (World Health Organization) along with FIND (Foundation for Innovative New Diagnostics) have started evaluations of rapid diagnostic tests (RDTs) since 2008 in order to provide for fast, accurate, sensitive and affordable tools for the

instant evaluation of blood samples in the field. In 2010 from the 29 diagnostic tests submitted for analysis 15 have met the minimum performance criteria as per WHO guidelines based on RDTs[21,22]. The RDTs are based on the detection of *plasmodium* specific antigens in the whole blood specimens. These are available in dipstick, cassette or card format and contain bound antibodies to specific antigens such as histidine-rich proteins-2 (HRP2) (specific to *P.falciparum*), pan specific or species specific plasmodium lactate dehydrogenase (pLDH) or aldolase (specific to all major plasmodium species : *P.falciparum*, P.*vivax*, P.*ovale*, P.*malariae*)[15]. These RDTs are sensitive towards test environment and conditions. The existing diagnostic tests for malaria along with my proposed method have been summarized in the Table 3[23].

Methods for Malaria and Drug Resistance Diagnosis

Factors	In vivo response	In vitro microscopy	In vitro radioactive hypo-xanthine	Polymerase chain reaction PCR	Rapid diagnostic tests (RDTs)	**Fluorescent anti-malarial probes**[23]
Cost	High	Low	High	High	Low	**Low**
Time for results	Days	48 hrs	48 hrs	12 hrs	0.5 hrs	**4 hrs**
Skill level	High	High	High	Moderate	Moderate	**Moderate**
Sensitivity	++	++	+++	+++	++	**+++**
Resources	Human subjects	Microscope	Scintillation counter	PCR machine	Visual based technique	**Flow Cytometer**
MDRSs ID	Yes	No	Yes	Yes	No	**YES**
Portability	NA	No	No	No	Yes	**YES**

++ - low sensitivity

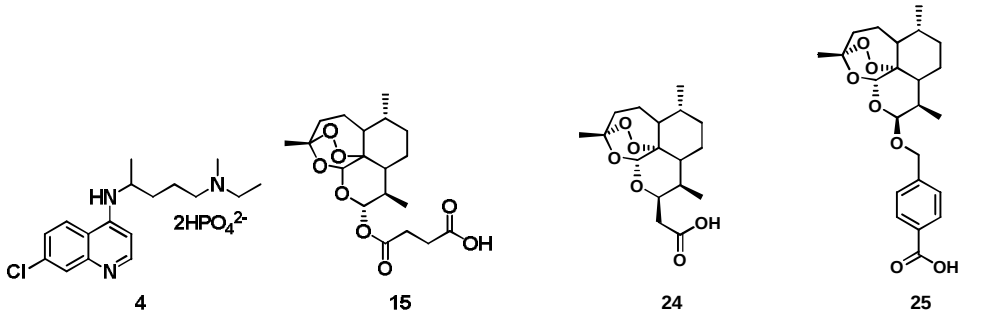
+++ - high sensitivity

Table 3: Comparison of methods for malaria and drug resistance diagnosis.

HYPOTHESIS & OBJECTIVES -

This thesis covers the design, synthesis and biological applications of fluorescent anti-malarial probes[24,25,26], by addressing the key requirements for robust, sensitive, fast and portable diagnostic. The idea of using fluorescent drug probe has not gained popularity due to change in the final pharmacophore thus influencing the binding property of the final molecular structure[24]. Chloroquine is very well known for its lysosomotropic (accumulation in the food vacuole of the parasite) and heme-binding pathway of action within the chloroquine sensitive parasite. However these features have never been exploited towards diagnosis for identifying resistant strain[26]. Artemisinin and its derivatives have a wide range of mode of action within the parasite. There are still ongoing debates on the modes of action of artemisinin and its bio-activation pathways within the plasmodium parasite. Meshnick's heme-iron triggered bio-activation of trioxanes to form reactive oxygen species[27], Posner and Jefford's reductive scission model[28], Haynes and co-workers open peroxide model[29] and iron vs heme dependant bio-activation[30], inside parasite cells all suggests that there is not a single pathway for the activation of artemisinin. The binding site of artemisinin is not clearly understood and is proposed to inhibit the sarcoplasmic reticulum Ca^{2+} - transporting ATPases (SERCAs) specifically within the parasites (PfATP6)[31-33]. Artemisinin has also been found to have increasing importance for their application as anti-cancer drug that act upon drug and radiation resistant tumour cell lines. The mode of action is again proposed to be endo-peroxide mediated with the end result of decreased proliferation, increased oxidative stress, induction of apoptosis and inhibition of angiogenesis thus leading to cytotoxicity in tumour cells[27,34]. Clearly with the recent discovery of artemisinin sensitive strains it would be increasingly important to understand the mode of action of artemisinin within the

resistant parasites. Thus, chloroquine and artemisinin (mainly Artesunate, Artelinic acid and Deoxocarbaartemisinin) analogue based probes were synthesized for diagnostic and bio-imaging application as shown in Fig. 6



Chloroquine diphosphate | α–Artesunate | β-Deoxocarba-artemisinin | β-Artelinic acid

Fig.6 Molecular structures of parent drug molecules.

The model for design and synthesis of fluorescent anti-malarial probe can be described as below.

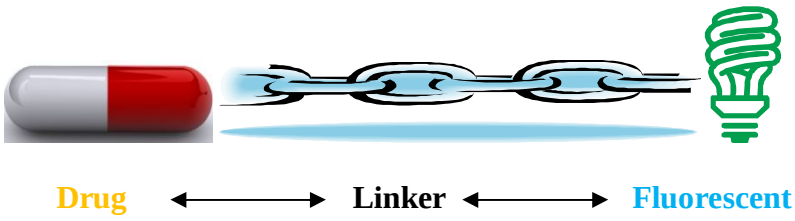


Fig.7 Diagrammatic representation of fluorescent drug probes.

My method of utilizing fluorescent anti-malarial probe for diagnosis provides the health worker on the field with a portable tool for malaria detection and identification of multi-drug resistant strains (MDRSs). This would help in reliable data collection and administration of the appropriate drug regime based on the type of drug resistance identified in the parasite. It would also provide personal healthcare, reduce the burden of drug inventory in hospitals and control the further spread of MDRSs, thus modestly contributing towards WHOs elimination of Malaria goal of 2015.

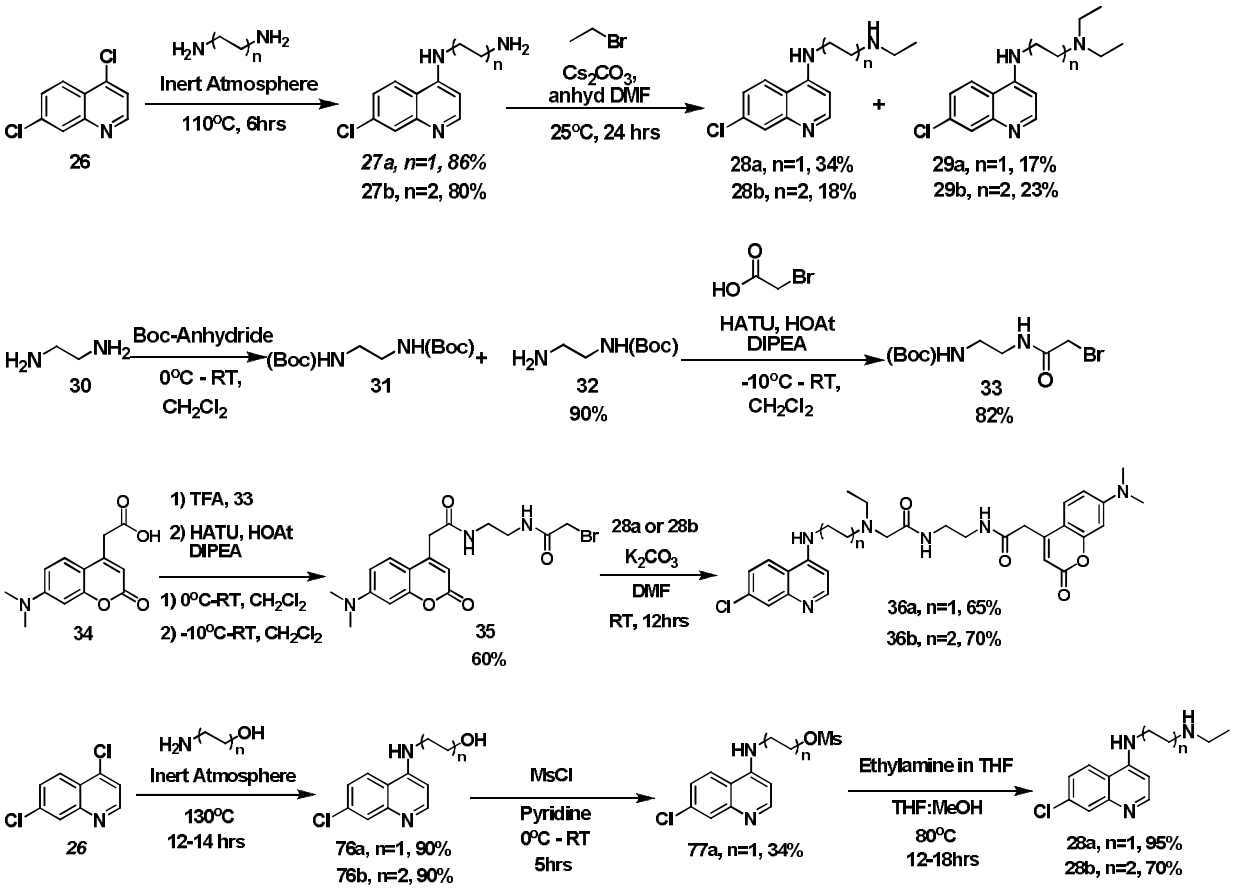
RESULTS AND DISCUSSIONS

3.1 Proposed Synthetic route –

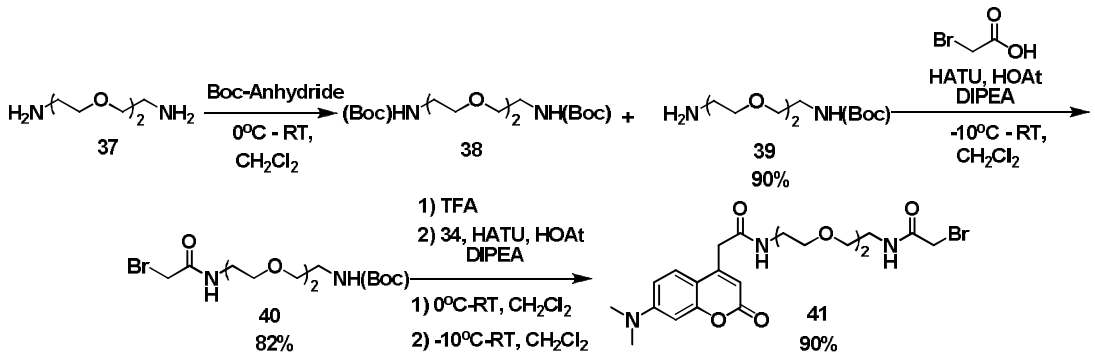
3.1.1 Synthesis of probes **36a**, **36b** and **42**[35-37] –

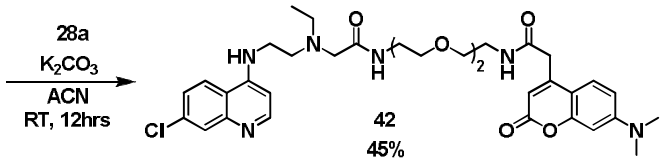
The synthesis of probes **(36a, 36b and 42)** is divided into synthesis of the chloroquine precursor **(28a, 28b)** and the coumarin precursor **(35, 41)**. Nucleophilic substitution on 4,7-dichloroquinoline **(26)** using 1,2-diaminoethane and 1,4-diamino butane gave analogues **(27a)** and **(27b)** respectively. Due to the quinoline structure it is easy to replace the labile chlorine atom at 4-position compared to the one at the 7-position. Further addition reactions using bromo ethane gave the chloroquine precursor **(28a and 28b)** and diethyl **(29a and 29b)** precursor of chloroquine. Direct addition of bromoethane led to diethyl chloroquine analogues in reasonable yields. Hence slow addition and dilution of bromoethane in anhydrous DMF is an important step to increase the yields of formation of the desired chloroquine precursor versus the diethyl analogues. An alternative technique for synthesis of chloroquine precursor **(28a and 28b)** was defined and scale up synthesis up to 1gm with almost 90% yields was achieved. Mono-boc analogue of 1,2-diaminoethane was synthesized by slow addition and dilution (in anhydrous CH_2Cl_2) of boc-anhydride into excess of diamine (also diluted in anhydrous CH_2Cl_2). Activation of the carboxylic group in bromoacetic acid using reagents 2-(1H-7-Azabenzotriazol-1-yl)--1,1,3,3-tetramethyl uronium hexafluorophosphate methanaminium (HATU)) + 1-Hydroxy-7-Azabenzo- triazole (HOAt) in presence of base diisopropylethylamine (DIPEA) and coupling to mono boc protected 1,2-ethanediamine gave the acetamido analogue **(33)**. HATU + HOAt reagents for activation of carboxylic group were preferred over DCC + HOBt or other similar reagents, due to high yields and ease of work-up. The linker **(33)** is further deprotected using trifluoroacetic acid in anhydrous CH_2Cl_2 to give the trifluoroacetic

salt of the amine, which upon neutralization with excess DIPEA is again coupled with coumarin-4-acetic acid **(34)** using HATU + HOAt reagents to give the coumarin precursor **(35)**. This bromo acetamido coumarin precursor **(35)** is purified by column chromatography and used immediately without storage due to its inherent instability. Finally nucleophilic substitution of the labile bromine atom by the amine group **(28a, 28b)** in the presence of dry potassium carbonate and anhydrous acetonitrile (ACN) yielded the probes **36a, 36b** and **42**.



Scheme 1 – Chloroquine-coumarin probe synthesis 1

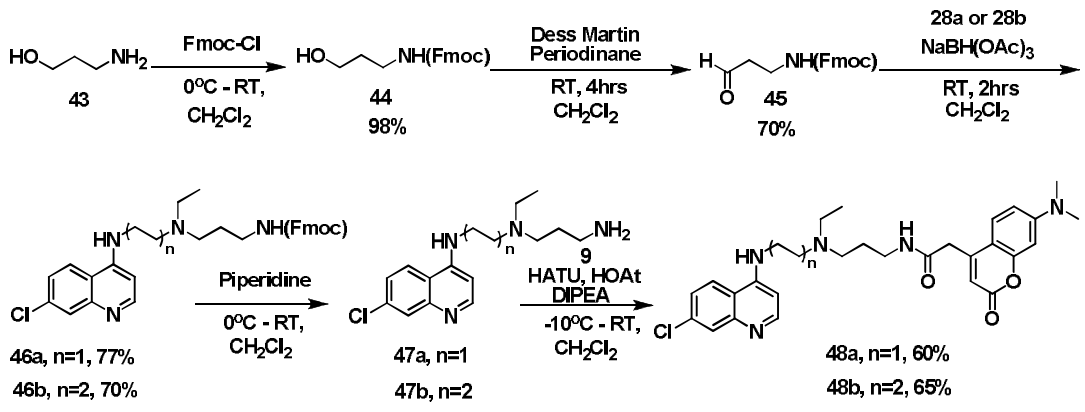




Scheme 2 – Chloroquine-coumarin probe synthesis 2

3.1.2 Synthesis of probes **(48a** and **48b)**[38] –

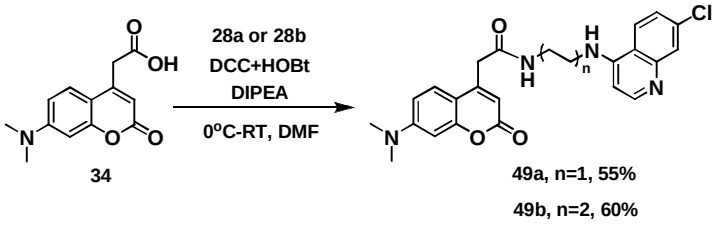
Dess-Martin Periodinane reagent was used for reduction of fmoc protected 3-amino propanol because it was found to be milder method over chromium based reductions, ease of work-up and sensitivity of the aldehyde precursor **(45)**. Sodium triacetoxyborohydride in anhydrous CH_2Cl_2 was used for reductive amination reaction between aldehyde **(45)** and amine analogue of chloroquine **(28a, 28b)**. The reaction progresses by formation of imine upon addition of aldehyde and this intermediate is reduced to the desired product **(46a, 46b)** on addition of $NaBH(OAc)_3$. Sodium triacetoxyborohydride is a mild reducing agent and excess reagent can easily quenched with methanol, which affords cleaner work up and high yields of the desired product in comparision to other hydride reducing agents. Upon fmoc de-protection the amine was directly used after short column purification for the final coupling process, due to its high affinity towards the silica column. The low yield of probes **(48a, 48b)** was possibly due to the mild coupling method adopted and low reactivity between **(47a, 47b)** and coumarin-4-acetic acid **(34)**.



Scheme 3 – Chloroquine-coumarin probe synthesis 3

3.1.3 Synthesis of probes **49a** and **49b** –

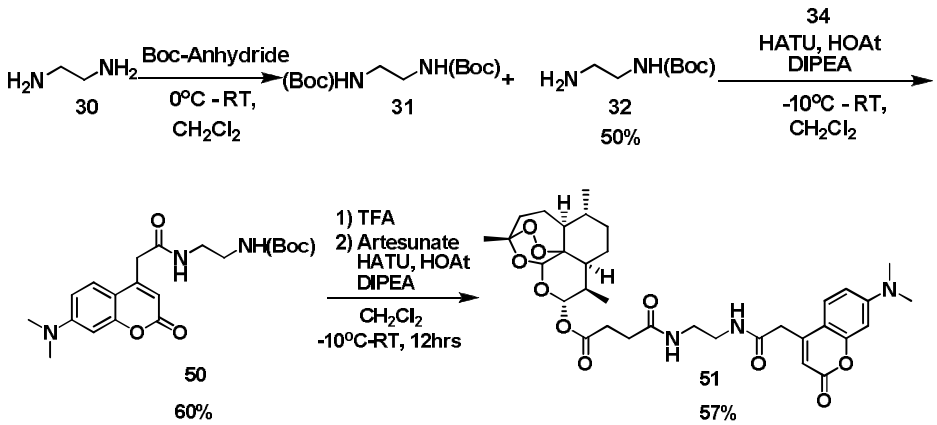
Dicyclohexylcarbodiimide (DCC) + hydroxybenzotriazole (HOBt) with DIPEA in anhydrous DMF as solvent gave good yields of probes **49a** (55%) and **49b** (60%) in comparision to HATU + HOAt reagents. The above reagents follow the same mechanism of formation of activated carboxylic acid ester, which upon reaction with amine **(28a, 28b)** gave the desired product **(49a, 49b)**.



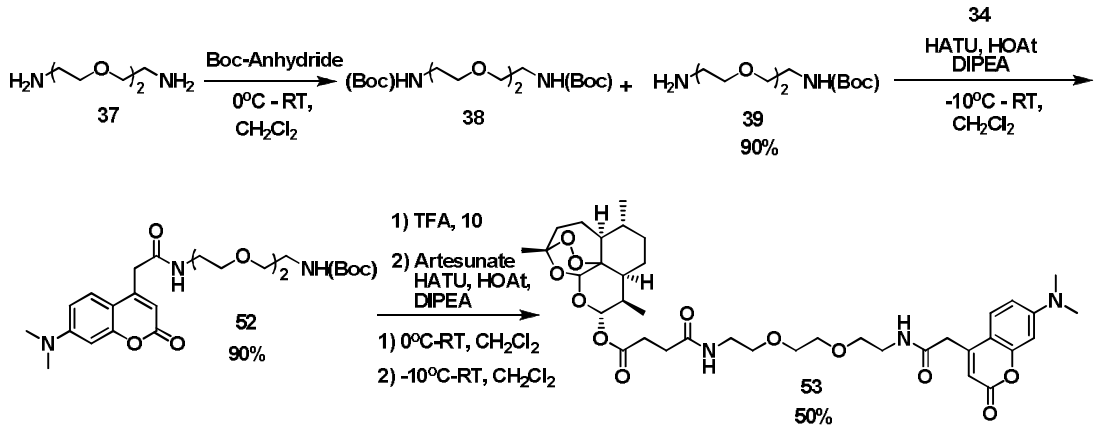
Scheme 4 – Chloroquine-coumarin probe synthesis 4

3.1.4 Synthesis of probe **51, 53, 55**[39] –

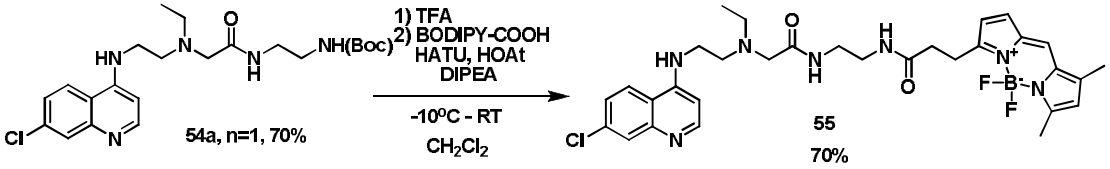
Amide coupling method used for synthesis of probes 48a, 48b was used for synthesis of probes **51, 53, 55.** In the case of probe 55a and 55b, the amine was isolated by treatment of the TFA salt with bicarbonate solution at 0°C and then coupled with BODIPY-COOH using HATU+HOAt coupling technique.



Scheme 5 – Artesunate-coumarin probe synthesis 1



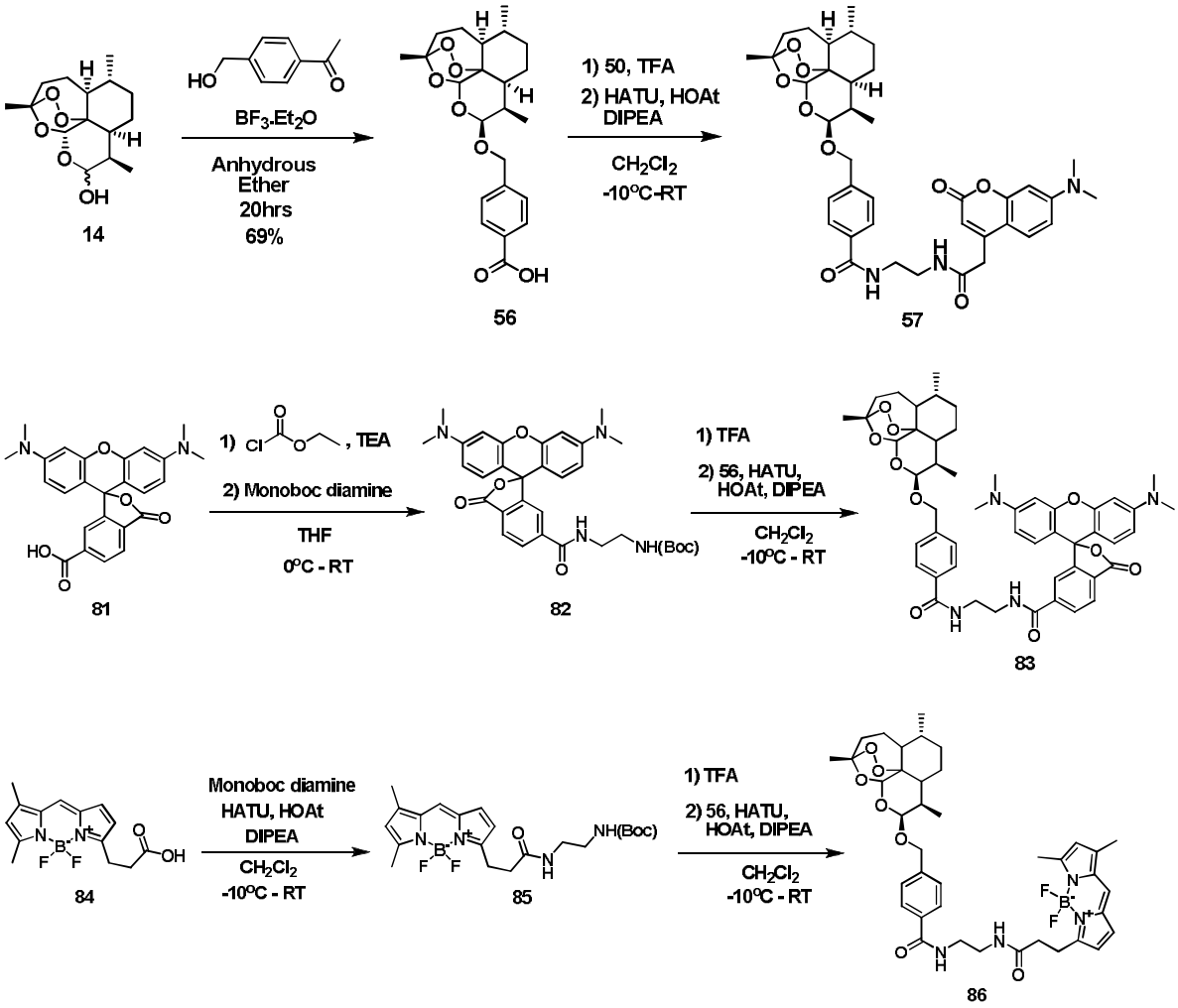
Scheme 6 – Artesunate-coumarin probe synthesis 2



Scheme 7 – Chloroquine-BODIPY based probes.

3.1.5 Synthesis of artelinic acid probe **(57, 83, 86)**[40] –

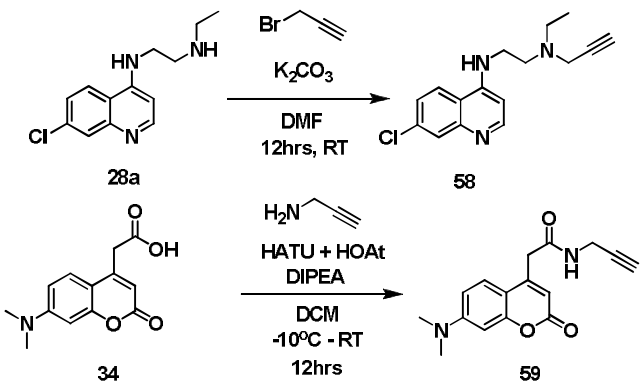
Mixture of dihydroartemisinin epimers was reacted with 4-(hydroxy methyl) benzoic acid in the presence of Lewis acid boron trifluoride etherate to preferentially give the β–Artelinic acid **(56)**. The reaction proceeds via formation of oxy-carbenium species on addition of boron trifluoroetherate. 4-(hydroxymethyl) benzoic acid is only able to approach the oxy-carbenium ion from the 10β-position due to possible steric hindrance by the endoperoxide arrangement. Thus it selectively yielded β–Artelinic acid **(56)**. Method adopted for synthesis of artesunate probes **(51**, **53)** was used for synthesis of artelinic acid based probe **(57).** However TAMRA analogue for coupling with artelinic acid was synthesized using mixed anhydride method as shown below followed by HATU+HOAt coupling to give TAMRA-Artelinic probe **(83)**.

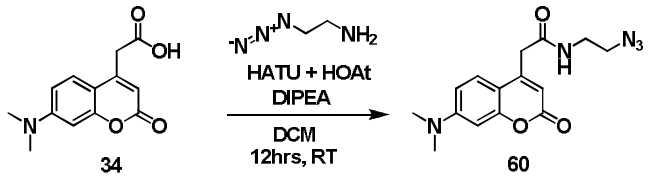


Scheme 8 – Artelinic acid based probes.

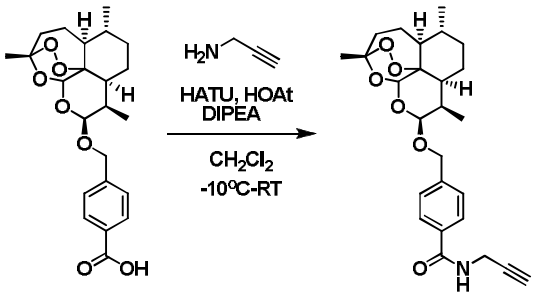
3.1.6 Synthesis[41] of click probes **58-60, 61-64, 87-90, 104-105** –

Amide coupling method used for synthesis of probes **48a, 48b** was used for synthesis of probes **59-62**. Probes **58, 63a, 63b, 64a and 64b** utilized similar nucleophilic substitution method as adopted during synthesis of probes **36a and 36b**.

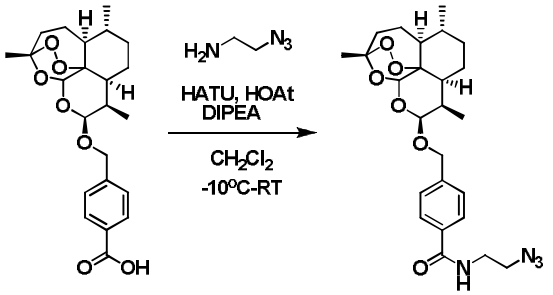




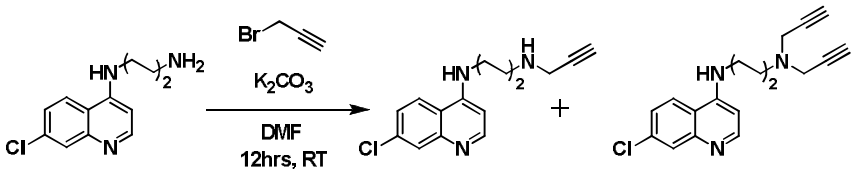
34 60



56 61



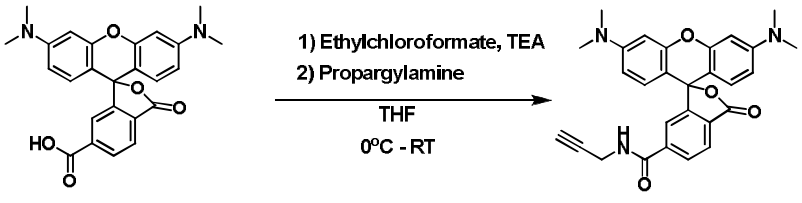
56 62



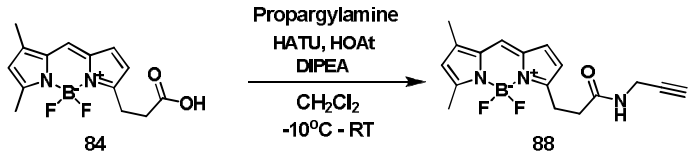
27a, n=1
27b, n=2

63a, n=1, 20%
63b, n=2, 35%

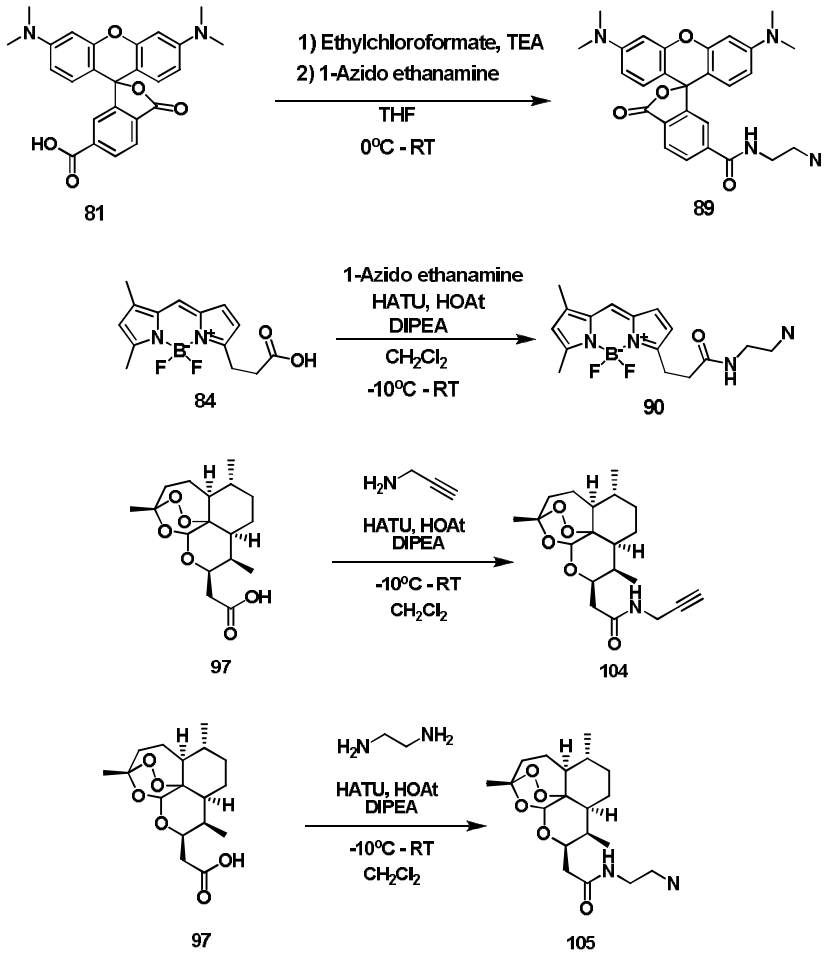
64a, n=1, 65%
64b, n=2, 70%



81 87



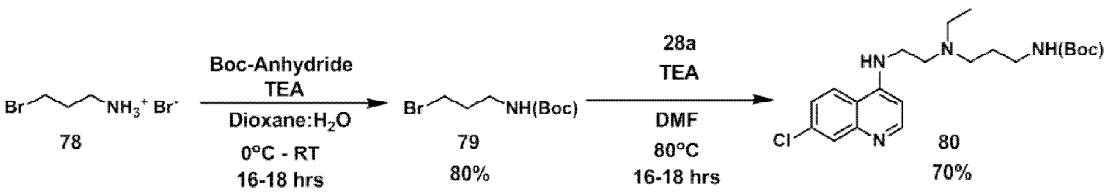
84 88

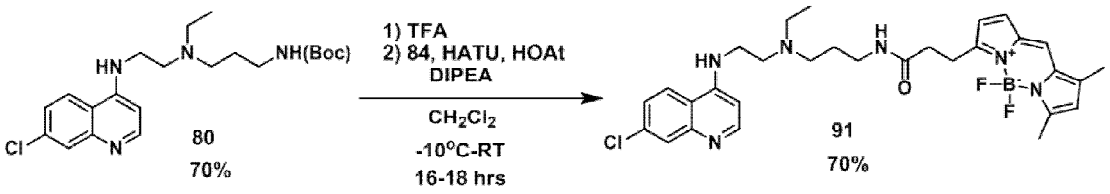


Scheme 9 – Click chemistry enabled probes.

3.1.7 Synthesis of BODIPY fluorescent probes **91** –

Due to the problems associated with the isolation of amine precursor after Fmoc deprotection, a second strategy of synthesis of boc analogue **(80)** of chloroquine precursor was adopted. This was further deprotected with trifluoroacetic acid at 0^{o}C and then the salt obtained was directly used for further coupling reaction by neutralization with DIPEA at 0^{o}C as shown in **Scheme 10.**

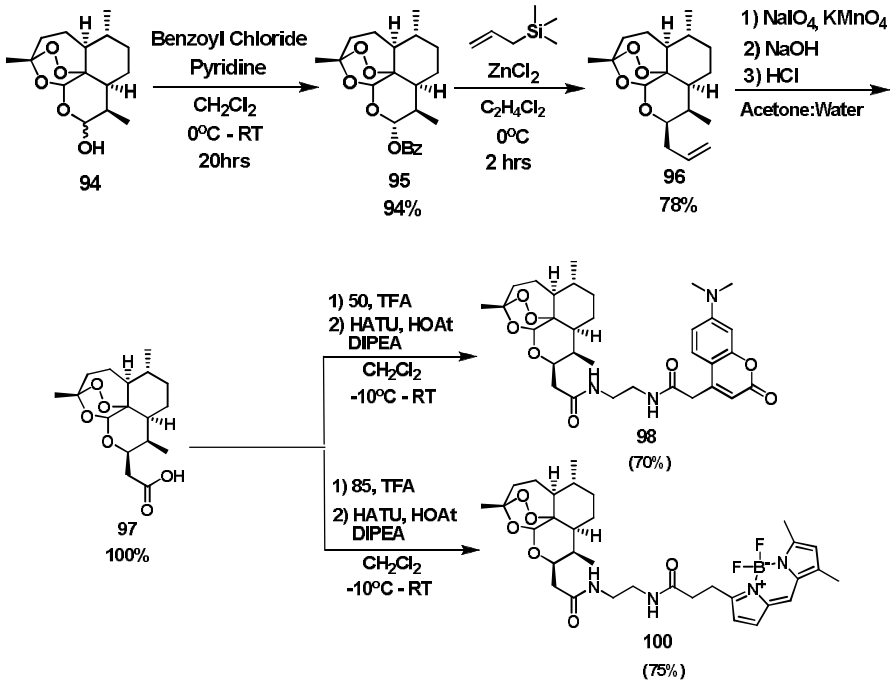




Scheme 10 –BODIPY chloroquine probe

3.1.8 Synthesis of Deoxocarbartemisinin probes **(98 and 100)** –

Deoxocarbaartemisinin intermediate was synthesized as per procedure enclosed in literature. The synthesis of final probes follows the same procedure as used for synthesis of artelinic acid probes. The detailed synthesis is shown in **Scheme 11**.



Scheme 11 – Deoxocarbaartemisinin probes

3.2 Drug design rationale and IC_{50} values –

The proposed drug design was expected to have the following properties

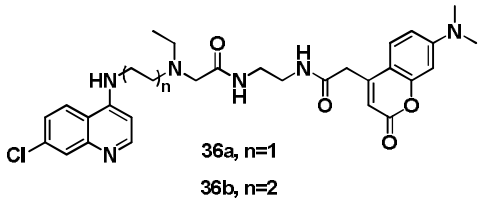
1) Minimal modification of the parent drug molecule structure.

2) Efficacy of the final probe would be similar to the parent drug molecule.

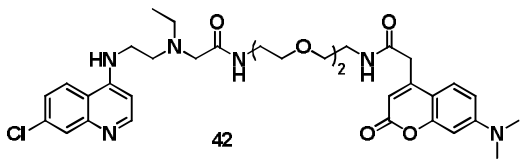
3) Fluorescent dyes selected would not show any activity with the *Plasmodium falciparum* parasite as cultured in the lab. Coumarin-4-acetic acid **34**, Borondipyrro methane carboxylic acid (BODIPY-COOH) **84**, Tetraaminomethylrhodamines carboxylic acid (5-TAMRA COOH and 6-TAMRA COOH **81**) have no activity within the parasite.

4) High thermal and hydrolytic stability for applications in biological systems.

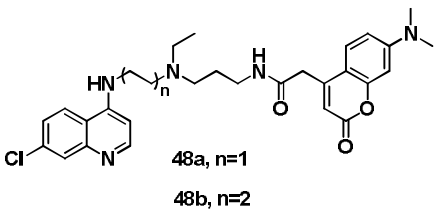
5) High fluorescence quantum yields confer live-cell imaging capability[42,43].



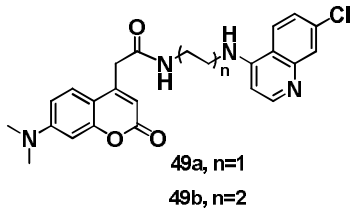
Probe design 1



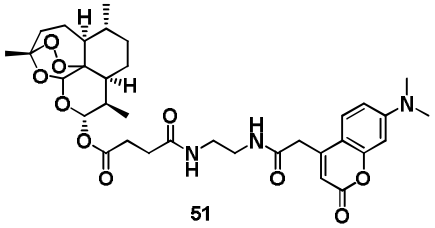
Probe design 2



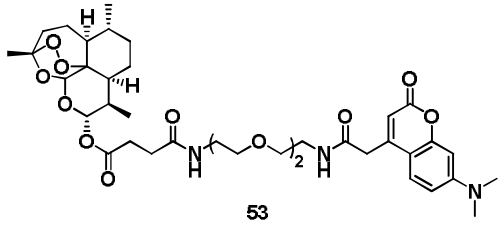
Probe design 3



Probe design 4

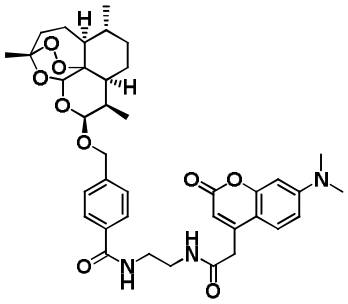


α-Artesunate probe design 1



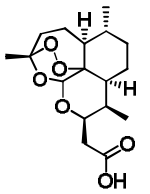
α-Artesunate probe design 2

Fig.8 Proposed drug design for probes



57

β-Artelinic acid probe design



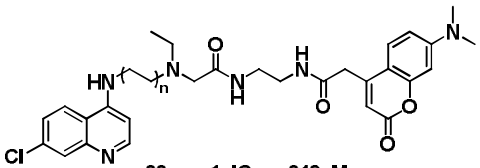
65

β-Deoxocarbaartemisinin carboxylic acid

Fig.8 Proposed drug design for probes

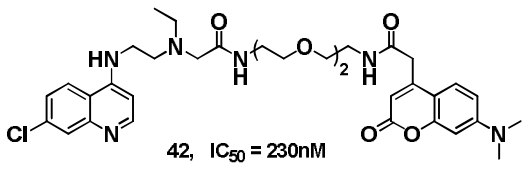
The proposed drug design was analyzed for its IC_{50} activity on chloroquine sensitive *Plasmodium falciparum* (3D7) as cultured in lab. As expected from the proposed design the tertiary amine functionality of the chloroquine was critical to the activity inside the parasite thus probes **36a** (IC_{50}= 349nM)**, 36b** (IC_{50}= 230nM)**, 48a** (IC_{50}= 85nM) have values closer to the parent drug molecule (chloroquine diphosphate **(4)**, IC_{50} = 23nM). Variation was also observed when the chain length was increased from 2 carbon **48a** (IC_{50}= 85nM) to 4 carbon **48b** (IC_{50}= 1200nM). The dioxaoctane linker **42** (IC_{50}= 230nM)**,** also lead to difference in IC_{50} values. This design could assist in fishing out enzymes using immunoprecipitation technique due to the large spacing between drug and the fluorescent-affinity probe but cannot be utilized in diagnosis.

IC_{50} values of other probes are summarized in Fig. 9 below.

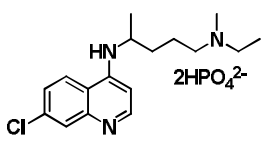


36a, n=1, IC_{50} = 349nM

36b, n=2, IC_{50} = 230nM



42, IC_{50} = 230nM



4, IC_{50} = 23nM

Chloroquine diphosphate

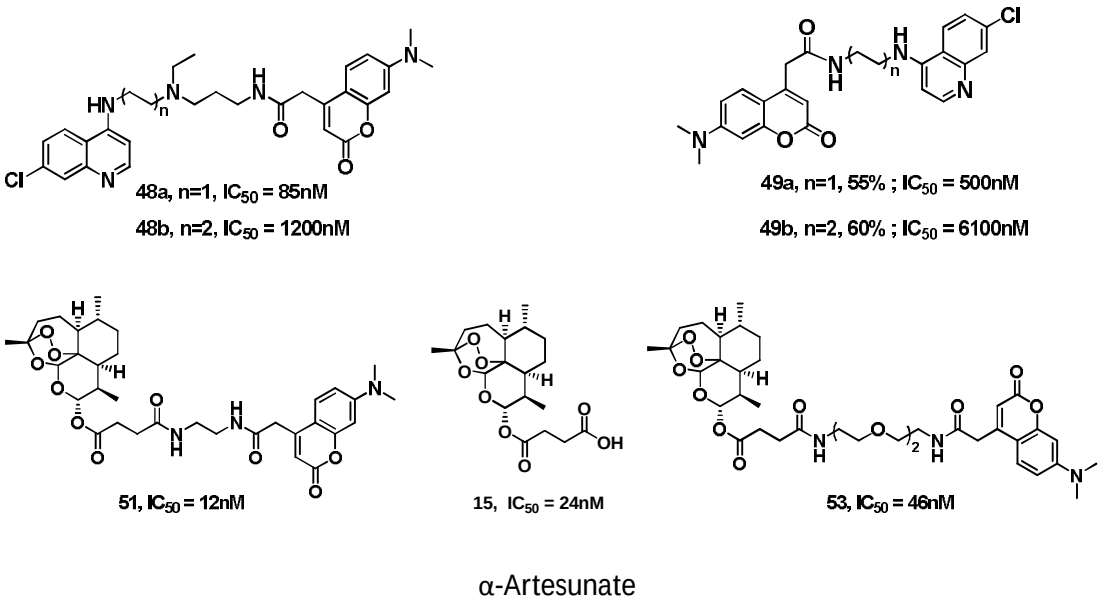
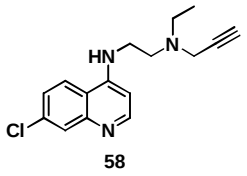


Fig.9 IC_{50} values of proposed probes.

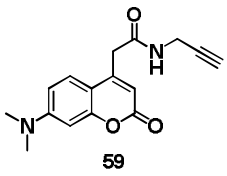
Thus, as per the above proposal, chloroquine probe designs 1 and 3 are best suited for diagnostic applications. Artesunate probe design 1 showed good activity within both *P.falciparum* and cancer cell lines (discussed in NCI studies section 3.6). However it was observed by LCMS (as observed during thermal stability studies section 3.3) that there was a distinct possibility of cleavage of the fluorescent tag within the parasite. This has also been confirmed by reports that artesunate has a relatively short half life (~10min) and that the dihydroartemisinin fragment is the actual active component (half life ~ 1hr). Thus I further proposed two modifications to the artesunate based structure : one by replacing succinate fragment in artesunate with a para benzoxy carboxylic acid fragment also known as β- Artelinic acid **56** and the other by replacing the oxygen at 10 position with carbon also known as Deoxocarbaartemisinin carboxylic acid **65**. These intermediates have been thoroughly studied for their hydrolytic stability (β- Artelinic acid **56** has half life of 13hrs in acidic pH; Deoxocarbaartemisinin carboxylic acid **65** has a half life of 300hrs in acidic pH) stability[44,45]. However the biological activity against the sensitive 3D7 strain of

parasite for the above molecules has not been studied. The design of the final probes (57, 83, 86, 92.93) is based on the above discussed parent molecules.



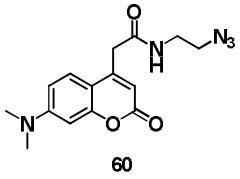
58

Alkyne - Chloroquine



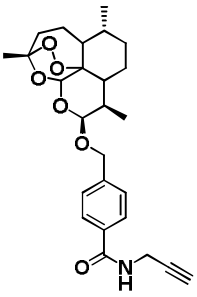
59

Alkyne – Coumarin



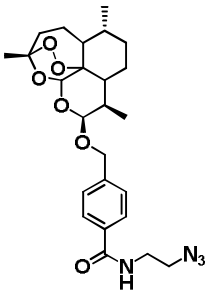
60

Azide – Coumarin



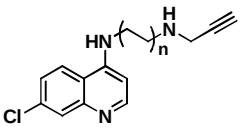
61

Alkyne - β-Artelinic acid



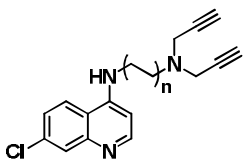
62

Azide - β-Artelinic acid



63a, n=1

63b, n=2



64a, n=1

64b, n=2

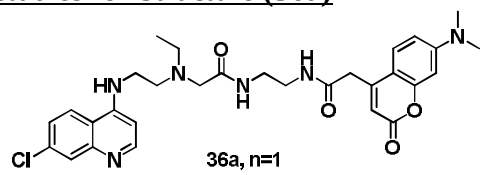
Fig. 10 – Probe Design for Click Chemistry

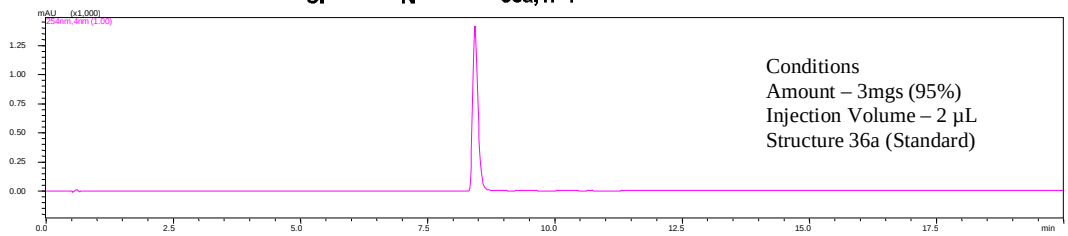
Click chemistry enabled probes have also been synthesized for bio-imaging, binding studies and enzyme fishing for in-vitro applications. Examples of click enabled molecules are shown in Fig. 10. Later sections in this thesis show that short term thermal stability (4days) of the probes presented in Fig. 10 have shown that they are resilient for applications in many biological protocols. Thus, the above drug design covers a considerable range of molecules to assist in diagnostic, bio-imaging and pathway elucidation studies for understanding diseases within *P.falciparum,* cancer cell lines and other cell lines.

3.3 Thermal Stability Protocol for Drug Probes –

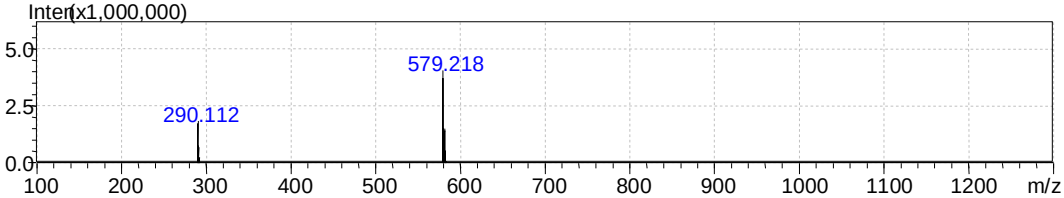
Thermal stability study was designed based on the ICH guidelines for pharmaceutical drug analysis[46]. The above studies clearly indicate thermal stability for **36a, 36b** and **48a** probe structures. (The shift in retention time can be attributed to the pressure imbalance in the column but the overall mass values for the peaks are consistent across all tests). However the artesunate based probes are not as stable as the chloroquine based probes as observed in both thermal and hydrolytic studies. Nevertheless the thermal stability tests established that the probes (**36a, 36b** and **48a)** are stable under normal packaging conditions and are suitable for field requirements. The integration area under the curve represents 95-98% of the probe concentrations and is consistent as compared with the standard. Data for 4 days thermal stability studies on the artelinic acid **(56)**, artelinic acid based probe (**57**) and click chemistry probes (**58–64**) are enclosed in Appendix 1. Although the above probes have shown excellent thermal stability for 4 days, long term stability data (6months, 1year) needs to be established for understanding packaging requirements for the same.

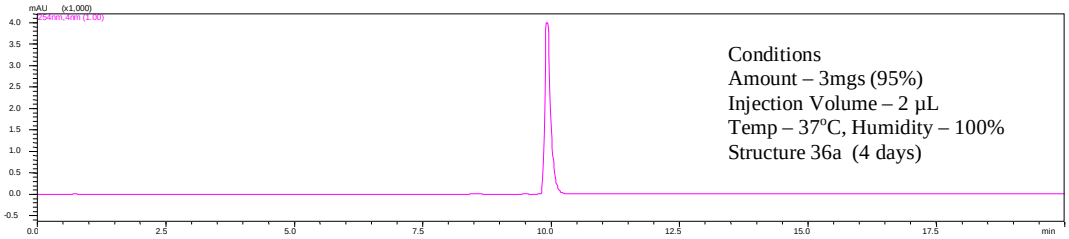
3.3.1 Thermal Stability studies for Structure **(36a)** –



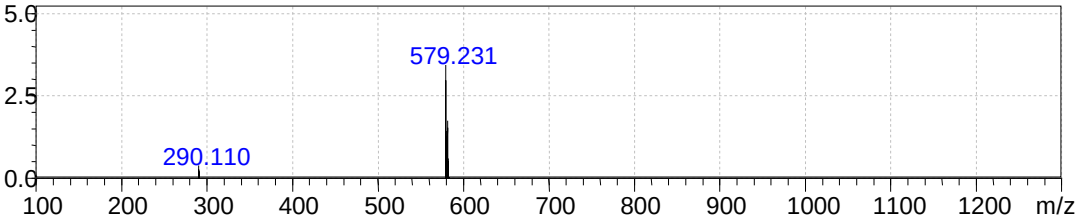


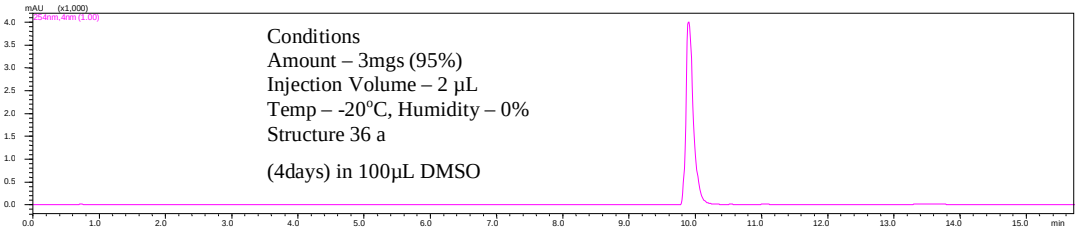
MS (E+) Ret. Time: 8.460 -> 8.473 - 8.333 <-> 8.753



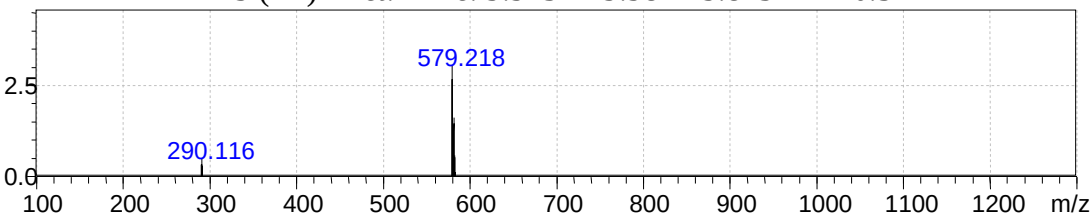


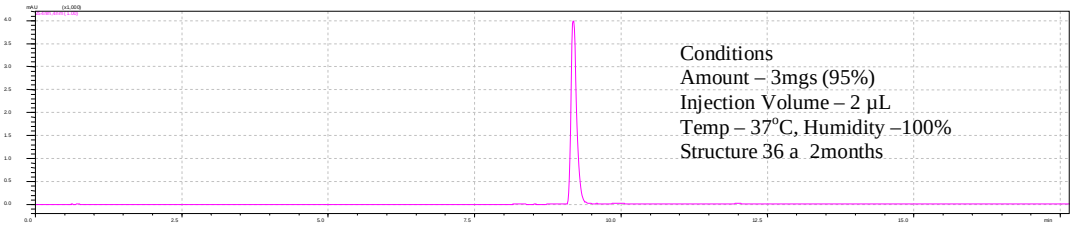
MS (E+) Ret. Time: 10.000 -> 10.013 - 9.827 <-> 10.720



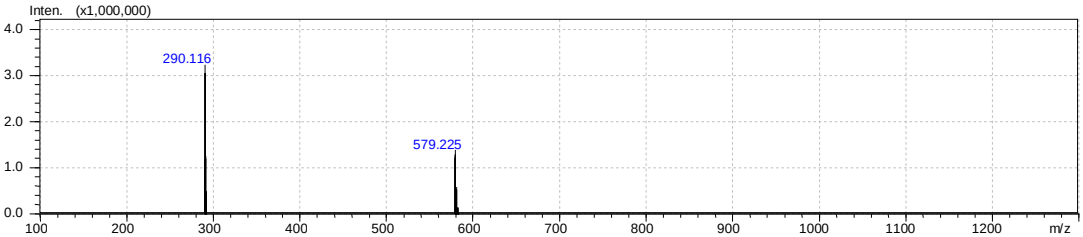


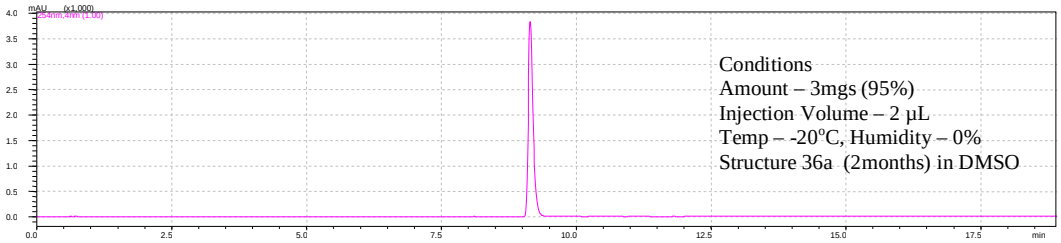
MS (E+) Ret. Time: 9.973 -> 9.987 - 9.813 <-> 10.547



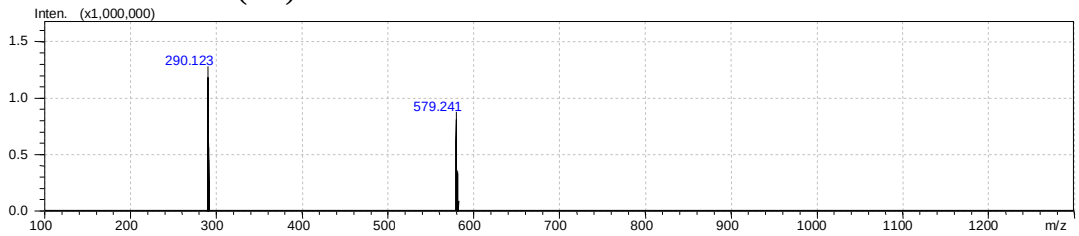


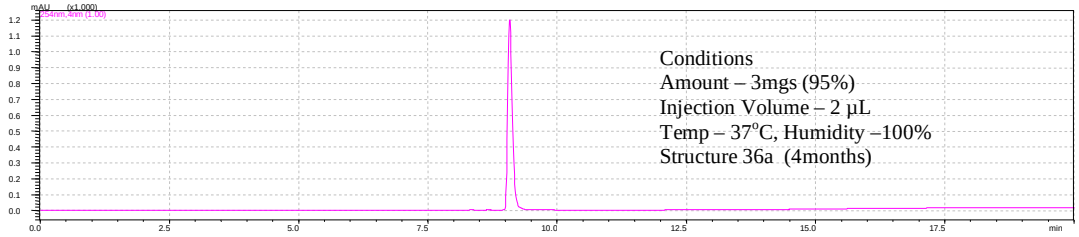
MS(E+) Ret. Time : 9.267 -> 9.280 - 9.120 <-> 9.567



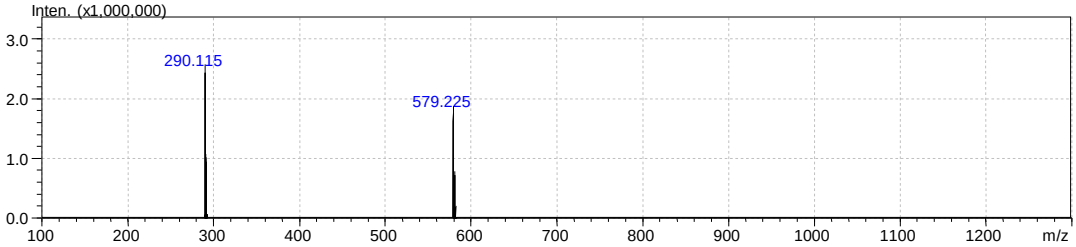


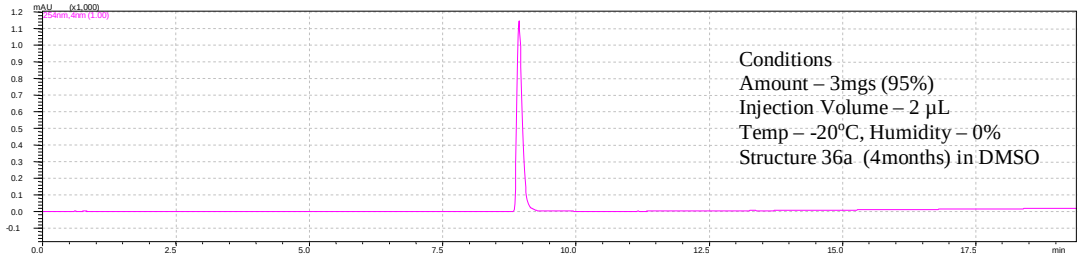
MS(E+) Ret. Time : 9.193 -> 9.207 - 9.087 <-> 9.447





MS(E+) Ret. Time : 9.153 -> 9.167 - 9.020 <-> 9.540





MS(E+) Ret. Time : 8.940

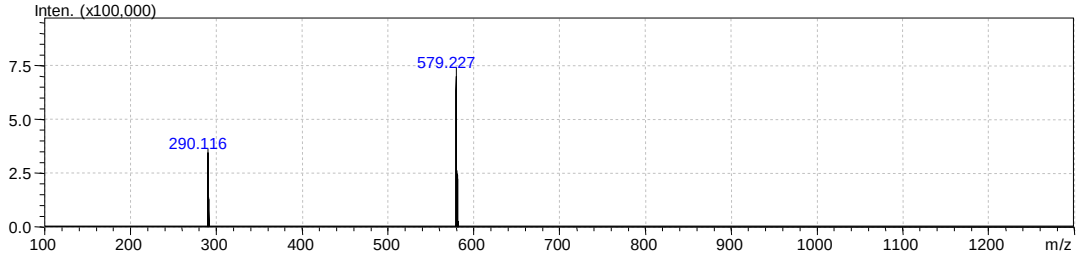
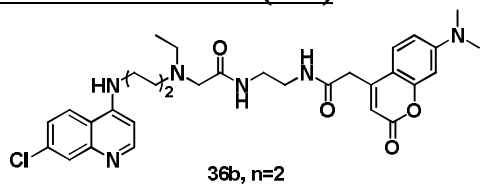
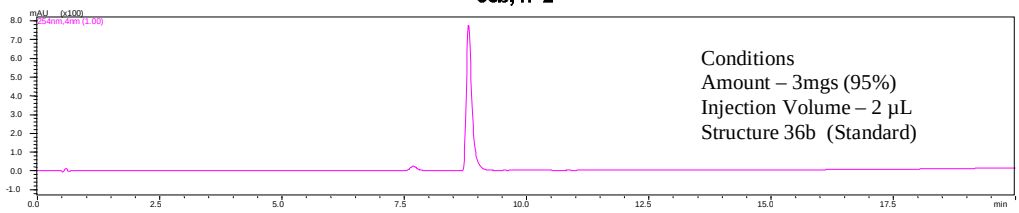


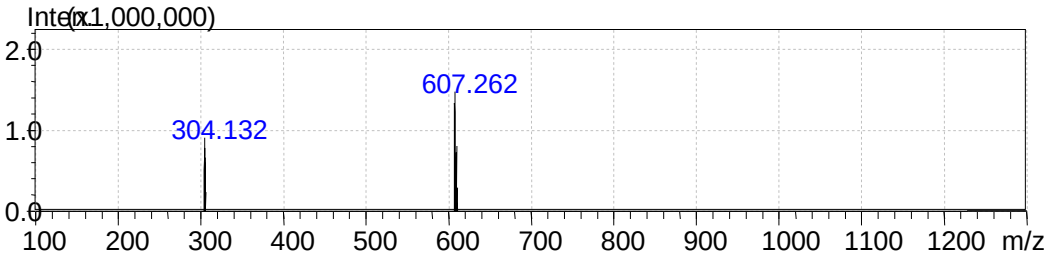
Fig. 11 – LCMS Analysis probe 36a (Eluent – ACN +0.1% TFA: H_2O + 0.1% TFA)

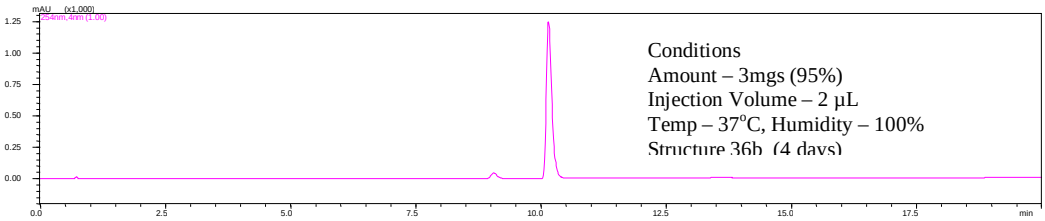
3.3.2 Thermal Stability Studies on Structure **(36b)** –



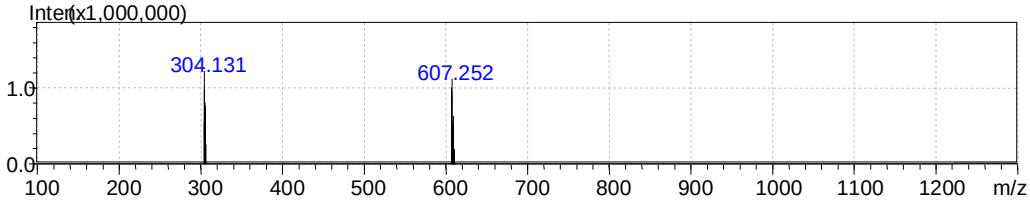


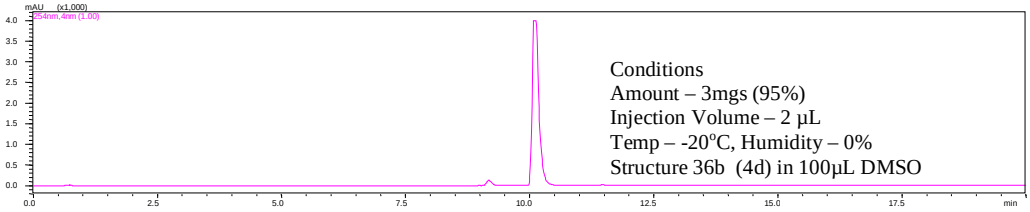
MS (E+) Ret. Time: 8.880 -> 8.893 - 8.727 <-> 9.207



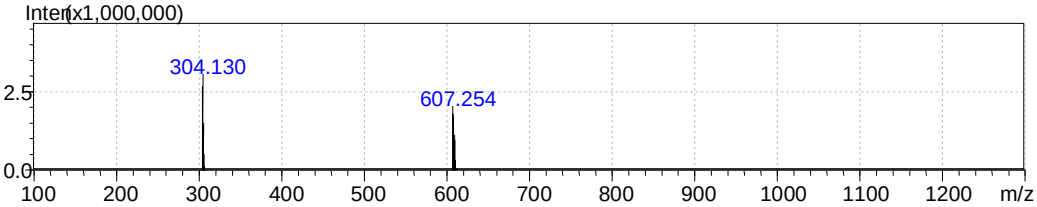


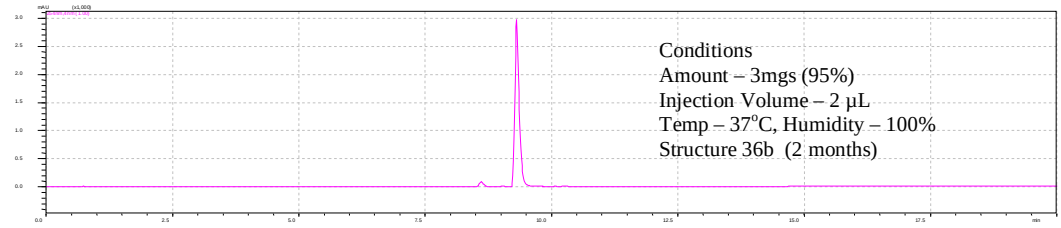
MS (E+) Ret. Time: 10.227 -> 10.240 - 10.060 <->10.540



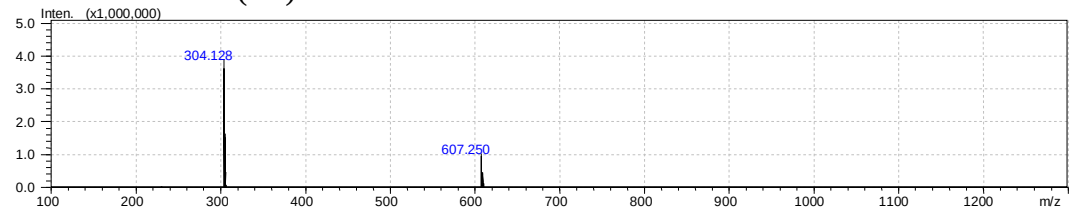


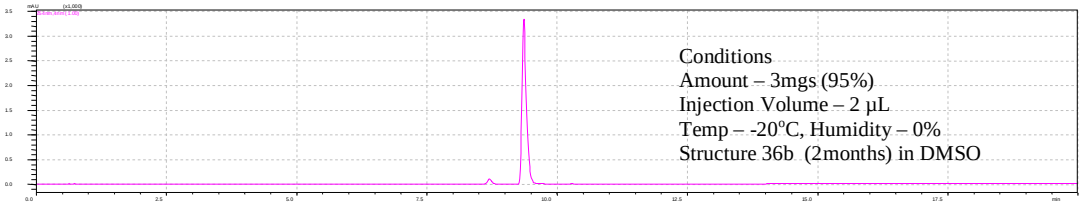
MS (E+) Ret. Time: 10.173 -> 10.187 - 10.000 <-> 10.760



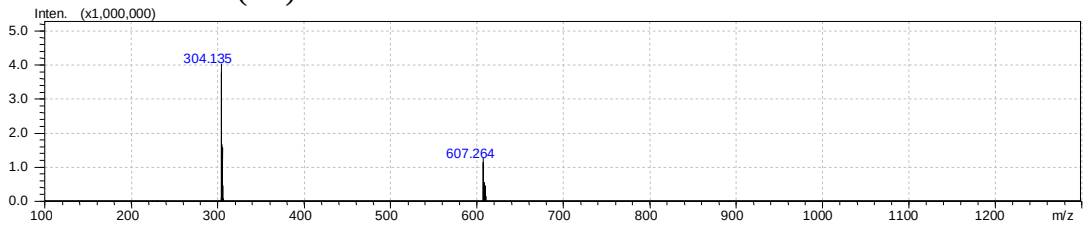


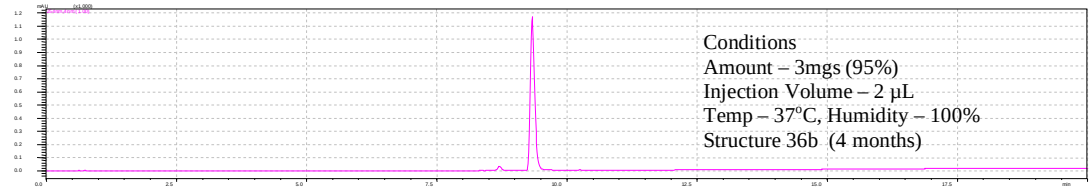
MS(E+) Ret. Time : 9.380 -> 9.393 - 9.260 <-> 9.653



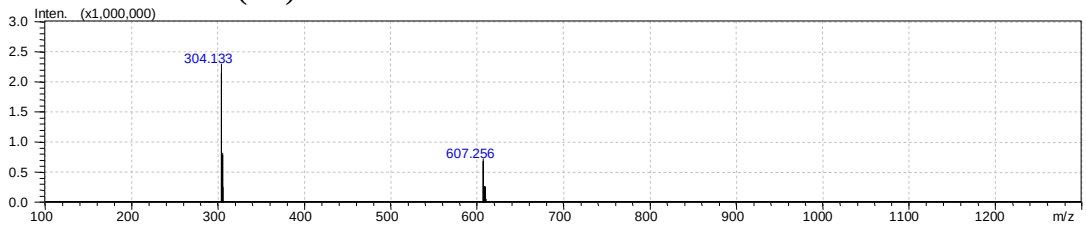


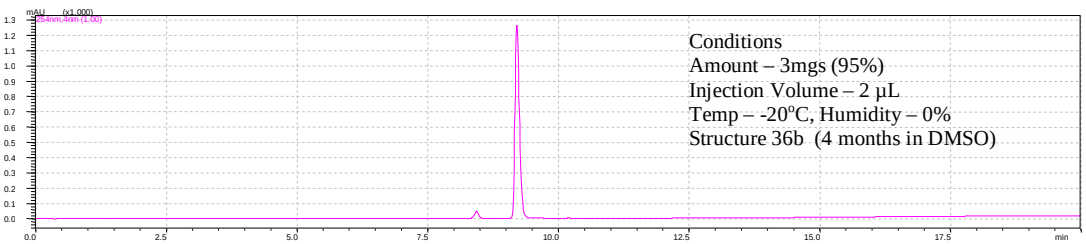
MS(E+) Ret. Time : 9.433 -> 9.447 - 9.293 <-> 9.880





MS(E+) Ret. Time : 9.380 -> 9.393 - 9.287 <-> 9.660





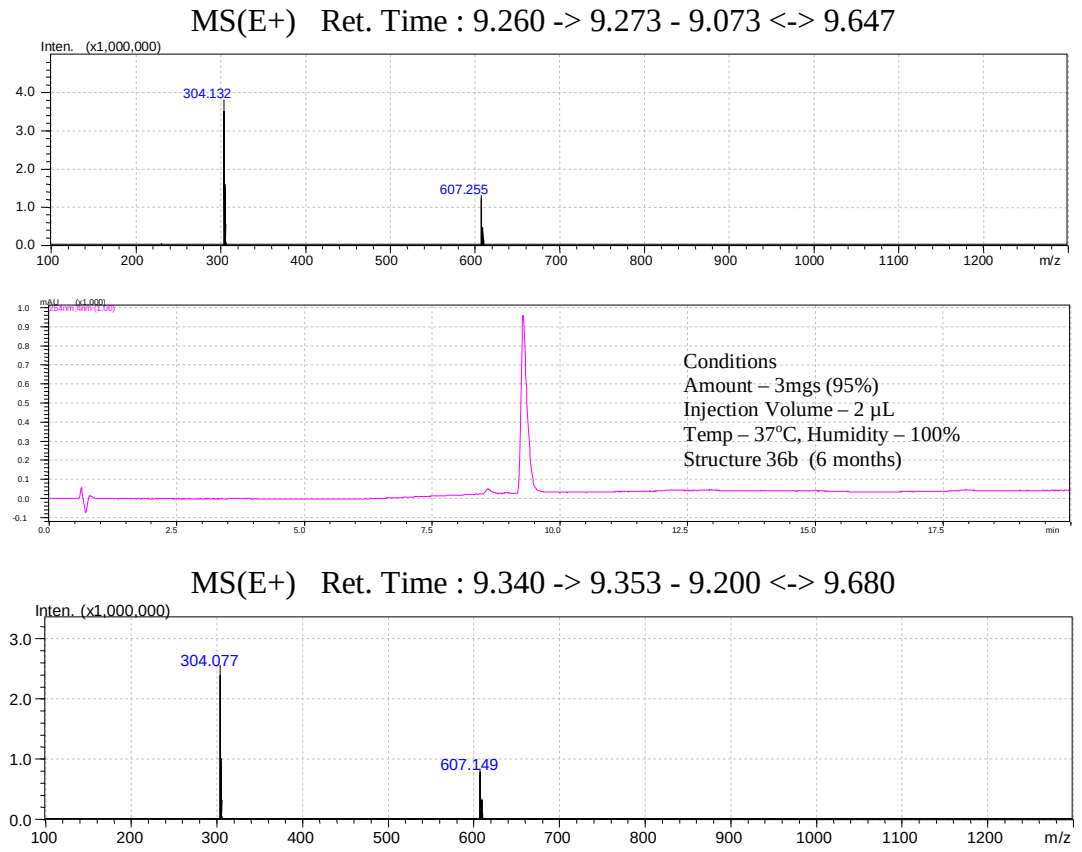


Fig. 12 – LCMS Analysis probe 36b (Eluent – ACN +0.1% TFA: H_2O + 0.1% TFA)

3.3.3 Thermal Stability Studies on Structure **48a –**

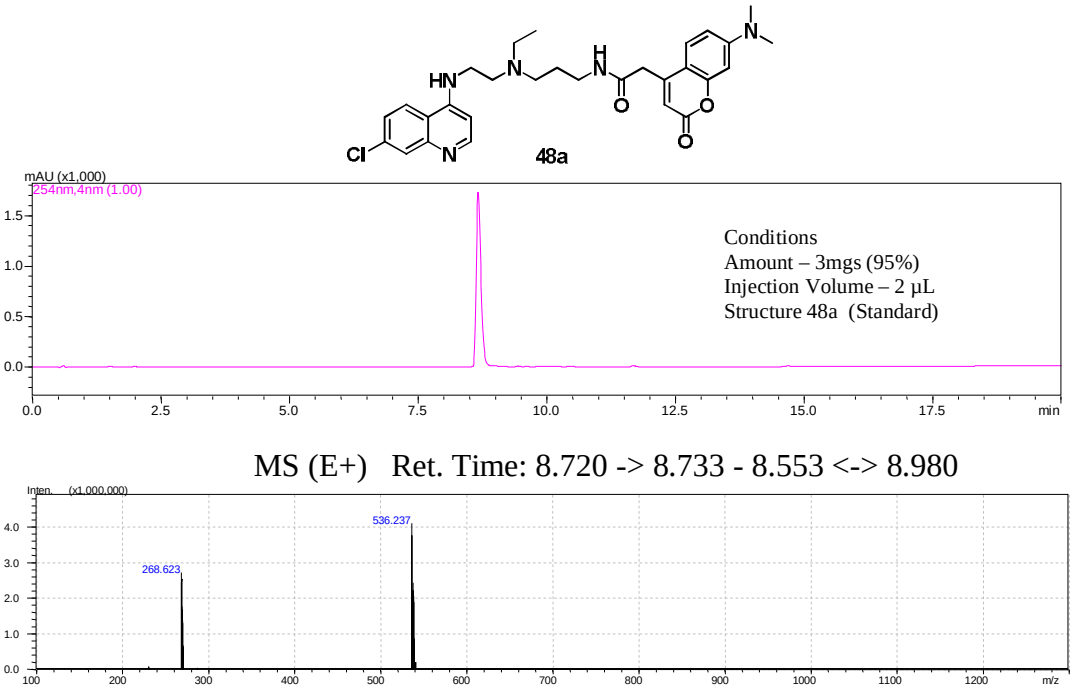
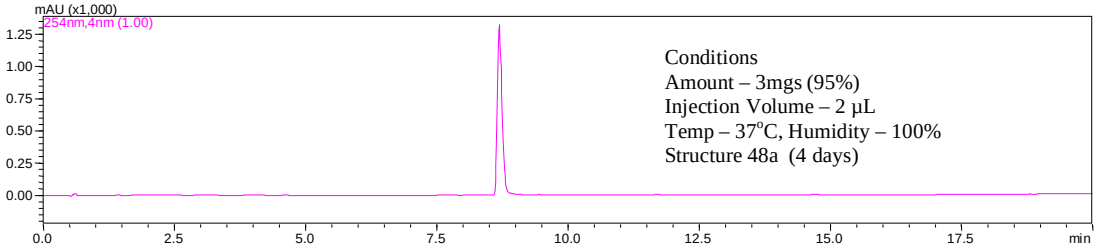
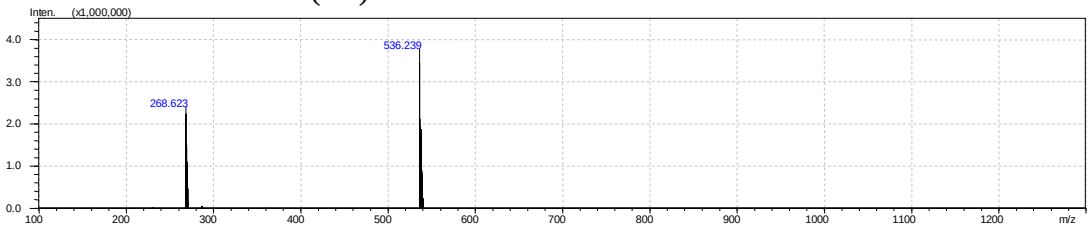
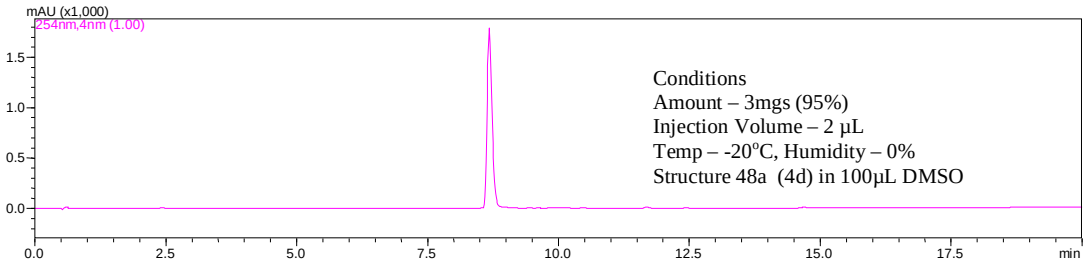


Fig. 13 – LCMS Analysis probe **48a** (Eluent – ACN +0.1% TFA: H_2O + 0.1% TFA)

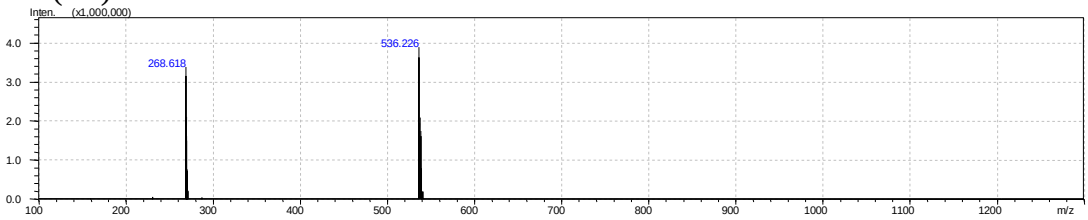


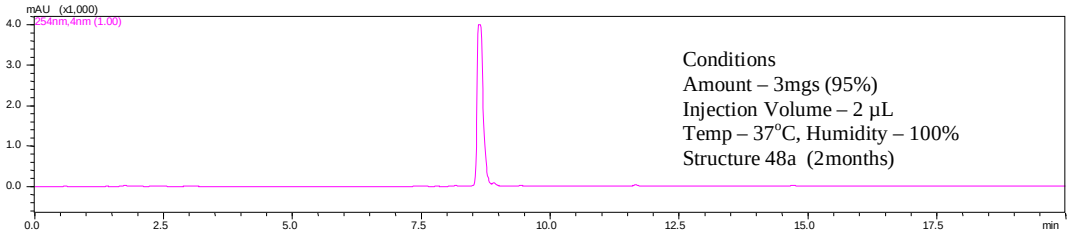
MS (E+) Ret. Time: 8.747 -> 8.760 - 8.587 <-> 9.240





MS(E+) Ret. Time : 8.720 -> 8.733 - 8.593 <-> 9.033





MS(E+) Ret. Time : 8.687 -> 8.700 - 8.487 <-> 9.280

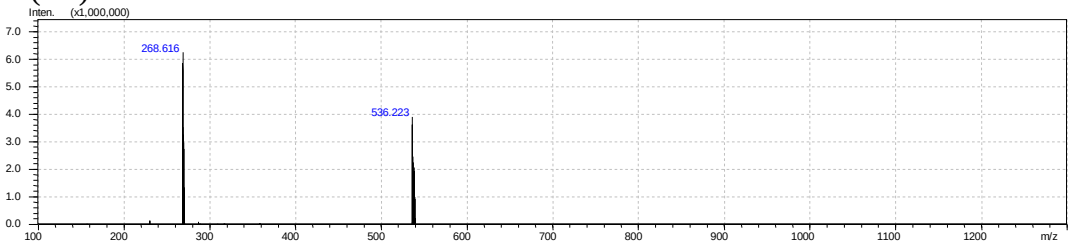
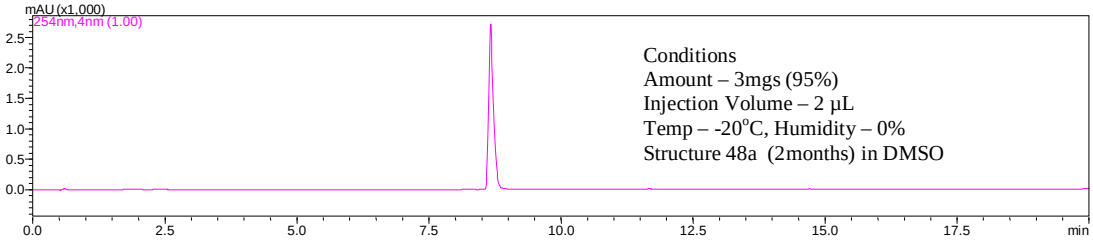
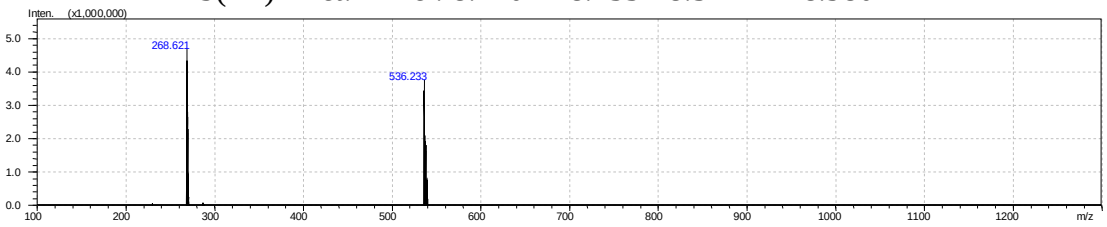
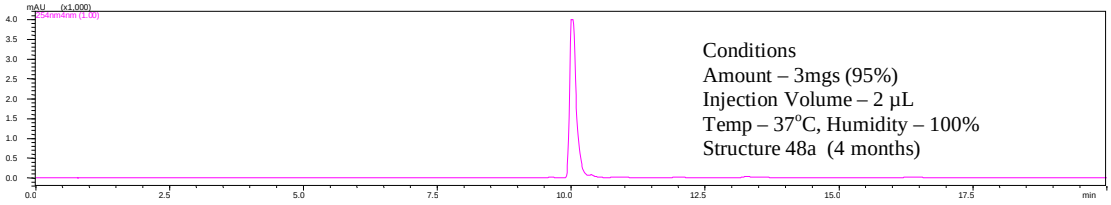


Fig. 13 – LCMS Analysis probe **48a** (Eluent – ACN +0.1% TFA: H_2O + 0.1% TFA)

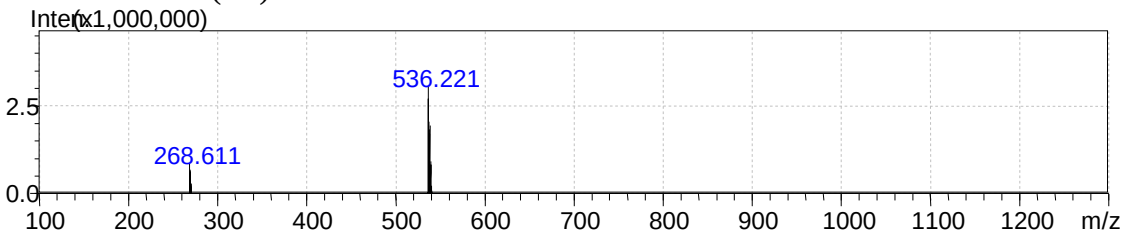


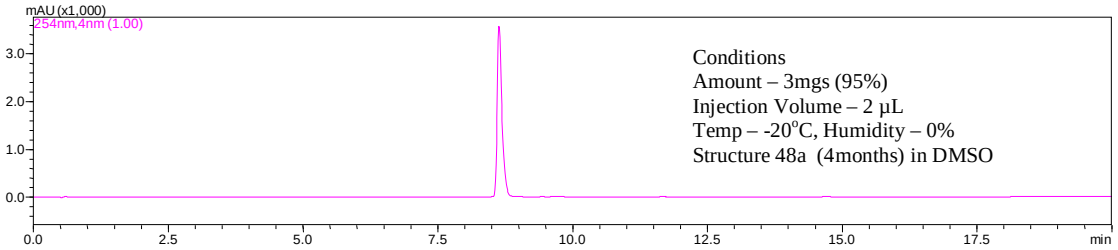
MS(E+) Ret. Time : 8.720 -> 8.733 - 8.547 <-> 8.980



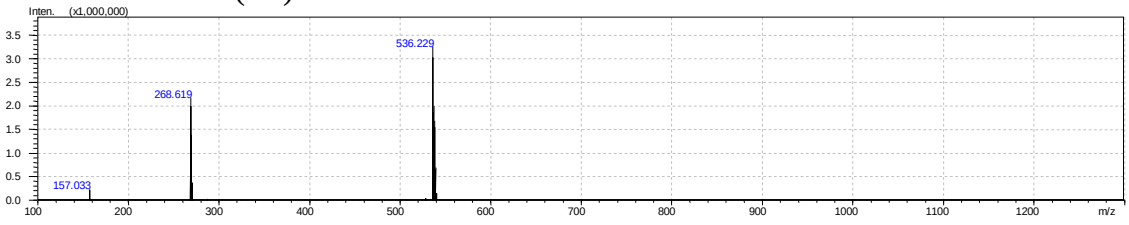


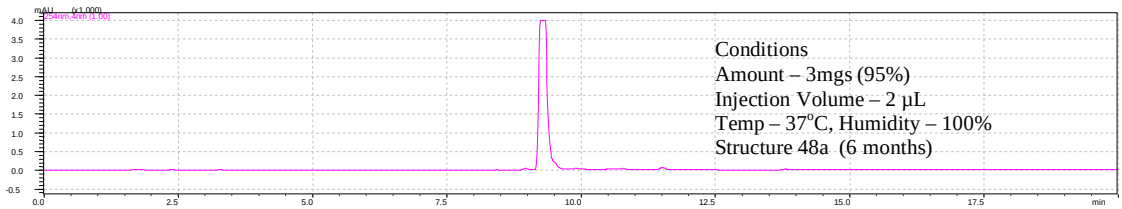
MS(E+) Ret. Time : 10.107 -> 10.120 - 9.933 <-> 10.987





MS(E+) Ret. Time : 8.693 -> 8.707 - 8.553 <-> 9.340





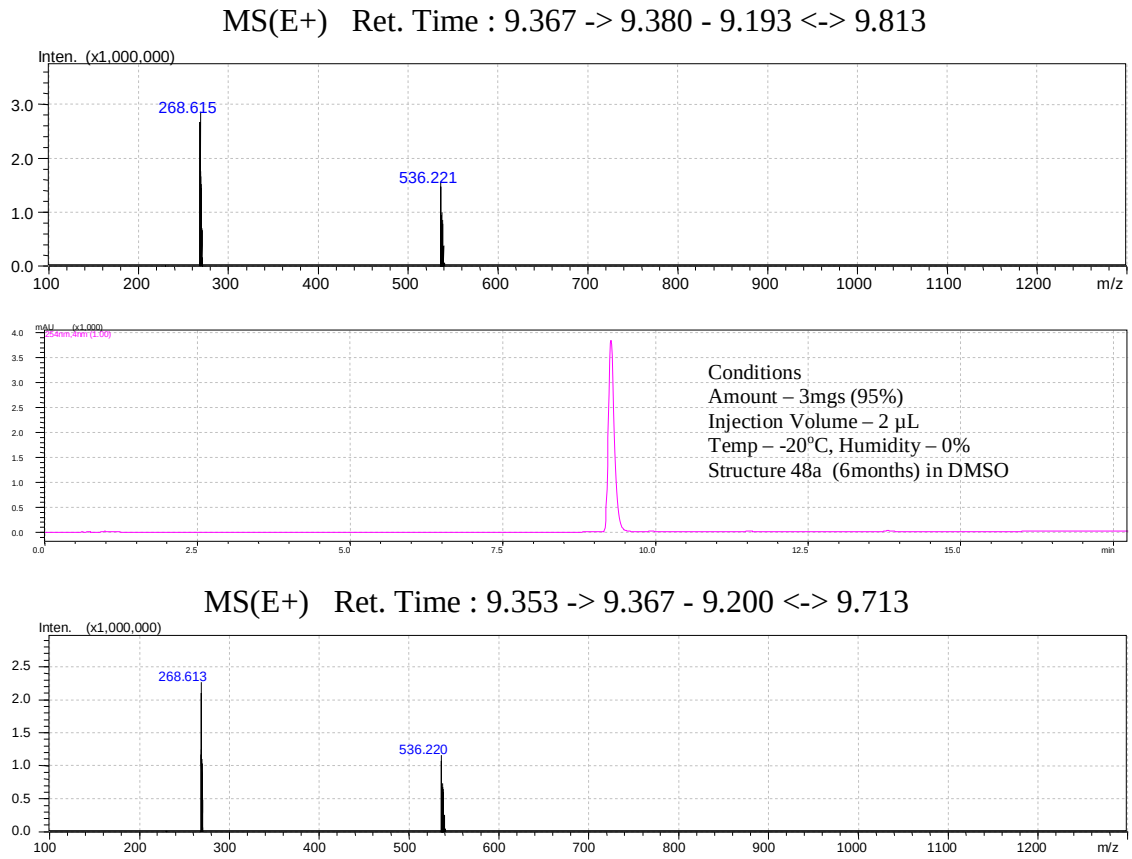
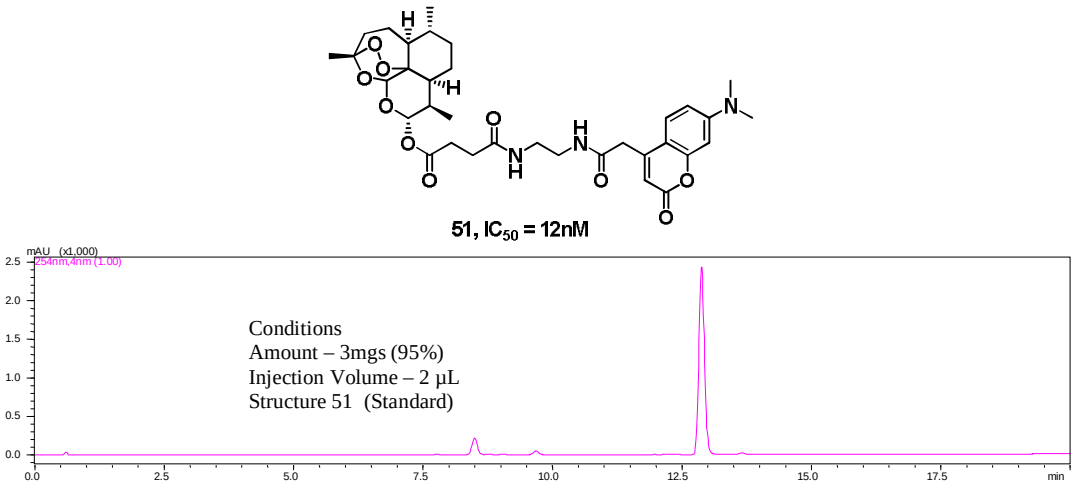


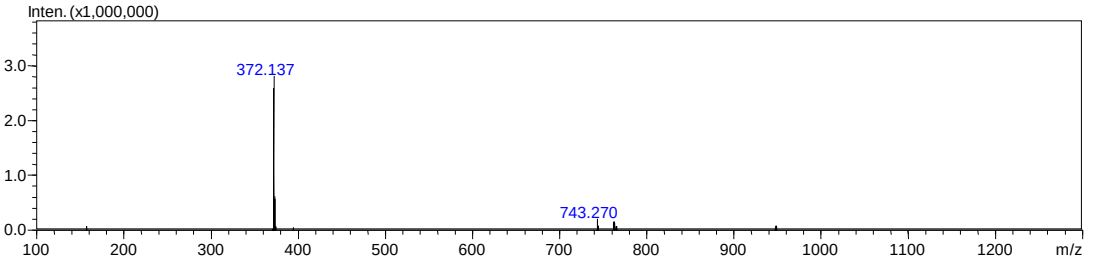
Fig. 13 – LCMS Analysis probe **48a** (Eluent – ACN +0.1% TFA: H_2O + 0.1% TFA)

3.3.4 Thermal Stability Studies on Structure **51** –

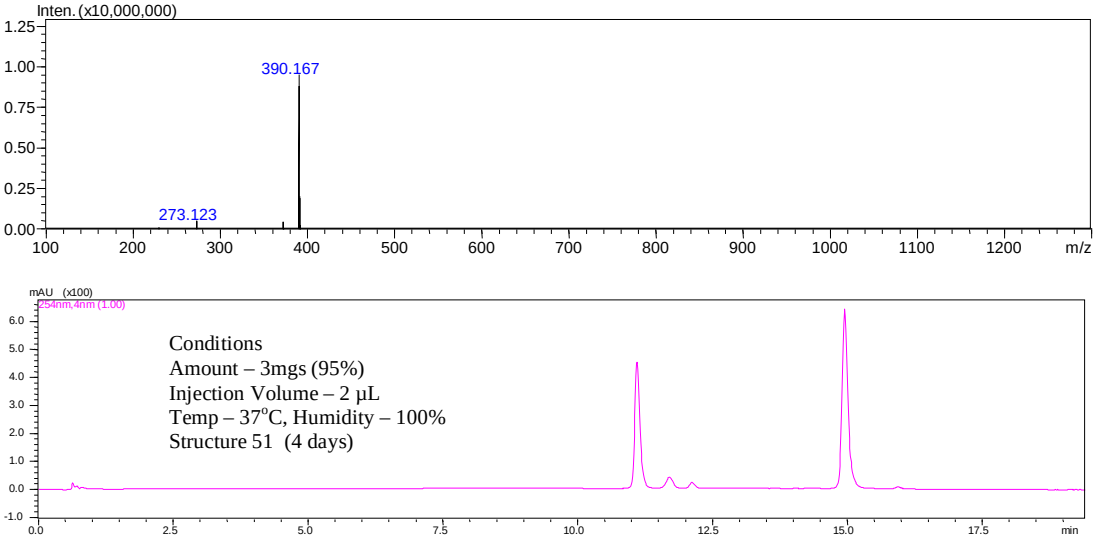
As shown by thermal studies on artesunate probe **51**, the probe is unstable after 4 days of heat treatment as per standard conditions. This was expected due to the acid labile nature of the ester bond between artesunate and coumarin linker precursor **(50).**



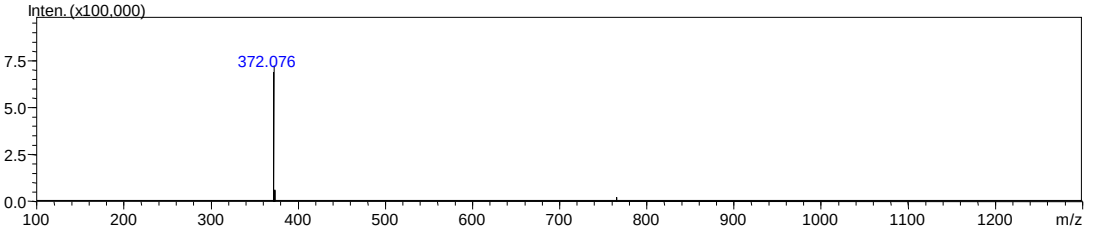
MS(E+) Ret. Time : 8.533 -> 8.547 - 8.413 <-> 8.773



MS(E+) Ret. Time : 12.860 -> 12.873 - 12.740 <-> 12.887.



MS(E+) Ret. Time : 11.107 -> 11.120 - 10.967 <-> 11.193



MS(E+) Ret. Time : 14.953 -> 14.967 - 14.807 <-> 15.000

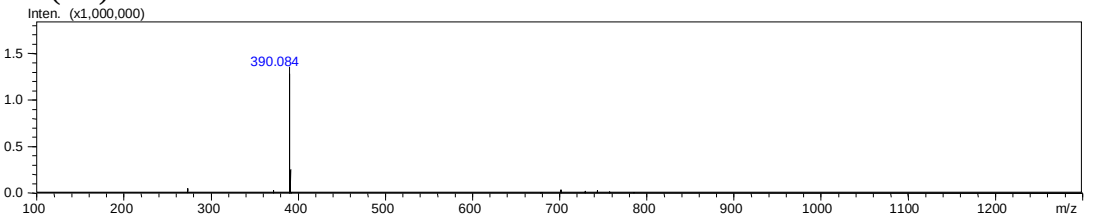


Fig. 14 – LCMS Analysis probe **51** (Eluent – ACN: H_2O)

Thus I have proposed and synthesized artelinic acid and β–Deoxocarbaartemisinin carboxylic acid based probes **(57, 83. 86, 98, 100)**, which should have high thermal and hydrolytic stability, compared to the above artesunate based probe 51. Thermal stability data for remaining probes is enclosed in Appendix 1.

3.4 Plasmodium studies –

The probe **36b** was analyzed for its ability to differentiate between chloroquine sensitive versus chloroquine drug resistant strains as shown in Fig.15.

The chloroquine resistant strains do not uptake chloroquine probe **36b** as compared to chloroquine sensitive ones. This selective efflux is due to mutations in PfCRT (*Plasmodium falciparum* chloroquine resistant transporter) in the drug resistant strains[47]. This establishes a crtical component for diagnosis of drug sensitive over resistant strains.

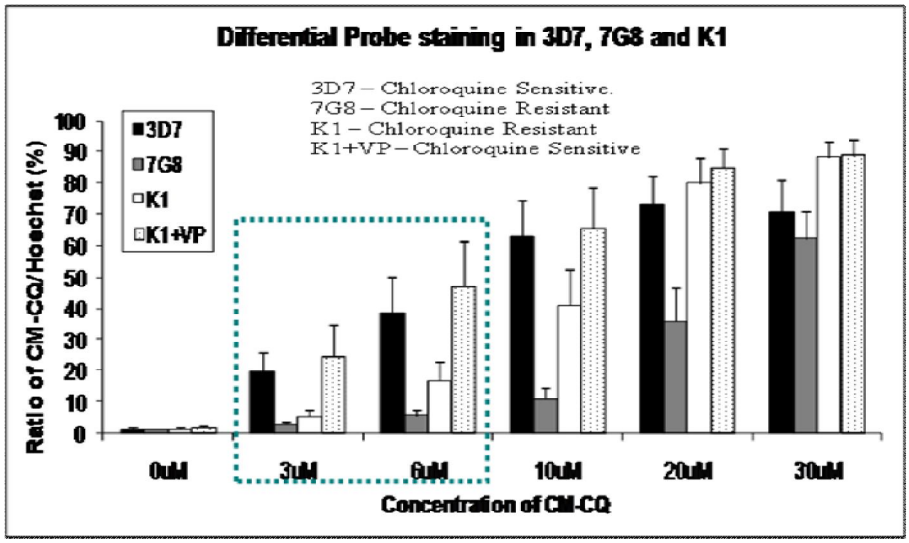


Fig. 15 – Differential staining[47] by structure **36b** in 3D7 (chloroquine sensitive), 7G8, K1 and K1+VP strains of *Plasmodium falciparum* Fig.15 clearly shows that

the anti-malarial fluorescent probe can selectively identify chloroquine sensitive strains of *P.falciparum* by higher % of staining vs Hoechst stain with increasing concentrations of the probe. The optimal working concentrations for the probe were identified to be 3µM to 6 µM. The above flow cytometry data was supported by confocal imaging study on the probe. Thus the lysosomotropic property of the probe can be effectively utilized for diagnosis. The imaging studies[47] on the chloroquine sensitive 3D7 *P.falciparum* strain using chloroquine probe **36b** are shown in Fig.16

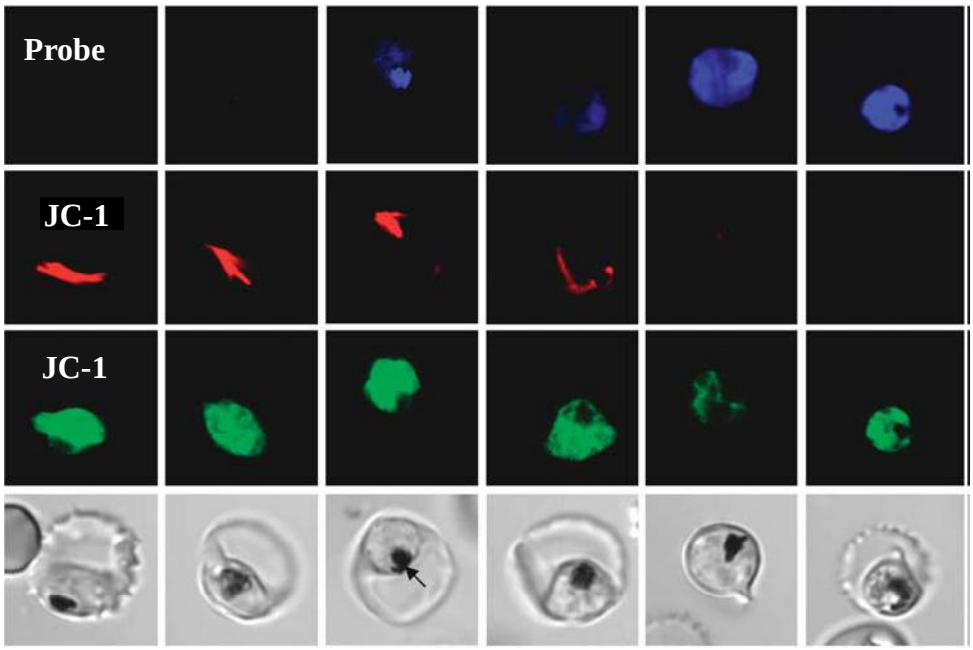


Fig. 16 – Imaging[47] of Chlorquine probe **36b** at different concentrations

The confocal imaging studies show that the chloroquine probe **36b** accumulates in the food vacuole of the parasite at low concentrations (mainly 300nM to 3μM) as compared to JC-1 red and JC-1 green probes, which stain the other cell components of the parasite. The above concentrations can be considered to be the optimum for confocal imaging for the identification of chloroquine sensitive versus chloroquine resistant strains of *P.falciparum*. However, at high concentrations (mainly 30 μM and 300 μM) the probe starts to stain the entire parasite non-selectively. Thus we could effectively analyse parasite strains at a lower concentration of probe. However, coumarin based probes cannot be used for live-cell imaging studies due to the sensitivity of the probe and the cells to blue laser irradiation. Chloroquine –BODIPY based probe **55** was synthesized and used for live-cell bio-imaging study on *P.falciparum* 3D7 strain as shown in Fig. 17. This experiment was performed on the trophozoite stage of *P.falciparum* 3D7 strain. Few concentrations of the Chloroquine

– BODIPY probe **55** were used for varying time periods to study the effects of the concentrations on the digestive vacuole of the parasite. The results show that 2µM is the optimal concentration of the probe at which the digestive vacuole integrity remains intact despite 4hrs of incubation with the fluorescent probe **55**. Higher concentrations lead to disruption of the food vacuole, in turn leading to parasite death. Thus probe **55** is an efficient substitute for the probes like **36a, 36b, 48a** and further enables live cell imaging.

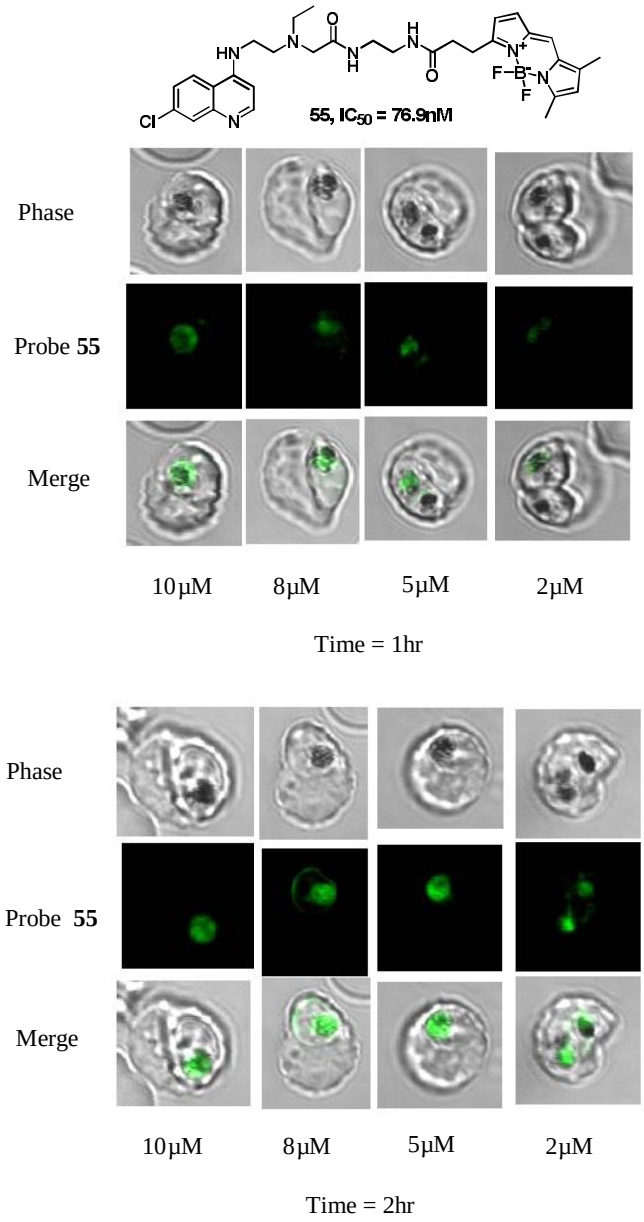


Fig. 17 – Live-cell imaging studies on *P.falciparum* using probe **55**

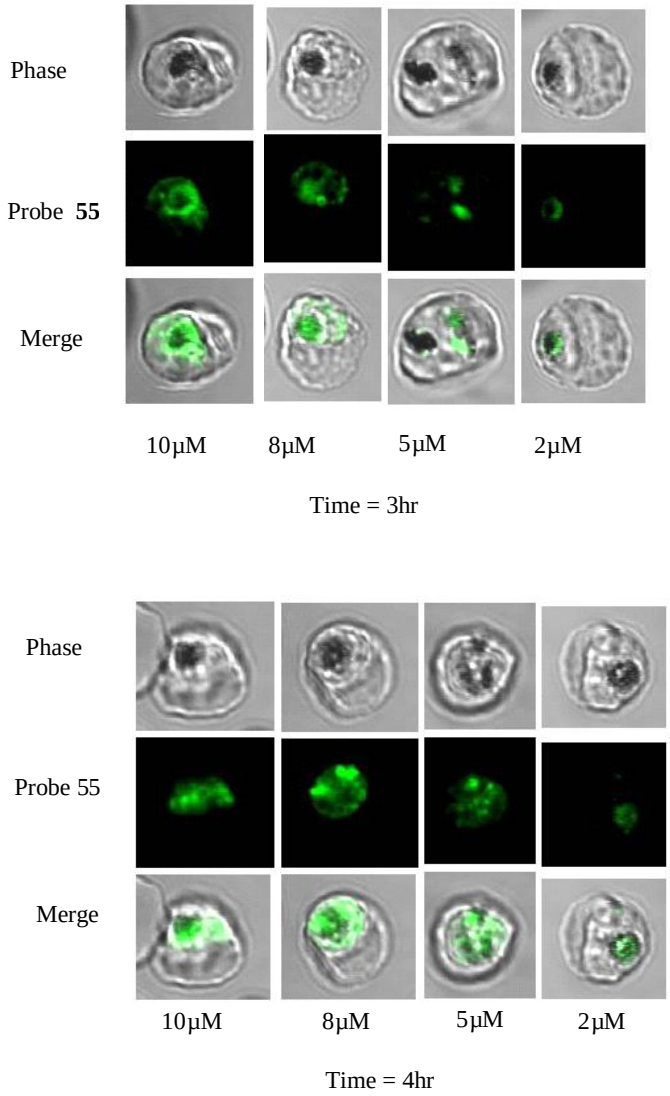


Fig. 17 – Live-cell imaging studies on *P.falciparum* using probe **55**

3.5 Macrophage study –

Chloroquine is also used as an anti-inflammatory agent for the treatment of rheumatoid arthritis[48,49,50]. Its lysosomotropic property decreases the production of the pro-inflammatory cytokines IFN-γ, tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) in LPS- or phyto-hemagglutininstimulated peripheral blood mononuclear cells and the augmented LPS-induced expression of TNF- α, IL-1 α, IL-1β and IL-6 in monocytic and microglial cells. The chloroquine probe **48a** was studied for its lysosomotropic mode of action within macrophages using confocal

microscopy and flow cytometry techniques. It was confirmed that the probe exhibits lysosomotropic property by accumulating in the lysosomal compartments within macrophages in turn exerting their anti-inflammatory or pro-inflammatory effects. However the artesunate based probe **51** does not show specific localization in the lysosomes. Fig. 18, 19 and 20 show a comparision between flow cytometry (shown in four quadrant window) and confocal microscopy (10x magnification) for increasing concentration of probes. The four quadrants represent the distribution of cells extreme left bottom (unstained cells and live), extreme left top (dead cells stained by Propidium iodide (PI)), extreme right bottom (live cells stained with probe), extreme right top (semi-live and dead cells co-staining with propidium iodide and probes).

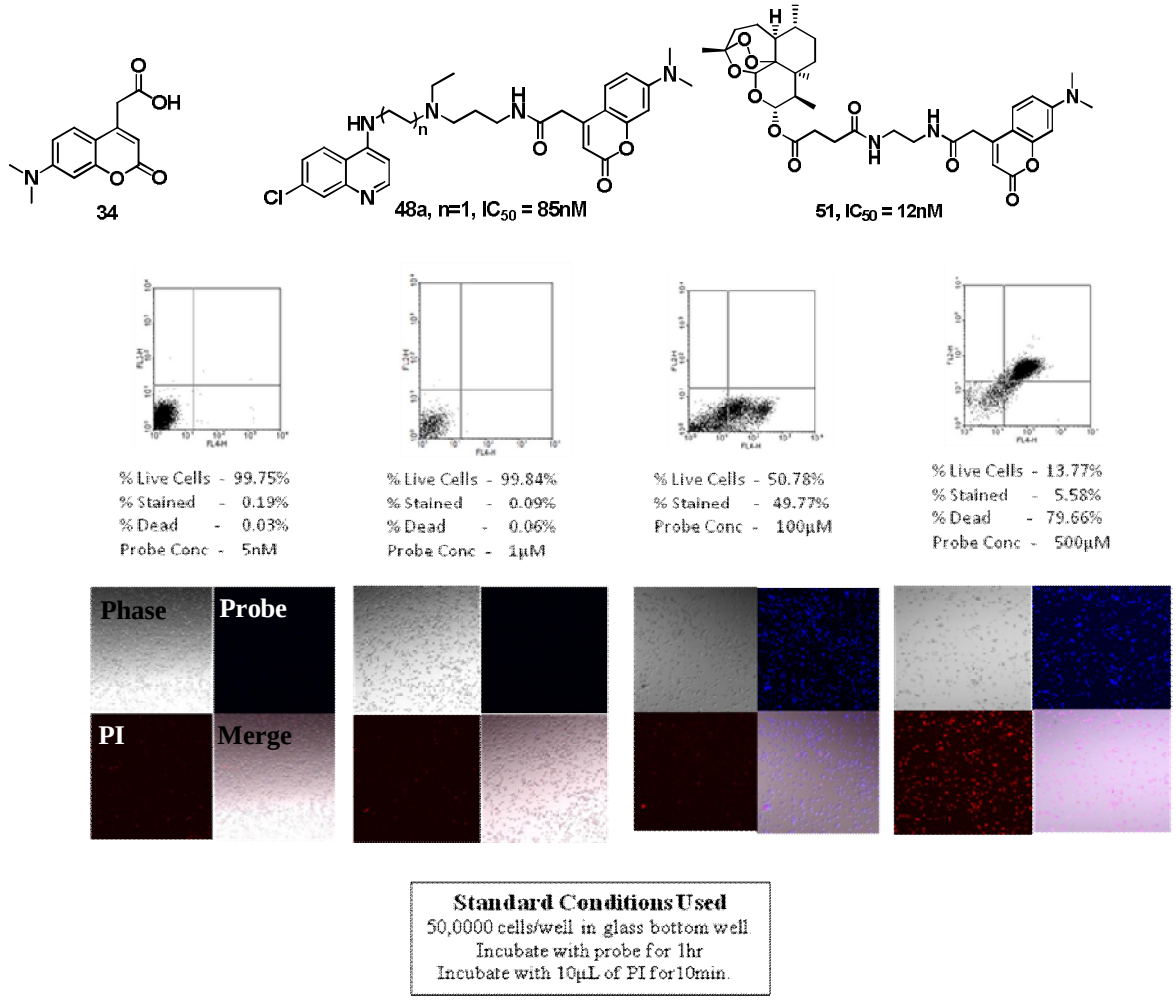


Fig. 18 – Flow cytometry with confocal microscopy data for probe **48a**

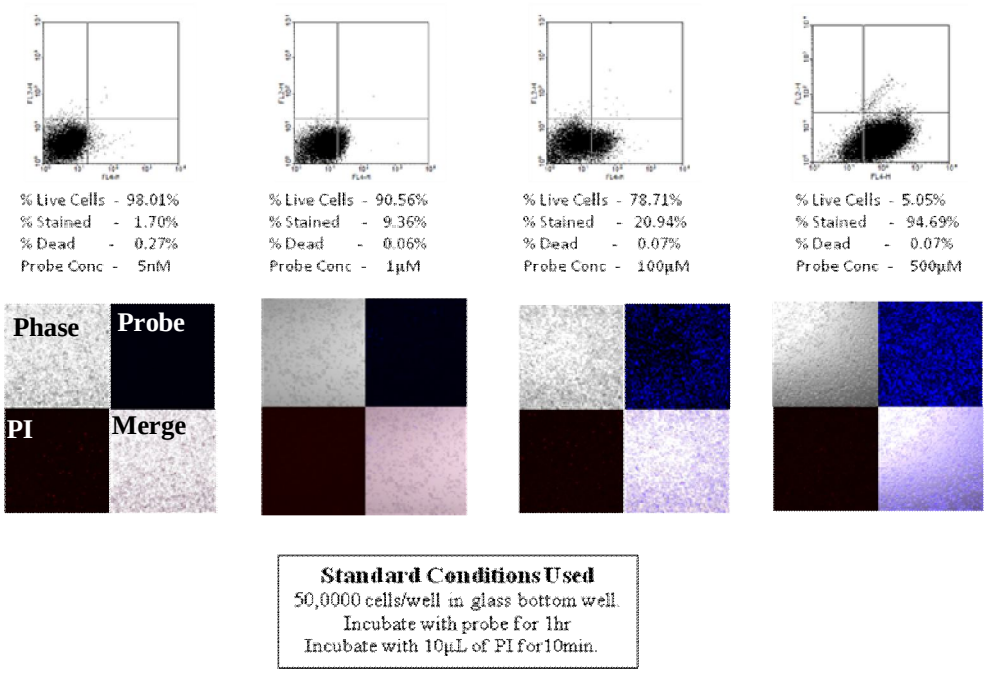


Fig. 19 – Flow cytometry with confocal microscopy data for probe **51**

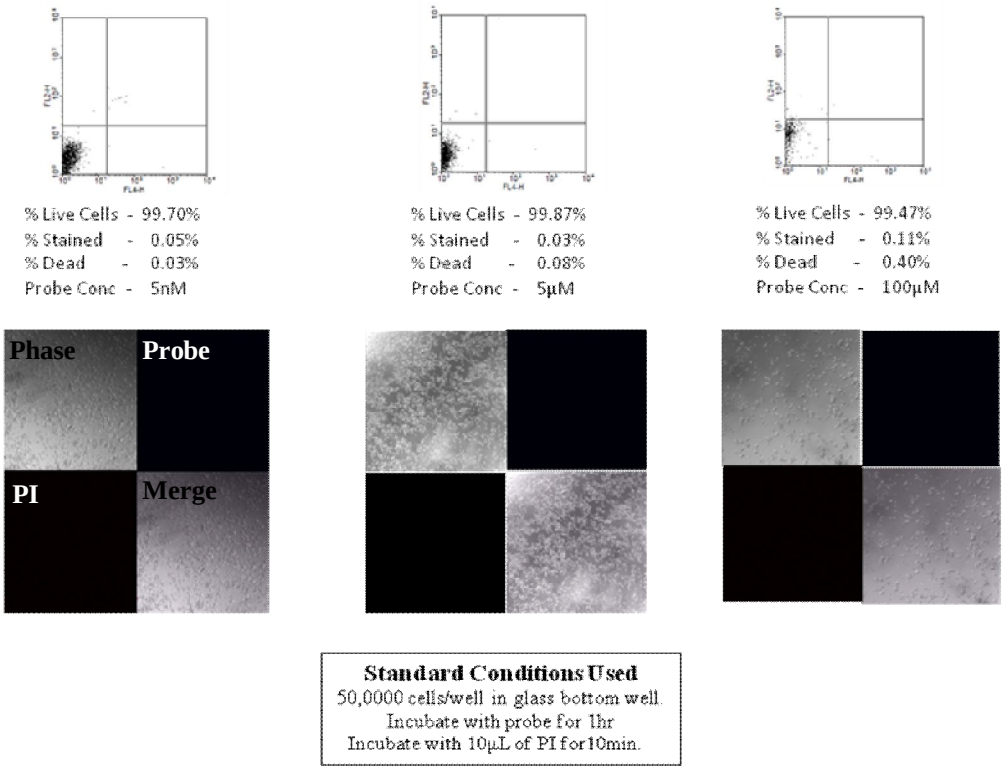


Fig. 20 – Flow cytometry with confocal microscopy data for coumarin **34**

Thus the crude working concentrations for probe **48a** and probe **51** which lies between 100 and 500µM. This concentration range shows staining of a maximum no of cells with minimal cell death. Three concentrations 50, 100 and 250µM were chosen for localization studies on the probes at higher magnification. Since initial data

confirms that coumarin-4-acetic acid **34** on its own does not stain macrophages at 5nM, 5µM and 100µM concentrations, higher concentrations were not considered in the study. The graphical representation of flow cytometry results are shown below. Fig. 21 shows that the chloroquine probe **48a** gives maximum staining of cells with minimal cell death (as measured by propidium iodide (PI) stain) at 100µM. Above 100µM, the effect of DMSO along with the probe leads to higher cell death along with higher staining. Upon comparision with the coumarin fluorescent dye **(34)** alone for staining of macrophages, it was observed that probe 34 does not selectively stain macrophages and at concentrations above 100µM leads to cell death. This confirms that the staining of cells is primarily due to the chloroquine component of probe **48a**. It also concludes that the probe has lysosomotropic properties like the parent drug chloroquine. The staining of cells is also observed at 100µM for artesunate probe **51** but the results of the confocal imaging show interesting differences between probes **48a** and **51**.

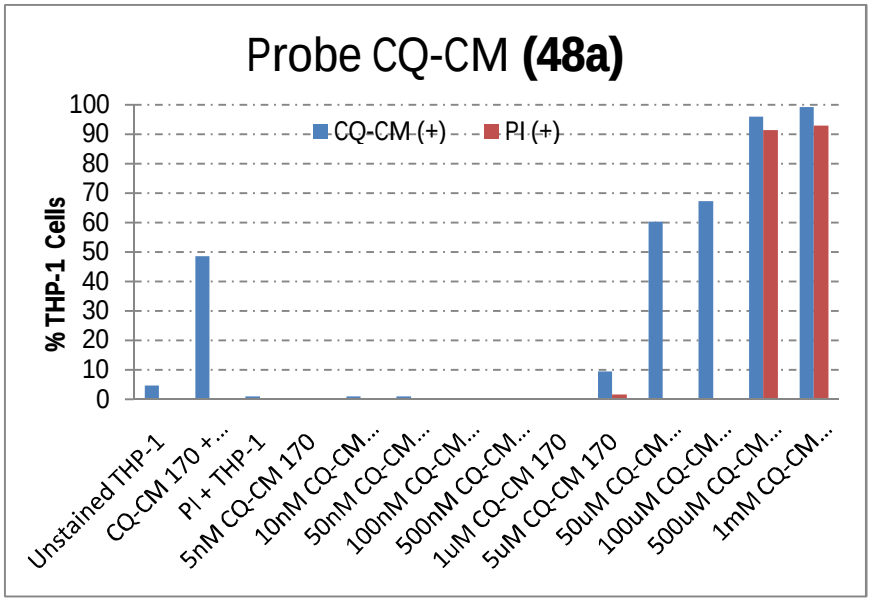


Fig. 21 – Comparision of graphical Flow Cytometry results **48a**

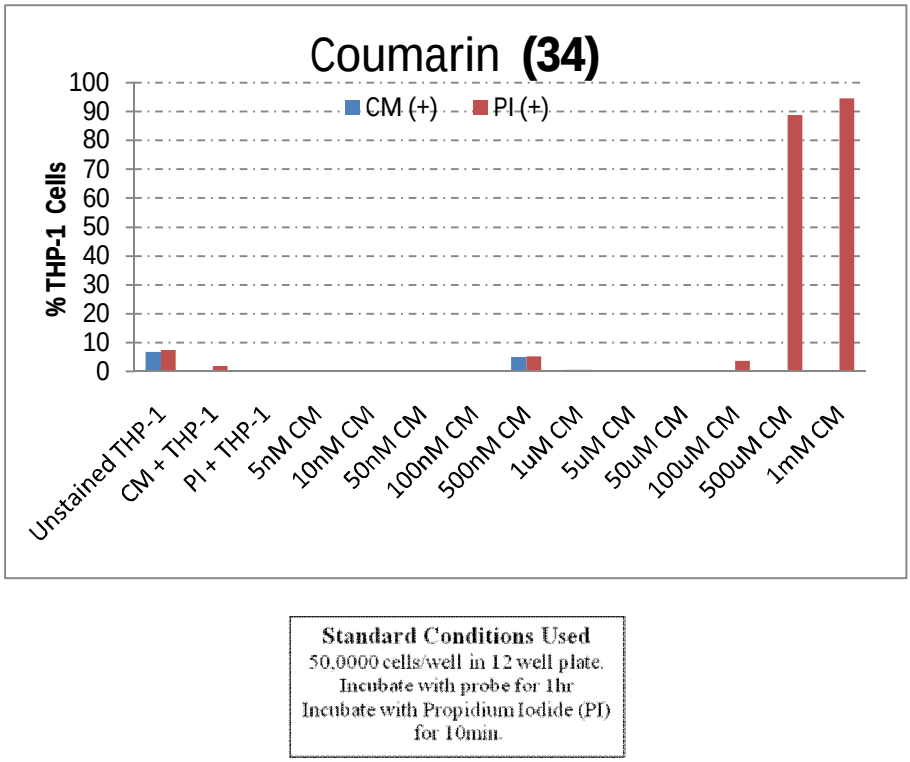
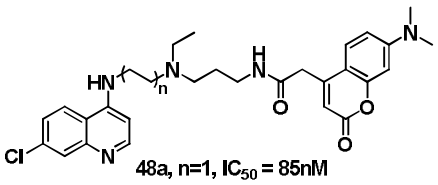


Fig. 22 – Comparision of graphical Flow Cytometry results 34

Upon observing macrophages incubated with the chloroquine probe **48a** under standard conditions and at 60x magnification 2.5 optical zoom (Fig. 23), it was clear that the probe localizes within lysosomal compartment of the macrophages (confirmed against Lyso-Tracker red stain). This further double confirms the lysosomotropic nature of the probe thus confirming its action is similar to the parent molecule chloroquine diphosphate **4**. In comparision, macrophages incubated with probe **51** did not show specific staining of lysosomal compartments of the macrophages, thus confirming the non-specific cell staining nature of artesunate or artesunate based probes. This also conforms that artesunate has multiple pathways of interaction within cells as hypothesized by Meschnick, Posner, Haynes and co-workers[27-30].



48a, n=1, IC_{50} = 85nM

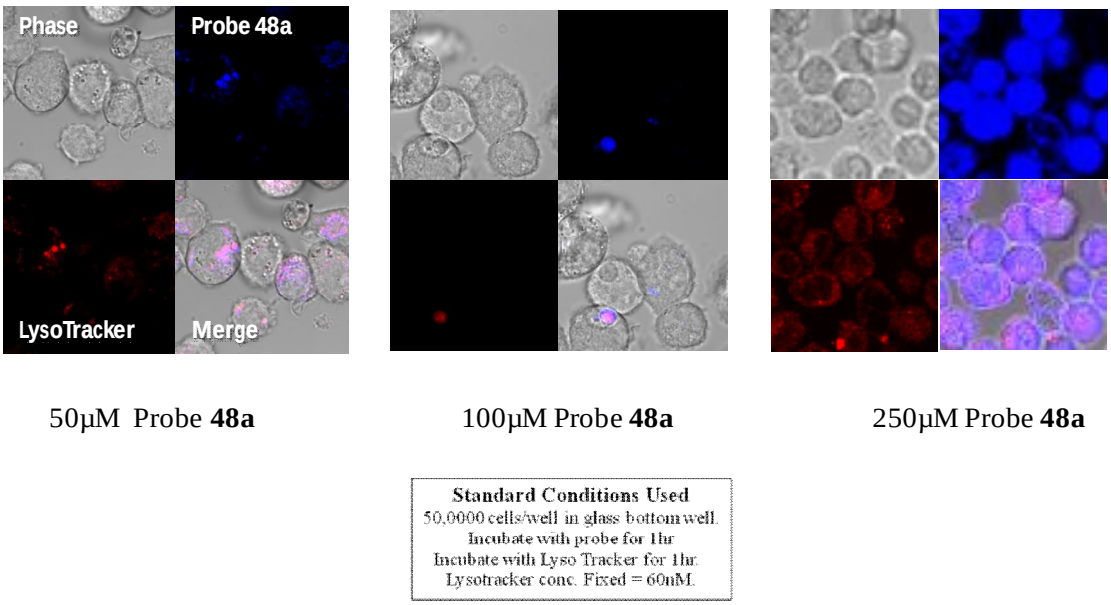


Fig. 23 – Confocal microscopy results of chloroquine probe **48a** vs lyso tracker red.

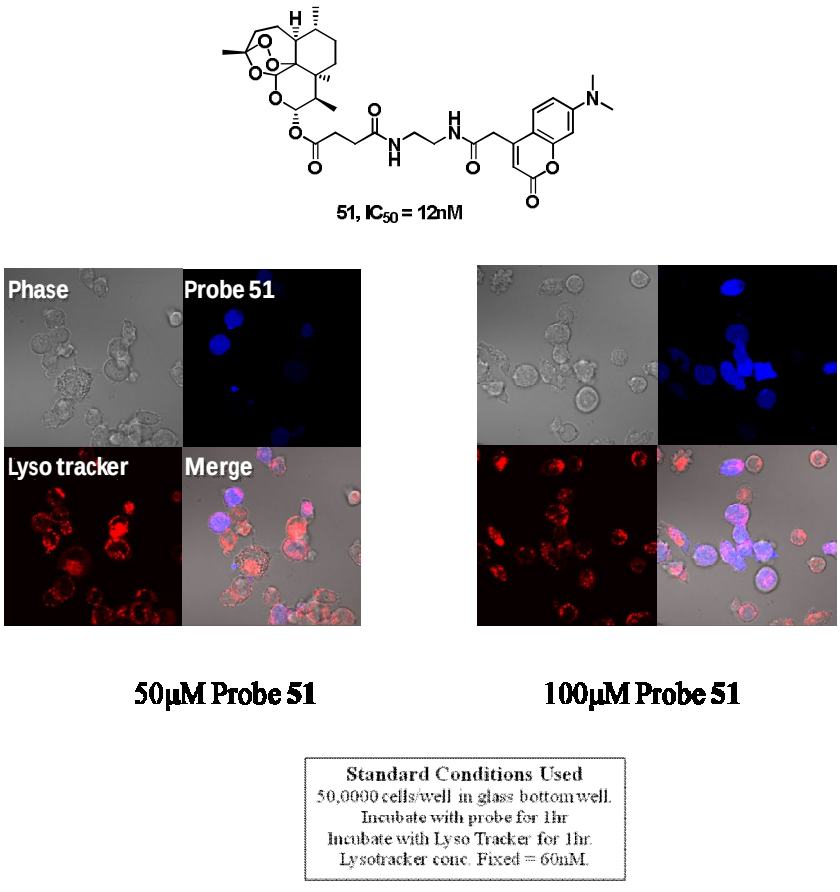
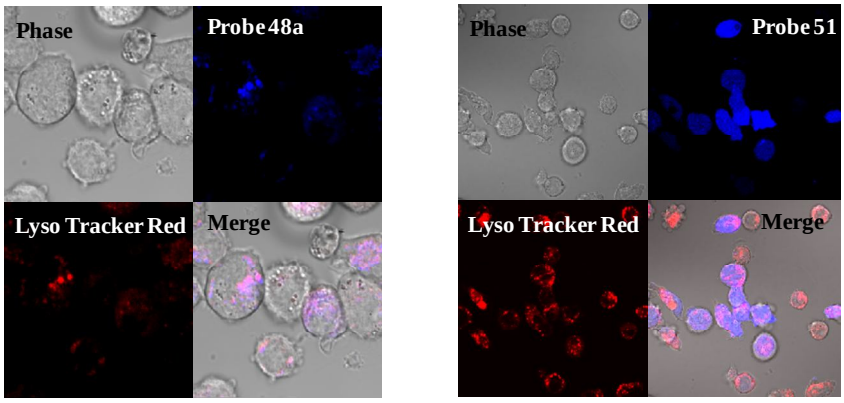


Fig. 24 – Confocal microscopy results of artesunate probe **51** vs lyso tracker red.



Standard Conditions Used
50,00,000 cells/well in glass bottom well
Incubate with probe 23a for 1hr
Incubate with Lysotracker for 1hr.
Lysotracker conc. fixed at 60nM.
Probe 48a Conc - 100µM
Confocal Objective – 60X (Water)
Digital Zoom – 2.5

Standard Conditions Used
50,00,000 cells/well in glass bottom well
Incubate with probe 27 for 1hr
Incubate with Lysotracker for 1hr.
Lysotracker conc. fixed at 60nM.
Probe 48a Conc - 100µM
Confocal Objective – 60X (Water)

Fig. 25 – Localization studies results of chloroquine probe **48a** (left) and artesunate probe **51** (right) vs lyso tracker red.

3.6 NCI 60 Cancer Cell Line studies[51,52] –

3.6.1 Single dose study results on artesunate probe **(51)** – The chart below shows 60 cancer cell lines and the effect of probe **51** on the growth of the cancer cells. The above results show the inhibitory effects on the majority of cancer cell lines, which can be further interpreted for its mode of action via the five dose studies results summarized in Fig. 26 and 28.

3.6.2 Single and five dose study results on artesunate probe **(51)** –

It can be concluded from the graphs summarized in Fig. 26 and 27 that artesunate probe **51** is potent against majority of cancer cell lines at concentrations of 10^{-8}M to 10^{-4}M as compared against the standard cell line growth. Mean graph data compares growth inhibition "GI_{50}" (drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation) ; total growth inhibition "TGI" (drug concentration resulting in total growth inhibition) and lethal concentration "LC_{50}" (drug concentration resulting in a

50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning indicating a net loss of cells). As observed from Fig. 27 and 28, artesunate probe **51** inhibits the proliferation of majority of cancer cell lines at 10^{-5} M mean concentration but does not lead to net loss of cells or cell death in the concentration range of 10^{-8} to 10^{-4} M. The above results confirms to the study of effects of artemisinins on cancer cell lines conducted by Woerdenbag et.al[69]. The above probe would be a useful tool to understand the modes of action within cancer cells

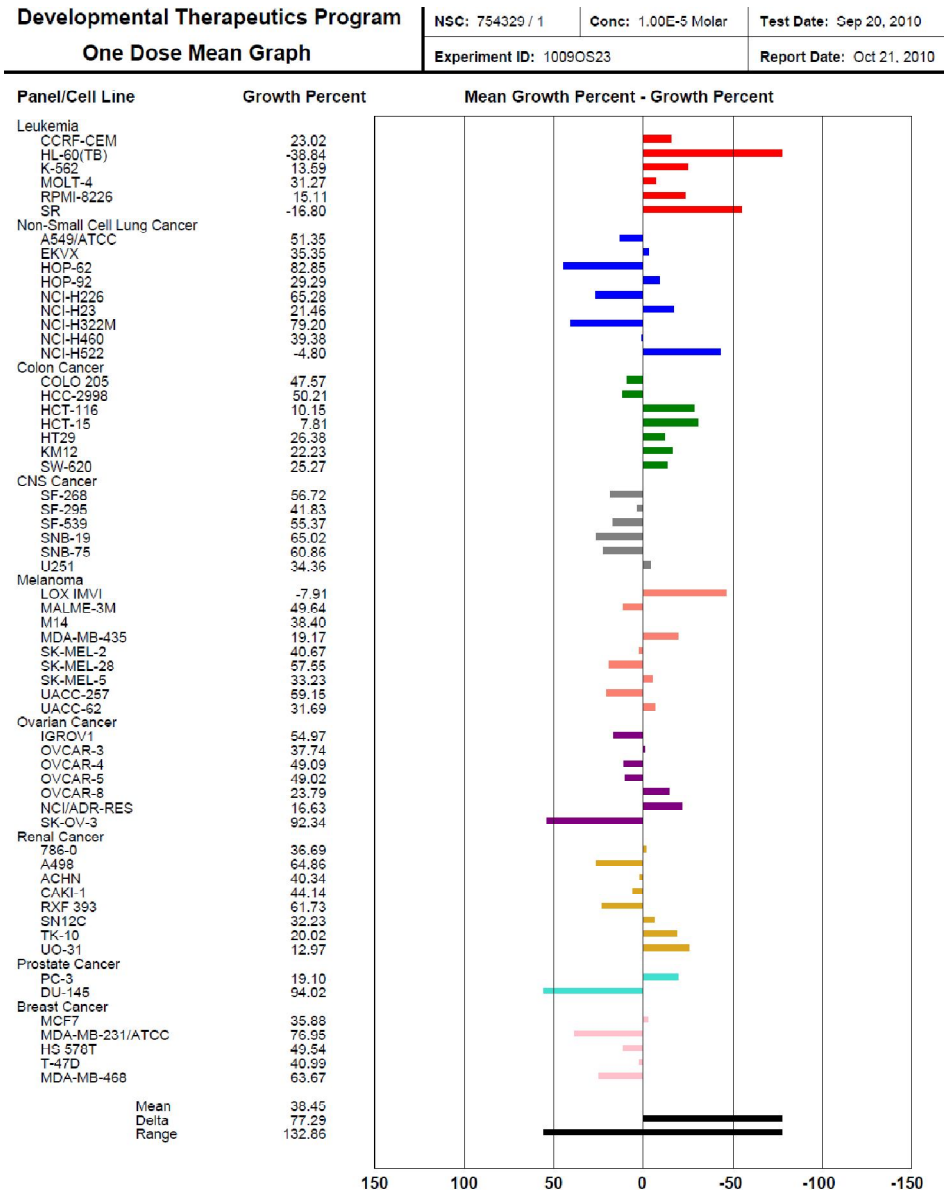


Fig. 26 – NCI 60 cancer cell line data for artesunate probe **51** (Single Dose Data).

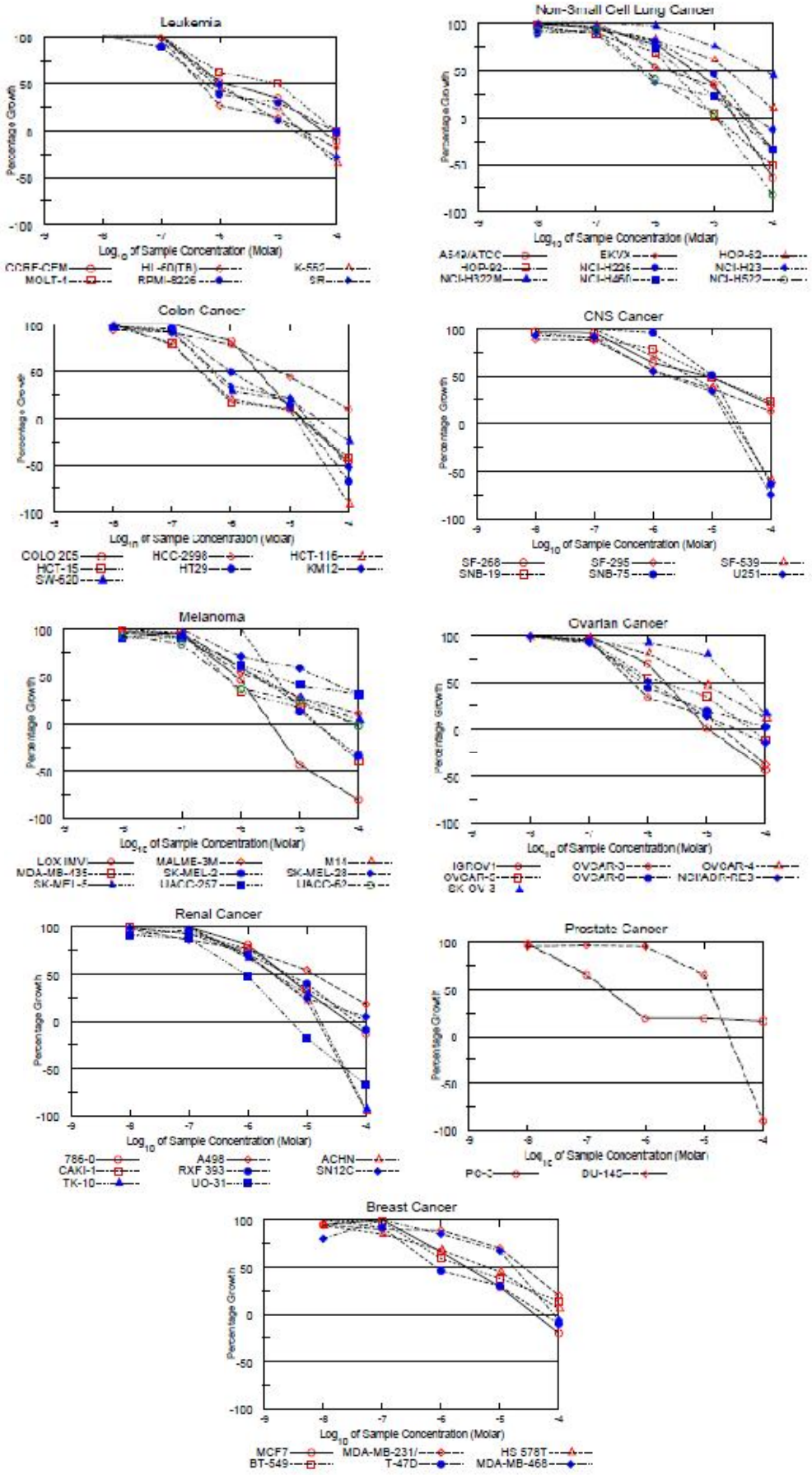


Fig. 27 – NCI 60 Cancer Cell Line data for Artesunate probe **51** (Five Dose Data).

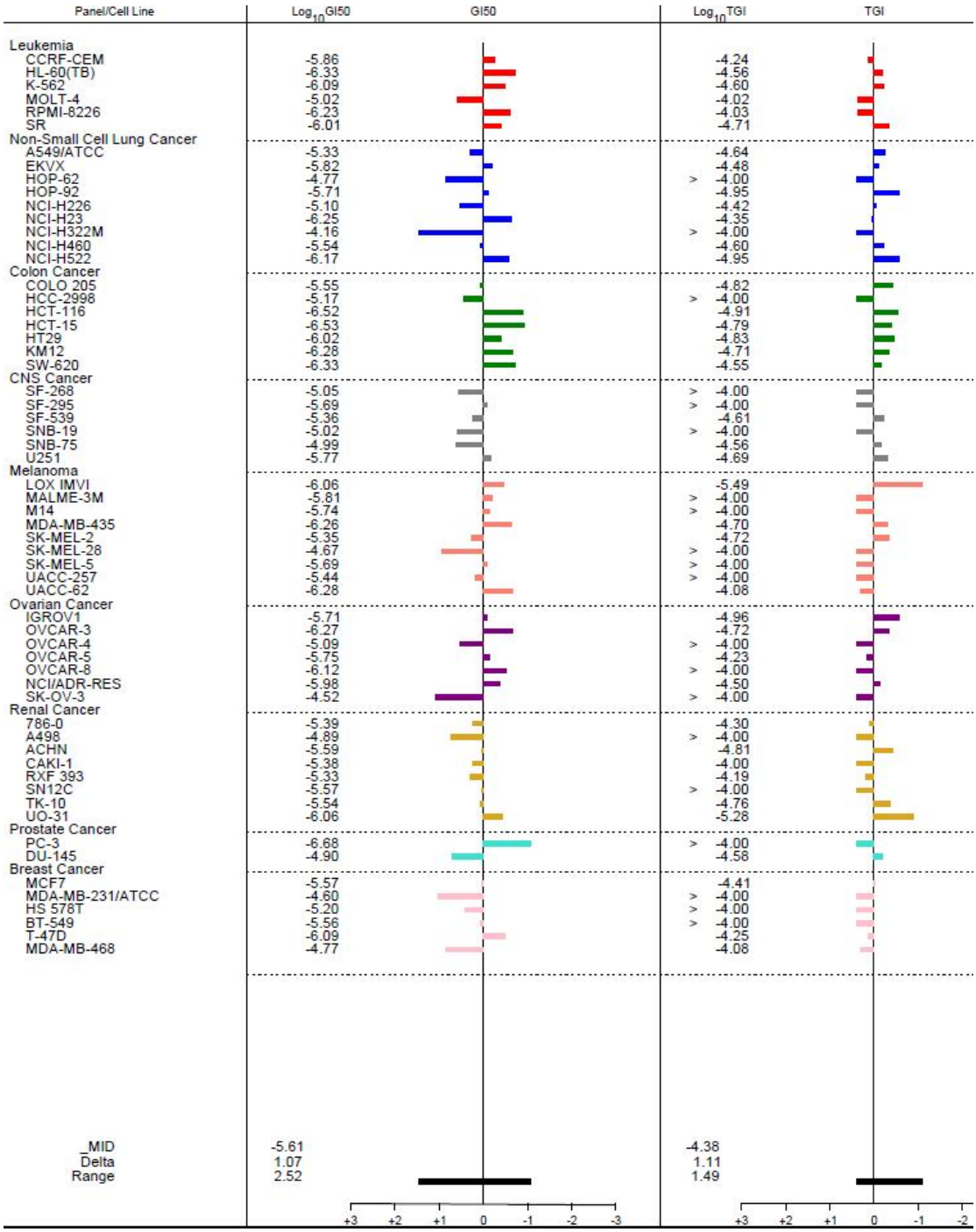


Fig. 28 – NCI 60 artesunate probe **51** (Mean Graph Data).

3.6.3 Comparision of mean graph data of Probe **51** with Artesunate –

A comparision of five dose data with artesunate parent molecule shows that the artesunate probe **51** maintains the same efficacy as the parent molecule respite the attachment of the fluorescent molecule. Supporting data is enclosed in Appendix 3.

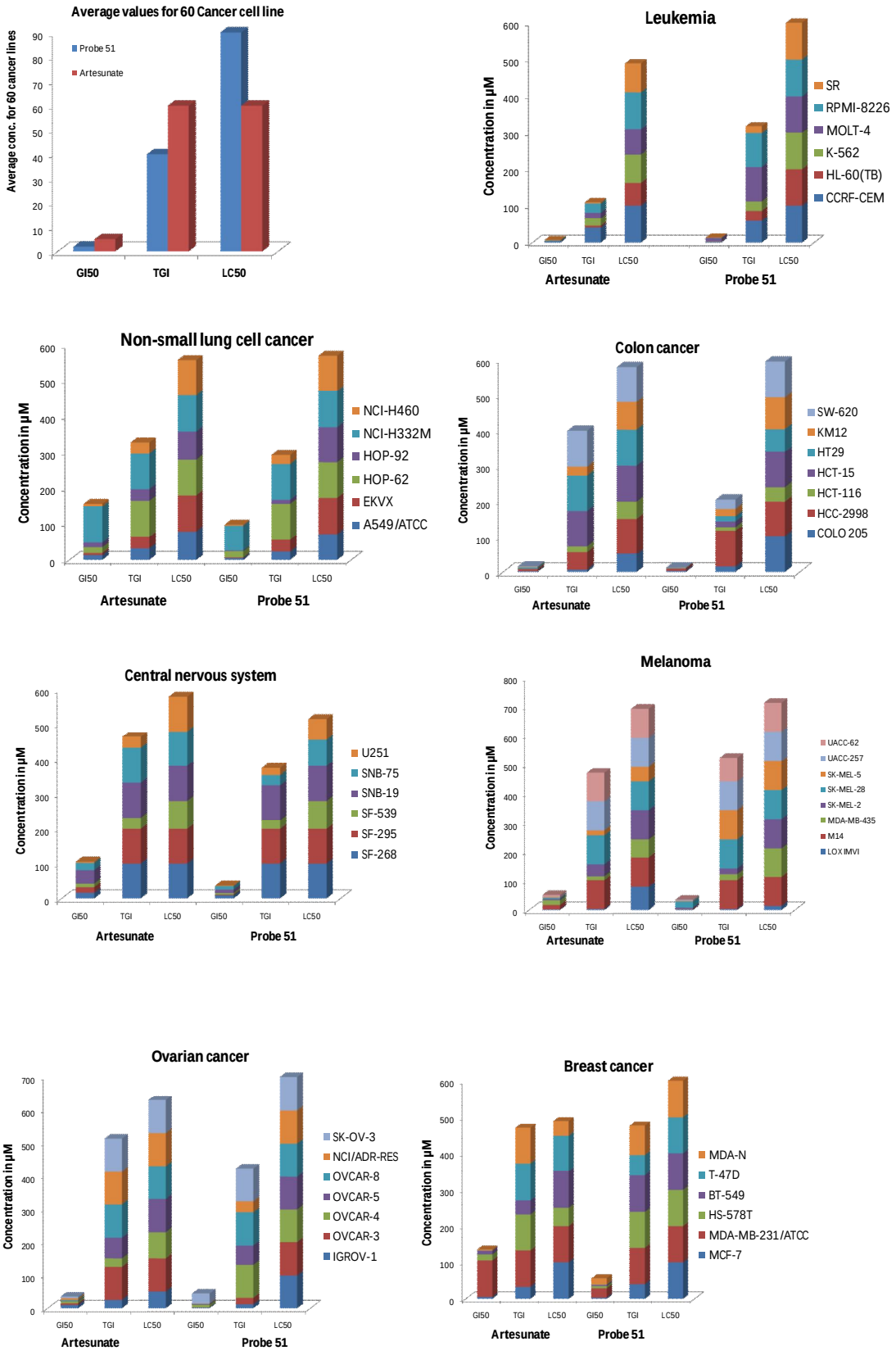


Fig. 29 – Comparision of mean graph data for Probe 51 vs Artesunate.

3.6.4 Single dose study results on chloroquine probe (**48a**) – The data in Fig. 30 below shows that chloroquine probe (**48a**) has little toxic or inhibitory effect on majority of cancer cell lines at the single dose concentration of 10^{-5}M.

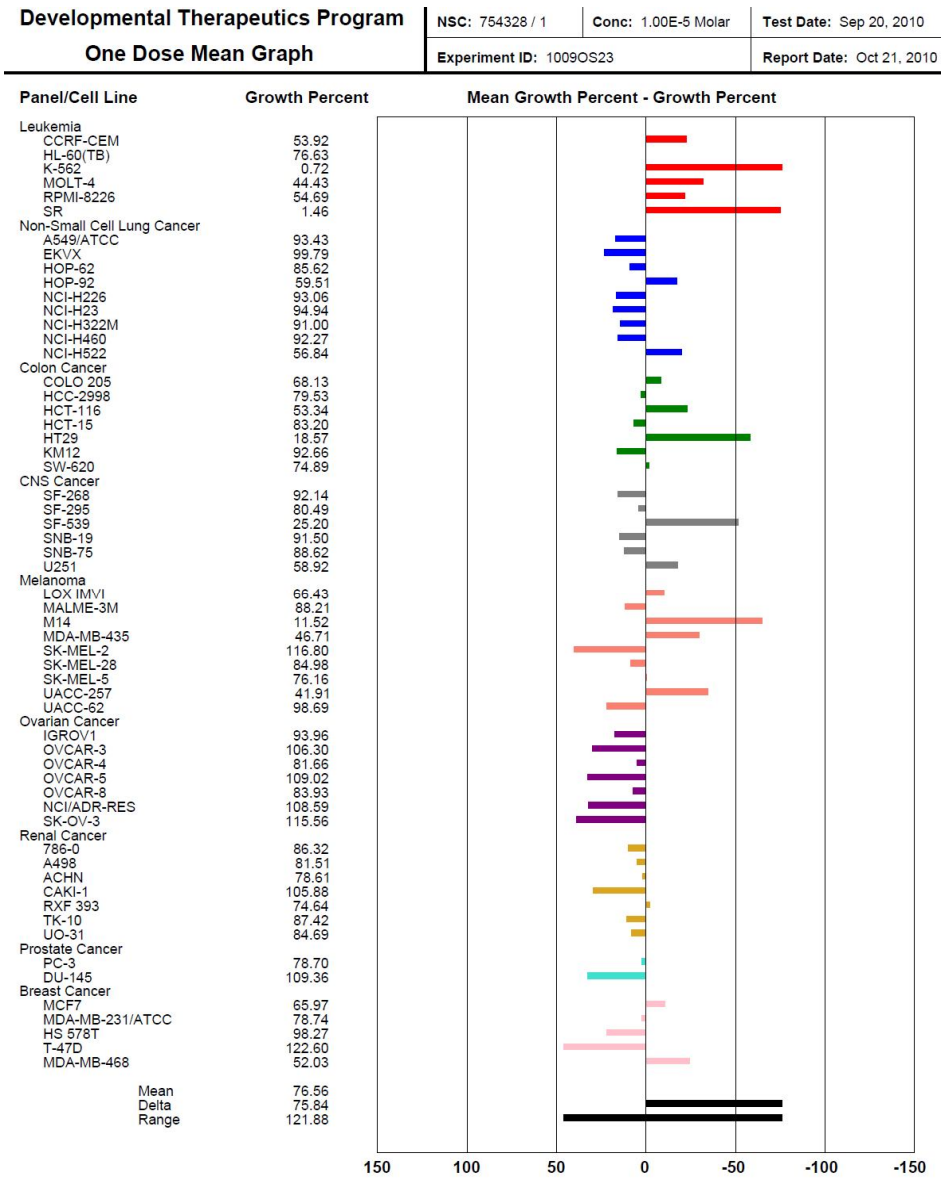


Fig. 30 – NCI 60 cancer cell line data for chloroquine probe **48a** (Single Dose Data).

3.6.5 Five dose study results on chloroquine probe **(48a)** – However the mean 5-dose data enclosed below in Fig. 31 and 32 showed some interesting results. As in Fig. 31, the chloroquine probe **(48a)** shows growth inhibition (GI_{50}) of cell lines at 10^{-5}M concentration but total growth inhibition of cell lines is only observed above 10^{-4}M

concentrations, which conforms to a number of reports on use of chloroquine as an anti-cancer agent[53].

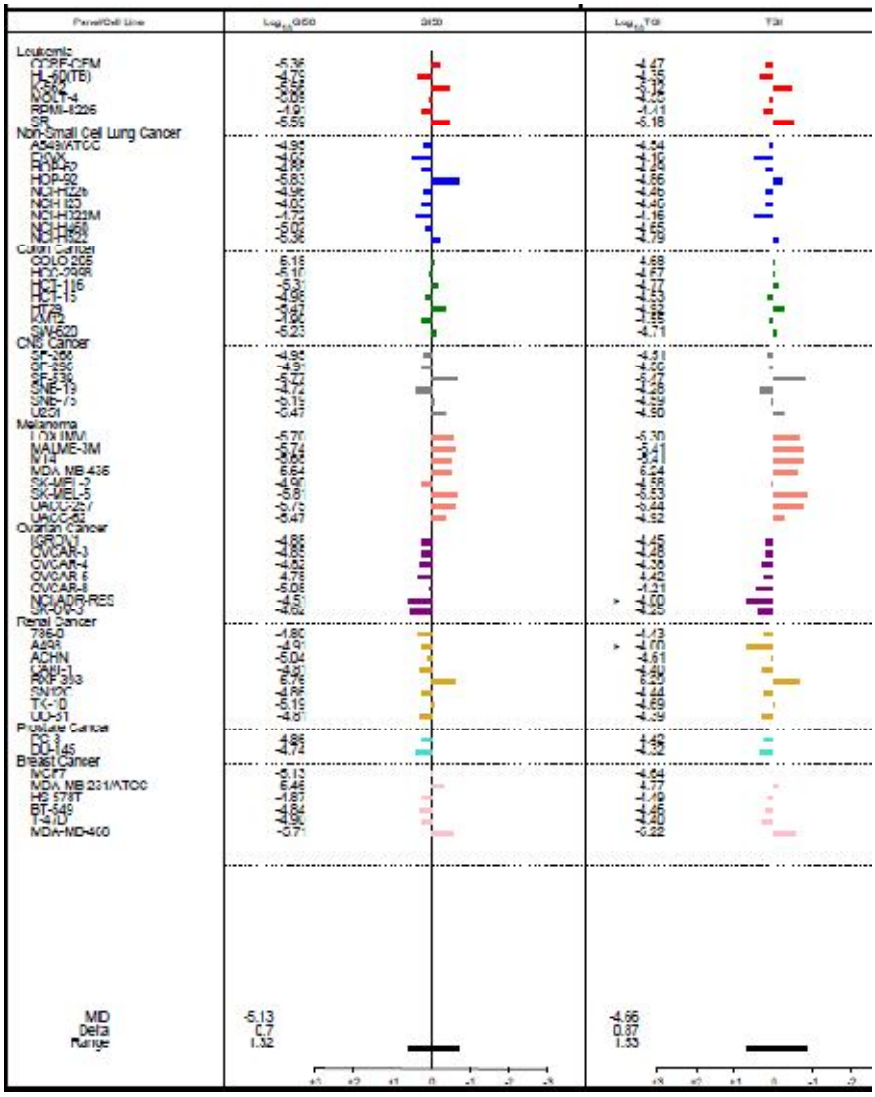


Fig. 31 – NCI 60 cancer cell line data for chloroquine probe **48a** (mean graph).

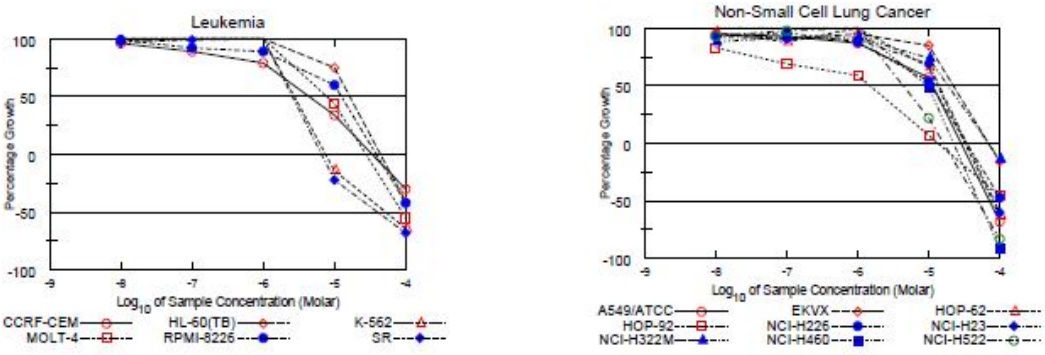


Fig. 32 – NCI 60 cancer cell line data for chloroquine probe **48a** (5- Dose Data).

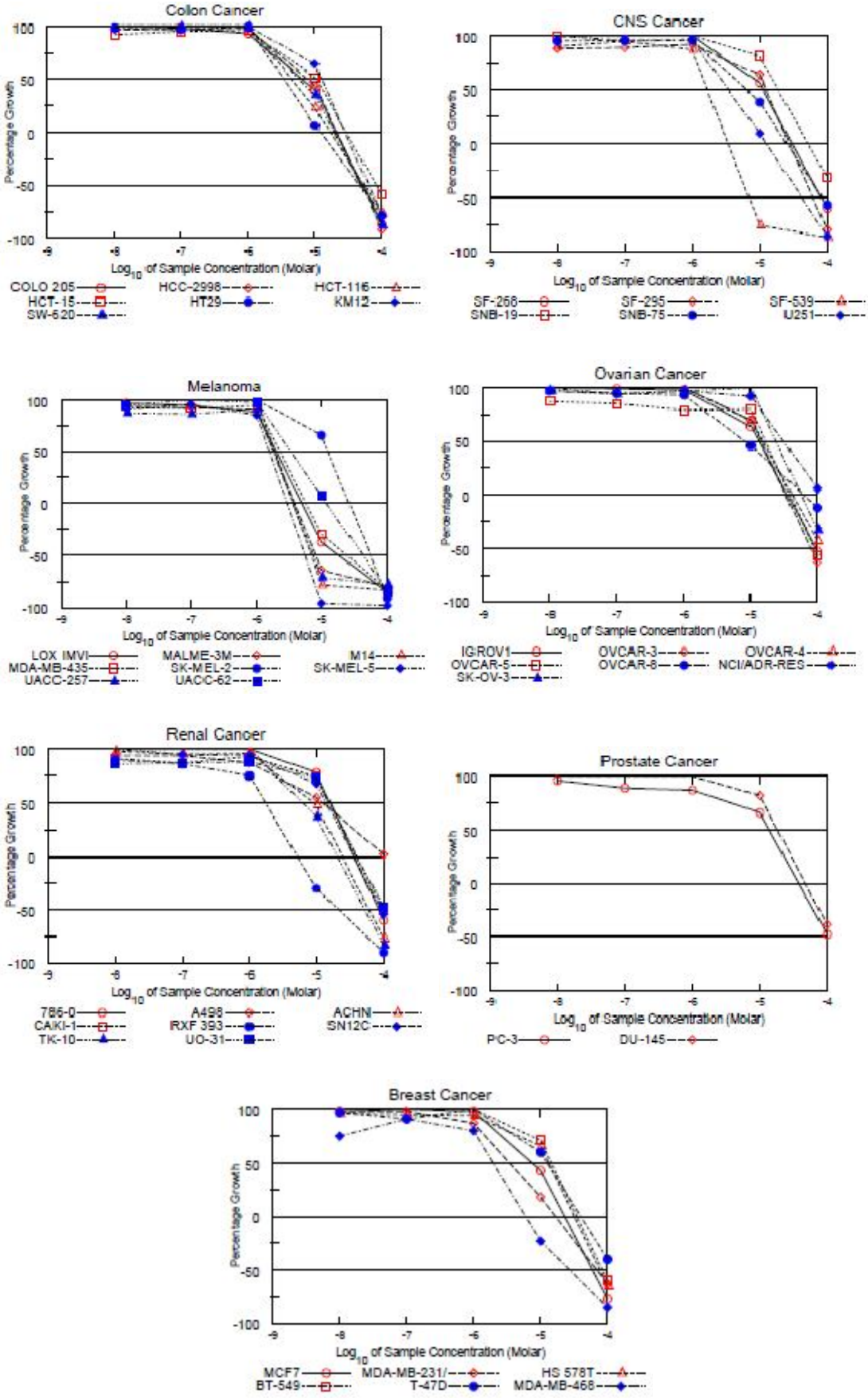


Fig. 32 – NCI 60 cancer cell line data for chloroquine probe **48a** (5- Dose Data).

3.6.6 Comparision of mean graph data of probe **(48a)** with parent molecule –

A comparision of five dose data with chloroquine parent molecule shows that the chloroquine probe **(48a)** maintains the same efficacy as the parent molecule respite the attachment of the fluorescent molecule as shown in Fig.33 (Supporting data Appendix 3).

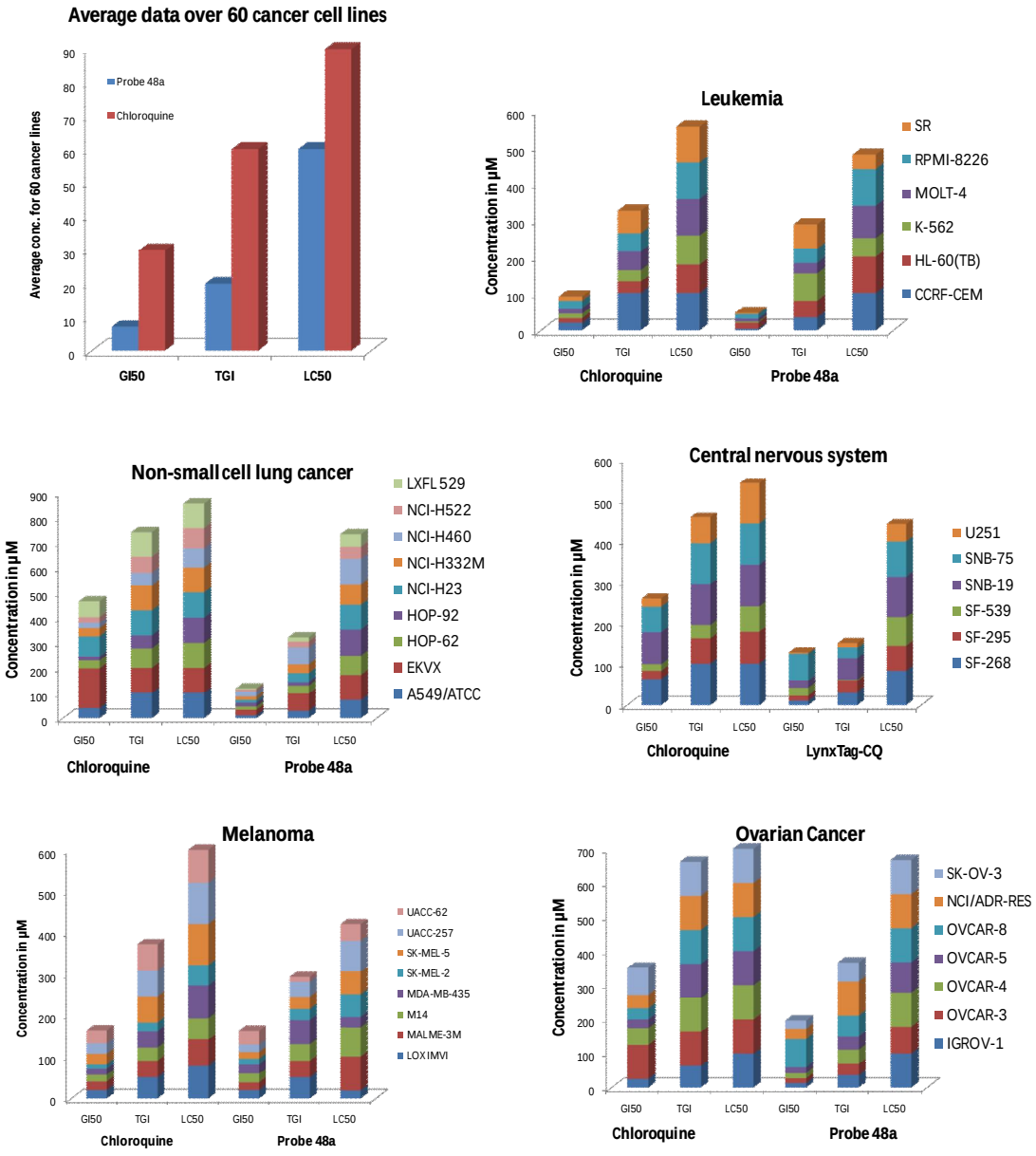


Fig. 33 – Comparision of mean graph data for Probe **(48a)** vs Chloroquine.

Thus the above probes can also be used for understanding the modes of action of the drugs within cancer cell lines thus expanding the applications beyond Malaria.

CONCLUSION

Thus I have synthesized chloroquine based probes **36b, 48a** and artesunate based probes **51** with applications into malaria diagnostic, cancer bio-imaging study and other auto-immune diseases. Probe **36b** as seen from the results (section 3.4) was able to clearly distinguish between chloroquine sensitive and chloroquine resistant strain of *P.falciparum* and coupled with its steadfast thermal stability (section 3.3.2) has established a robust diagnostic tool, fulfilling WHOs requirements for an fast, robust and effective diagnostic tool for malaria. Probe **48a** (section 3.5) expands the applications of chloroquine probes for bio-imaging studies of auto-immune diseases. It was able to establish the lysosomotropic nature of the drug due to its localization within lysosomal compartment within the mammalian cell lines. Ultimately probe **48a** (sections 3.6.3, 3.6.4) encompasses bio-imaging of cancer cell lines showing characteristics similar to the parent chloroquine diphosphate molecule. Probe **51** has also shown diverse applications from bio-imaging of auto-immune diseases (section 3.5) to extremely high potency within cancer cell lines (sections 3.6.1, 3.6.2). It still needs to be evaluated for its activity within artemisinin resistant *P.falciparum* parasites.

However probe **36b** and **48a** cannot be effectively used for in-vitro live cell imaging studies due to the detrimental effects of blue laser radiation. This is where chloroquine based probes (**55, 83, 86, 92**) have provided an advantage (section 3.4) due to sample & probe friendly green laser radiation and the high extinction coefficient of Borondipyrromethane (BODIPY). The chloroquine-bodipy probe **55** has show excellent resilience under green laser radiation while maintaining the efficacy and lysosomotropic properties of the parent drug molecule.

Although artesunate based probe **51** shows excellent anti-cancer activity, it has severe limitations due to its inherent poor hydrolytic stability and thermal stability (section 2.3.4). Hence it was difficult to ascertain whether the fluorescent tag would sustain enzymatic cleavage within the cells. This limitation was overcome by the artelinic based probes **(57, 83, 86, 98 and 100)** which has shown excellent short term thermal resilience (appendix A1). However, long term stability (12months) of these probes, along with their biological activity is under study. All of the click probes (**58-64, 87-90**) have show excellent short term thermal stability (4days), they still need to be evaluated for their ability to undergo click reaction in-situ in the cell. Further the final click product needs to be evaluated for its ease of separation and binding ability on its target for immunological study. Overall I have established the molecular tool for bio-imaging and diagnostic application in malaria, cancer and mammalian cell lines. They have provided a clear picture of the mode of action of the drugs confirming with the hypothesis of lysosomotropic mode of action presented by many researchers in past papers[27-30, 53-57].

FUTURE WORK

We have already observed the selective efflux of chloroquine probes from chloroquine resistant *P.falciparum* strains cultured in lab. The limitations of the chromophore have been surmounted by synthesis of superior BODIPY and TAMRA based probes **(83, 86, 92, 95, 98, 100)**. Rising healthcare costs[61] and increasing cases of cancer worldwide[62] have stimulated focus of researchers on personalized medicine with the promise of effective and individualized healthcare where drugs would be administered as per individual patient responses. However this field which evaluates genetic data of individuals to address the right drug treatment is based on probabilistic model, is highly labor intensive and expensive[63].

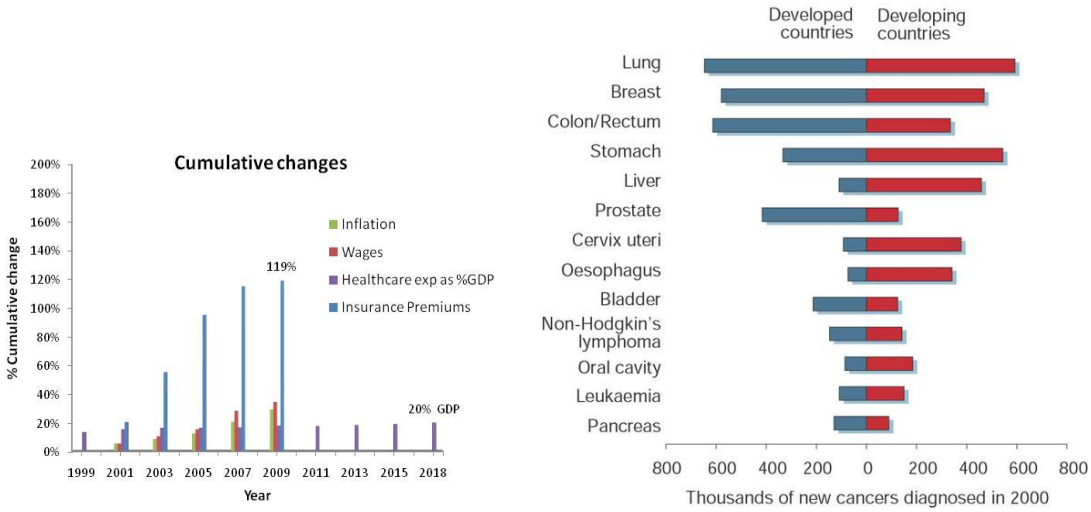
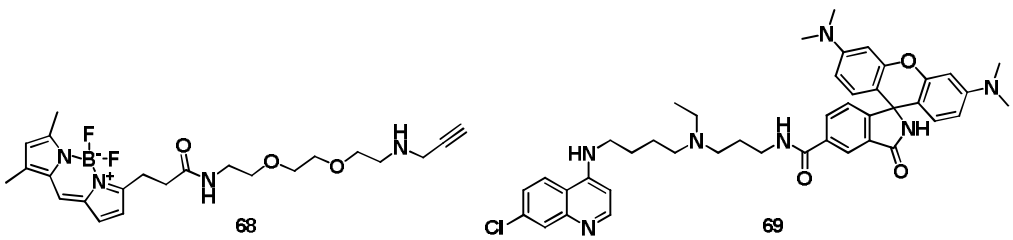


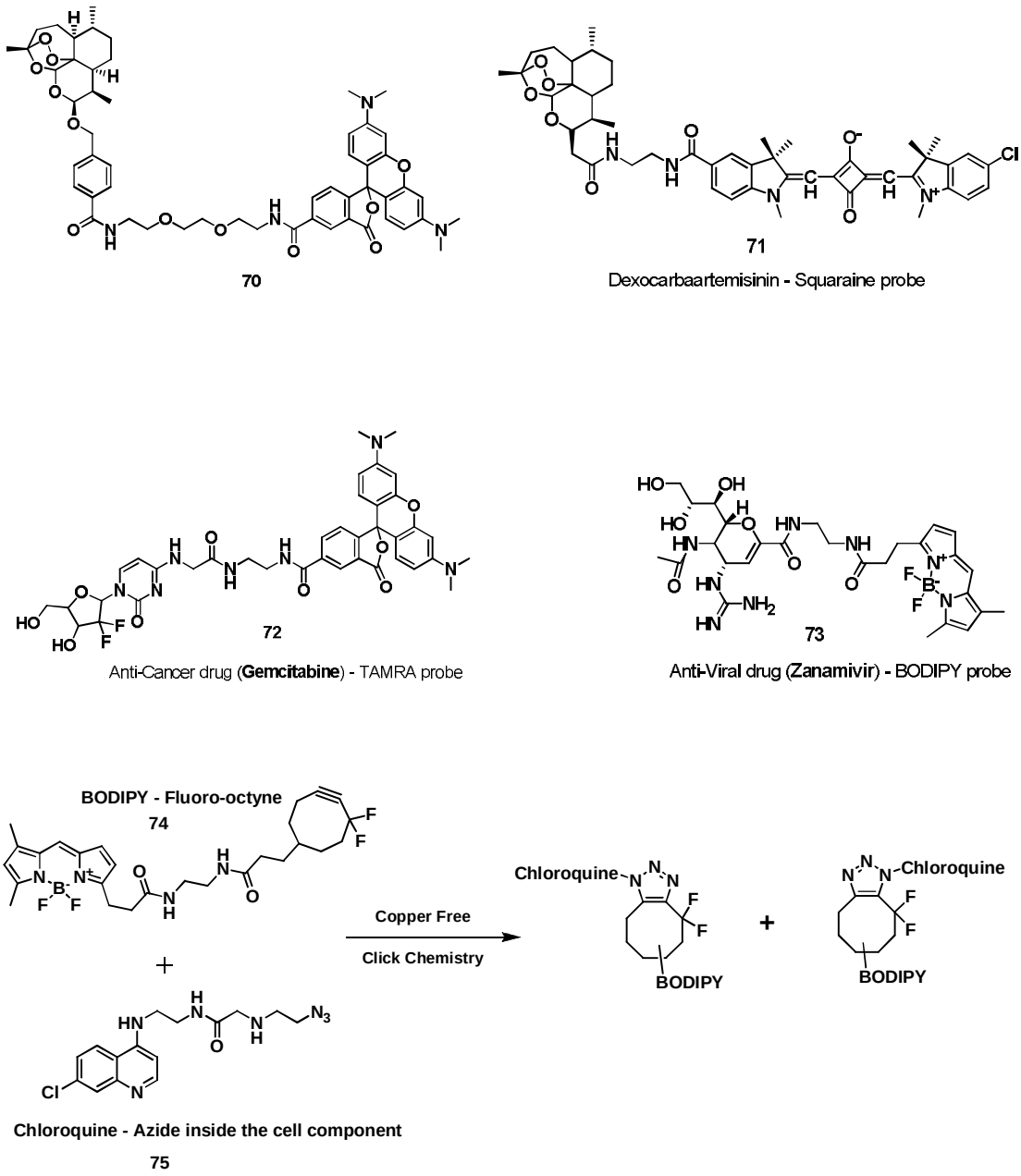
Fig. 34 – Healthcare costs (left) and Cancer incidences worldwide (right).

The concept of fluorescent drug probe as addressed in my thesis for malaria and cancer can be expanded to include other diseases, giving rise to the field of *"Medicinal Probes"*.

In comparision with the pharmacogenomics approach, *medicinal probes* could lead to a more direct method for visualization of individual drug response analysis using existing technology and instrumentation. Thus the future of drug probes lies into

1) In-vitro live cell imaging capable probes (**68-70**) for malaria, cancer and other diseases.
2) In-vivo probes[58] **(71)** for the study of mode of drug action within animal models.
3) Tagging anti-cancer **(72)**, anti-viral **(73)** and other drugs[59] with probes with possible applications into bio-imaging or diagnostic studies.
4) Copper free click chemistry probes (**74, 75)**.[60] for In-Vivo applications.





Copper free click chemistry probes

Fig. 35 – Future applications for probes

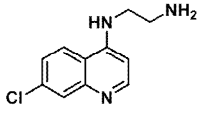
EXPERIMENTAL WORK

All chemicals were purchased from the Sigma-Aldrich Chemical Co. or Alfa Aesar and were used without further purification, unless indicated otherwise. Experiments were conducted at ambient temperature, unless noted otherwise. Analytical thin layer chromatography (TLC) was performed using a Merck 60 F254 pre-coated silica gel plate. Subsequent to elution, plates were visualized using UV radiation (254 nm). Further visualization was possible by staining with a Ninhydrin, Ceric ammonium molybdate (CAM) or Potassium permanganate ($KMnO_4$) or 2,4-Dinitrophenol (2,4-DNP) solutions. Silica gel 60 (230–400 mesh ASTM) was used for flash column chromatography. All the yields noted are for 96% purity of the compounds as analyzed by ^{1}H, ^{13}C NMR spectra (enclosed in Appendix A.2), LCMS for purity for biotesting submission (enclosed in Appendix A.4), IR and HRMS. Infrared spectra was recorded on a Bio-Rad FTS 165 FTIR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Brücker AMX 300 or 500M*Hz* spectrometer. The NMR samples were prepared in a $CDCl_3$, DMSO-d_6 or MeOD-d_4 solution. Chemical shifts are reported as δ in units of parts per million (ppm), calibrated based on the different solvents used (CDCl3 s 7.26; DMSO-d6 m 2.50; MeOD-d4 d 3.31).Multiplicities are given as: s (singlet); d (doublet); t (triplet) or m (multiplets), b (broad) and sex (sextet). Coupling constants (*J*) are represented in *Hz*. Purity and Thermal stability results were recorded on Shimadzu LC-IT-TOF spectrometer. High Resolution Mass Spectroscopy was recorded for new molecules using Finnigan LCQ mass spectrometer. Pre-calibrated weighing balance (for weighing samples for thermal stability studies), oven (maintained at 37^{o}C) and freezer (maintained at -20^{o}C) were used for thermal stability studies which were established using the ICH guidelines.

5.1 Synthesis scheme[35-41]

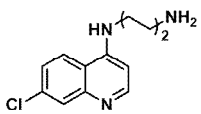
5.1.1 General experimental procedure for the synthesis of N-(7-Chloro-4-quinolyl)-1,n-diaminoalkanes **(27a, 27b)**

A mixture of 4,7-dichloroquinoline **(26)** (3.0gms, 15.2 mmol, 1equiv.) and ethylenediamine (**30**) (4.55 gms, 75.7 mmol, 5 equiv) was heated to 110 °C for 6 h under inert atmosphere and then cooled to room temperature. Aqueous NaOH (0.61gms, 15.2 mmol, 1equiv,(1N soln., in 10mL)) was then added and the mixture was extracted with CH_2Cl_2. The organic layers were washed with water (50mL), brine (25mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to get N-(7-Chloro-4-quinolyl)-1,n-diaminoalkanes.



27a, n=1, 86%

N-(7-Chloro-4-quinolyl)-1,2-diaminoethane **(27a)** - Following the above procedure the compound was obtained as pale-yellow crystals with 86% yield. This was used without further purification. $\mathbf{R_f}$ (1:9 MeOH: CH_2Cl_2+0.1%TEA) = 0.09. **IR** (KBr)/ cm^{-1} – 3248, 2916, 1581. ^{1}**H** NMR (500M*Hz*, MeOD) δ 8.35(1H, d, -N=CH-, *J*=9.45*Hz*), 8.04(1H, d, Ar-CH, *J*=9.45*Hz*), 7.76(1H, d, Ar-H, *J*=2.5*Hz*), 7.38(1H, dd, Ar-H, *J*=2*Hz* 6.95*Hz*), 6.56(1H, d, Ar-H, *J* = 5.7*Hz*), 3.45(2H, t, -NH-*CH_2*-,*J* = 6.3*Hz*), 2.93(2H, t, -NH_2-*CH_2*-, *J* = 6.3*Hz*) ; ^{13}**C** NMR (500 M*Hz*, MeOD) δ 152.6, 152.4, 149.7, 136.4, 127.6, 126.1, 124.2, 118.8, 99.7, 50.3, 44.2 .**MS**(ES^+): m/z (%): 222.1 $[M+H]^+$.

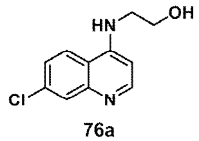


27b, n=2, 80%

N-(7-Chloro-4-quinolyl)-1,4-diaminobutane **(27b)** – Employing the procedure 80% yield of pale-yellow crystals was obtained. R_f (1:9 MeOH: CH_2Cl_2+0.1%TEA) = 0.03. **IR** (KBr)/cm^{-1} – 3255, 2932, 1582. **^{1}H** NMR(500M*Hz*, MeOD) δ 8.34(1H, d, -N=CH-, *J*=9.45*Hz*), 8.10(1H, d, Ar-CH, *J*=9. 5*Hz*), 7.77(1H, d, Ar-H, *J*=2.5*Hz*), 7.39(1H, dd, Ar-H, *J*=2.5*Hz*,6.3*Hz*), 6.52(1H, d, Ar-H, *J* = 5.7*Hz*), 3.38(2H, t, -NH-*CH_2*-,*J* = 7.6*Hz*), 2.71(2H, t, -NH_2-*CH_2*-, *J* = 6.9*Hz*), 1.78(2H, m, -NH-*CH_2*-*CH_2*-), 1.62(2H, m, -NH_2-*CH_2*-*CH_2*-); **^{13}C** NMR (500 M*Hz*, MeOD) δ 152.7, 152.4, 149.7, 136.2, 127.6, 125.8, 124.3, 118.8, 99.6, 43.9, 42.3, 31.3, 26.8. **MS** (ES^+): m/z (%): 250.1 $[M+H]^+$.

5.1.2 General experimental procedure for the synthesis of 2-(7-chloroquinolin-4-ylamino)ethanol and 4-(7-chloroquinolin-4-ylamino)butan-1-ol **(76a, 76b)**[65]

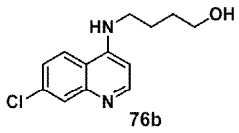
A mixture of 4,7-dichloroquinoline **(26)** (4.0gms, 20.2mmol, 1equiv.), 1-aminobutanol (24.71gms, 403.9 mmol, 20equiv) and TEA (0.62gms, 6.06 mmol, 0.3 equiv.) was heated to 130 °C for 12-14 h under inert atmosphere and then cooled to room temperature. Water was added and the mixture was extracted with CH_2Cl_2. The organic layers were washed with water (50mL), brine (25mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to get 2-(7-chloroquinolin-4-ylamino)ethanol. The compound was directly used without purification.



76a

2-(7-chloroquinolin-4-ylamino)ethanol **(76a)** – Yields – 80%, **R_f** (15% MeOH:CH_2Cl_2) = 0.25 . **^{1}H** NMR(500M*Hz*, MeOD) δ 8.35(1H, d, -N=CH-, *J*=9.45*Hz*), 8.05(1H, d, Ar-CH, *J*=9.45*Hz*), 7.39(1H, dd, Ar-H, *J*=2*Hz*, 6.9*Hz*), 7.78(1H, d, Ar-H, *J*=2.5*Hz*), 6.57(1H, d, Ar-H, *J* = 5.7*Hz*), 3.84(2H, t, -OH-*CH_2*-, *J* =

5.7*Hz*), 3.50(2H, t, -NH_2-CH_2-, *J* = 5.7*Hz*); **^{13}C** NMR(500 M*Hz*, MeOD) δ 152.9, 152.4, 149.7, 136.4, 127.6, 126.0, 124.2, 118.8, 111.6, 99.7, 60.8, 46.3. **MS** (ES^+): m/z (%): 222.8 $[M]^+$.



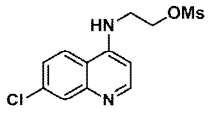
76b

4-(7-chloroquinolin-4-ylamino)butan-1-ol **(76b)** – Yields – 100%, **R_f** (15% MeOH:CH_2Cl_2) = 0.2 . **^{1}H** NMR(500M*Hz*, MeOD) δ 8.32(1H, d, -N=CH-, *J*=9.45*Hz*), 8.07(1H, d, Ar-CH, *J*=9. 5*Hz*), 7.76(1H, d, Ar-H, *J*=2.5*Hz*), 7.36(1H, dd, Ar-H, *J*=2.5*Hz,* 11.5*Hz*), 6.49(1H, d, Ar-H, *J* = 5.7*Hz*), 3.34-3.65(4H, t, -OH-CH_2-, -NH-CH_2-), 1.58-1.85(4H, m, -NH_2-CH_2-CH_2-CH_2-CH_2-OH); **^{13}C** NMR(500 M*Hz*, MeOD) δ 152.7, 152.4, 149.7, 136.3, 133.5, 131.8, 127.6, 125.9, 124.3, 120.3, 118.8, 25.9, 99.6, 62.6, 43.9, 31.1. **MS** (ES^+): m/z (%): 251.14 $[M+H]^+$. **HRMS**(ES^+) – obsvd. – 251.0953 ; calc. - 251.0946.

5.1.3 General experimental procedure for the synthesis of 2-(7-chloroquinolin-4-yl amino)ethylmethanesulfonate, 4-(7-chloroquinolin-4-ylamino)butylmethanesulfonate **(77a, 77b)**[65]

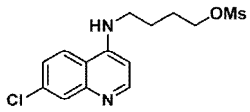
A mixture of 2-(7-chloroquinolin-4-ylamino)ethanol **(76a)** (1.0gms, 4.5 mmol, 1 equiv.) in 13ml of pyridine is stirred for 30min. Then MsCl (1.54gm, 13.5mmol, 3equiv) in 3ml of pyridine is added at 0^oC under inert atmosphere. The reaction mixture is stirred at this temperature for 1hr, then warmed to room temperature and stirred for 5hrs. After confirmation with TLC, the mixture is transfered to a bigger round bottom flask kept at 0^oC and about 5-10ml of 25% Ammonia solution is added slowly with stirring till solution turns basic. Water was added and the mixture till precipitation was observed. The mixture was extracted with CH_2Cl_2. The organic layers were washed with water (25mL), brine (15mL), dried over anhydrous Na_2SO_4,

and evaporated under reduced pressure till thick slurry is obtained. To this slurry 20ml diethyl ether and about 40ml hexane is added to precipitate out the product. The precipitate is separated from the solution and then dried thoroughly under vacuum to get 2-(7-chloroquinolin-4-yl amino)ethylmethanesulfonate.



77a

2-(7-chloroquinolin-4-yl amino)ethylmethanesulfonate **(77a)** – Yields-91%, $\mathbf{R_f}$ (15% MeOH:CH_2Cl_2) = 0.4 . **^{1}H** NMR(500M*Hz*, $CDCl_3$) δ 8.53(1H, d, -N=CH-, *J*=9.45*Hz*), 7.96(1H, d, Ar-CH, *J*=9.45*Hz*), 7.77(1H, d, Ar-H, *J*=2.5*Hz*), 7.39(1H, dd, Ar-H, *J*=2*Hz*, 6.9*Hz*), 4.59(2H, t, -OH-CH_2-, *J* = 5.7*Hz*), 6.41(1H, d, Ar-H, *J* = 5.7*Hz*), 3.48(2H, t, -NH_2-CH_2-, *J* = 5.7*Hz*) 3.08(3H, OSO_2-CH_3); **^{13}C** NMR(500 M*Hz*, $CDCl_3$) δ 151.36, 149.49, 148.53, 135.48, 128.29, 125.98, 121.38, 117.15, 98.94, 66.84, 42.49, 37.81. **MS** (ES^+): m/z (%): 301.1 $[M+H]^+$.



77b

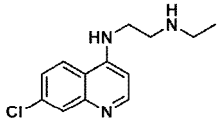
4-(7-chloroquinolin-4-ylamino)butylmethanesulfonate **(77b)** – Yields – 92%, $\mathbf{R_f}$ (15% MeOH: CH_2Cl_2) = 0.35 . **IR** (KBr)/cm^{-1} – 3421, 2935, 1610, 1541, 1352, 1575. **^{1}H** NMR(500M*Hz*, $CDCl_3$) δ 8.46(1H, d, -N=CH-, *J*=9.45*Hz*), 7.91(1H, d, Ar-CH, *J*=9.45*Hz*), 7.78(1H, d, Ar-H, *J*=2.5*Hz*), 7.33(1H, dd, Ar-H, *J*=2*Hz*, 6.3*Hz*), 6.39(1H, d, Ar-H, *J* = 5.7*Hz*), 5.61(1H, b, -NH-), 4.31(2H, t, -CH_2-OSO_2CH_3, *J* = 5.7*Hz*), 3.39(2H, t, -NH_2-CH_2-, *J* = 5.7*Hz*), 3.01(3H, OSO_2-CH_3), 1.68-1.91(4H, m, -NH_2-CH_2-CH_2-CH_2-CH_2-OH); **^{13}C** NMR(500 M*Hz*, $CDCl_3$) δ 151.7, 150.9, 136.0, 128.5, 126.2, 122.3, 117.7, 99.6, 70.1, 43.3, 38.2, 27.6, 25.5. **MS**(ES^+): m/z (%): 329.1 $[M+H]^+$. HRMS (ES^+) – observed – 329.0735 ; calculated. - 329.0721.

5.1.4a General experimental procedure for the synthesis of N-(7-chloro-4-quinolyl)-N′,N′-diethyl-1,(n)-diaminoalkanes (Structures 29a,29b) and N-(7-chloro-4-quinolyl)-N′-ethyl-1,n-diaminoalkanes (Structures **28a**, **28b**)

To a solution of N-(7-chloro-4-quinolyl)-1,2-diaminoethane (**27a**) (1.0 g, 4.5 mmol, 1 equiv.) in anhydrous DMF (10mL) was added Cs_2CO_3 (4.41 gms, 13.5 mmol, 3 equiv). The solution was stirred at 25 °C for 0.5 h and ethyl bromide (0.49g, 4.5 mmol, 1 equiv.) (diluted in 10mL anhydrous DMF) was added dropwise for 2hrs and then the reaction mixture is stirred at 25 °C for 24 h. DMF was removed in vacuum. The residue was dissolved in CH_2Cl_2, washed with water (10mL), brine (10mL) dried over anhydrous Na_2SO_4, and the organic extracts were removed under reduced pressure. Flash chromatography (2%-8% MeOH in CH_2Cl_2+0.1% TEA) allowed isolation of 0.21 g (16.8% yield) of N-(7-chloro-4-quinolyl)-N′,N′-diethyl-1,2-diaminoethane (**29a**) and 0.38 g (33.7% yield) of N-(7-chloro-4-quinolyl)- N′-ethyl-1,2-diaminoethane (**28a**) as pale-yellow crystals.

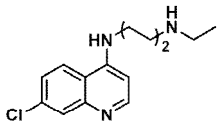
5.1.4b Alternate general experimental procedure for the synthesis of N-(7-chloro-4-quinolyl)-N′-ethyl-1,n-diaminoalkanes (Structures 28a,28b)

To a mixture of 2-(7-chloroquinolin-4-yl amino)ethylmethanesulfonate **(77a)** (1.2gms, 4mmol, 1equiv.) in THF: MeOH (5:2) is added 2M ethylamine solution in THF (26.97gms (30mL), 598mmol, 150 equiv.). The solution is heated to reflux under inert atmosphere for 12-16hrs. After confirmation with TLC, the solution is cooled down. The product is isolated by removing the solvent under vacuum and further column with 10% MeOH in CH_2Cl_2 + 0.1% TEA. The diethyl amine by product is thus avoided and the yields are quantitative.



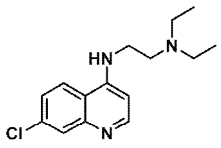
28a, n=1, 34%

N-(7-Chloro-4-quinolyl)-N′-ethyl-1,2-diaminoethane **(28a)** – Yields – 34% (as per procedure 4.1.4a) ; 90% (as per procedure 4.1.4b), $\mathbf{R_f}$ (1:9 MeOH:CH_2Cl_2 +0.1% TEA) = 0.23 . **IR** (KBr)/cm^{-1} – 3271, 2963, 2847, 1582. ^{1}**H** NMR(500M*Hz*, MeOD) δ 8.37(1H, d, -N=*CH*-, *J*=5.7*Hz*), 8.10(1H, d, Ar-H, *J*=8.8*Hz*), 7.78(1H, d, Ar-H, *J* =2.6*Hz*), 7.41(1H, dd, Ar-H, *J*=2.5*Hz*, 6.3*Hz*), 6.56(1H, d, Ar-H, *J* =5.7*Hz*), 3.51(2H, t, -NH-*CH_2*-, *J* = 6.3*Hz*), 2.96(2H, t, -NH-*CH_2*-, *J* = 7.0*Hz*), 2.72(2H, q, -*CH_2*-CH_3-, *J*=7.0*Hz*), 1.16(3H, t, -CH_2-*CH_3*-, *J*=7.6*Hz*); ^{13}**C** NMR(500 M*Hz*, MeOD) δ 152.8, 152.5, 149.7, 136.4, 127.6, 126.1, 124.3, 118.8, 99.7, 44.7, 43.2, 14.6. **MS** (ES^+): m/z (%): 250.1 $[M+H]^+$.



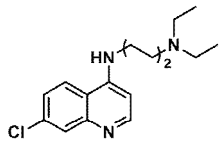
28b, n=2, 18%

N-(7-Chloro-4-quinolyl)-N′-ethyl-1,4-diaminobutane **(28b)** – Following procedure described above and purification by flash chromatography (2%-8% MeOH in CH_2Cl_2+0.1% TEA) gave 18% yield(procedure 4.1.4a), 80% yield(procedure 4.1.4b) of pale-yellow crystals. $\mathbf{R_f}$ (1:9 MeOH:CH_2Cl_2+0.1%TEA) = 0.2 **IR** (KBr)/cm^{-1} – 3289, 3279, 1582. ^{1}**H** NMR(500M*Hz*, MeOD) δ 8.34(1H, d, -N=*CH*-, *J*=5.1*Hz*), 8.09(1H, d, Ar-H, *J*=8.9*Hz*), 7.77(1H, d, Ar-H, *J*=2.0*Hz*), 7.39(1H, dd, Ar-H, *J*=2.0*Hz*, 6.9*Hz*), 6.52(1H, d, Ar-H, *J* = 5.7*Hz*), 3.30(2H, t, -NH-*CH_2*-, *J* = 7.0*Hz*), 2.67(4H, q, -*CH_2*-CH_3-, *J* = 7.0*Hz*), 1.79(2H, m, -NH-CH_2-*CH_2*-), 1.66(2H, m, -NH_2-*CH_2*-CH_2-), 1.13(3H, t, -CH_2-*CH_3* -, *J*=7.0*Hz*); ^{13}**C** NMR(500 M*Hz*, MeOD) δ 152.7, 152.4, 149.7, 136.2, 127.6, 125.9, 124.3, 118.8, 99.6, 49.9, 44.5, 43.8, 27.9, 27.2, 14.5. **MS** (ES^+): m/z (%): 278.1 $[M+H]^+$.



29a, n=1, 17%

N-(7-Chloro-4-quinolyl)-N′,N′-diethyl-1,2-diaminoethane **(29a)** – **R_f** **(**1:9 MeOH:CH_2Cl_2 +0.1 %TEA) = 0.3 . **IR**(KBr)/cm^{-1} – 3233, 2970, 1582. **^{1}H** NMR(500M*Hz*, MeOD) δ 8.37(1H, d, -N=*CH*-, *J*=5.1*Hz*), 8.04(1H, d, Ar-H, *J*=8.8*Hz*), 7.78(1H, d, Ar-H, *J*=2.0*Hz*), 7.41(1H, dd, Ar-H, *J*=2.0*Hz*, 6.9*Hz*), 6.55(1H, d, Ar-H, *J* = 5.1*Hz*), 3.48(2H, m, -NH-*CH_2*-, *J* = 6.3*Hz*), 2.82(2H, m, -N-*CH_2*-, *J* = 7.0*Hz*), 2.67(4H, q, -*CH_2*-CH_3-, *J*=7*Hz*), 1.34(6H, t, -CH_2-*CH_3*-, *J*=7.6*Hz*); **^{13}C** NMR(500 M*Hz*, MeOD) δ 152.6, 152.5, 149.7, 136.4, 127.7, 126.1, 124.1, 118.8, 99.7, 49.5, 48.1, 41.4, 11.6. **MS** (ES^+): m/z (%): 278.1 $[M+H]^+$. **LCMS** Purity -96%. Rt = 0.75 min. Eluent system – 0% - 100% ACN/H_2O+0.1% TFA

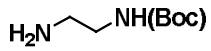


29b, n=2, 23%

N-(7-Chloro-4-quinolyl)-N′,N′-diethyl-1,4-diaminobutane **(29b)** – Following procedure described and purification by flash chromatography (2%-8% MeOH in CH_2Cl_2+0.1% TEA) gave 23% yield of pale-yellow crystals. **R_f** (MeOH:CH_2Cl_2+0.1%TEA) = 0.36. **IR** (KBr)/cm^{-1} – 3217, 2932, 2793, 1582. **^{1}H** NMR(500M*Hz*, $CDCl_3$) δ 8.51(1H, d, -N=*CH*-, *J*=5.7*Hz*), 7.94(1H, d, Ar-H, *J*=2.0*Hz*), 7.73(1H, d, Ar-H, *J*=9.5*Hz*), 7.34(1H, dd, Ar-H, *J*=2.0*Hz*, 6.9*Hz*), 6.38(1H, d, Ar-H, *J* = 5.1*Hz*), 6.00(1H, s, -NH-), 3.30(2H, t, -NH-*CH_2*-, *J* = 6.3*Hz*), 2.60(4H, q, -CH_2-CH_3-, *J* = 7.0*Hz*), 2.53(2H, m, -N-*CH_2*-), 1.84(2H, m, -NH-*CH_2*-CH_2-), 1.69(2H, m, -NH_2-CH_2-*CH_2*-), 1.05(6H, t, -CH_2-*CH_3*-, *J*=7.0*Hz*); **^{13}C** NMR(500 M*Hz*, $CDCl_3$) 152.7, 152.4, 149.7, 136.3, 127.6, 125.9, 124.3, 118.8, 99.7, 53.5, 47.8, 43.8, 27.5, 24.9, 11.1. **MS** (ES^+): m/z (%): 306.1 $[M+H]^+$. LCMS Purity -98%. Rt = 10.3min. Eluent system – 0% - 100% ACN/H_2O+0.1% TFA

5.1.5 General Experimental procedure for the synthesis of Monoboc diamine (**31, 38**)

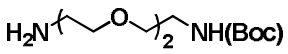
To a solution of the ethylene diamine **(30)** (3gms, 49.9mmol, 5equiv.) in (100ml) dry CH_2Cl_2 was added dropwise dilute solution (100ml of dry CH_2Cl_2) of boc anhydride (2.18gms, 9.99mmol, 1equiv.) under rigorous stirring in ice-water over a period of 40min under Ar atmosphere. The reaction is stirred for 12hrs and checked using TLC (9:1 CH_2Cl_2: MeOH +0.1% TEA). The reaction mixture is diluted with 50ml of dichloromethane and then washed with distilled water (100mL) and brine (50mL) to remove un-reacted diamine in a separating funnel. The CH_2Cl_2 layer is dried over Na_2SO_4 and concentrated in vacuum. The product can be directly used without further purification as viscous oil in 90% yield which was stored under Ar atmosphere at 20°C.



31

90%

1-(tert-Butyloxycarbonyl) ethyldiamine **(31)** – The residue can be used directly after work-up with **31** as viscous oil in 90% yield which was stored under Ar atmosphere at 20°C. $\mathbf{R_f}$ (9:1 CH_2Cl_2: MeOH +0.1% TEA) = 0.22 . **IR** (NaCl)/ cm^{-1} – 3364, 2878, 2932, 1697, 1528, 1172.7. ^{1}**H** NMR(500M*Hz*, $CDCl_3$) δ 4.92(1H, b, -*NH*-), 3.20(2H, t, *J*= 5*Hz*, -CH_2-NH-), 2.79(2H, t, *J*= 5*Hz*, -CH_2-NH-), 1.43(9H, s, -C$(CH_3)_3$). ^{13}**C** NMR(500 M*Hz*, $CDCl_3$) δ 156.2, 79.5, 43.3, 41.8, 28.3. **MS**(ES^+): m/z (%): 160.8 $[M+H]^+$, 201 $[M+CH_3CN]^+$.



38

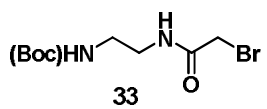
90%

N-Boc-1,8-diamino-3,6-dioxaoctane **(38)** – Following procedure product was obtained in 90% yield. $\mathbf{R_f}$ (9:1 CH_2Cl_2: MeOH +0.1% TEA) = 0.26 . **IR**(NaCl)/ cm^{-1} –

3372, 2924, 2870, 1697. 1528, 1173, 1111. **^{1}H** NMR(500M*Hz*, $CDCl_3$) δ 3.50(2H, d, *J*=28*Hz*, -CH_2-NHBoc), 3.42(2H, t, *J*=9.6*Hz*, *J*=9.0*Hz* m, -O-CH_2CH_2-O-), 3.19(2H, t, *J*= 8.3*Hz*, -O-CH_2CH_2-), 2.76(2H, t, *J*= 8.5*Hz*, -CH_2-NH_2-), 1.32(9H, s, -C$(CH_3)_3$). **^{13}C** NMR(500 M*Hz*, $CDCl_3$) δ 155.8, 78.8, 72.9, 69.9, 41.6, 40.0, 28.3. **MS**(ES^+): m/z (%): 249 $[M+H]^+$.

5.1.6 General experimental procedure for the synthesis of N-(tert-Butyloxycarbonyl)amino ethyl-2-bromoacetamide (**33,40**)

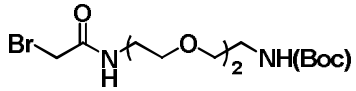
Bromoacetic acid (44mgs, 0.31mmol, 1equiv) was added to a single neck flask containing freshly distilled CH_2Cl_2 (10 ml). The solution was cooled to -10^{o}C using ice-methanol mixture. Di-isopropylethylamine (DIPEA) (81mgs, 0.62 mmol, 2equiv) was added immediately at this followed by addition (2-(7-Aza-1H-benztriazole-1-yl))-1,1,3,3,-tetramethylammonium hexafluorophosphate (HATU) (142mgs, 0.37mmol, 1.2equiv) and 3H-[1,2,3]triazolo[4,5-b] pyridine-3-ol (HOAt) (51mgs, 0.37mmol, 1.2 equiv) and after 10min of stirring 1-(tert-Butyloxycarbonyl) ethyldiamine (**31**) (50 mgs, 0.31mmol, 1equiv) is added in the sequence respectively. The reaction is maintained under Ar atmosphere and -10^{o}C till the addition of the above components is complete. The reaction is cooled to room temperature gradually and stirring is continued at room temperature till TLC shows the product spot. The product was isolated by washing the reaction mixture with water and separating the dichloromethane layer. Dichloromethane was distilled under vacuum and the residue was purified by flash chromatography (2% - 4% MeOH in$CHCl_3$+ 0.1% TEA) in 80% yield.



33

82%

N-(tert-Butyloxycarbonyl) aminoethyl-2-bromoacetamide **(33)** – Employing the procedure the residue was purified by flash chromatography (2% - 4% MeOH in$CHCl_3$+ 0.1% TEA) in 80% yield. $\mathbf{R_f}$ (MeOH:CH_2Cl_2+0.1%TEA) = 0.33 . **^{1}H** NMR(500M*Hz*, $CDCl_3$) δ 7.09(1H, b, -*NH*-CO-), 4.88(1H, b, -*NH*(Boc)), 3.86(2H, s, -CO-*CH_2*-Br), 3.40(2H, m, -NH-*CH_2*-), 3.29(2H, m, -*CH_2*-NH(Boc)), 1.45(9H, s, -C*$(CH_3)_3$*). **^{13}C** NMR(500 M*Hz*, $CDCl_3$) δ 166.3, 157.8, 79.9, 41.6, 39.8, 28.9, 28.4. **MS**(ES^+): m/z (%): 304.9 $[M+Na+H]^+$.HRMS (m/z) calc. - 303.0314, observed – 303.0314.



40

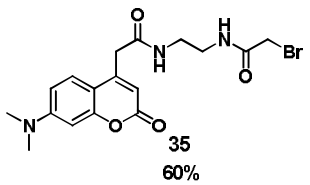
82%

N-(tert-Butyloxycarbonyl)aminoethoxyethoxyethyl-2-bromoacetamide**(40)** – Employing the above procedure and purification by flash chromatography in 90% yield. $\mathbf{R_f}$ (MeOH:CH_2Cl_2+0.1%TEA) = 0.36 . **^{1}H** NMR(500M*Hz*, $CDCl_3$) δ 1.43(9H, s, -C*$(CH_3)_3$*), 2.79(2H, s, -*CH_2*-NHCO-), 3.52(10H, m , -NH(Boc)-*CH_2*-*CH_2*-*O*-*CH_2*-*CH_2*-*O*-*CH_2*-), 4.04(1H, b, -CO-*CH_2*-Br), 4.98(1H, b, -*NH*(Boc)) 6.94(1H, b, -*NH*-CO-) . **^{13}C** NMR(500 M*Hz*, $CDCl_3$) δ 165.7, 155.9, 79.9, 70.3, 70.2, 70.1, 69.4, 40.3, 39.9, 29.0, 28.4. **MS** (ES^+): m/z (%): 390.9 $[M+Na]^+$.

5.1.7 General experimental procedure for the synthesis of (7-Coumarin-acetamide)aminoethyl -2-bromoacetamide (**35**)

N-(tert-Butyloxycarbonyl)aminoethyl-2-bromoacetamide (**33**) (60mgs, 0.21mmol, 1 equiv.) was dried overnight under vacuum and then placed under Ar atmosphere. 1ml of freshly distilled dichloromethane was added to above reactant. The reaction mixture was cooled to 4^{o}C using Ice bath and then Trifluoroacetic acid (TFA) (243mgs, 2.13mmol, 10equiv.) was added immediately under Ar atmosphere using a

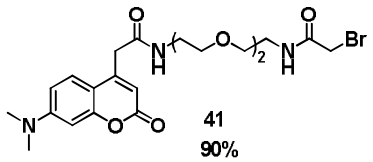
syringe. The reaction was checked after 1hr by TLC (9:1 CH_2Cl_2:MeOH + 0.1% TEA) for absence of starting material. After confirmation by TLC the reaction mixture is distilled under vacuum at 16-18^oC to remove excess TFA and CH_2Cl_2. The residue is flushed with argon to release the vacuum and then 2-4ml of freshly distilled dry ether is added to observe precipitation of the TFA salt of the amine. If no precipitation is observed then the ether is removed under vacuum and after flushing the TFA salt with Argon the residue is kept under vacuum line for 10min. The residue is placed under Ar atmosphere using a balloon and then 10-12ml of Dichloromethane (dry freshly distilled) was added. The reaction mixture is cooled to -10^oC using ice-methanol mixture. Di-isopropylethylamine (82.7 mgs, 0.64 mmol, 3equiv) was added immediately at this followed by addition of 7-Dimethyl coumarin-4-acetic acid **(34)** (53mgs, 0.21mmol, 1equiv), HATU (98mgs, 0.26 mmol, 1.2equiv) and HOAt (35 mgs, 0.26mmol, 1.2equiv) is added in the sequence respectively. The reaction is maintained under Ar atmosphere and -10^oC till the addition of the above components is complete. The reaction is cooled to room temperature gradually and stirring is continued at room temperature till TLC shows the product spot. The product was isolated by washing the reaction mixture with water and separating the dichloromethane layer. Dichloromethane was distilled under vacuum and the residue was purified by flash chromatography (2% - 4% MeOH in$CHCl_3$+ 0.1% TEA) in 60% yield.



35

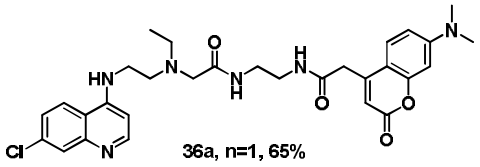
60%

(7-Coumarin-acetamide) aminoethyl-2-bromoacetamide **(35) –** Yield – 70% Compound is unstable and is to be used directly for next step. Hence characterization was not done on this molecule. **MS** (ES^+): m/z (%): 431.96 $[M+Na]^+$



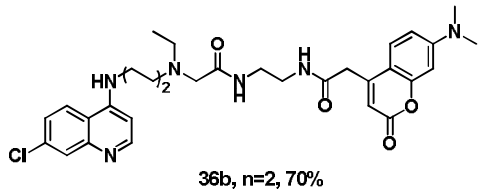
(7-Coumarin-acetamide) aminoethoxyethoxyethyl-2-bromoacetamide **(41)** - was Obtained following the above process and flash chromatography in 90% yield. Compound is unstable and is to be used directly for next step. Hence characterization was not done on this molecule.

5.1.8 General experimental procedure for the synthesis of Chloroquine based probes (**36a, 36b, 42**) - N-(7-Chloro-4-quinolyl)-N′-ethyl-1,2-diaminoethane (**28a**) (16.8mgs, 0.07mmol, 1equiv.) and freshly dried K_2CO_3 (18.6mgs, 0.135mmol, 2equiv.) were stirred together in a single neck flask under Ar atmosphere in 2-3ml of dry Acetonitrile. (7-Coumarin-acetamide)aminoethyl-2-bromo acetamide (**35**) (27.6mgs, 0.07mmol, 1equiv.) was added directly in dried powder form to the above reaction mixture at 4^oC (Ice water). Ice Water is removed after the addition is done and the reaction is stirred at room temperature for 12hrs. After confirmation of formation of product by TLC (9:1 CH_2Cl_2:MeOH+0.1%TEA). The reaction mixture is columned through celite and 50ml of Acetonitrile is added to extract the product from the column. Acetonitrile is distilled under vacuum and the residue is purified by flash chromatography (2% -6% MeOH in $CHCl_3$+0.1%TEA).



2-((2-(7-chloroquinolin-4-ylamino)ethyl)(ethyl)amino)-N-(2-(2-(7-(dimethylamino)-2-oxo-2H-chromen-4yl) acetamido) ethyl) acetamide **(36a)** – Following the above procedure and purification by flash chromatography (2% - 4% MeOH in

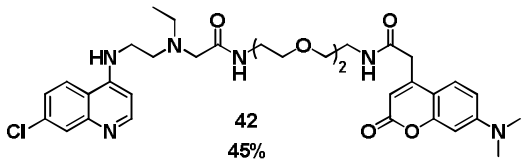
$CHCl_3$+0.1%TEA) the compound was obtained in 65% yield. **R_f** (1:9 MeOH:CH_2Cl_2+0.1%TEA) = 0.41. **IR**(KBr)/cm^{-1} – 3364, 2924, 2855, 1659, 1613, 1582. **^{1}H** NMR(500M*Hz*, MeOD) δ 8.29(1H, d, Chlor-*CH*-, *J*= 5.7*Hz*), 8.02(1H, d, Chlor-*CH*-, *J*= 9.5*Hz*), 7.71(1H, s, Cl-*C*=*CH*- , *J*= 2*Hz*), 7.41(1H, d, Cou-*CH*-, *J*=8.9*Hz*), 7.23(1H, dd, Ar-H, *J*=2.0*Hz*, 8.9*Hz*), 6.55(1H, dd, Ar-H , *J*=2.6*Hz*, 9.5*Hz*), 6.44(1H, d, Cou-*CH*, *J*= 5.7*Hz*), 0.95(3H, t, -CH_2-CH_3, *J*= 7.0*Hz*), 6.23(1H, d, -N=*CH*=*CH*-, *J*= 2.5*Hz*), 5.98(1H, s, Cou-*CH*), 3.38(6H, m, -NHCO-CH_2-N(CH_2CH_3), -CH_2-NH-, -CH_2-NHCO), 3.30(2H, t, -CH_2-NHCO-, *J*= 5.7*Hz*), 3.08(2H, s, Coum-CH_2-CONH-), 2.94(6H, s, -N$(CH_3)_2$), 2.70(2H, t, -N-CH_2-, *J*= 6.3*Hz*), 2.53(2H, q, -CH_2CH_3, *J*= 7.6*Hz*); **^{13}C** NMR(500 M*Hz*, MeOD) δ 174.9, 172.1, 164.1, 157.0, 153.0, 152.6, 151.5, 148.6, 136.7, 126.9, 126.7, 126.1, 124.8, 118.6, 110.5, 110.3, 109.8, 99.5, 98.5, 59.0, 53.7, 50.3, 41.2, 40.1, 40.0, 14.5, 12.2. **MS** (ES^+): m/z (%): 579.3 $[M+H]^+$. **HRMS** (ES^+) m/z calc. - 579.2497, observed – 579.2500. **LCMS** Purity - 96%. Rt = 8.33-8.47 min. Eluent system – 10% ACN/H_2O+0.1%TFA – 100% ACN/H_2O+0.1%TFA.



36b, n=2, 70%

2-((4-(7-chloroquinolin-4-ylamino)butyl)(ethyl)amino)-N-(2-(2-(7-(dimethylamino)-2-oxo-2H-chromen-4-yl)acetamido) ethyl) acetamide **(36b) –** Following the above procedure and purification by flash chromatography (2% -6% MeOH in $CHCl_3$+0.1%TEA) the compound was obtained in 70% yield. **R_f** (1:9 MeOH:CH_2Cl_2+0.1%TEA) = 0.44. **IR** (NaCl) / cm^{-1} – 3333, 2932, 2862, 1713, 1613, 1582, 1528. **^{1}H** NMR(500M*Hz*, MeOD) δ 8.29 (1H, d, Ar-H, *J*= 5.8 *Hz*), 7.99 (1H, Ar-H, d, *J*= 9.1 *Hz*), 7.71 (1H, d, Ar-H, *J*= 2.0 *Hz*), 7.42 (1H, d, Ar-H, *J*= 8.8 *Hz*),

7.25 (1H, dd, Ar-H, *J*= 2.0 *Hz*, 8.8 *Hz*), 6.55 (1H, dd, Ar-H, *J*=2.6 *Hz*, 9.1 *Hz*), 6.43 (1H, d, Ar-H, *J*= 5.8 *Hz*), 6.34 (1H, d, Ar-H, *J*= 2.3*Hz*), 5.98 (1H, s), 3.63 (2H, s), 3.38-3.40 (4H, m), 3.21 (2H, m), 2.97 (8H, bs), 2.41 (2H , t, *J*= 7.0 *Hz*), 2.49 (2H, q, *J*= 7.0 *Hz*), 1.62-1.74 (2H, -*CH*$_2$-*CH*$_2$-*CH*$_2$-*CH*$_2$-N((CH$_2$CH$_3$)(CH$_2$)), m), 1.52-1.57 (2H, m, NH-*CH*$_2$-*CH*$_2$-*CH*$_2$-), 0.87 (3H, t, -CH$_2$*CH*$_3$, *J*= 6.4 *Hz*),; **^{13}C** NMR(500 M*Hz*, MeOD) δ 175.2, 171.5, 164.2, 157.1, 154.6, 152.2, 149.4, 152.7, 136.2, 127.3, 126.8, 125.8, 124.5, 118.7, 110.5, 110.3, 109.7, 99.6, 98.6, 58.7, 55.7, 50.0, 47.4, 44.0, 40.2, 40.1, 40.0, 27.1, 26.2, 12.1. **MS** (ES$^+$): m/z (%): 608.3 [M+2H]$^+$. **HRMS** (ES$^+$) m/z calculated 608.2848, observed - 608.2847. **LCMS** Purity -96%. Rt = 8.73-8.89 min. Eluent system – 10% ACN/H$_2$O+0.1%TFA – 100% ACN/H$_2$O+0.1%TFA.



42

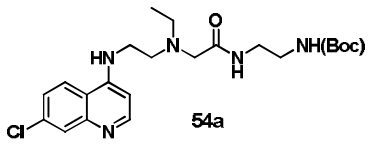
45%

N-(1-(7-chloroquinolin-4-ylamino)-3-ethyl-5-oxo-9,12-dioxa-3,6-diazatetradecan-14-yl)-2-(7-(dimethylamino)-2-oxo-2H-chromen-4-yl)acetamide **(42)** – Following the above procedure and purification by flash chromatography (2% - 4% MeOH in CHCl$_3$+0.1%TEA) the compound was obtained in 60% yield. **R$_f$** (1:9 MeOH:CH$_2$Cl$_2$+0.1%TEA) = 0.56. **IR** (NaCl)/cm^{-1} – 3279, 3071, 2924, 2862, 1713, 1613, 1528. **^{1}H** NMR (500M*Hz*, CDCl$_3$) δ 8.29(2H, d, Chlor-*CH*-, d, , *J*= 5.7*Hz*), 7.76(1H, d, Chlor-*CH*-, *J*= 1.9*Hz*), 7.52(2H, dd, Cou-*CH*-, Cl-C-*CH*$_2$-, *J*= 1.9*Hz*, 8.8*Hz*), 6.68(1H, dd, Cou-*CH*-, Cou-*CH*-, *J*= 2.5*Hz*, 2.6*Hz*), 6.41(1H, d, -N=*CH*=*CH*- , *J*= 2.5*Hz*), 6.01(1H, s, Cou-*CH*-), 3.55(16H, m), 3.25 (2H, s, Coum-*CH*$_2$-CONH-), 3.03(6H, s, -N*(CH$_3$)$_2$*), 2.89 (2H, t, -N-*CH*$_2$-, *J*= 6.3*Hz*), 2.69 (2H, q, -*CH*$_2$CH$_3$, *J*= 7.0*Hz*), 1.07 (3H, t, -CH$_2$*CH*$_3$, *J*= 7.5*Hz*); **^{13}C** NMR(500 M*Hz*, CDCl$_3$) δ 172.0, 168.3, 162.1, 155.9, 153.1, 126.8, 125.8, 124.1, 116.1, 109.5, 109.3, 108.3, 98.0, 97.9, 70.2,

69.6, 69.5, 57.2, 52.2, 48.7, 42.1, 40.1, 39.5, 39.1, 12.0. **MS**(ES^+): m/z (%): 667.4 $[M]^+$. **HRMS** (ES^+) m/z calc. - 668.3100, obsvd. – 668.3098. **LCMS** Purity -96%. Rt = 8.83-8.87 min. Eluent system– 10% ACN/H_2O+0.1%TFA – 100% ACN/H_2O+0.1%TFA.

5.1.9 General procedure for synthesis of tert-butyl 2-(2-((2-(7-chloroquinolin-4-ylamino)ethyl)(ethyl)amino)acetamido)ethylcarbamate and tert-butyl 2-(2-((4-(7-chloroquinolin-4-ylamino)butyl)(ethyl)amino)acetamido)ethylcarbamate **(54a, 54b)**

N-(7-Chloro-4-quinolyl)-N′-ethyl-1,2-diaminoethane (**28a**) (100mgs, 0.4mmol, 1equiv.) and freshly dried K_2CO_3 (166mgs, 1.2mmol, 3equiv.) were stirred together in a single neck flask under Ar atmosphere in 2-3ml of dry Acetonitrile. N-(tert-Butyloxycarbonyl) aminoethyl-2-bromoacetamide **(33)** (170mgs, 0.6mmol, 1.5equiv.) was added as Acetonitrile solution to the above reaction mixture at 0^oC (Ice water). Ice Water is removed after the addition is done and the reaction is stirred at room temperature for 12hrs. After confirmation of formation of product by TLC (10% MeOH: CH_2Cl_2). The reaction mixture is columned through celite and 50ml of Acetonitrile is added to extract the product from the column. Acetonitrile is distilled under vacuum and the residue is purified by flash chromatography (6% -8% MeOH in $CHCl_3$+0.1%TEA).

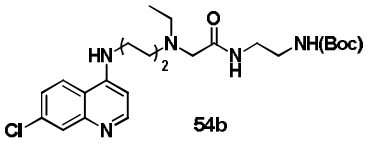


54a

tert-butyl 2-(2-((2-(7-chloroquinolin-4-ylamino)ethyl)(ethyl)amino)acetamido) ethyl carbamate **(54a)** –

Yields – 70%. $\mathbf{R_f}$ (1:9 MeOH:CH_2Cl_2+0.1%TEA) = 0.45. IR (KBr)/cm^{-1} – 3306, 2972, 2939, 1694, 1668, 1613, 1541, 1451. ^{1}**H** NMR(500M*Hz*, MeOD) δ 8.37(1H, d,

-N=*CH*-, *J*=5.7*Hz*), 8.31(1H, d, Ar-H, *J*=8.9*Hz*), 7.80(1H, d, Ar-H, *J*=2.0*Hz*), 7.51(1H, dd, Ar-H, *J*=2.0*Hz*, 6.9*Hz*), 6.63(1H, d, Ar-H, *J* = 5.7*Hz*), 2.87-3.25 (10H, m, -CH_2-NH(Boc), NH-CH_2-, -NH_2-CH_2-CH_2-, -NH-CH_2-CH_2-, -CO-CH_2-N), 2.67(4H, q, -CH_2-CH_3-, *J* = 7.0*Hz*), 1.40(9H, s, -C$(CH_3)_3$),1.07(3H, t, -CH_2-CH_3 -, *J*=7.0*Hz*); **^{13}C** NMR(500 M*Hz*, MeOD) δ 174.6, 154.1, 149.9, 147.0, 137.8, 126.9, 125.5, 125.1, 118.3, 99.7, 80.1, 58.4, 53.6, 50.0, 42.6, 40.9, 40.6, 28.8, 12.2. **MS** (ES^+): m/z (%): 450.1 $[M+H]^+$. **HRMS** (ES^+) m/z calc. - 450.2266, obsvd. – 450.2284.



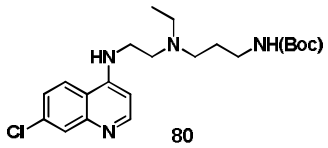
54b

tert-butyl 2-(2-((4-(7-chloroquinolin-4-ylamino)butyl)(ethyl)amino)acetamido)ethyl carbamate **(54b)** –

Yields – 70%, **R_f** (1:9 MeOH:CH_2Cl_2+0.1%TEA) = 0.4. IR (KBr)/cm^{-1} – 3306, 2957, 2924, 2853, 1695, 1676, 1655, 1582, 1533, 1451. **^{1}H** NMR(500M*Hz*, $CDCl_3$) δ 8.39(1H, d, -N=CH-),7.85(1H, d, Ar-CH, *J*=9.45*Hz*), 7.57(1H, d, Ar-H, *J*=2.5*Hz*), 7.22(1H, dd, Ar-H, *J*=2*Hz*,8.2*Hz*), 6.29(1H, d, Ar-H, *J* = 5.7*Hz*), 3.22-3.32 (6H, m), 2.98(2H, s, -CO-CH_2-N-), 2.47(4H, m), 1.50-1.73(4H, m, -NH_2-CH_2-CH_2-CH_2-CH_2-OH), 1.37(9H, s, -C$(CH_3)_3$), 0.96(3H, t, -CH_2-CH_3 -, *J*=7.0*Hz*), ; **^{13}C** NMR(500 M*Hz*, $CDCl_3$) δ 173.3, 157.2, 152.1,150.9, 149.3, 135.4, 128.5, 125.6, 122.6, 117.9, 99.3, 80.1, 58.4, 55.1, 54.0, 49.7, 43.7, 41.1, 39.9, 29.0, 28.9, 26.9, 25.7, 12.6. **MS**(ES^+): m/z (%): 478.1 $[M+H]^+$. HRMS (ES^+) – observed – 478.2585; calculated. - 478.2579.

5.1.10 General procedure for synthesis of tert-butyl 3-((2-(7-chloroquinolin-4-ylamino)ethyl)(ethyl)amino)propylcarbamate**(80)** - N-(7-Chloro-4-quinolyl)-N′-ethyl-1,2-diaminoethane (**28a**) (400mgs, 1.6mmol, 1equiv.) and TEA (242mgs, 3.2mmol,

2.0equiv.) and tert-butyl 3-bromopropylcarbamate (649mgs, 2.4mmol, 1.5equiv.) were stirred together in a single neck flask under Ar atmosphere in 3ml of dry DMF. The reaction mixture was heated to 80^{o}C under inert atmosphere for 12-16hrs. After confirmation of formation of product by TLC (10% MeOH:CH_2Cl_2), DMF was removed by evaporation under vacuum. The residue was purified by flash chromatography (6% -8% MeOH in $CHCl_3$+0.1%TEA).



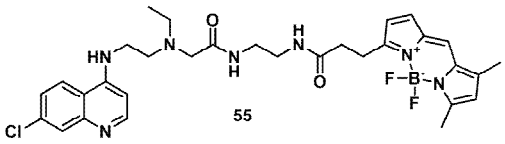
80

tert-butyl3-((2-(7-chloroquinolin-4-ylamino)ethyl)(ethyl)amino)propylcarbamate**(80)**– Yields – 85%, $\mathbf{R_f}$ – 0.55 (10% MeOH:CH_2Cl_2), **IR** (KBr)/cm^{-1} – 3421.72, 2974.23, 2973.59, 2756.28, 2738.92, 2490.1, 1689.64, 1614.42, 1581.63. ^{1}**H** NMR(500M*Hz*, MeOD) δ 8.28(1H, d, -N=*CH*-, *J*=5.1*Hz*), 7.99(1H, d, Ar-H, *J*=8.9*Hz*), 7.69(1H, d, Ar-H, *J*=2.0*Hz*), 7.32(1H, dd, Ar-H, *J*=2.0*Hz*,11.6*Hz*), 6.47(1H, d, Ar-H, *J* = 5.7*Hz*), 3.50(1H, m), 2.50-3.24(10H, m, -*CH*$_2$-CH$_3$-, *J* = 7.0*Hz*), 1.53-1.60(2H, m), 1.31(9H, s, -C(*CH*$_3$)$_3$), 0.99(3H, t, -CH$_2$-*CH*$_3$ -, *J*=7.0*Hz*),; ^{13}**C** NMR(500 M*Hz*, MeOD) δ 158.5, 152.9, 151.7, 148.8, 136.7, 127.0, 126.3, 124.3, 118.6, 99.8, 89.0, 79.9, 52.5, 51.9, 41.5, 39.5, 28.8, 28.0, 11.7. **MS** (ES$^+$): m/z (%): 407.1[M+H]$^+$. **HRMS** (ES$^+$) m/z calc. – 407.2222 , observed – 407.2225.

5.1.11 General procedure for synthesis of **(55 and 92)**

Tert-butyl 2-(2-((2-(7-chloroquinolin-4-ylamino)ethyl)(ethyl)amino)acetamido) ethyl carbamate (**54a**) (100 mgs, 0.22 mmol, 1.0 equiv.) is added to flame dried single neck flask under argon containing dried 1mL dichloromethane. To this solution trifluoroacetic acid (634 mgs(414 µL), 5.6mmol, 25.0 equiv.) is added at 0^{o}C and the reaction mixture is stirred till all the boc amine is converted to trifluoroacetate salt of

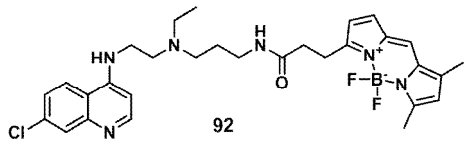
amine as per TLC. After confirmation by TLC, the solvent is removed under vacuum and vacuum is released under Ar atmosphere. Dry ether is added to precipitate the free amine. In case no precipitation is observed the solution is dried under vacuum for 15min and then kept under argon. Fresh dried dichloromethane is added to the reaction mixture and then its cooled to -10^{o}C. DIPEA (718 mgs(970µL), 5.6mmol, 25equiv.) is added at -10^{o}C followed by HATU (126mgs, 0.33mmol, 1.5equiv) and HOAt (45mgs, 0.33mmol, 1.5equiv) and finally BODIPY (**84**) (65 mgs, 0.22 mmol, 1equiv) is added to the reaction mixture at -10^{o}C. The reaction mixture is warmed to room temperature and stirred at this temperature for 12-16hrs. Upon confirmation by TLC the solvent is removed under vacuum and the crude is purified by column chromatography (4% -8% MeOH in $CHCl_3$).



55

3-(3-(2-(2-((2-(7-chloroquinolin-4-ylamino)ethyl)(ethyl)amino)acetamido) ethylamino)-3-oxopropyl)-5,5-difluoro-7,9-dimethyl-5H-dipyrrolo[1,2-c:1',2'-f][1,3,2]diazaborinin-4-ium-5-uide **(55)** – Following the above procedure and purification by flash chromatography (4% - 8% MeOH in CH_2Cl_2) the compound was obtained in 70% yield. $\mathbf{R_f}$ (1:9 MeOH:CH_2Cl_2) = 0.45. **IR** (KBr)/cm^{-1} – 3412, 2950, 1608, 1448, 1383, 1252, 1136, 1084. ^{1}**H** NMR (500M*Hz*, MeOD) δ 8.33(1H, d, *J*= 9*Hz*, Ar-H), 8.25(1H, d, *J*= 6.3*Hz*, Ar-H), 7.69(1H, d, *J*= 2*Hz*, Ar-H), 7.42 (1H, dd, *J*= 2*Hz*, 6.9*Hz*, Ar-H), 7.26(1H, s, Ar-H), 6.90(1H, d, *J*= 4*Hz*, Ar-H), 6.58(1H, d, *J*=6.95*Hz*, Ar-H), 6.23(1H, s, Ar-H), 6.10(1H, s, Ar-H), 3.68-3.73(2H, m), 3.46(2H, t, *J*= 6.4*Hz*, NHCO-CH_2-*CH_2*-), 3.30-3.35(4H, m & s, N-*CH_2*-CO-), 3.14-3.23(2H, m), 2.82(2H, t, *J*= 6.3*Hz*, NHCO-CH_2-*CH_2*-), 2.59-2.66(4H, m, N-*CH_2*-CH_3-), 2.39(3H, s, BODIPY-*CH_3*), 2.20(3H, s, BODIPY-*CH_3*), 1.03(3H, t, *J*= 6.9*Hz*, N-CH_2-*CH_3*-); ^{13}**C**

NMR (500M*Hz*, MeOD) δ 175.2, 174.6, 161.2, 158.3, 155.1, 147.5, 145.7, 144.2, 138.6, 136.4, 134.7, 129.5, 127.3, 125.7, 125.6, 123.3, 121.3, 117.7, 117.1, 99.6, 58.5, 55.8, 53.4, 50.1, 43.8, 42.9, 40.6, 39.9, 35.5, 25.4, 18.0, 14.9, 13.2, 12.2, 11.3. **MS** (ES^+): m/z (%): 624.25 $[M+H]^+$. **HRMS**(ES^+) m/z calc. – 624.2837 , observed – 624.2861. **LCMS** Purity -96%. Rt = 8.73-8.89 min. Eluent system – 10% ACN/H_2O+0.1%TFA – 100% ACN/H_2O+0.1%TFA.

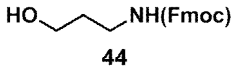


92

3-(3-(3-((2-(7-chloroquinolin-4-ylamino)ethyl)(ethyl)amino)propylamino)-3-oxopropyl)-5,5-difluoro-7,9-dimethyl-5H-dipyrrolo[1,2-c:1',2'-f][1,3,2]diazaborinin-4-ium-5-uide (**92**) – Following the above procedure and purification by flash chromatography (6% - 10% MeOH in $CHCl_3$+0%TEA) the compound was obtained in 65% yield. $\mathbf{R_f}$ (1:9 MeOH: CH_2Cl_2) = 0.40. **IR** (KBr)/cm^{-1} – 2954, 2920, 2851, 1608, 1465, 1251, 1136. ^{1}**H** NMR(500M*Hz*, MeOD) δ 8.32(1H, d, -N=*CH*-, *J*=6.3*Hz*), 8.14(1H, d, Ar-H, *J*=8.9*Hz*), 7.74(1H, d, Ar-H, *J*=2.0*Hz*), 7.45(1H, dd, Ar-H, *J*=2.0*Hz, J*=6.9*Hz*), 7.27(1H, s, Ar-H), 6.94(1H, d, Ar-H, *J* = 4*Hz*), 6.59(1H, d, Ar-H, *J* = 5.7*Hz*), 6.30(1H,d, Ar-H, *J*= 4*Hz*), 6.09(1H, s, Ar-H), 3.49(2H, t, *J*=6.3*Hz*), 3.18-3.27 (4H, m), 2.60-2.78(6H,m), 2.43 (3H, s, BODIPY-*CH_3*), 2.15 (3H, s, BODIPY-*CH_3*), 1.74(2H, q, -*CH_2*-*CH_3*-, *J*=7.0*Hz*), 1.08(3H, t, -CH_2-*CH_3*-, *J*=7.0*Hz*); ^{13}**C** NMR(500 M*Hz*, MeOD) δ 174.9, 161.4, 158.3, 154.1, 149.3, 146, 145.8, 136.5, 134.8, 129.5, 127.1, 125.6, 125.0, 124.9, 118.1, 117.3, 99.8, 83.6, 71.3, 55.8, 54.8, 52.2, 51.7, 43.8, 41.3, 38.1, 35.6, 30.7, 27.1, 25.6, 18.0, 14.9, 13.2, 11.2, 11.1. **MS** (ES^+): m/z (%): 581.23 $[M+H]^+$. **HRMS**(ES^+) m/z calc. – 581.2778 , observed – 581.2777. **LCMS** Purity -96%. Rt = 11.9-12.2 min. Eluent system – 0% ACN/H_2O+0.1%TFA – 100% ACN/H_2O+0.1%TFA.

5.1.12 General experimental procedure for synthesis of (9H-fluoren-9-yl)methyl 3-hydroxy propylcarbamate (**44**)

To a solution of the 3-amino propanol (**43**) (3.76gms, 50.0mmol, 1equiv.) in (10ml) dry CH_2Cl_2 was added dropwise Fmoc-OSu (8.92gms, 50.0 mmol, 1equiv.) under rigorous stirring in ice-water over a period of 40min under Ar atmosphere. The reaction is stirred for 12hrs and checked using TLC (9:1 CH_2Cl_2: MeOH +0.1% TEA). The reaction mixture is diluted with 50ml of dichloromethane and then washed with distilled water (50mL) and brine water (25mL) to remove un-reacted amine in a separating funnel. The CH_2Cl_2 layer is dried over Na_2SO_4 and concentrated in vacuum to obtain viscous oil which is directly used for the next step without purification. The product is obtained as white solid (**44**) 70% yield.



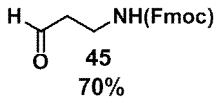
44

90%

(9H-fluoren-9-yl)methyl 3-hydroxypropylcarbamate **(44)** - The product is obtained as white solid in 70% yield. $\mathbf{R_f}$ (1:9 MeOH:CH_2Cl_2+0.1%TEA) = 0.75. **IR** (KBr)/cm^{-1} – 3327, 2947, 2885, 1692, 1543, 1445, 1267. ^{1}**H** (300M*Hz*, $CDCl_3$) δ 7.76(2H, d, Ar-*CH*-, *J*= 4.6*Hz*), 7.59(2H, d, Ar-*CH*-, *J*= 4.6*Hz*), 7.40(2H, m, Ar-*CH*-, *J*= 7.4*Hz*), 7.32(2H, m, Ar-*CH*-, *J*= 7.4*Hz*), 5.00(1H, b, -*NH*-), 4.44(2H, d, *J*= 6.6*Hz*), 4.22(1H, t, -CO_2-CH_2-*CH*-, *J*=6.6*Hz*), 3.65(2H, d, -CONH-*CH_2*-, *J*= 5.4*Hz*), 3.36(2H,m, -*CH_2*-CH_2-OH), 2.46(1H, b, -*OH*-), 1.70(2H, m, -CH_2-*CH_2*-OH); ^{13}**C** (300 M*Hz*, $CDCl_3$) δ 157.4, 143.9, 141.3, 127.7, 127.0, 125.0, 120.0, 66.7, 59.5, 47.3, 37.6, 32.6. **MS** (ES^+): m/z (%): 297.9 $[M]^{+,}$ 320.18 $[M+Na]^+$.

5.1.13 General experimental procedure for synthesis of (9H-fluoren-9-yl)methyl 3-oxo propyl carbamate (**45**)

(9H-fluoren-9-yl) methyl 3-oxopropylcarbamate (**44**) (0.10gms, 0.34 mmol, 1equiv.) was added to 2 ml of freshly distilled dry dichloromethane and Dess-Martin periodinane (0.357gms, 0.84mmol, 2.5equiv.) was added to the reaction mixture at room temperature. The reaction mixture is monitored by TLC till the presence of aldehyde is observed. After reaction is complete the reaction mixture is filtered and the dichloromethane extract is vacuum distilled to obtain a white solid which is further purified by flash chromatography (25% - 50% EtOAc in Hexane) in 70% yield.



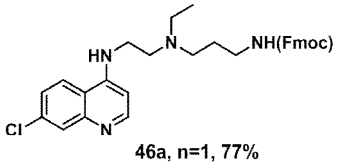
45
70%

(9H-fluoren-9-yl)methyl 3-oxopropylcarbamate **(45)** – $\mathbf{R_f}$ (1:1 EtOAc:Hex) = 0.88. **IR** (KBr)/cm^{-1} – 3329, 2957, 2925, 2855, 1725, 1691, 1543, 1269. ^{1}**H** (300M*Hz*, $CDCl_3$) δ 9.81(1H, s, -*CH*O-), 7.76(2H, d, Ar-*CH*-, *J*= 7.4*Hz*), 7.56(2H, d, Ar-*CH*-, *J*= 7.5*Hz*), 7.40(2H, t, Ar-*CH*-, *J*= 7.4*Hz*), 7.31(2H, t, Ar-*CH*-, *J*= 7.4*Hz*), 5.51(1H, b, -NH-), 4.39(2H, d, *J*= 6.9*Hz*), 4.20(1H, t, -O-CH_2-*CH*-, *J*=6.9*Hz*), 3.49(2H, m, -CH_2-CHO, *J*= 3.4*Hz*), 2.75(2H, t, -CONH-CH_2-, *J*=3.4*Hz*), ;^{13}**C** (300 M*Hz*, $CDCl_3$) δ 201.2, 156.3, 143.9, 141.3, 127.7, 127.0, 125.0, 120.0, 66.7, 47.3, 44.1, 34.5. **MS** (ES^+): m/z (%): 296.00 $[M+H]^+$.

5.1.14 General experimental procedure for synthesis of (9H-fluoren-9-yl) methyl 3-((2-(7-chloroquinolin-4-ylamino)ethyl)(ethyl) amino) propylcarbamate (**46a. 46b**)

(9H-fluoren-9-yl)methyl 3-oxopropylcarbamate (**45**) (0.036gms, 0.12 mmol, 2 equiv.) was dried under vacuum overnight and then about 5-8ml of freshly distilled dried CH_2Cl_2 was added to the single neck flask kept under Ar atmosphere. N-(7-Chloro-4-

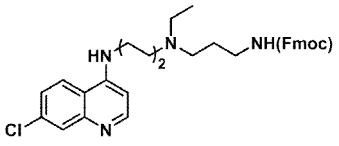
quinolyl)-N′-ethyl-1,2-diamino ethane (**28a**) (0.015gms, 0.06 mmol, 1 equiv.) was added to the reaction mixture. The reaction mixture was cooled to 4^{o}C (using Ice Water) and then $NaBH(OAc)_3$ (0.025gms, 0.12 mmol, 2.0) was weighed quickly and added immediately to the reaction mixture. The reaction mixture was stirred at room temperature till TLC shows formation of product. After the reaction is complete distilled water is added to the reaction mixture and then 4-5ml of saturated $NaHCO_3$ is added to the reaction mixture. The mixture is then extracted with ethyl acetate and the ethyl acetate extract is vacuum distilled and further the residue is purified by flash chromatography (2% - 4% MeOH in $CHCl_3$+ 0.1% TEA) to obtain the product in (0.025) 70% yield.



46a, n=1, 77%

(9H-fluoren-9-yl) methyl 3-((2-(7-chloroquinolin-4-ylamino)ethyl)(ethyl) amino) propylcarbamate **(46a)** – The residue is purified by flash chromatography (2% - 4% MeOH in $CHCl_3$+ 0.1% TEA) to obtain the product in 70% yield. $\mathbf{R_f}$ (1:9 MeOH:CH_2Cl_2+0.1%TEA) = 0.35 . **IR** (KBr)/cm^{-1} – 3384, 2959, 2925, 1645, 1457, 1378. ^{1}**H** (500M*Hz*, $CDCl_3$) δ 8.51(1H, d, Ar-*CH*, *J*= 4.7*Hz*), 7.94(1H, d, Ar-*CH*, *J*= 1.9*Hz*), 7.74(2H, d, Ar-*CH*, *J*= 7.6*Hz*), 7.69(1H, d, Ar-*CH*, *J*=8.9*Hz*), 7.54(2H, d, Ar-*CH*, *J*= 7.6*Hz*), 7.38(2H, t, Ar-*CH*, *J*= 7.6*Hz*), 7.30(1H, dd, Ar-*CH*, *J*=2.6, 9.1*Hz*), 7.27(2H, t, Ar-*CH*, *J*= 7.6*Hz*), 6.35(1H, d, Ar-CH, *J*=5.1*Hz*), 5.87(1H, b, -*NH*-Fmoc), 5.14(1H, b, -*NH*-Chloroquine), 4.39(2H, d, -O-*CH_2*-CH-, *J*= 6.9*Hz*), 4.17(1H, t, -O-CH_2-*CH*-, *J*=6.9*Hz*), 3.27(2H, m, -*CH_2*-NH(Fmoc), *J*=5.7*Hz*), 2.79(2H, m, NCH_2CH_3-*CH_2*-, *J*=5.7*Hz*), 2.59(2H, m, -*CH_2*-NCH_2CH_3, *J*=7.6*Hz*), 2.56(2H, q, -N*CH_2*CH_3, *J*=6.9*Hz*, 12.6*Hz*), 1.69(4H, m, -NH-*CH_2*-*CH_2*-NCH_2CH_3), 1.65 (2H, m, -CONH-*CH2*CH_2*CH_2*-), 1.05(3H, t, -NCH_2*CH_3*, *J*=6.9*Hz*); ^{13}**C**(500 M*Hz*, $CDCl_3$) δ

156.4, 152.0, 149.7, 149.1, 143.9, 141.3, 134.8, 128.7, 127.6, 127.0, 125.3, 124.9, 121.3, 119.4, 117.4, 99.2, 66.4, 51.5, 50.8, 47.3, 46.8, 40.1, 39.5, 27.4, 11.7. **MS** (ES^+): m/z (%): 529.2 $[M]^+$. **HRMS** (ES^+) m/z calc. - 529.2378, observed – 529.2377.

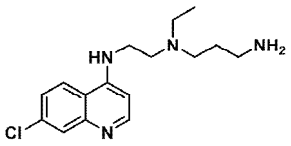


46b, n=2, 70%

(9H-fluoren-9-yl)methyl3-((4-(7-chloroquinolin-4-ylamino)butyl)(ethyl)amino)propyl carbamate **(46b)** – Following the above procedure using N-(7-Chloro-4-quinolyl)-N′-ethyl-1,4-diaminobutane (**28b**) (0.015gms, 0.05 mmol, 1 equiv.) and purification by flash chromatography (2% -6% MeOH in $CHCl_3$+0.1%TEA) gave 77% yield. $\mathbf{R_f}$ (1:9 MeOH:CH_2Cl_2+0.1%TEA) = 0.26. **IR** (KBr)/cm^{-1} – 3384, 2958, 2924, 1645, 1564, 1456, 1378. ^{1}**H** (500M*Hz*, $CDCl_3$) δ 8.49(1H, d, Ar-*CH*, *J*= 5.1*Hz*), 7.94(1H, d, Ar-*CH*, *J*= 1.9*Hz*), 7.74(2H, d, Ar-*CH*, *J*= 7.6*Hz*), 7.54(2H, d, Ar-*CH*, *J*= 7.6*Hz*), 7.38(2H, t, Ar-*CH*, *J*= 7.6*Hz*), 7.30(1H, dd, Ar-*CH*, *J*=2.6, 9.1*Hz*), 7.27(2H, t, Ar-*CH*, *J*= 7.6*Hz*), 6.35(1H, d, Ar-*CH*, *J*=5.1*Hz*), 5.14(1H, b, -*NH*-Fmoc), 5.14(1H, b, -*NH*-Chloroquine), 4.39(2H, d, -O-CH_2-CH-, *J*= 5.7*Hz*), 4.17(1H, t, -O-CH_2-*CH*-, *J*=6.9*Hz*), 3.26(2H, m, -CH_2-NH(Fmoc), *J*= 5.7*Hz*), 2.62(2H, m, NCH_2CH_3-CH_2- , *J*=5.7*Hz*), 2.56(2H, q, -NCH_2CH_3, *J*=6.9, 12,6*Hz*), 2.48(4H, m, -CH_2-NCH_2CH_3), 1.69(2H, m, -NH-CH_2-CH_2-N CH_2CH_3, *J*=6.3*Hz*), 1.65 (2H, m, -CONH-$CH_2CH_2CH_2$-), 1.05(3H, t, -NCH_2CH_3, *J*=6.9*Hz*); ^{13}**C** (500 M*Hz*, $CDCl_3$) δ 156.4, 152.0, 149.8, 149.1, 144.0, 141.3, 134.7, 128.7, 127.6, 127.0, 126.9, 125.0, 124.9, 121.1, 120.0, 98.9, 66.4, 53.0, 52.4, 47.3, 46.2, 43.2, 40.8, 26.8, 26.5, 25.1, 11.5. **MS** (ES^+): m/z (%): 557.3 $[M]^+$. **HRMS** (ES^+) m/z calc. - 557.2691, observed – 557.2691.

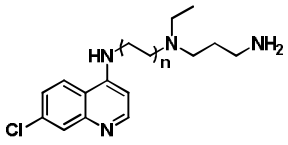
5.1.15 General experimental procedure for synthesis of N1-(2-(7-chloroquinolin-4-ylamino)ethyl)-N1-ethylpropane-1,3-diamine (**47a. 47b**)

(9H-fluoren-9-yl)methyl3-((2-(7-chloroquinolin-4-ylamino)ethyl)(ethyl)amino)propyl carbamate (**46a**) (0.025gms, 0.01 mmol, 1 equiv.) was dried overnight under vacuum and then about 1-2ml of freshly distilled dry CH_2Cl_2 was added to the single neck flask under Ar atmosphere. The reaction mixture was cooled to 4^oC (using Ice water) and then piperidine (0.035gms, 0.05 mmol, 5 equiv.) was added to the reaction mixture immediately with vigorous stirring. The reaction mixture was stirred at room temperature till TLC shows product formation. After completion of the reaction as observed by TLC the reaction mixture was distilled under vacuum to remove piperidine and then the compound is purified by flash chromatography 15-25% MeOH in $CHCl_3$+0.1%TEA to get 13mgs of crude product.



47a, n=1

N1-(2-(7-chloroquinolin-4-ylamino)ethyl)-N1-ethylpropane-1,3-diamine **(47a)** - The crude is purified by flash chromatography 15-25% MeOH in $CHCl_3$+0.1%TEA to get 85% yield of crude product. **^{1}H** (500M*Hz*, $CDCl_3$) δ 8.51(1H, d, Ar-*CH*, *J*= 5.7*Hz*), 7.92(1H, d, Ar-*CH*, *J*= 1.9*Hz*), 7.74(1H, d, Ar-*CH*, *J*= 8.8*Hz*), 7.35(1H, dd, Ar-*CH*, *J*=2.6, 9.1*Hz*), 6.35(1H, d, Ar-*CH*, *J*=5.1*Hz*), 3.30(2H, m, NCH_2CH_3-CH_2-, *J*=5.7*Hz*), 2.82(2H, t, -CH_2-N(CH_2 CH_3), *J*=4.4*Hz*), 2.60(6H, m, -NCH_2CH_3, -NH-CH_2-CH_2-NCH_2CH_3, -CH_2-NH_2), 1.65 (2H, m, -CONH-$CH_2CH_2CH_2$-), 1.07(3H, t, -NCH_2CH_3, *J*=6.9*Hz*); **^{13}C** (500 M*Hz*, $CDCl_3$) δ 152.0, 150.0, 149.0, 134.8, 128.6, 125.3, 121.4, 117.5, 99.2, 51.3, 51.2, 47.1, 40.5, 40.1, 30.0, 11.8. **MS**(ES^+): m/z (%): 307.6 $[M+H]^+$.



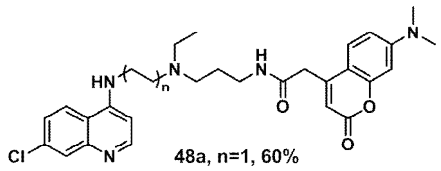
47b, n=2

N1-(3-aminopropyl)-N4-(7-chloroquinolin-4-yl)-N1-ethylbutane-1,4-diamine **(47b)** – Following the above procedure using (9H-fluoren-9-yl)methyl3-((4-(7-chloroquinolin-4-ylamino) butyl)(ethyl)amino)propylcarbamate **(46b)** (0.025gms, 0.01 mmol, 1 equiv.) and the compound was obtained in 80% crude product. **^{1}H** (500M*Hz*, $CDCl_3$) δ 8.51(1H, d, Ar-*CH*, *J*= 5.7*Hz*), 7.92(1H, d, Ar-*CH*, *J*= 1.9*Hz*), 7.74(1H, d, Ar-*CH*, *J*= 8.8*Hz*), 7.35(1H, dd, Ar-*CH*, *J*=2.6, 9.1*Hz*), 6.35(1H, d, Ar-*CH*, *J*=5.1*Hz*), 3.30(2H, m, NCH_2CH_3-CH_2-, *J*=5.7*Hz*), 2.82(2H, t, -CH_2-N(CH_2 CH_3), *J*=4.4*Hz*), 2.60(6H, m, -NCH_2CH_3, -NH-CH_2-CH_2-NCH_2CH_3, -CH_2-NH_2), 1.65 (2H, m, -CONH-$CH_2CH_2CH_2$-), 1.07(3H, t, -NCH_2CH_3, *J*=6.9*Hz*); **^{13}C** (500 M*Hz*, $CDCl_3$) δ 152.0, 150.0, 149.0, 134.8, 128.6, 125.3, 121.4, 117.5, 99.2, 51.3, 51.2, 47.1, 40.5, 40.1, 30.0, 11.8. **MS** (ES^+): m/z (%): 335.2 $[M]^+$. **HRMS** (ES^+) m/z calc. - 335.1997, observed – 335.1988.

5.1.16 General experimental procedure for synthesis of N-(3-((2-(7-chloroquinolin-4-ylamino)ethyl)(ethyl)amino)propyl)-2-(7-(dimethylamino)-2-oxo-2H-chromen-4-yl)acetamide tert-butyl (**48a, 48b**)

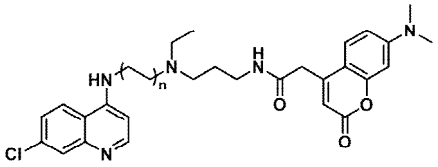
7-Dimethyl coumarin-4-acetic acid **(34)** (8mgs, 0.03mmol, 1equiv.) was placed under Ar atmosphere and then 10-12ml of Dichloromethane (dry freshly distilled) was added. The reaction mixture is cooled to -10^{o}C using ice-methanol mixture. DIPEA (10.0 mgs, 0.06 mmol, 2 equiv) was added immediately followed by addition of N1-(2-(7-chloroquinolin-4-ylamino)ethyl)-N1-ethylpropane-1,3-diamine**(47a)**(10mgs, 0.03mmol, 1 equiv), HATU (15 mgs, 0.04 mmol, 1.2 equiv) and HOAt (8 mgs, 0.04

mmol, 1.2 equiv) is added in the sequence respectively. The reaction is maintained under Ar atmosphere and -10^{o}C till the addition of the above components is complete. The reaction is cooled to room temperature gradually and stirring is continued at room temperature till TLC shows the product spot. The product was isolated by washing the reaction mixture with water (10mL) and separating the dichloromethane layer. Dichloromethane was distilled under vacuum and the residue was purified by flash chromatography (2% - 4% MeOH in$CHCl_3$+ 0.1% TEA) to obtain (7mgs) 40% yield of pure product.



48a, n=1, 60%

N-(3-((2-(7-chloroquinolin-4-ylamino)ethyl)(ethyl)amino)propyl)-2-(7-(dimethyl amino)-2-oxo-2H-chromen-4-yl)acetamide tert-butyl **(48a) –** The residue was purified by flash chromatography (2% - 4% MeOH in$CHCl_3$+ 0.1% TEA) to obtain 60% yield of pure product. $\mathbf{R_f}$ (1:9 MeOH: CH2Cl2+0.1%TEA) = 0.36. **IR** (KBr)/cm^{-1} - 3383, 2957, 2925, 1646, 1565, 1455, 1377. ^{1}**H** (500M*Hz*, $CDCl_3$) δ 8.33(1H, d, Chlor-*CH*, *J*= 5.7*Hz*), 8.06(1H, d, Chlor-*CH*, *J*= 9.5*Hz*), 7.75(1H, s, Cl-C=*CH*-), 7.53(1H, d, Cou-*CH*-, *J*=8.9*Hz*), 7.35(1H, dd, Cl-*CH*, *J*=2.0*Hz*, 8.9*Hz*), 6.68(1H, d, -N=C=*CH*-, *J*= 2.5*Hz*), 6.45(1H, dd, $(CH_3)_2$N-CH_2-CH_2- , *J*=2.6*Hz*, 9.5*Hz*), 6.03(1H, s, Cou-*CH*), 3.30(2H, s, Coum-CH_2-CONH-), 2.94(6H, s, -N$(CH_3)_2$), 2.4-2.8(6H, m), 1.72(2H, q, -CH_2CH_3, *J*= 7.6*Hz*), 0.95(3H, t, -CH_2CH_3, *J*= 7.6*Hz*),; ^{13}**C** (500 M*Hz*, MeOD) δ 171.1, 164.2, 157.2, 154.8, 152.8, 152.8, 152.7, 152.2, 149.4, 136.4, 127.4, 126.9, 125.9, 124.4, 118.7, 110.5, 110.3, 109.7, 99.7, 98.7, 54.0, 51.9, 47.6, 43.8, 40.2, 39.1, 24.9, 11.2. **MS** (ES^{+}): m/z - 536.3 $[M+H]^{+}$. **HRMS** (ES^{+}) m/z - calc. 536.2445, obsvd. – 536.2441. **LCMS** Purity -96%. Rt = 8.55-8.73 min. Eluent system – 10% ACN/H_2O+0.1%TFA – 100% ACN/H_2O+0.1%TFA.



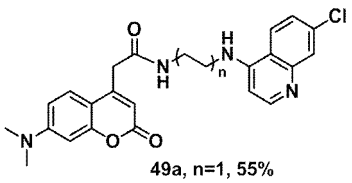
48b, n=2, 65%

N-(3-((4-(7-chloroquinolin-4-ylamino)butyl)(ethyl)amino)propyl)-2-(7-(dimethyl amino)-2-oxo-2H-chromen-4-yl)acetamide **(48b)** **–** Following the above procedure and purification by flash chromatography (2% - 4% MeOH in $CHCl_3$+0.1%TEA) the compound was obtained in 65% yield. $\mathbf{R_f}$ (1:9 MeOH:CH_2Cl_2+0.1%TEA) = 0.30. **IR** (KBr)/cm^{-1} - 3384, 2958, 2924, 1645, 1564, 1456, 1378. ^{1}**H** (500M*Hz*, MeOD) δ 8.33(1H, d, Chlor-*CH*, *J*= 5.7*Hz*), 8.06(1H, d, Chlor-*CH*, *J*= 9.5*Hz*), 7.75(1H, s, Cl-C=*CH*-), 7.53(1H, d, Cou-*CH*-, *J*=8.9*Hz*), 7.35(1H, dd, Cl-*CH*-, *J*=2.0*Hz*, 8.9*Hz*), 6.68(1H, d, -N=C=*CH*-, *J*= 2.5*Hz*), 6.45(1H, dd, $(CH_3)_2$N-CH_2-CH_2- , *J*=2.6*Hz*, 9.5*Hz*), 6.03(1H, s, Cou-*CH*), 3.30(2H, s, Coum-CH_2-CONH-), 2.94(6H, s, -N$(CH_3)_2$), 2.4-2.8(6H, m), 1.72(6H, m), 0.95(3H, t, -CH_2CH_3, *J*= 7.6*Hz*); ^{13}**C** (500 M*Hz*, MeOD) δ 171.1, 164.2, 157.2, 154.8, 152.8, 152.8, 152.7, 152.2, 149.4, 136.4, 127.4, 126.9, 125.9, 124.4, 118.7, 110.5, 110.3, 109.7, 99.7, 98.7, 54.0, 51.9, 47.6, 43.8, 40.2, 39.1, 27.7, 26.9, 24.9, 11.2, 9.9. **MS** (ES^+): m/z - 564.3 $[M+H]^+$. **HRMS** (ES^+) m/z - calc. 564.2376, obsvd. – 564.2723. **LCMS** Purity -96%. Rt = 9.2-9.22 min. Eluent system – 10% ACN/H_2O+0.1%TFA – 100% ACN/H_2O+0.1%TFA.

5.1.17 General experimental procedure for synthesis of N-(2-(7-chloroquinolin-4-yl amino) ethyl)-2-(7-(dimethylamino)-2-oxo-2H-chromen-4-yl)acetamide (**49a, 49b**)

N-(7-Chloro-4-quinolyl)-1,2-diaminoethane (**27a**) (30mgs, 0.12mmol, 1 equiv.) was added under Ar atmosphere and 2.5ml of anhydrous DMF was added to above reactant. The reaction mixture was cooled to 4^{o}C using Ice bath and then 7-Dimethyl coumarin-4-acetic acid (**34**) (53 mgs, 0.21mmol, 1 equiv), DCC (27 mgs, 0.13 mmol,

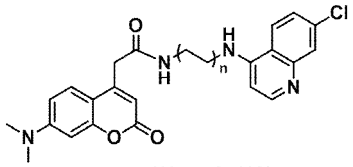
1.1 equiv) and HOBt (18 mgs, 0.13 mmol, 1.2 equiv) was added in the sequence respectively. The reaction is maintained under Ar atmosphere and 4^{o}C till the addition of the above components is complete. The reaction is cooled to room temperature gradually and stirring is continued at room temperature till TLC shows the product spot. The product was isolated by removing the reaction mixture under vacuum and then the residue was extracted with cold ethyl acetate separating the dicyclohexylurea by-product which precipitates out from the cold ethyl acetate. Ethyl acetate filtrate was distilled under vacuum and the residue was purified by flash chromatography (2% - 4% MeOH in$CHCl_3$+ 0.1% TEA) (41mgs) **(49a)** 67% yield.



49a, n=1, 55%

N-(2-(7-chloroquinolin-4-ylamino)ethyl)-2-(7-(dimethylamino)-2-oxo-2H-chromen-4-yl)acetamide **(49a)** – The residue was purified by flash chromatography (2% - 4% MeOH in$CHCl_3$+ 0.1% TEA) to get 55% yield of product. $\mathbf{R_f}$ (1:9 MeOH:CH_2Cl_2+0.1%TEA) = 0.21. **IR** (KBr)/cm^{-1} – 3325, 3063, 2924. 1705, 1620, 1535. ^{1}**H** (500M*Hz*, DMSO-d6) δ 8.39(1H, d, Chlor-*CH*, *J*= 5.7*Hz*), 8.36(1H, b, -*NH*CO-), 8.06(1H, d, Chlor-*CH*, *J*= 8.8*Hz*), 7.78(1H, d, Cl-C=*CH*- , *J*= 2.5*Hz*), 7.46(1H, b, Chlor-*NH*-), 7.42(1H, dd, Cl-C-*CH*-, *J*=2.0*Hz*, 8.9*Hz*), 7.40(1H, s, Cl-C-*CH*-), 6.56(1H, dd, $(CH_3)_2$N-C-*CH*- , *J*=2.5*Hz*, 8.8*Hz*),, 6.43(1H, d, Cou-*CH*, *J*= 2.5*Hz*), 6.41(1H, d, -N=C=*CH*-, *J*= 7.0*Hz*), 6.02(1H, s, Cou-*CH*), 3.62(2H, s, Cou-CH_2), 3.37-3.41(4H, m, -NH-CH_2-, -CH_2-NHCO), 2.97(6H, s, N$(CH_3)_2$); ^{13}**C** (500 M*Hz*, DMSO-d6) δ 168.7, 168.6, 160.6, 155.3, 152.5, 150.9, 150.8, 150.3, 147.1, 134.1, 126.0, 125.7, 124.4, 124.0, 116.9, 109.6, 108.6, 108.0, 98.5, 97.2, 42.3, 40.0, 37.5, 37.4. **MS** (ES$^+$): m/z (%): 451.2 $[M+H]^+$. **HRMS** (ES$^+$) m/z calc. - 473.1351,

observed – 473.1361. **LCMS** Purity -96%. Rt = 8.8-8.99min. Eluent system – 10% ACN/H_2O+0.1%TFA – 100% ACN/H_2O+0.1%TFA.



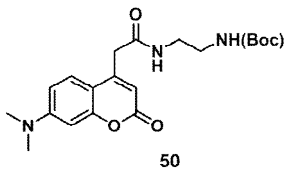
49b, n=2, 60%

N-(4-(7-chloroquinolin-4-ylamino)butyl)-2-(7-(dimethyl amino)-2-oxo-2H-chromen-4-yl)acetamide **(49b)** – Following the above procedure and purification by flash chromatography (2% -6% MeOH in $CHCl_3$+0.1%TEA) the compound was obtained in 60% yield. $\mathbf{R_f}$ (MeOH:CH_2Cl_2+0.1%TEA) = 0.20. **IR** (KBr)/cm^{-1} – 3487, 3363, 2924, 2862, 1689, 1612, 1589, 1535. ^{1}**H** (500M*Hz*, DMSO-d6) δ 8.37(1H, d, Chlor-*CH*, *J*= 5.7*Hz*), 8.26(1H, d, Chlor-*CH*, *J*= 9.5*Hz*), 8.18(1H, b, -*NH*CO-), 7.53(1H, d, Cou-*CH*-, *J*=8.9*Hz*), 7.77(1H, d, Cl-C=*CH*- , *J*= 2.0*Hz*), 7.43(1H, dd, Cl-C-*CH*-, *J*=2.0*Hz*, 8.9*Hz*), 7.30(1H, b, Chlor-*NH*-), 6.65(1H, dd, $(CH_3)_2$N-C-*CH*- , *J*=2.5*Hz*, 8.8*Hz*), 6.53(1H, d, Cou-*CH*, *J*= 2.6*Hz*), 6.46(1H, d, -N=C=*CH*-, *J*= 5.1*Hz*), 5.99(1H, s, Cou-*CH*), 3.58(2H, s, Cou-CH_2), 3.25(2H, t, -CH_2-NHCO-, *J*= 7.0*Hz*), 3.13(2H, t, -NH-CH_2-, *J*= 7.0*Hz*), 2.97(6H, s, N$(CH_3)_2$), 1.62-1.68(2H, m, -CH_2-CH_2-), 1.50-1.56(2H, m, -CH_2-CH_2-); ^{13}**C** (500 M*Hz*, DMSO-d6) δ 167.6, 160.6, 155.3, 152.7, 151.3, 150.2, 148.7, 133.4, 127.2, 125.9, 124.1, 124.0, 117.3, 109.3, 108.9, 108.1, 98.6, 97.4, 42.0, 38.4, 26.6, 25.1. **MS** (ES^+): m/z (%): 479.2 $[M+H]^+$. **HRMS** (ES^+) m/z calc. - 479.1861, observed – 479.1858. **LCMS** Purity -96%. Rt = 9.69-9.73 min. Eluent system – 10% ACN/H_2O+0.1%TFA – 100% ACN/H_2O+0.1%TFA.

5.1.18 General experimental procedure for the synthesis of tert-butyl 2-(2-(7-(dimethylamino)-2-oxo-2H-chromen-4-yl)acetamido)ethylcarbamate (**50,52, 82, 85**).

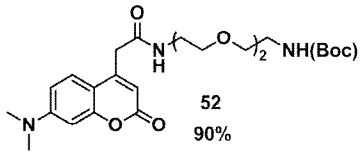
1-(tert-Butyloxycarbonyl) ethyldiamine (**31**) (130mgs, 0.81mmol, 1equiv) was added to a single neck flask containing freshly distilled CH_2Cl_2 (10 mL). The solution was

cooled to -10^{o}C using ice-methanol mixture. 7-Dimethyl coumarin-4-acetic acid (**34**) (200mgs, 0.81mmol, 1equiv) was added followed by di-isopropylethylamine (210 mgs, 1.62mmol, 2equiv), HATU (370mgs, 0.97mmol, 1.2equiv) and HOAt (130 mgs, 0.97mmol, 1.2equiv) in the sequence respectively. The reaction is maintained under Ar atmosphere and -10^{o}C till the addition of the above components is complete. The reaction is cooled to room temperature gradually and stirring is continued at room temperature till TLC shows the product spot. The product was isolated by washing the reaction mixture with water and separating the dichloromethane layer. Dichloromethane was distilled under vacuum and the residue was purified by flash chromatography (2% - 4% MeOH in$CHCl_3$+ 0.1% TEA) in 60% yield. This method was primarily used to synthesize 50,52 and 85. In case of TAMRA (**81**) based intermediates the procedure enclosed below is followed. 6-TAMRA-COOH (**81**) (100 mgs, 0.23 mmol, 1.0equiv) is added to flame dried single necked flask containing dry THF 5mL. The reaction mixture was cooled to -10^{o}C and then ethylchloroformate (38 mgs (33µL), 0.35 mmol, 1.5equiv) and TEA (35 mgs (48 µL), 0.35 mmol, 1.5equiv). The reaction mixture is stirred for 1hr at this temperature and then 1-(tert-Butyloxycarbonyl) ethyldiamine (**31**) (56 mgs, 0.35mmol, 1.5equiv) is added in liquid form directly to the reaction mixture at -10^{o}C. The reaction mixture is gradually cooled to room temperature and stirred for 16-18hrs. After confirmation with TLC, the solvent is removed under vacuum and then the crude is purified by column chromatography (10% -15% MeOH in $CHCl_3$) to get (**82**).



50
60%

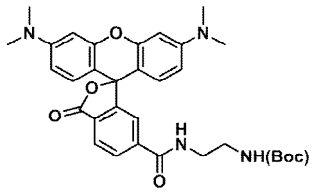
tert-butyl 2-(2-(7-(dimethylamino)-2-oxo-2H-chromen-4-yl) acetamido) ethyl carbamate **(50)** – Following the above procedure and purification by flash chromatography (2% -6% MeOH in $CHCl_3$+0.1%TEA) the compound was obtained in 60% yield. $\mathbf{R_f}$ (MeOH:CH_2Cl_2+0.1%TEA) = 0.48. (500M*Hz*, $CDCl_3$) $^{\mathbf{1}}\mathbf{H}$ (500M*Hz*, $CDCl_3$) δ 7.46 (1H, d, *J* =9.5*Hz*, Coum-*H*), 6.6 (1H, dd, *J* = 2.5, 8.8*Hz*, Coum-*H*), 6.48 (1H, d, *J* = 8.8*Hz*, Coum-*H*), 6.04 (1H, s, Coum-*H*), 4.89(1H, bs, -*NH*), 3.61 (2H, s, -CH_2-CO-), 3.33 (4H, m), 3.01 (6H, s,-N$(CH_3)_2$), 1.04 (9H, s, O=C-O-C$(CH_3)_3$); $^{\mathbf{13}}\mathbf{C}$ (500 M*Hz*, $CDCl_3$) δ 168.6, 161.7, 156.0, 153.1, 149.5, 125.6, 110.6, 109.1, 108.4, 98.3, 79.8, 45.8, 41.3, 40.7, 40.1, 28.3. **MS**(ES^+): m/z (%): 412.2 $[M+Na]^+$. **HRMS**(ES^+) m/z calc. – 412.1867 , observed – 412.1857 .



52

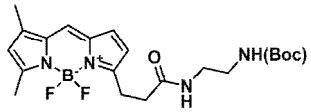
90%

tert-butyl2-(2-(2-(7-(dimethylamino)-2-oxo-2H-chromen-4-yl)acetamido)ethoxy)ethyl carbamate **(52)** **–** Following the above procedure and purification by flash chromatography (2% -6% MeOH in $CHCl_3$+0.1%TEA) the compound was obtained in 99% yield. $\mathbf{R_f}$ (MeOH:CH_2Cl_2+0.1%TEA) = 0.30. $^{\mathbf{1}}\mathbf{H}$ (500M*Hz*, $CDCl_3$) δ 7.46 (1H, d, *J* =9.5*Hz*, Coum-*H*), 6.57 (1H, dd, *J* = 2.5, 8.8*Hz*, Coum-*H*), 6.44 (1H, d, *J* = 8.8*Hz*, Coum-*H*), 6.03 (1H, s, Coum-*H*), 5.04(1H, br, -*NH*-), 3.5 (14H, m), 3.01 (6H, s,-N$(CH_3)_2$), 1.04 (9H, s, O=C-O-C$(CH_3)_3$), $^{\mathbf{13}}\mathbf{C}$(500 M*Hz*, $CDCl_3$) δ 168.6, 161.7, 155.9, 155.8, 152.9, 149.9, 125.6, 110.1, 109.0, 98.1, 79.5, 70.2, 70.1, 69.5, 40.3, 39.9, 39.5, 28.3. **MS**(ES^+) m/z (%): 500.2 $[M+Na]^+$. **HRMS**(ES^+) m/z calc. – 500.2381, obsvd. – 500.2375.



82

tert-butyl 2-(3',6'-bis(dimethylamino)-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthene]-6-ylcarboxamido)ethylcarbamate **(82)** – Following the above procedure and purification by flash chromatography (10% -15% MeOH in $CHCl_3$+0.1%TEA) the compound was obtained in 70% yield. $\mathbf{R_f}$ (9:1 MeOH: CH_2Cl_2) = 0.30. **IR**(KBr)/ cm^{-1} – 3421, 2922, 1647, 1597, 1491, 1366, 1348. ^{1}**H** (500M*Hz*, DMSO-d_6) δ 8.69(1H, b,-NH-), 8.05-8.14 (2H, m,Ar-H), 7.62 (1H, s, Ar-H), 6.84 (1H, b,-NH-), 6.5 (6H, s, Ar-H), 3.04-3.21 (4H, m), 2.96 (12H, s,(-N*(CH₃)₂)₂*), 1.29 (9H, s, O=C-O-C*(CH₃)₃*). ^{13}**C** (500 M*Hz*, DMSO-d_6) δ 164.8, 155.6, 152.2, 129.2, 128.6, 97.8, 77.6, 28.1. **MS** (ES^+) m/z (%): 573.1 $[M+H]^+$. **HRMS** (ES^+) m/z calc. – 573.2708, obsvd. – 573.2722



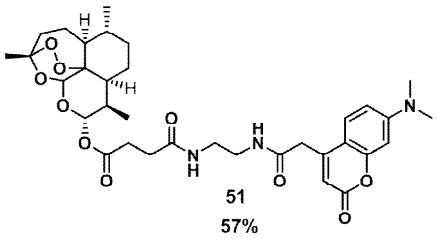
85

3-(3-(2-(tert-butoxycarbonylamino)ethylamino)-3-oxopropyl)-5,5-difluoro-7,9-dimethyl-5H-dipyrrolo[1,2-c:1',2'-f][1,3,2]diazaborinin-4-ium-5-uide **(85)** – Following the above procedure and purification by flash chromatography (6% -8% MeOH in $CHCl_3$) the compound was obtained in 70% yield. $\mathbf{R_f}$ (50% EtAc:Hex) = 0.50. **IR**(KBr)/cm^{-1} – 3422, 3395, 2972, 2916, 1695, 1653, 1523. ^{1}**H** (500M*Hz*, MeOD) δ 7.42(1H, s, N-C-*CH*-C-N^+) , 7.00(1H, m, Ar-*CH*-), 6.32(1H, m, Ar-*CH*-), 6.20(1H, s, Ar-*CH*-), 3.10-3.25(4H, t, NH-C-CH-*CH*-CO-, NH-*CH_2*-*CH_2*-NH(Boc), *J*=6.9), 2.61(2H, t, NH-C-*CH_2*-CH-CO-, *J*=6.9*Hz*), 2.51 (3H, s, -*CH_3*-), 2.27 (3H, s, -*CH_3*-), 1.42(9H, s, O-*(CH₃)₃*-); ^{13}**C** (500 M*Hz*, MeOD) δ 174.9, 161.3, 158.5, 145.8, 136.5, 134.9, 129.6, 125.8, 121.3, 117.6, 99.9, 80.1, 40.9, 40.5, 36.1, 28.7, 25.6, 14.9,

11.2. **MS** (ES^+) m/z (%): 457.11 $[M+Na]^+$. **HRMS** (ES^+) m/z calc.–457.2222, obsvd. – 457.2209.

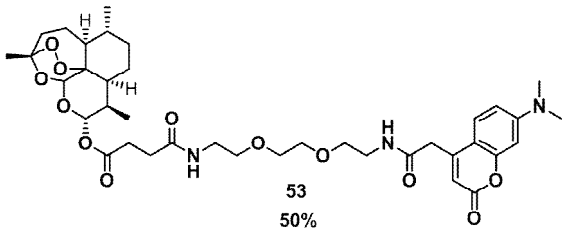
5.1.19 General experimental procedure for synthesis of Artemisinin based probes (**51, 53, 57, 83, 86, 98-100**) Tert-butyl2-(2-(7-(dimethylamino)-2-oxo-2H-chromen-4-yl) acetamido) ethylcarbamate (**50**) (10mgs, 0.026mmol, 1equiv.) was dried overnight under vacuum and then placed under Ar atmosphere. 1ml of freshly distilled dichloromethane was added to above reactant. The reaction mixture was cooled to 4^oC using Ice bath and then Trifluoroacetic acid (TFA) (29mgs, 0.26mmol, 10equiv.) was added immediately under Ar atmosphere using a syringe. The reaction was checked after 1hr by TLC (9:1 CH_2Cl_2:MeOH + 0.1% TEA) for absence of boc product. After confirmation by TLC the reaction mixture is distilled under vacuum at 16-18^oC to remove excess TFA and CH_2Cl_2. The residue is flushed with argon to release the vacuum and then 2-4ml of freshly distilled ether is added to observe precipitation of the TFA salt of the amine. If no precipitation is observed then the ether is removed under vacuum and after flushing the TFA salt with Argon the residue is kept under vacuum line for 10min. The residue is placed under Ar atmosphere using a balloon and then 10-12ml of Dichloromethane (dry freshly distilled) was added. The reaction mixture is cooled to -10^oC using ice-methanol mixture. DIPEA (10 mgs, 0.077 mmol, 3 equiv) was added immediately at this followed by addition of Artesunate (10 mgs, 0.026mmol, 1equiv), HATU (10.7 mgs, 0.028 mmol, 1.1equiv) and HOAt (38.5 mgs, 0.028 mmol, 1.1equiv) is added in the sequence respectively. The reaction is maintained under Ar atmosphere and -10^oC till the addition of the above components is complete. The reaction is cooled to room temperature gradually and stirring is continued at room temperature till TLC shows the product spot. The product was isolated by washing the reaction mixture with water and separating the

dichloromethane layer. DCM was distilled under vacuum and the residue was purified by flash chromatography (2% - 4% MeOH in$CHCl_3$+ 0.1% TEA) in 60% yield.



51
57%

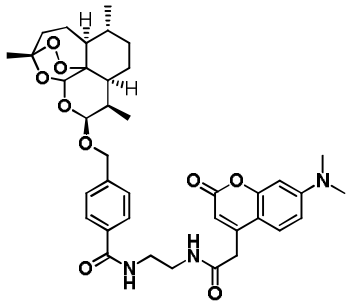
Artesunate probe **(51)** – Following the above procedure and purification by flash chromatography (2% -6% MeOH in $CHCl_3$+0.1%TEA) the compound was obtained in 99% yield. **R_f** (MeOH:CH_2Cl_2+0.1%TEA) = 0.20. **IR**(KBr)/cm^{-1} – 3325, 2878, 2484, 1604, 1404, 1165. **^{1}H**(500M*Hz*,$CDCl_3$) δ 7.56(1H, d, Ar-*CH*, *J*= 7.4*Hz*), 6.74(1H, dd, Ar-*CH* ,*J*=2.6*Hz*, 9.5*Hz*), 6.52(1H, d, Ar-*CH*, *J*=2.5*Hz*), 6.02(1H, b, -*NH*-), 5.63(1H, d, O-*CH*-OCO, *J*= 9.45*Hz*, α- epimer Artesunate), 5.45(1H, s, Artesunate-O-*CH*-O), 5.16(1H, s, Ar-*CH*-), 3.65(2H, m, Cou-NHCO-CH_2-CH_2-NHCO-Artesunate, *J*=4.4*Hz*), 3.30(4H, m, Coum-CH_2-CONH-, Cou-NHCO-CH_2-CH_2-NHCO-Artesunate), 3.04(6H, s, -N$(CH_3)_2$), 0.78-2.73(25H, m, Artesunate protons),; **^{13}C** (500 M*Hz*, MeOD) δ 174.88, 173.53, 171.4, 164.3, 157.3, 154.8, 152.8, 127.2, 110.8, 110.6, 110.0, 105.6, 98.8, 93.8, 92.8, 81.3, 54.8, 52.8, 46.5, 40.9, 40.2, 39.5, 38.2, 37.2, 35.2, 32.9, 31.2, 30.4, 25.9, 25.7, 22.8, 20.5, 12.3. **MS** (ES^+): m/z - 678.2 $[M+Na]^+$. **HRMS** (ES^+) m/z - calc. 678.2997, obsvd. – 678.2994. **LCMS** Purity -96%. Rt = 8.41-8.55 min. Eluent system – 0% ACN/H_2O – 100% ACN/H_2O.



53
50%

Artesunate probe **(53)** – Following the above procedure and purification by flash chromatography (2% -6% MeOH in $CHCl_3$+0.1%TEA) the compound was obtained

in 99% yield. **R_f** (MeOH:CH_2Cl_2+0.1%TEA) = 0.40. **IR**(KBr)/cm^{-1} – 3496, 3318, 2924, 2878, 1713, 1659, 1612, 1535, 1404, 1010. **^{1}H** (500M*Hz*, $CDCl_3$) δ 7.51(1H, d, Chlor-*CH*, *J*= 9.1*Hz*), 6.72(1H, b, $COCH_2CH_2CO$-NH-*CH*-), 6.65(1H, dd, $(CH_3)_2$N-C-*CH*- , *J*=2.6*Hz*, 9.5*Hz*), 6.48(1H, d, Cou-*CH*, *J*= 5.7*Hz*), 6.38(1H, b, CO-*NH*-CH_2CH_2-), 6.18(1H, s, Cou-OCO-*CH*-CH-CH_2), 5.67(1H, d, -O-*CH*-OCO, *J*= 9.45*Hz*, α-epimer Artesunate), 5.44(1H, s, O-*CH*-O Artesunate), 3.61(2H, s, Coum-CH_2-CONH-), 3.3-3.5 (12H, m), 3.31(6H, s, -N*(CH_3)$_2$*), 0.81-2.80(25H, m, Artesunate protons); **^{13}C**(500 M*Hz*, $CDCl_3$) δ – 171.9, 171.4, 168.2, 161.9, 156.0, 153.1, 150.1, 125.8, 110.2, 109.1, 108.5, 104.4, 98.2, 92.2, 91.5, 80.1, 70.3, 70.2, 70.2, 70.2, 69.8, 69.5, 68.7, 53.4, 51.5, 45.2, 40.4, 40.1, 39.6, 39.3, 37.2, 36.2, 34.1, 31.8, 30.7, 29.7, 25.9, 24.5, 21.9, 20.2, 12.0. **MS** (ES^+): m/z (%): 766.2 $[M+Na]^+$. **HRMS** (ES^+) m/z calc. – 766.3521 , observed – 766.3521. LCMS Purity -96%. Rt = 12.2-12.4 min. Eluent system – 0% ACN/H_2O – 100% ACN/H_2O.

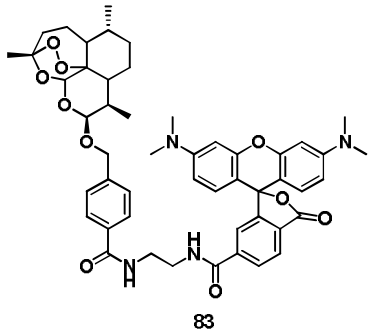


57

Artelinic acid-Coumarin probe **(57)** – Following the above procedure and purification by flash chromatography (2% -6% MeOH in $CHCl_3$+0.1%TEA) the compound was obtained in 80% yield. **R_f** (MeOH:CH_2Cl_2+0.1%TEA) = 0.75. **IR** (KBr)/cm^{-1} – 3400, 2450, 2400, 1700, 1650, 1625, 1525. **^{1}H** (300M*Hz*, MeOD) δ – 7.66(2H, d, Ar-*CH*, *J*=8.22*Hz*), 7.34-7.68(1H, d, Ar-H, *J*=8.88*Hz*), 7.35(2H, d, Ar-*CH*, *J*=8.22*Hz*), 6.43-6.56(1H,dd, Ar-H, *J*=2.6*Hz*, 6.6*Hz*), 6.44(1H,d, Ar-H, *J*=2.6*Hz*), 6.04(1H,s, Ar-H), 5.45(1H, s, -O-*CH*-O), 4.55(1H, 4.58(1H, d, β-CH-O, *J*=12.7*Hz*), 3.66(2H, s, -

NHCO-*CH*$_2$-), 3.45-3.51(4H, m, -NH-*CH*$_2$-*CH*$_2$-NH-), 2.99(6H,s, N*(CH*$_3$*)*$_2$), 0.87-2.63 (20H, m, Artemisinin-*CH*-); **^{13}C** (300 M*Hz*, MeOD) δ 171.8, 170.2,164.23, 157.1, 154.6, 152.5, 143.4, 134.5, 128.4, 128.3, 126.7, 110.8, 110.6, 109.7, 105.5, 102.7, 98.7, 89.4, 82.2, 70.3, 53.9, 48.2, 45.8, 40.9, 40.3, 40.2, 38.7, 37.4, 35.8, 32.4, 26.1, 25.9, 25.8, 20.7, 13.5, 6.02. **MS** (ES$^+$): m/z (%): 712.1 [M+Na]$^+$. **HRMS** (ES$^+$) m/z calc. – 712.3205, observed – 712.3231. **LCMS** Purity -96%. Rt = 15.9 - 16.1 min. Eluent system – 10% ACN/H_2O – 100% ACN/H_2O.

Artelinic-TAMRA probe **(83)**

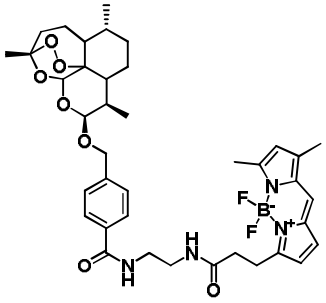


83

Following the above procedure and purification by flash chromatography (10% -20% MeOH in $CHCl_3$) the compound was obtained in 70% yield. **R_f** (MeOH:CH_2Cl_2) = 0.25. **IR** (KBr)/cm^{-1} – 3446, 2918, 2848, 1647, 1595, 1346, 1188, 1136. **^{1}H** (500M*Hz*, MeOD) δ 8.07-8.17(2H, d, Ar-H, *J*=8.2*Hz*, 8.2*Hz*), 7.62-7.72(4H, m, Ar-H), 7.32(2H, d, Ar-*CH*, *J*=8.20*Hz*), 7.19(2H, d, Ar-*CH*, *J*=9.45), 6.90-6.99(3H, m, Ar-H), 5.43(1H, s, -O-*CH*-O), 4.52(1H, d, β-CH-O, *J*=12.6*Hz*), 4.22(1H,m, Artemisinin-*CH*-), 3.60(4H,m, -NH-*CH*$_2$-*CH*$_2$-NH-), 3.30(12H,s, (-NH*(CH*$_3$*)*$_2$)$_2$), 1.35-2.69 (23H, m, Artemisinin-*CH*-); **^{13}C** (500 M*Hz*, MeOD) δ 170.3, 170.1, 169.3. 169.1, 159.1, 158.8, 143.4, 136.9, 134.7, 133.6, 132.6, 132.4, 131.4, 129.9, 129.7, 129.6, 128.5, 128.4, 115.1, 114.9, 105.5, 102.5, 97.4, 89.4, 82.2, 69.0, 54.0, 45.8, 41.1, 40.9, 40.7, 40.2, 38.7, 37.4, 35.8, 32.3, 31.6, 30.1, 26.1, 25.9, 25.7, 25.0, 24.0, 20.7, 14.4, 13.4, 11.4. **MS** (ES$^+$): m/z (%): 873.3 [M]$^+$. **HRMS** (ES$^+$) m/z calc. – 873.4069, observed –

873.4062. **LCMS** Purity -96%. Rt = 15.1 - 15.4 min. Eluent system – 0% ACN/H_2O – 100% ACN/H_2O.

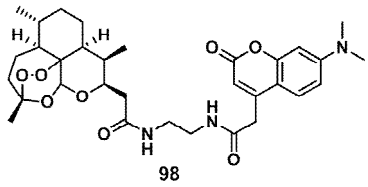
Artelinic acid-BODIPY probe **(86)**



86

Following the above procedure and purification by flash chromatography (10% -20% MeOH in $CHCl_3$) the compound was obtained in 80% yield. $\mathbf{R_f}$ (MeOH:CH_2Cl_2) = 0.55. **IR** (pellet)/cm^{-1} – 3379, 2922, 2853, 1714, 1657, 1606, 1537, 1447, 1252. **^{1}H** (500M*Hz*, MeOD) δ 7.77(2H, d, Ar-*CH*, *J*=8.2*Hz*), 7.41(2H, d, Ar-*CH*, *J*=8.2), 7.34(1H, s, N-C-*CH*-C-N^+), 6.89(1H, d, Ar-*CH*-, *J*=4.4*Hz*), 6.28(1H, d, Ar-*CH*-, *J*=4*Hz*), 6.20(1H, s, Ar-*CH*-), 5.43(1H, s, Artelinic-CH), 4.82(2H, s, O-CH_2-Ph), 4.54(1H, d, *J*=12.6*Hz*, β-isomer, O-*CH*-O), 3.40-3.50(4H, m, -CONH-CH_2-CH_2-NH-), 3.23(2H, t, BODIPY-CH_2-, *J*= 7.6*Hz*), 2.64(2H, t, BODIPY-CH_2-, *J*= 7.6*Hz*), 1.19-2.56(27H, m,s,s, Artelinic acid-*CH*-, BODIPY-CH_3); **^{13}C** (500 M*Hz*, MeOD) δ 175.4, 170.2, 158.4, 145.7, 143.4, 134.7, 132.4, 129.6, 128.5, 128.4, 125.8, 121.4, 117.5, 105.4, 102.7, 89.3, 82.2, 70.3, 69.1, 65.3, 61.3, 53.9, 45.8, 43.2, 41.2. 40.2, 39.9, 38.6, 37.4, 35.9, 35.7, 32.3, 31.3, 30.7, 30.3, 30.1, 28.7, 26.1, 25.8, 25.7, 25.6, 24.9, 24.2, 24.0, 20.7, 14.9, 14.4, 13.4, 11.4, 11.2. **MS** (ES^+): m/z (%): 757.19 $[M+Na]^+$. **HRMS** (ES^+) m/z calc. – 757.3561 , observed – 757.3575. **LCMS** Purity -96%. Rt = 17.2-17.6 min. Eluent system – 0% ACN/H_2O – 100% ACN/H_2O.

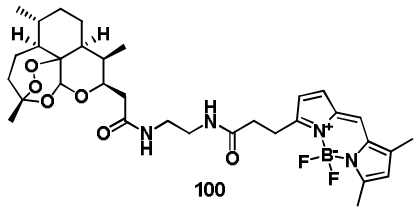
Deoxocarbaartemisinin-Coumarin probe **(98)**



98

Following the above procedure and purification by flash chromatography (10% -20% MeOH in $CHCl_3$) the compound was obtained in 70% yield. $\mathbf{R_f}$ (9:1 MeOH:CH_2Cl_2) = 0.55. **IR**(KBr)/cm^{-1} – 3294, 2957, 2926, 2857, 1719, 1618, 1533,1375, 1278, 1128, 1042. ^{1}**H** (500M*Hz*, MeOD) δ – 7.71(1H, d, Ar-*CH*, *J*=8.88*Hz*), 6.77(1H,dd, Ar-H, *J*=2.6*Hz*, 6.6*Hz*), 6.55(1H,d, Ar-H, *J*=2.6*Hz*), 6.05(1H,s, Ar-H), 5.25(1H, s, -O-*CH*-O), 3.8 (1H, m, β-CH-O, *J*=12.7*Hz*), 3.69(2H, s, -NHCO-CH_2-), 3.25-3.50(4H, m, -NH-CH_2-CH_2-NH-), 3.09(6H,s, N$(CH_3)_2$), 0.77-2.60 (23H, m, Artemisinin-*CH*-); ^{13}**C** (300 M*Hz*, MeOD) δ 174.0, 171.3, 164.2, 157.2, 154.8, 152.8, 126.9, 126.8, 110.6, 110.2, 105.6, 98.8, 93.2, 82.3, 73.2, 53.1, 47.2, 41.0, 40.5, 40.3, 40.2, 39.9, 38.4, 37.2, 35.2, 32.6, 26.2, 25.9, 22.2, 20.6, 14.1. **MS** (ES^+): m/z (%): 620.2 $[M+Na]^+$. **HRMS** (ES^+) m/z calc. – 598.3123 , observed – 598.3119 $[M+H]^+$. **LCMS** Purity -96%. Rt = 14.95 min. Eluent system – 0% ACN/H_2O – 100% ACN/H_2O +0.01%TFA.

Deoxocarbaartemisinin-BODIPY probe **(100)**

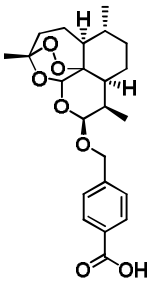


100

Following the above procedure and purification by flash chromatography (10% -20% MeOH in $CHCl_3$) the compound was obtained in 70% yield. $\mathbf{R_f}$ (9:1 MeOH:CH_2Cl_2) = 0.55. **IR** (KBr)/cm^{-1} – 3350, 2954, 2926, 1724, 1647, 1607, 1529, 1437, 1252, 1134. ^{1}**H** (500M*Hz*, MeOD) δ 7.45(1H, s, N-C-*CH*-C-N^+), 7.01(1H, d, Ar-*CH*-, *J*=3.8*Hz*),

6.31(1H, d, Ar-*CH*-, *J*=3.8*Hz*), 6.21(1H, s, Ar-*CH*-), 5.24(1H, s, Artelinic-CH), 3.85(1H, sex, *J*=12.6*Hz*, β-isomer, O-*CH*-O), 3.15-3.55(6H, m, -CONH-CH_2-CH_2-NH-, BODIPY-CH_2-), 0.79-2.70(33H, m,s,s, Artemisinin-*CH*-, BODIPY-CH_3, BODIPY-CH_2-); **^{13}C** (500 M*Hz*, MeOD) δ 174.9, 174.0, 161.2, 158.9, 145.7, 136.5, 134.9, 129.8, 125.8, 121.4, 117.8, 105.6, 104.6, 93.2, 90.8, 82.3, 82.1, 73.7, 73.3, 53.6, 53.2, 47.2, 45.4, 41.3. 40.2, 39.9, 39.8, 39.4, 38.7, 38.3, 38.2, 37.4, 37.2, 36.4, 36.2, 35.6, 35.2, 32.9, 31.5, 26.1, 25.9, 25.7, 25.68, 24.65, 22.2, 20.6, 20.5, 14.9, 14.0, 13.0, 11.2. **MS** (ES^+): m/z (%): 665.2 $[M+Na]^+$. **HRMS** (ES^+) m/z calc. – 643.3479, observed – 643.3508 $[M+H]^+$. **LCMS** Purity -96%. Rt = 16.33 min. Eluent system – 0% ACN/H_2O – 100% ACN/H_2O +0.01%TFA..

5.1.20 Synthesis of Artelinic Acid –

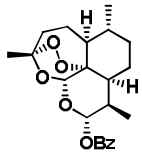


56

β-Artelinic Acid **(49)** – Following the procedure for synthesis of artelinic acid as shown in literature and purification by flash chromatography (50% EtOAc in Hexane) the compound was obtained in 65% yield. **R_f** (50% EtOAc in Hexane) = 0.5. **IR** (KBr)/cm^{-1} – 3500, 3000, 1750, 1390, 1350. **^{1}H** (300M*Hz*, MeOD) δ 8.00(2H, d, Ar-*CH*, *J*=8.37*Hz*), 7.43(2H, d, Ar-*CH*, *J*=8.37), 5.44(1H, s, -O-*CH*-O), 4.58(1H, d, β-*CH*-O, *J*=12.8*Hz*), 0.9-2.62 (22H, m, Artemisinin-*CH*-, -*CH3*-), **^{13}C** (300 M*Hz*, MeOD) δ 145.0, 130.9, 128.3, 105.5, 102.8, 89.4, 82.2, 70.3, 57.4, 53.9, 45.8, 38.7,

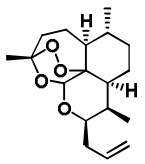
37.4, 32.4, 26.1, 25.8, 25.7, 20.7, 13.4. **MS** (ES^+): m/z (%): 440.94 $[M+Na]^+$. **LCMS** Purity -98%. Rt = 16.4 - 16.6 min. Eluent system – 0% ACN/H_2O – 100% ACN/H_2O.

5.1.21 Synthesis of β–Deoxocarbaartemisinin (**97**)[64] –



95

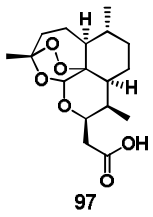
Dihydroartemisinin 10α- benzoate **(95)** – Following the procedure for synthesis of Dihydroartemisinin 10α- benzoate as shown in literature[64] and purification by flash chromatography (10% EtOAc in Hexane) the compound was obtained in 92% yield. **R_f** (10% EtOAc in Hexane) = 0.3. **^{1}H** (500M*Hz*, $CDCl_3$) δ 8.13 (2H, d, Ar-*CH*-, *J*= 7.6 *Hz*), 7.57 (1H, m, Ar-*CH*-, *J*= 7.6 *Hz*), 7.45 (2H, m, aromatic, *J*= 8.2 *Hz*), 6.05 (1H, d, *J*= 10*Hz*, α-epimer), 5.53 (1H, s), 2.76 (1H, m), 2.40 (1H, m), 0.93-2.07 (19 H, m); **^{13}C** (500M*Hz*, $CDCl_3$) δ 165.9, 133.9, 130.8, 130.3, 128.9, 105.1, 93.2, 92.3, 80.9, 52.3, 46.0, 37.9, 36.9, 34.8, 32.7, 26.6, 25.3, 22.7, 20.9, 13.9. **MS** (ES^+): m/z (%): 410.96 $[M+Na]^+$.



96

10-β -Allyldeoxoartemisinin **(96)** – Following the procedure for synthesis of 10-β - Allyldeoxoartemisinin as shown in literature[64] and purification by flash chromatography (10% EtOAc in Hexane) the compound was obtained in 70% yield. **R_f** (10% EtOAc in Hexane) = 0.5. **^{1}H** (500M*Hz*, $CDCl_3$) δ 5.91 (1H, m), 5.30 (1H, s, -O-*CH*-O), 5.08(2H,m), 4.29(1H,m, *J*= 12.6*Hz*, β-*CH*-O, β-epimer), 2.68 (1H, sex, *J*=6.3*Hz*), 0.89-2.43 (21H, m, Artemisinin-*CH*), δ_c (500M*Hz*, $CDCl_3$) - 137.2, 116.8,

103.8, 89.8, 81.8, 75.4, 53.0, 45.0, 38.2, 37.3, 35.2, 34.9, 30.9, 26.8, 25.6, 25.4, 20.9, 13.7. **MS** (ES^+): m/z (%): 331.02 $[M+Na]^+$.



97

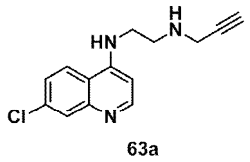
10-β -Deoxocarbaartemisinin carboxylic acid **(97)** – Following the procedure for synthesis of 10-β -Allyldeoxoartemisinin as shown in literature[64] and purification by flash chromatography (10% EtOAc in Hexane) the compound was obtained in 90% yield. R_f (10% MeOH/CH_2Cl_2) = 0.5. **^{1}H** (500M*Hz*, $CDCl_3$) δ 5.37 (1H, s, -O-*CH*-O), 4.87(1H,m, *J*= 12.6*Hz*, β-*CH*-O, β-epimer), 0.89-2.72 (21H, m, Artemisinin-*CH*-, -*CH_3*-); **^{13}C** (500M*Hz*, $CDCl_3$) δ 176.2, 103.9, 90.2, 81.5, 71.1, 52.7, 44.4, 38.2, 37.1, 36.5, 34.9, 30.5, 29.9, 26.5, 25.4, 25.3, 20.7, 13.3. **MS** (ES^+): m/z (%): 349.2 $[M+Na]^+$. **LCMS** Purity -96%. Rt = 15.5min. Eluent system – 0% ACN/H_2O – 100% ACN/H_2O.

5.1.22 General experimental procedure for synthesis of click probes (**58-64**)

Alkyne linkage using 3-bromo-prop-1-yne –

N-(7-Chloro-4-quinolyl)-N′-ethyl-1,2-diaminoethane (**27a**) (100mgs, 0.05mmol, 1equiv.) and freshly dried K_2CO_3 (125mgs, 0.09mmol, 2equiv.) were stirred together in a single neck flask under Ar atmosphere in 2-3ml of dry Acetonitrile. 3-bromo-prop-1-yne (54mgs, 0.05mmol, 1equiv.) was added directly to the above reaction mixture at 4^{o}C (Ice water). Ice Water is removed after the addition is done and the reaction is stirred at room temperature for 12hrs. After confirmation of formation of product by TLC (9:1 CH_2Cl_2:MeOH+0.1%TEA). The reaction mixture is columned through Celite and 50ml of Acetonitrile is added to extract the product from the

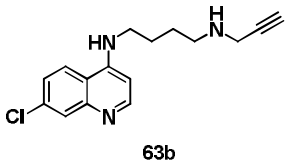
column. Acetonitrile is distilled under vacuum and the residue is purified by flash chromatography (2% -6% MeOH in $CHCl_3$+0.1%TEA).



63a

N1-(7-chloroquinolin-4-yl)-N2-(prop-2-ynyl)ethane-1,2-diamine **(63a)** –

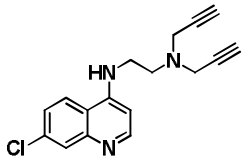
Following the above procedure and purification by flash chromatography (4% -8% MeOH in $CHCl_3$+0.1%TEA) the compound was obtained in 30% yield. R_f (MeOH:CH_2Cl_2+0.1%TEA) = 0.35. IR (KBr)/cm^{-1} – 3200, 3100, 1575. **^{1}H** (300M*Hz*, MeOD) δ – 8.29(1H, d, -N=CH-, *J*=5.7*Hz*), 8.03(1H, d, Ar-CH, *J*=8.8*Hz*), 7.77(1H, d, Ar-H, *J*=2.6*Hz*), 7.33(1H, dd, Ar-H, *J*=2.5*Hz*,*J*=6.93*Hz*), 6.52(1H, d, Ar-H, *J* = 5.7*Hz*), 3.39-3.46(2H, t, NH-*CH_2*-CH_2-NH, *J*=6.4*Hz;* 2H, m, -*CH_2*-Alkyne, *J*=2.6*Hz*), 2.96(2H, t, NH-CH_2-*CH_2*-NH,, *J*=6.4*Hz*), 2.56(1H, m, Alkyne-*CH*-, *J*=2.6*Hz*); **^{13}C** (500 M*Hz*, MeOD) δ 152.9, 152.2, 149.4, 136.5, 127.4, 126.2, 124.4, 99.7, 81.9, 73.5, 47.3, 43.2, 42.1, 38.3. **MS** (ES^+): m/z (%): 260.1 $[M+H]^+$. **HRMS** m/z calc. – 260.0949 , observed – 260.0955. **LCMS** Purity -96%. Rt = 10.0 - 10.3 min. Eluent system – 0% ACN/H_2O – 100% ACN/H_2O.



63b

N1-(7-chloroquinolin-4-yl)-N4-(prop-2-ynyl)butane-1,4-diamine **(63b)** – Following the above procedure and purification by flash chromatography (4% -8% MeOH in $CHCl_3$+0.1%TEA) the compound was obtained in 25% yield. **R_f** (MeOH:CH_2Cl_2+0.1%TEA) = 0.35. **IR** (KBr)/cm^{-1} – 3200, 3100, 1575. **^{1}H**(300M*Hz*, MeOD) δ 8.22(1H, d, -N=CH-, *J*=5.7*Hz*), 7.98(1H, d, Ar-CH, *J*=8.8*Hz*), 7.65(1H, d,

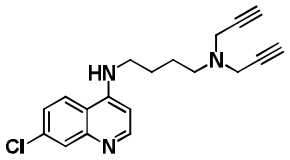
Ar-H, *J*=2.6*Hz*), 7.27(1H, dd, Ar-H, *J*=2.5*Hz, J*=6.9*Hz*), 6.39(1H, d, Ar-H, *J* = 5.7*Hz*), 3.21-3.30(2H,m,-*CH*$_2$-Alkyne, *J*=2.6*Hz*; 2H, t, -NH-*CH*$_2$-(CH$_2$)$_2$-CH$_2$-NH-, *J*=6.4*Hz*), 2.51-2.65 (2H, t, -NH-CH$_2$-(CH$_2$)$_2$-*CH*$_2$-NH-, *J*=6.4*Hz;* 1H, s, Alkyne-*CH*-), 1.49-1.71(4H, m, -NH-CH$_2$-*(CH*$_2$*)*$_2$-CH$_2$-NH-); **^{13}C** (500 M*Hz*, MeOD) δ 152.9, 152.1, 149.3, 136.4, 127.3, 126.9, 124.4, 118.7, 99.6, 81.9, 73.3, 43.8, 38.4, 27.8, 27.1. **MS** (ES$^+$): m/z (%): 288.1 [M+H]$^+$. **HRMS** m/z calc. – 288.1262 , observed – 288.1267. **LCMS** Purity -96%. Rt = 4.0 - 4.1 min. Eluent system – 0% ACN/H$_2$O – 100% ACN/H$_2$O.



64a

N1-(7-chloroquinolin-4-yl)-N2,N2-di(prop-2-ynyl)ethane-1,2-diamine **(64a)** –

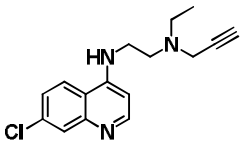
Following the above procedure and purification by flash chromatography (2% -4% MeOH in CHCl$_3$+0.1%TEA) the compound was obtained in 70% yield. R$_f$ (MeOH:CH$_2$Cl$_2$+0.1%TEA) = 0.7. **IR**(KBr)/cm^{-1} – 3200, 1625, 1600, 1575. **^{1}H** (300M*Hz*, MeOD) δ 8.27(1H, d, -N=CH-, *J*=5.7*Hz*), 7.93(1H, d, Ar-CH, *J*=8.8*Hz*), 7.30(1H, dd, Ar-H, *J*=2.5*Hz*), 7.68(1H, d, Ar-H, *J*=2.6*Hz*), 6.46(1H, d, Ar-H, *J* = 5.7*Hz*), 2.60(2H, t, Alkyne-*CH*-), 3.46 (4H, m, -*CH*$_2$-Alkyne, *J*=2.3*Hz*), 3.40 (4H, t,-NH-CH$_2$-*CH*$_2$-NH-, *J*=6.8*Hz*), 2.85(2H, t, -NH-*CH*$_2$-CH$_2$-NH-, *J*=6.8*Hz),* 2.60(2H, m, Alkyne-*CH*-, *J*=2.3*Hz*); **^{13}C**(500 M*Hz*, MeOD) δ 152.5, 149.6, 136.4, 127.6, 126.1, 124.1, 118.7, 99.8, 79.2, 75.0, 51.7, 43.1, 41.42. **MS** (ES$^+$): m/z (%): 298.1 [M+H]$^+$. **HRMS** m/z calc. – 298.1106 , observed – 298.1110. **LCMS** Purity -96%. Rt = 14.9 - 15.2 min. Eluent system – 0% ACN/H$_2$O – 100% ACN/H$_2$O.



64b

N1-(7-chloroquinolin-4-yl)-N4,N4-di(prop-2-ynyl)butane-1,4-diamine **(64b)** –

Following the above procedure and purification by flash chromatography (2% -4% MeOH in $CHCl_3$+0.1%TEA) the compound was obtained in 70% yield. $\mathbf{R_f}$ (9:1 MeOH:CH_2Cl_2+0.1%TEA) = 0.7. **IR** (pellet)/cm^{-1} – 3200, 1625, 1600, 1575. ^{1}**H** (300M*Hz*, MeOD) δ 8.24(1H, d, -N=CH-, *J*=5.7*Hz*), 7.97(1H, d, Ar-CH, *J*=8.8*Hz*), 7.66(1H, d, Ar-H, *J*=2.6*Hz*), 7.28(1H, dd, Ar-H, *J*=2.5*Hz*, *J*=6.8*Hz*), 6.42(1H, d, Ar-H, *J* = 5.7*Hz*), 3.46 (4H, m, -CH_2-Alkyne, *J*=2.3*Hz*), 3.22 (4H, t,-NH-CH_2-$(CH_2)_2$-CH_2-NH-, *J*=6.8*Hz*), 2.47-2.55 (2H, t, -NH-CH_2-$(CH_2)_2$-CH_2-NH-, *J*=6.8*Hz*; 2H, m, Alkyne-*CH*-, *J*=2.3*Hz*), 1.38-1.72 (4H, t, -NH-CH_2-$(CH_2)_2$-CH_2-NH-, *J*=6.8*Hz*); ^{13}**C** (500 M*Hz*, MeOD) δ 152.7, 152.3, 149.6, 136.3, 127.5, 125.9, 124.3, 118.7, 99.7, 79.1, 74.9, 74.8, 53.4, 43.7, 42.7, 27.0, 25.6. **MS** (ES^+): m/z (%): 326.1 $[M+H]^+$. **HRMS** m/z calc. – 326.1419, observed – 326.1424. **LCMS** Purity -96%. Rt = 9.2 – 9.3 min. Eluent system – 0% ACN/H_2O – 100% ACN/H_2O.



58

N1-(7-chloroquinolin-4-yl)-N2-ethyl-N2-(prop-2-ynyl)ethane-1,2-diamine **(58)** –

Following the above procedure and purification by flash chromatography (2% -4% MeOH in $CHCl_3$+0.1%TEA) the compound was obtained in 60% yield. $\mathbf{R_f}$ (9:1 MeOH:CH_2Cl_2+0.1%TEA) = 0.65. **IR** (KBr)/cm^{-1} – 3200, 3100, 1575. ^{1}**H** (300M*Hz*, MeOD) δ 8.27(1H, d, -N=CH-, *J*=5.7*Hz*), 7.93(1H, d, Ar-CH, *J*=8.8*Hz*), 7.68(1H, d, Ar-H, *J*=2.6*Hz*), 7.30(1H, dd, Ar-H, *J*=2.5*Hz*, *J*=6.9*Hz*), 6.46(1H, d, Ar-H, *J* =

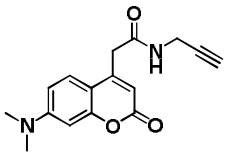
5.7*Hz*), 3.36-3.45(2H, m, -CH_2-Alkyne, *J*=2.5*Hz*; 2H, t, NH-CH_2-CH_2-NH, *J*=6.8*Hz*), 2.55-2.82(2H, t, NH-CH_2-CH_2-NH, *J*=6.8*Hz*; 4H, m, Alkyne-*CH*-, N-CH_2-CH_3-), 1.01(3H, t, -CH_2-CH_3-, *J*=7.1*Hz*),; **^{13}C** (300 M*Hz*, MeOD) δ 152.5, 152.4, 149.6, 136.4, 127.6, 126.1, 124.1, 118.7, 99.8, 78.7, 75.1, 52.2, 42.0, 41.5, 12.7. **MS** (ES^+): m/z (%): 288.1 $[M+H]^+$. **HRMS** m/z calc. – 288.1262, observed – 288.1263. **LCMS** Purity - 96%. Rt = 8.1 – 8.2 min. Eluent system – 0% ACN/H_2O+0.1%TFA – 100% ACN/H_2O+0.1%TFA.

Alkyne linkage using prop-2-yn-1-amine (**59, 61,86,87**) –

Coumarin-4-acetic acid (**34**) (50mgs, 0.2mmol, 1equiv.) was added under Ar atmosphere and 5 ml of anhydrous CH_2Cl_2 was added to above reactant. The reaction mixture was cooled to 4^{o}C using Ice bath and then HATU (8.5 mgs, 0.22 mmol, 1.1 equiv) and HOAt (3 mgs, 0.22 mmol, 1.1equiv), prop-2-yn-1-amine (11mgs, 0.2mmol, 1equiv) was added in the sequence respectively. The reaction is maintained under Ar atmosphere and 4^{o}C till the addition of the above components is complete. The reaction is cooled to room temperature gradually and stirring is continued at room temperature till TLC shows the product spot. The product was isolated by washing the reaction mixture with water and brine and collecting the organic fraction. The organic fraction was evaporated and the residue was purified by flash chromatography (2% - 4% MeOH in$CHCl_3$+ 0.1% TEA) (41mgs) 40% yield.

In case of TAMRA (**81**) based intermediates the procedure enclosed below is followed. 6-TAMRA-COOH (**81**) (100 mgs, 0.22 mmol, 1.0equiv) is added to flame dried single necked flask containing dry THF 5mL. The reaction mixture was cooled to -10^{o}C and then ethylchloroformate (33mgs, 0.35 mmol, 1.5equiv) and TEA(40mgs, 0.35 mmol, 1.5equiv). The reaction mixture is stirred for 1hr at this temperature and then prop-2-yn-1-amine (19 mgs, 0.44 mmol, 2 equiv) is added in

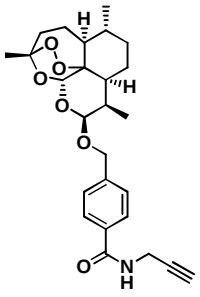
liquid form directly to the reaction mixture at -10^{o}C. The reaction mixture is gradually cooled to room temperature and stirred for 16-18hrs. After confirmation with TLC, the solvent is removed under vacuum and then the crude is purified by column chromatography (10% -15% MeOH in $CHCl_3$).



59

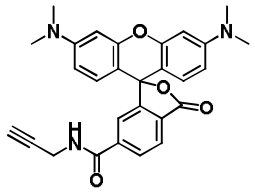
2-(7-(dimethylamino)-2-oxo-2H-chromen-4-yl)-N-(prop-2-ynyl)acetamide **(59)** –

Following the above procedure and purification by flash chromatography (4% -8% MeOH in $CHCl_3$+0.1%TEA) the compound was obtained in 40% yield. $\mathbf{R_f}$ (9:1 MeOH:CH_2Cl_2+0.1%TEA) = 0.45. **IR** (KBr)/cm^{-1} – 3400, 3300, 2900, 2850, 1700, 1650, 1625, 1525. **^{1}H** (300M*Hz*, DMSO-d6) δ 8.64(1H, bs, -NH-), 7.52(1H, d, Ar-CH-, *J*= 9.1*Hz*), 6.72(2H, dd, Ar-CH-, *J*=2.6*Hz*, *J*=6.4*Hz*), 6.55(2H, d, Ar-CH-*J*=2.6*Hz*), 5.76(1H, s, Ar-CH-), 3.87(2H, m, -*CH$_2$*-Alkyne,*J*=2.5*Hz*), 3.63(2H, s, -NHCO-*CH$_2$*-),3.14(1H, m, Alkyne-*CH*-, *J*=2.5*Hz*), 3.01(6H,s, N*(CH$_3$)$_2$*); **^{13}C**(300 M*Hz*, DMSO-d6) δ 177.1, 167.7, 160.6, 155.4, 152.8, 150.9, 125.9, 109.3, 109.0, 108.1, 97.5, 96.4, 85.4, 80.8, 73.3, 71.9, 28.2. **MS** (ES^{+}): m/z (%): 285.1 [M+H]$^{+}$. **HRMS** (ES^{+}) m/z calc. – 285.1240, observed – 285.1234. LCMS Purity - 96%. Rt = 11.6 – 11.7 min. Eluent system – 0% ACN/H_2O – 100% ACN/H_2O.



61

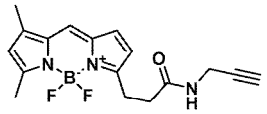
Artelinate Alkyne **(61)** – Following the above procedure and purification by flash chromatography (50% EtOAc in Hexane) the compound was obtained in 65% yield. R_f (50% EtOAc in Hexane) = 0.5. IR (KBr)/cm^{-1} – 3400, 2950, 1650. **^{1}H** (300M*Hz*, MeOD) δ 7.75(2H, d, Ar-*CH*, *J*=8.4*Hz*), 7.38(2H, d, Ar-*CH*, *J*=8.4*Hz*), 5.38(1H, s, -O-*CH*-O), 4.51(1H, d, β-*CH*-O, *J*=12.8*Hz*), 4.08 (2H, m, Alkyne-CH_2-, *J*=2.5*Hz*), 0.8-2.55 (25H, m, Artemisinin-*CH*-, Alkyne-CH_2-); **^{13}C** (300 M*Hz*, MeOD) δ 143.8, 134.4, 128.3, 105.5, 102.7, 101.7, 89.4, 82.2, 80.8, 72.0, 70.3, 53.9, 45.8, 38.7, 37.4, 35.8, 32.4, 26.1, 25.8, 25.7, 20.7, 13.4. **MS** (ES^+): m/z (%): 478 $[M+Na]^+$. **HRMS** (ES^+) m/z calc. – 478.2200 , observed – 478.2203 . **LCMS** Purity - 96%. Rt = 15.7-15.9 min. Eluent system – 5% ACN/H_2O – 100% ACN/H_2O.



87

TAMRA Alkyne (87) –

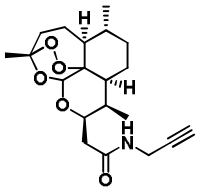
Following the procedure for TAMRA activation and coupling as described above and purification by flash chromatography (10% MeOH:CH_2Cl_2+0.1%TEA) the compound was obtained in 65% yield. **R_f**(10% MeOH:CH_2Cl_2+0.1%TEA)= 0.25. **IR** (KBr)/cm^{-1} – 3417, 1621, 1600, 1406, 1366, 1186, 1134. **^{1}H** (500M*Hz*, DMSO-d_6) δ 9.18(1H,b, -*NH*-), 8.08-8.19 (2H, d,d, Ar-CH, *J*=8.2*Hz*, *J*=8.2*Hz*), 7.68(1H, s, Ar-*CH*), 6.55(6H, bs, Ar-*CH*), 3.99(2H,m, Alkyne-CH_2, *J*=2.6*Hz*), 3.08(1H, s, Alkyne-*CH*, *J*=2.6*Hz*), 2.97(12H, s, (-NH-$(CH_3)_2)_2$); **^{13}C**(500M*Hz*, DMSO-d_6) δ 164.3, 129.2, 128.7, 97.8, 80.7, 73.0, 28.6, 8.4. **MS** (ES^+): m/z (%): 468.3 $[M+H]^+$. **HRMS** (ES^+) m/z calc. – 468.1918 , observed – 468.1931. **LCMS** Purity - 96%. Rt = 9.4-9.7 min. Eluent system – 0% ACN/H_2O – 100% ACN/H_2O.



88

BODIPY Alkyne (88) –

Following the above procedure and purification by flash chromatography (6% -8% MeOH in $CHCl_3$) the compound was obtained in 80% yield. $\mathbf{R_f}$ (50% EtAc:Hex) = 0.75. **IR**(KBr)/cm^{-1} – 3319, 3280, 2922, 2852, 1654, 1533, 1485. **1H** (500M*Hz*, MeOD) δ 7.42(1H, s, N-C-*CH*-C-N^+), 6.99(1H, d, Ar-*CH*-, *J*=4.0*Hz*), 6.32(1H, d, Ar-*CH*-,*J*=4.4*Hz*), 6.20(1H, s, Ar-*CH*-), 3.96(2H, m, Alkyne-CH_2, *J*=2.6*Hz*), 3.22(2H, t, BODIPY-CH_2, *J*=6.9), 2.57-2.63(2H, t, BODIPY-CH_2, *J*=6.9*Hz;* 1H, m, Alkyne-*CH*, *J*=2.6*Hz*), 2.51 (3H, s, BODIPY-CH_3), 2.27 (3H, s, BODIPY-CH_3); **^{13}C**(500 M*Hz*, MeOD) δ 174.2, 161.3, 158.4, 129.6, 125.8, 121.3, 117.7, 80.6, 72.2, 54.8, 35.7, 29.5, 25.4, 14.8, 11.2. **MS** (ES^+) m/z (%): 352.09$[M+Na]^+$. **HRMS** (ES^+) m/z calc. – 352.1406 , obsvd. – 352.1420. **LCMS** Purity - 96%. Rt = 13.9-14.2 min. Eluent system – 5% ACN/H_2O – 100% ACN/H_2O.



104

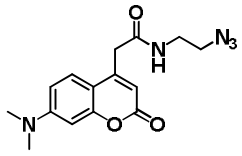
β-Deoxocarbaartemisinin alkyne (**104**) – Following the above procedure and purification by flash chromatography (6% -8% MeOH in $CHCl_3$) the compound was obtained in 77% yield. $\mathbf{R_f}$ (50% EtAc:Hex) = 0.65. **IR**(KBr)/cm^{-1} – 3361, 2955, 2859, 1676, 1526, 1458, 1383. **^{1}H** (500M*Hz*, $CDCl_3$) δ 5.39 (1H, s, -O-*CH*-O), 4.87(1H,m, *J*= 12.6*Hz*, β-*CH*-O), 4.05(2H, m, Alkyne-CH_2, *J*=2.9*Hz*), 0.89-2.72 (20H, m, Artesunate-*CH*-, -CH_3-*;* 1H, m, Alkyne-*CH*, *J*=2.9*Hz*); **^{13}C** (500M*Hz*, $CDCl_3$) δ 172.1, 103.6, 90.9, 81.5,80.5, 71.9, 69.7, 52.4, 43.9, 38.2, 38.1, 37.2, 34.9, 31.1, 29.6,

29.5, 25.5, 20.7, 12.6. **MS** (ES^+) m/z (%): 388.06 $[M+Na]^+$. **HRMS** (ES^+) m/z calc. – 364.2118, obsvd. – 364.2125 $[M+H]^+$. **LCMS** Purity - 96%. Rt = 14.7-14.9 min. Eluent system – 0% ACN/H_2O – 100% ACN/H_2O+0.1% TFA.

Azide linkage **(60, 62)** –

Coumarin acetic acid **(34)** (100mgs, 0.4mmol, 1equiv.) was added under Ar atmosphere and 2.5ml of anhydrous CH_2Cl_2 was added to above reactant. The reaction mixture was cooled to -10^oC using Ice bath and then DIPEA (209 mgs, 1.62mmol, 4 equiv), HATU (169mgs, 0.44 mmol, 1.1equiv), HOAt (61mgs, 0.44 mmol, 1.1 equiv) and 3-Azido propanamine (35mgs, 0.4mmol, 1equiv) was added in the sequence respectively. The reaction is maintained under Ar atmosphere and -10^oC till the addition of the above components is complete. The reaction is cooled to room temperature gradually and stirring is continued at room temperature till TLC shows the product spot. The product was isolated by removing the reaction mixture under vacuum and the residue was purified by flash chromatography (4% - 8% MeOH in$CHCl_3$+ 0.1% TEA) 70% yield.

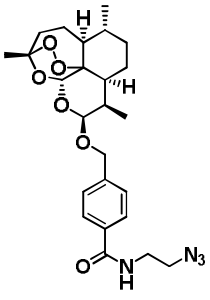
In case of TAMRA (**81**) based intermediates the procedure enclosed below is followed. 6-TAMRA-COOH (**81**) (53mgs, 0.16 mmol,1.0equiv) is added to flame dried single necked flask containing dry THF 5mL. The reaction mixture was cooled to -10^oC and then ethyl chloroformate (20mgs (33µL), 0.19 mmol, 1.5equiv) and TEA (35mgs (48µL), 0.19 mmol, 1.5equiv). The reaction mixture is stirred for 1hr at this temperature and then 2-Azidoethylamine (22mgs, 0.25mmol, 1.5equiv) is added in liquid form directly to the reaction mixture at -10^oC. The reaction mixture is gradually cooled to room temperature and stirred for 16-18hrs. After confirmation with TLC, the solvent is removed under vacuum and then the crude is purified by column chromatography (10% -15% MeOH in $CHCl_3$).



60

N-(2-azidoethyl)-2-(7-(dimethylamino)-2-oxo-2H-chromen-4-yl)acetamide **(60)** –

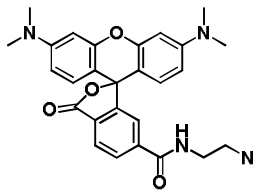
Following the above procedure and purification by flash chromatography (4% -8% MeOH in $CHCl_3$+0.1%TEA) the compound was obtained in 70% yield. $\mathbf{R_f}$ (10% MeOH:CH_2Cl_2) = 0.5. **IR** (KBr)/cm^{-1} – 3400, 3300, 2950, 1700, 1650, 1600, 1550. ^{1}**H** (300M*Hz*, MeOD) δ 7.47(1H, d, Ar-*CH*-, *J*=9*Hz*), 6.66 (1H, dd, Ar-*CH*-, *J*=2.5*Hz*, 6.6*Hz*), 6.46 (1H, d, Ar-*CH*-, *J*=2.6*Hz*), 5.97(1H, s, Ar-*CH*-), 3.62(2H, s, -NHCO-CH_2-), 3.20-3.3(4H, m, NH-CH_2-CH_2-N_3), 2.98 (6H,s, N$(CH_3)_2$); ^{13}**C** (300 M*Hz*, MeOD) δ 171.4, 164.3, 157.2, 154.8, 152.6, 130.1, 126.9, 110.6, 110.5, 109.8, 98.8, 51.5, 40.2, 40.2, 40.1. **MS** (ES^+): m/z (%): 316.1$[M+H]^+$ 338.0$[M+Na]^+$. **HRMS** (ES^+) m/z calc. – 338.1218 , observed – 338.1224. **LCMS** Purity - 96%. Rt = 11.9-12.1 min. Eluent system – 5% ACN/H_2O – 100% ACN/H_2O.



62

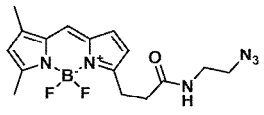
Artelinate Azide **(62)** – Following the above procedure and purification by flash chromatography (50% EtOAc in Hexane) the compound was obtained in 65% yield. $\mathbf{R_f}$ (50% EtOAc in Hexane) = 0.5. **IR** (KBr)/cm^{-1} – 3400, 2900, 2100, 1650, 1550. ^{1}**H** (300M*Hz*, MeOD) δ 7.74(2H, d, Ar-*CH*, *J*=6.6*Hz*), 7.38(2H, d, Ar-*CH*, *J*=8.8), 5.38(1H, s, -O-*CH*-O), 4.58(1H, d, β-*CH*-O, *J*=12.8*Hz*), 3.41-3.53(4H, -NH-CH_2-CH_2-N_3-), 0.79-2.58 (22H, m, Artemisinin-*CH*-); ^{13}**C** (300 M*Hz*, MeOD) δ 143.7,

128.6, 128.5, 105.5, 102.7, 89.4, 82.2, 70.3, 53.9, 51.4, 45.8, 40.6, 38.7, 37.4, 35.8, 32.4, 26.1, 25.8, 25.7, 20.7, 13.4. **MS** (ES^+): m/z (%): 509.1 $[M+Na]^+$. **HRMS** (ES^+) m/z calc. – 509.2368, observed – 509.2371. **LCMS** Purity - 96%. Rt = 15.9-16.2 min. Eluent system – 5% ACN/H_2O – 100% ACN/H_2O.



89

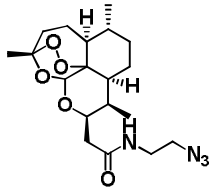
TAMRA azide **(89)** – Following the protocol for TAMRA based probes the above compound was obtained in 90% yields. R_f (10% MeOH/DCM) = 0.15. IR (KBr)/cm^{-1} – 3415, 2957, 2852, 2106, 1597, 1492, 1365. **^{1}H** (500M*Hz*, MeOD) δ 8.19-8.13 (2H, d, d, Ar-CH, *J*=8.2*Hz*, *J*=8.2*Hz*), 7.74(1H, d, Ar-*CH*, *J*= 2.0*Hz*), 7.27(2H, d, Ar-*CH*, *J*= 9.45*Hz*), 7.05(2H, dd, Ar-*CH*, *J*= 2.5*Hz*, *J*= 6.95*Hz*), 6.96(1H, d, Ar-H, *J*=2*Hz*), 3.30-3.34(4H, m, NH-CH_2-CH_2-N_3), 3.1(12H, s, (-NH-$(CH_3)_2)_2$) ; **^{13}C**(500 M*Hz*, MeOD) δ 159.1, 158.8, 132.6, 129.6, 115.2, 97.3, 49.5, 40.9, 39.9. **MS** (ES^+): m/z (%): 499.01 $[M+H]^+$. **HRMS** (ES^+) m/z calc. – 499.2088, observed – 499.2093. **LCMS** Purity - 96%. Rt = 12.89-12.93 min. Eluent system – 5% ACN/H_2O – 100% ACN/H_2O.



90

BODIPY azide **(90)** – Following the protocol for regular probes the above compound was obtained in 85% yields. **R_f** (10% MeOH/DCM) = 0.5. **IR** (KBr)/cm^{-1} – 3292, 2953, 2926, 2101, 1527, 1442. **1H** (500M*Hz*, MeOD) δ 7.37(1H, s, N-C-*CH*-C-N^+), 6.97(1H, d, Ar-*CH*-, *J*=3.8*Hz*), 6.31(1H, d, Ar-*CH*-,*J*=4.4*Hz*), 6.17(1H, s, Ar-*CH*-), 3.36(4H, m, NH-CH_2-CH_2-N_3), 3.22(2H, t, BODIPY-CH_2, *J*=7.6*Hz*), 2.61(2H, t,

BODIPY-*CH*$_2$, *J*=8.2*Hz*), 2.57-2.63(2H, t, BODIPY-*CH*$_2$, *J*=6.9*Hz;* 1H, m, Alkyne-*CH*, *J*=2.6*Hz*), 2.60 (3H, s, BODIPY-*CH*$_3$), 2.24 (3H, s, BODIPY-*CH*$_3$); **^{13}C**(500 M*Hz*, MeOD) δ 174.9, 161.3, 158.4, 145.8, 136.5, 134.9, 129.6, 125.7, 121.3, 117.6, 51.5, 39.9, 35.9, 25.5, 14.9, 11.2. **MS** (ES^+): m/z (%): 383.05 $[M+H]^+$. **HRMS** (ES^+) m/z calc. – 383.1577, observed – 383.1593. **LCMS** Purity - 96%. Rt = 14.0-14.4 min. Eluent system – 0% ACN/H_2O – 100% ACN/H_2O+0.1%TFA.

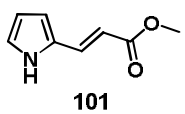


105

β-Deoxocarbaartemisinin azide **(105)** – Following the above procedure and purification by flash chromatography (6% -8% MeOH in $CHCl_3$) the compound was obtained in 77% yield. **R_f** (100% EtAc) = 0.68. **IR**(KBr)/cm^{-1} – 3361, 2955, 2859, 2092, 1674, 1522, 1381. **^{1}H** (500M*Hz*, $CDCl_3$) δ 5.39 (1H, s, -O-*CH*-O), 4.78(1H,m, *J*= 12.6*Hz*, β-*CH*-O), 3.58-3.28(1H, m, O-CH-*CH*$_2$-CO; 2H, m, NH-*CH*$_2$-CH_2-N_3; 1H, m, O-CH-*CH*$_2$-CO), 0.86-2.56 (22H, m, Artesunate-*CH*-, -*CH*$_3$-); **^{13}C** (500M*Hz*, $CDCl_3$) δ 172.8, 103.6, 90.9, 81.5, 70.2, 52.4, 51.4, 44.0, 39.5, 38.2, 38.1, 37.2, 34.9, 31.1, 26.4, 25.5, 20.7, 12.7. **MS** (ES^+) m/z (%): 417.06 $[M+Na]^+$. **HRMS** (ES^+) m/z calc. – 395.2289, obsvd. – 395.2292 $[M+H]^+$. **LCMS** Purity - 96%. Rt = 15.7-15.9 min. Eluent system – 5% ACN/H_2O – 100% ACN/H_2O.

5.1.23 – Synthesis of BODIPY-COOH[66] –

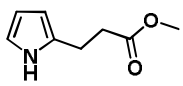
Following the procedure in literature[66] the characterization data for the intermediates and the final products is listed below.



101

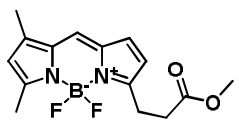
(E)-methyl 3-(1H-pyrrol-2-yl)acrylate (**101**) –

Following the procedure as discussed in literature[66], and purification by flash chromatography (10% EtOAc in Hexane) the compound was obtained in 90% yield. **R_f** (10% EtOAc in Hexane) = 0.15. **^{1}H** (300M*Hz*, $CDCl_3$) δ – 9.51(1H, b, -*NH*-), 7.62(1H, d, NH-C-CH-*CH*-CO-, *J*=26.6*Hz*, *E-enantiomer*), 6.93(1H, d, Ar-*CH*, *J*=1.9*Hz*), 6.57(1H, s, Ar-*CH*), 6.29(1H, m, Ar-*CH*), 6.13(1H, d, NH-C-*CH*-CH-CO-, *J*=15.9*Hz*), 3.79 (3H, s, -CO_2-CH_3-), 6.12(1H, d, NH-C-*CH*-CH-CO-, *J*=26.6*Hz*, *E-enantiomer*); **^{13}C** (300 M*Hz*, $CDCl_3$) δ – 168.7, 134.9, 128.3, 122.7, 114.5, 110.7, 110.3, 51.5. **MS** (ES^-): m/z (%): 150.1 $[M-H]^-$.



102

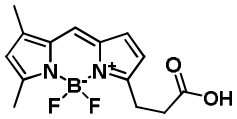
Methyl 3-(1H-pyrrol-2-yl)propanoate (**102**) - Following the procedure as discussed in literature[66], and purification by flash chromatography (10% EtOAc in Hexane) the compound was obtained in 95% yield. **R_f** (10% EtOAc in Hexane) = 0.35. **^{1}H** (300M*Hz*, $CDCl_3$) δ – 6.69(1H, m, Ar-*CH*-), 6.15(1H, m, Ar-*CH*-), 5.97(1H, m, Ar-*CH*-), 3.74 (3H, s, -CO_2-CH_3-), 2.95(2H, t, NH-C-CH-*CH*-CO-, *J*=6.9*Hz*), 2.68(2H, t, NH-C-CH_2-CH-CO-, *J*=6.9*Hz*); **^{13}C** (300 M*Hz*, $CDCl_3$) δ – 174.3, 130.7, 116.7, 107.9, 105.3, 51.6, 34.1, 22.4. **MS**(ES^-): m/z (%): 151.9 $[M-H]^-$. **HRMS**(ES^+) m/z calc. – 176.0682, observed – 176.0682$[M+Na]^+$.



103

5,5-difluoro-3-(3-methoxy-3-oxopropyl)-7,9-dimethyl-5H-dipyrrolo[1,2-c:1',2'-f][1,3,2]diazaborinin-4-ium-5-uide (**103**) - Following the procedure as discussed in literature[66], and purification by flash chromatography (25% EtOAc in Hexane) the

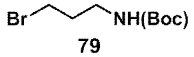
compound was obtained in 95% yield. **R_f** (25% EtOAc in Hexane) = 0.45. **^{1}H** (500M*Hz*, $CDCl_3$) δ – 7.06(1H, s, N-C-*CH*-C-N^+), 6.85(1H, d, Ar-*CH*-, *J*=3.8*Hz*), 6.24(1H, d, Ar-*CH*-, *J*=3.8*Hz*), 6.09(1H, s, Ar-*CH*-), 3.68 (3H, s, -CO_2-CH_3), 3.28(2H, t, NH-C-CH-*CH*-CO-, *J*=6.9 *Hz*), 2.76(2H, t, NH-C-CH_2-CH-CO-, *J*=6.9 *Hz*), 2.54 (3H, s, -CH_3-), 2.21 (3H, s, -CH_3-), ; **^{13}C** (500 M*Hz*, $CDCl_3$) δ – 172.9, 160.3, 156.9, 143.8, 135.1, 133.2, 128.0, 123.8, 120.4, 116.5, 51.6, 33.2, 29.6, 23.9, 14.9, 11.2. **MS** (ES^-): m/z (%): 329.05$[M+Na]^-$. HRMS (ES^+) m/z calc. – 329.1246, observed – 329.1249.



8

3-(2-carboxyethyl)-5,5-difluoro-7,9-dimethyl-5H-dipyrrolo[1,2-c:1',2'-f][1,3,2]diaza borinin-4-ium-5-uide (**84**) - Following the procedure as discussed in literature[66], and purification by flash chromatography (25% EtOAc in Hexane) the compound was obtained in 95% yield. **R_f** (25% EtOAc in Hexane) = 0.40. **^{1}H** NMR (500M*Hz*, MeOD) δ – 7.43(1H, s, N-C-*CH*-C-N^+), 7.01(1H, m, Ar-*CH*-), 6.34(1H, m, Ar-*CH*-), 6.21(1H, s, Ar-*CH*-), 3.20(2H, t, NH-C-CH-*CH*-CO-, *J*=6.9 *Hz*), 2.71(2H, t, NH-C-CH_2-CH-CO-, *J*=6.9*Hz*), 2.28 (3H, s, -CH_3-), 2.51 (3H, s, -CH_3-); **^{13}C** NMR (500M*Hz*, MeOD) δ – 176.2, 161.4, 158.4, 145.8, 134.9, 129.6, 125.8, 121.3, 117.5, 34.0, 25.0, 14.9, 11.2. **MS**(ES^-): m/z (%): 291.0 $[M-H]^-$.

5.1.24 – Synthesis of tert-butyl 3-bromopropylcarbamate[67] –

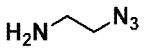


79

Following the procedure in literature[67] and purification by flash chromatography (25% Ethyl acetate/Hexane) the compound was obtained in 80% yield. R_f (25% Ethyl

acetate/Hexane) = 0.5. **^{1}H**(500M*Hz*, $CDCl_3$) δ – 4.66(1H, b, -*NH*-), 3.43(2H, m, B_{r}-CH_{2}-), 3.26(2H, m, Br-CH_2-*CH_2*-CH_2-NH), 2.04(2H, m, -*CH_2*-NH), 1.44(9H, s, -*$(CH_3)_3$*-); **^{13}C** (500 M*Hz*, $CDCl_3$) δ – 156.6, 80.14, 39.73, 33.42, 31.43, 29.06. **MS** (ES^+): m/z (%): 261.82 $[M+Na]^+$.

5.1.25 – Synthesis of 2-Azidoethylamine[68] –



2-Azido ethylamine

80%

Following the procedure in literature[68] and purification by work-up as shown in literature the compound was obtained in 80% yields. **R_f** (10% MeOH/CH_2Cl_2) = 0.5. **^{1}H** (500M*Hz*, $CDCl_3$) δ – 4.7(2H, bs, -*NH_2*-), 3.34(2H, t, -*CH_2*-N_3, *J*=9.1*Hz*), 2.85(2H, t, -*CH_2*-NH_2, *J*=9.1*Hz*); **^{13}C** (500 M*Hz*, $CDCl_3$) δ – 66.5, 55.3, 41.9, 24.9, 15.9. **MS** (ES^+): m/z (%): 86.82 $[M+H]^+$.

5.2 Thermal stability protocols –

A pre-calibrated oven maintained at 37^{o}C and 100% humidity was utilized for thermal stability study. Freezer at -20^{o}C and 0% humidity and AR grade DMSO was utilized for preparing DMSO solution (100µL added for all samples) and solid for cold storage conditions. Samples were placed in eppendorf tubes and lid was closed during storage in the oven and freezer. The samples were analyzed for stability after 4 days, 2months, 6months, 12months exposure to the above conditions using Shimadzu IT-TOF LCMS instrument. ACN + 0.1% TFA / H2O + 0.1% TFA was used as the eluent in the reverse phase short column and the polarity was increased from 0% - 100% in a period of 25min. 2µL of sample (sample diluted with 1.5ml of HPLC grade MeOH) was injected for analysis and compared with the standard sample (diluted with 1.5ml of MeOH when required). This standard is not subjected to any of the above conditions and kept in freezer at 4^{o}C.

5.3 Macrophage studies protocols –

Macrophages were prepared fresh by differentiation of THP-1 Monocytes using Phorbol 12-myristate 13-acetate (PMA) and used within 4 days for flow cytometry studies. Using the standard conditions as mentioned in Fig. 18-24, the cells were incubated with the probes and analyzed using Flow Cytometer (BD FACs Vantage SE). Varying concentrations of the probes from 5nM to 5µM were added to the fixed concentration of macrophages in cells along with 10µL of Propidium iodide (PI) to each cell for flow cytometry studies. The best concentrations of probes were chosen based on least cell death and maximum cell staining and used for confocal microscopy studies. Standard macrophage concentrations as described in Fig. 25 were prepared in glass base square shaped and the desired concentrations to be analyzed were added along with 60nM of Lyso-tracker red for confocal microscopy studies.

5.4 NCI 60 Cancer cell line protocols –

5.4.1 *Single Dose studies protocol* –

NCI 60 cancer cell line studies were done in collaboration with National Cancer Institute, USA[50-53] are based on single dose response of the probes (Structures **48a** and **51**). The concentration used for single dose testing is 10μM. The number reported for the one-dose assay is growth relative to the no-drug control, and relative to the time zero number of cells. This allows detection of both growth inhibition (values between 0 and 100) and lethality (values less than 0). E.g. - a value of 100 means no growth inhibition; 40 would mean 60% growth inhibition; 0 means no net growth over the course of the experiment. A value of -40 would mean 40% lethality.

5.4.2 *Five Dose studies protocol* [50-53] –

The human tumor cell lines of the cancer screening panel are grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells are inoculated into 96 well microtiter plates in 100 μL at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37° C, 5 % CO_2, 95 % air and 100 % relative humidity for 24 h prior to addition of experimental drugs.

After 24 h, two plates of each cell line are fixed *in situ* with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs are solubilised in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 μg/ml

gentamycin. Additional four, 10-fold or ½ log serial dilutions are made to provide a total of five drug concentrations plus control. Aliquots of 100 µl of these different drug dilutions are added to the appropriate microtiter wells already containing 100 µl of medium, resulting in the required final drug concentrations.

Following drug addition, the plates are incubated for an additional 48 h at 37°C, 5 % CO_2, 95 % air, and 100 % relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed *in situ* by the gentle addition of 50 µl of cold 50 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant is discarded, and the plates are washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 µl) at 0.4 % (w/v) in 1 % acetic acid is added to each well, and plates are incubated for 10 minutes at room temperature. After staining, unbound dye is removed by washing five times with 1 % acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50µl of 80 % TCA (final concentration, 16 % TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as:

[(Ti-Tz)/(C-Tz)] x 100 for concentrations for which Ti>/=Tz

[(Ti-Tz)/Tz] x 100 for concentrations for which Ti<Tz.

Three dose response parameters are calculated for each experimental agent. “Growth inhibition” of 50 % (GI_{50}) is calculated from [(Ti-Tz)/(C-Tz)] x 100 = 50, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in “total growth inhibition” (TGI) is calculated from Ti = Tz. The LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from [(Ti-Tz)/Tz] x 100 = -50. Values are calculated for each of these three parameters if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter is expressed as greater or less than the maximum or minimum concentration tested.

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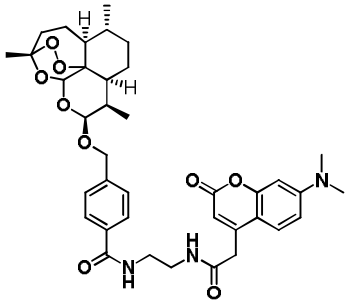
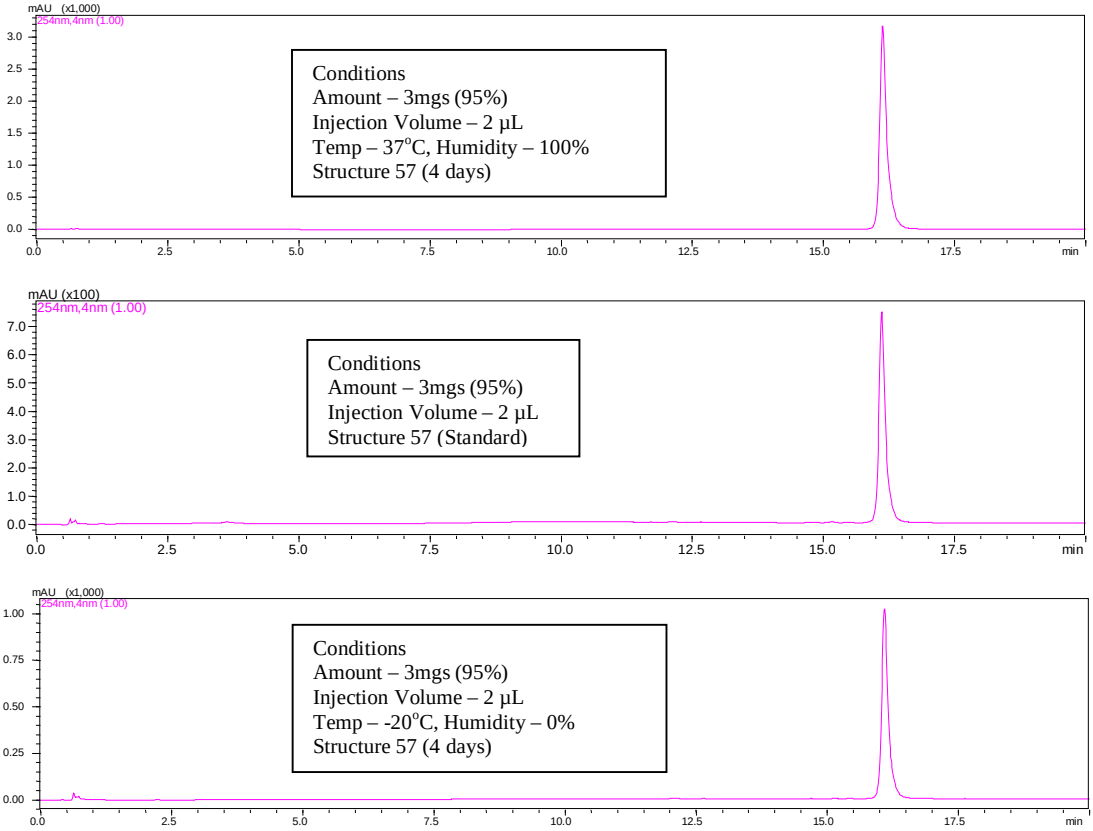
APPENDIX

Appendix A

A.1 Thermal stabilities of probes **57-63** –



57



MS(E+) Ret. Time : 16.193 -> 16.207 - 15.940 <-> 16.553

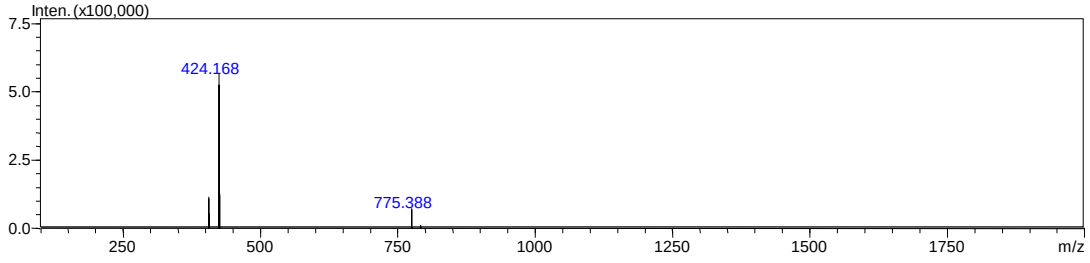
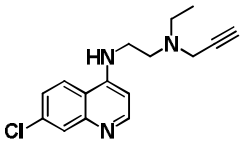
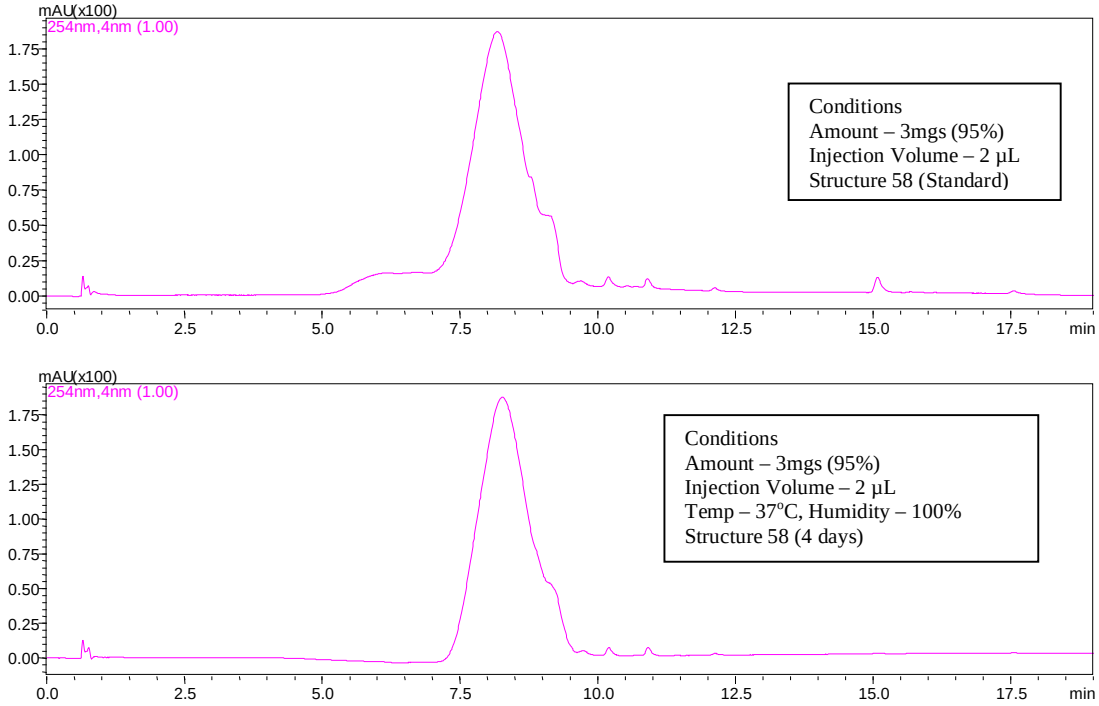


Fig. 34 – Artelinic acid probe **57** thermal stability studies (Eluent - ACN : H2O)



58



MS(E+) Ret. Time : 8.200 -> 8.213 - 8.153 <-> 8.280

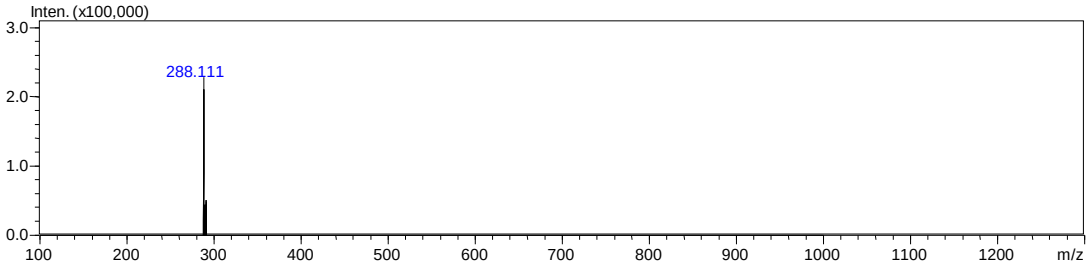
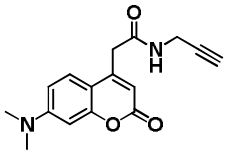
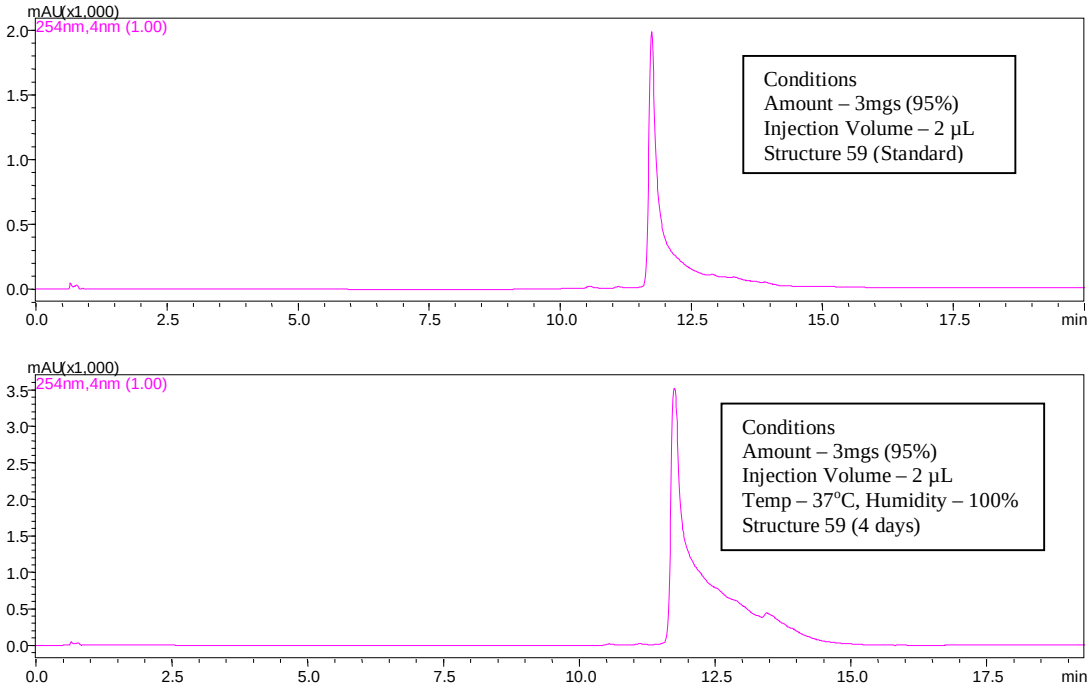


Fig. 35 – Probe **58** thermal stability studies (Eluent - ACN : H2O)



59



MS(E+) Ret. Time : 11.753 -> 11.767 - 11.600 <-> 11.840

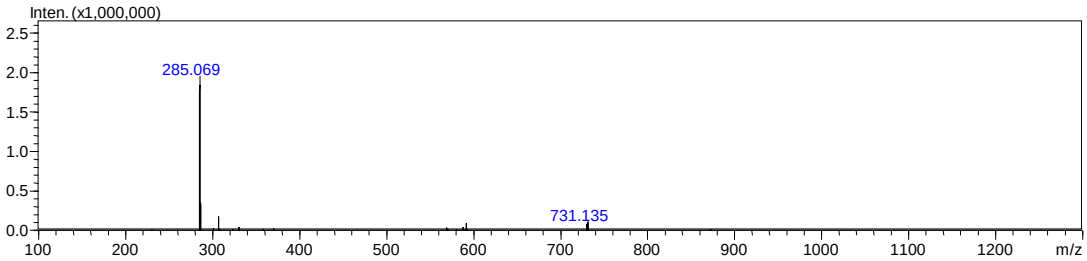
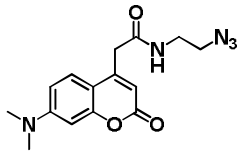
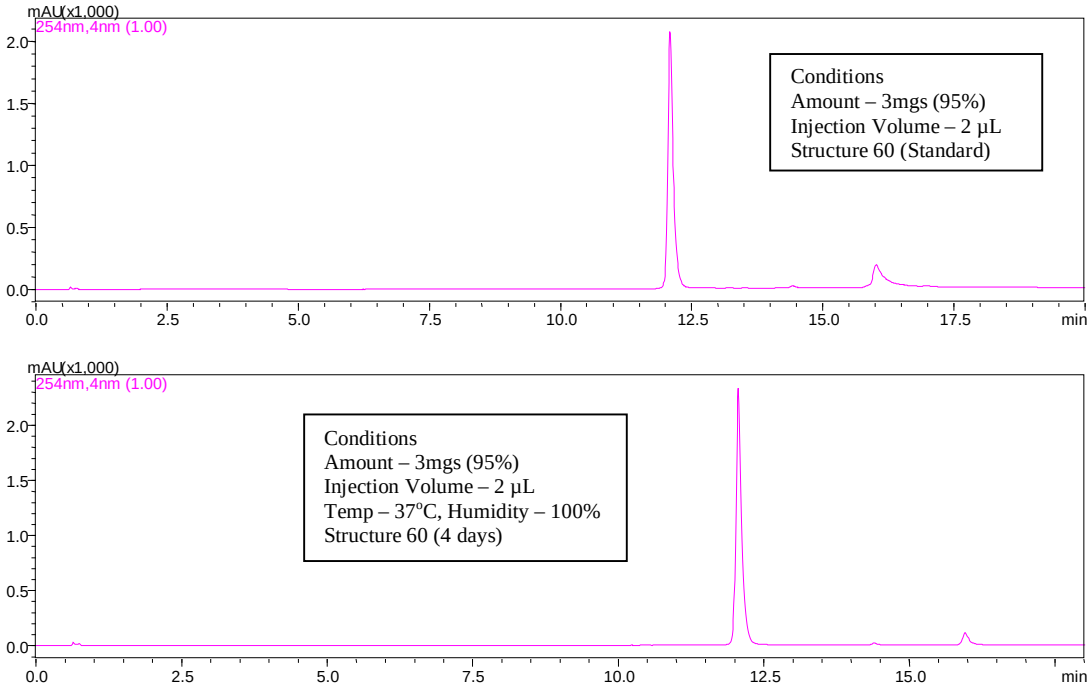


Fig. 36 – Probe **59** thermal stability studies (Eluent - ACN : H2O)



60



MS(E+) Ret. Time : 12.087 -> 12.100 - 11.960 <-> 12.120

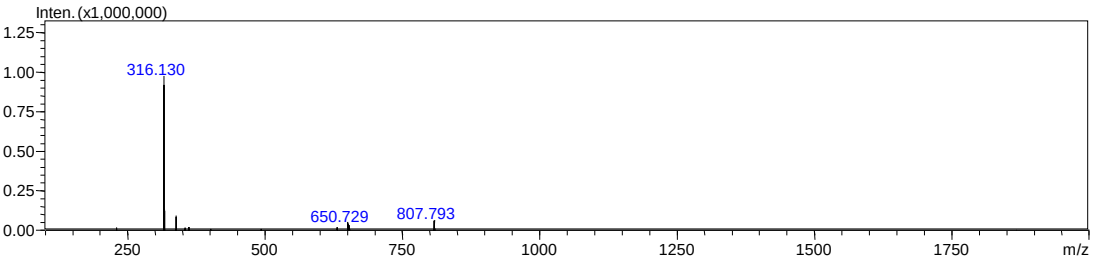
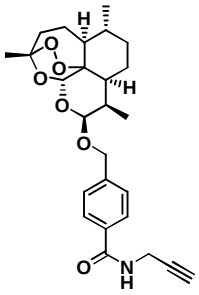


Fig. 37 – Probe **60** thermal stability studies (Eluent - ACN : H2O)



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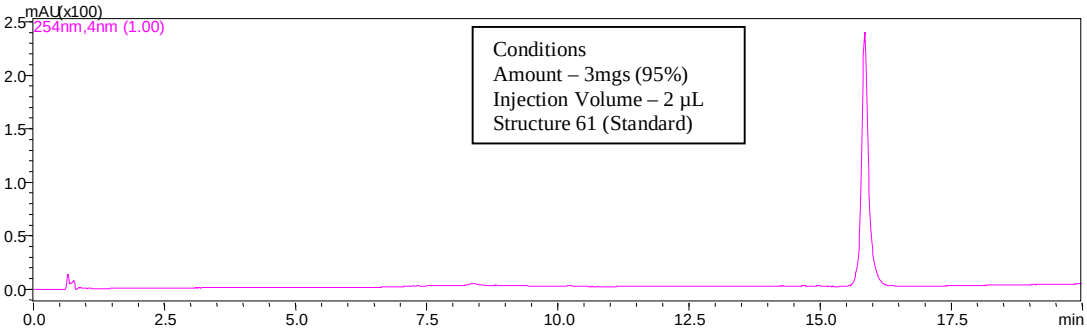
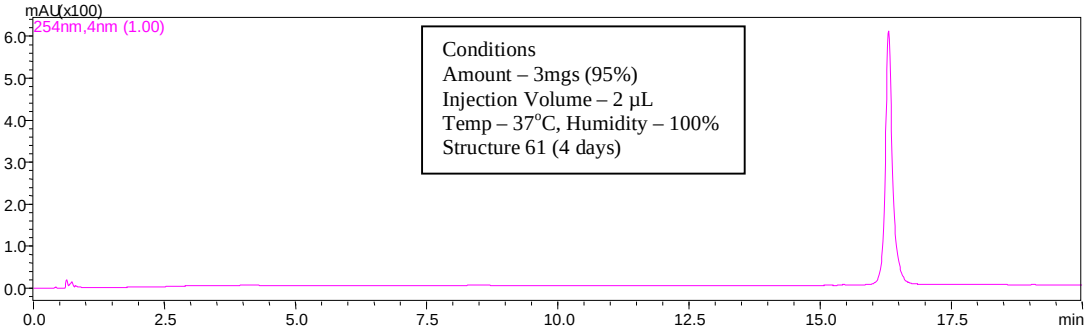




MS(E+) Ret. Time : 16.293 -> 16.307 - 16.120 <-> 16.367

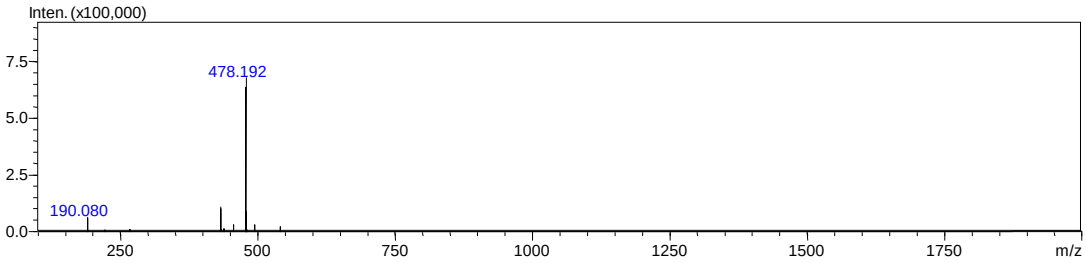
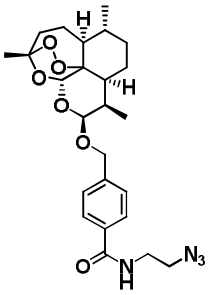


Fig. 38 – Probe **61** thermal stability studies (Eluent - ACN : H2O)



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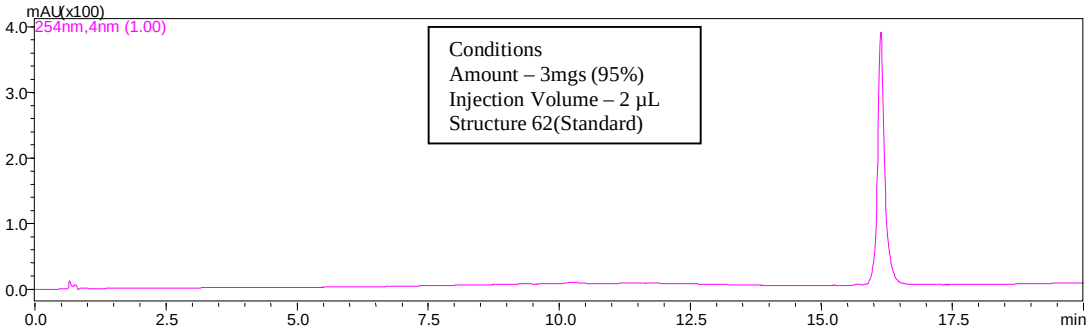
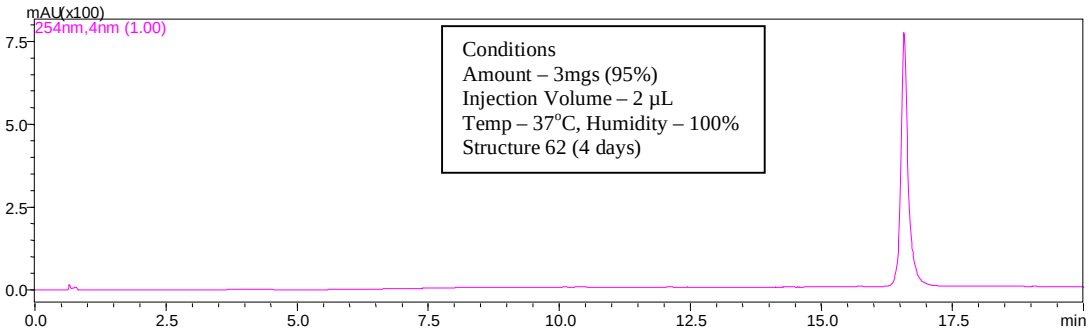




MS(E+) Ret. Time : 16.567 -> 16.580 - 16.413 <-> 16.660

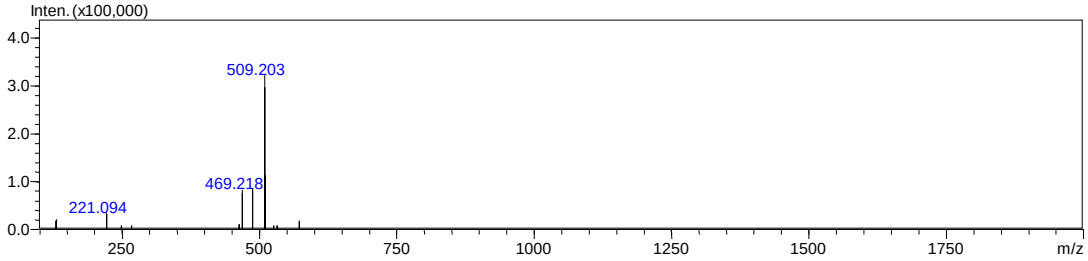
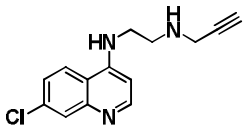
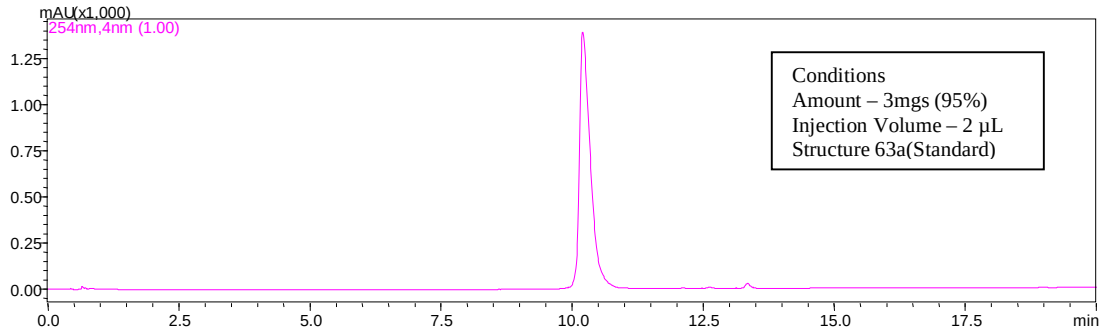
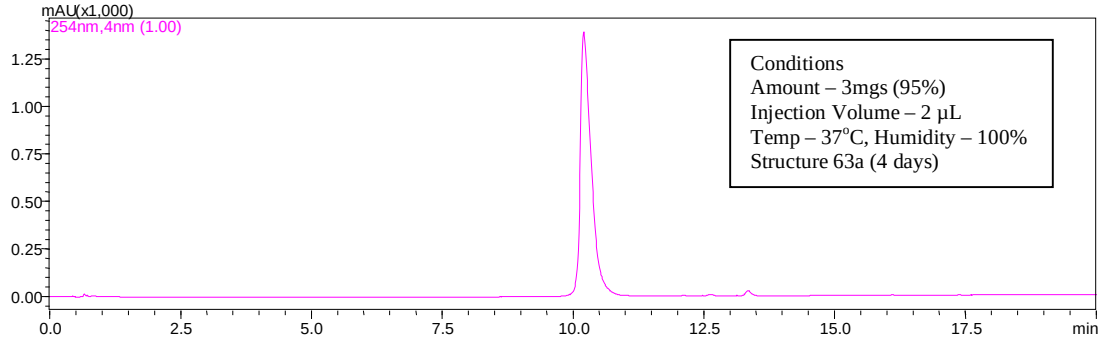


Fig. 39 – Probe **62** thermal stability studies (Eluent - ACN : H2O)



63a





MS(E+) Ret. Time : 10.267 -> 10.280 - 10.027

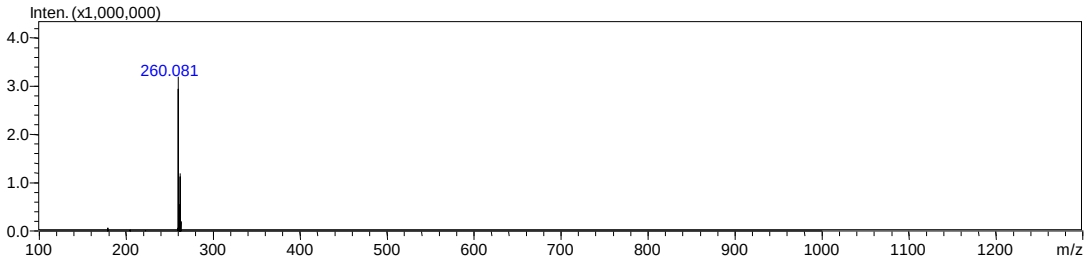
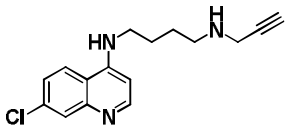
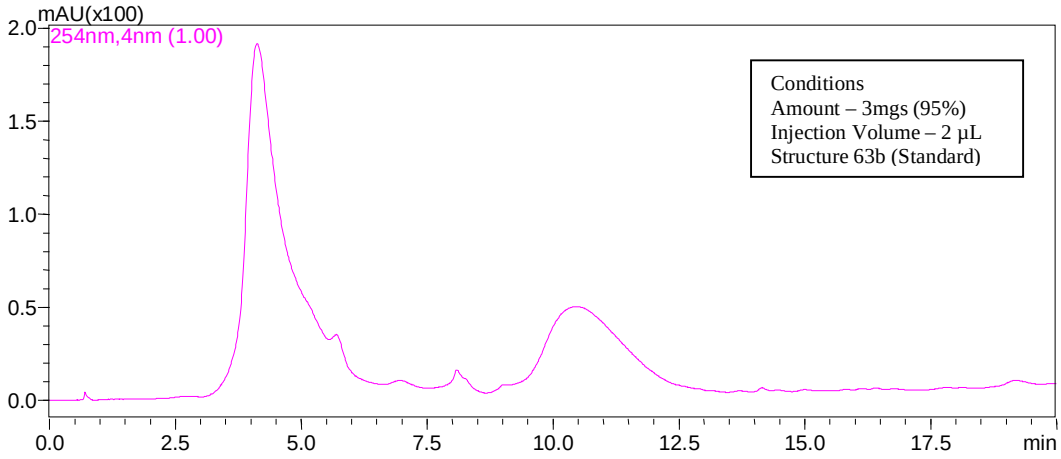
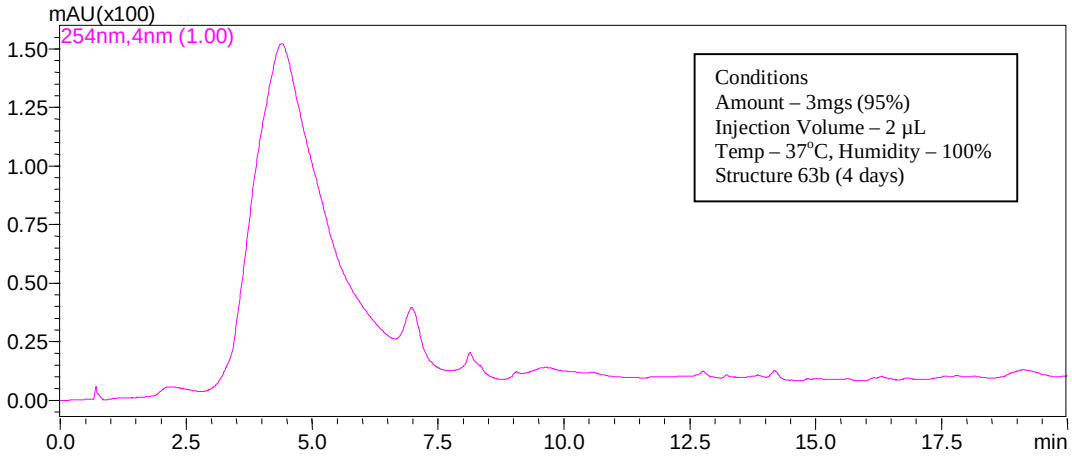


Fig. 40 – Probe **63a** thermal stability studies (Eluent - ACN : H2O)



63b





MS(E+) Ret. Time : 4.353 -> 4.367 - 4.313 <-> 4.413

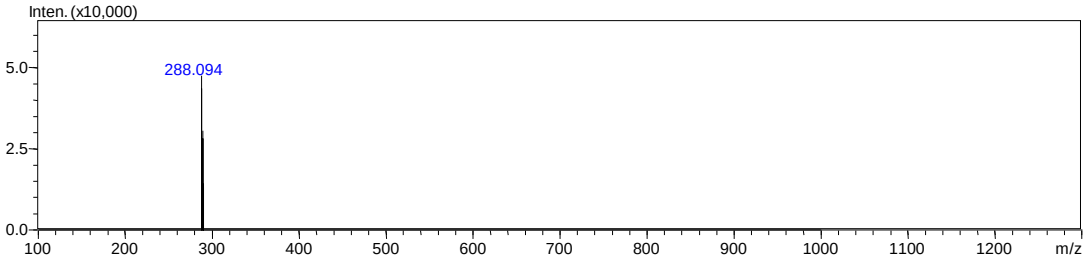
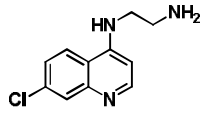


Fig. 41 – Probe **63b** thermal stability studies (Eluent - ACN : H2O)

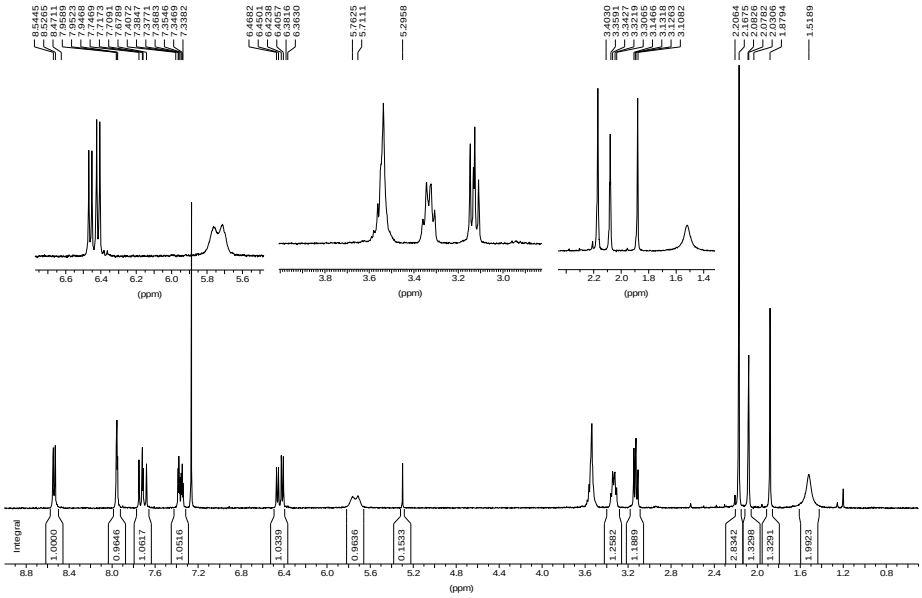
Appendix 2

A.2 NMR Data for molecules –

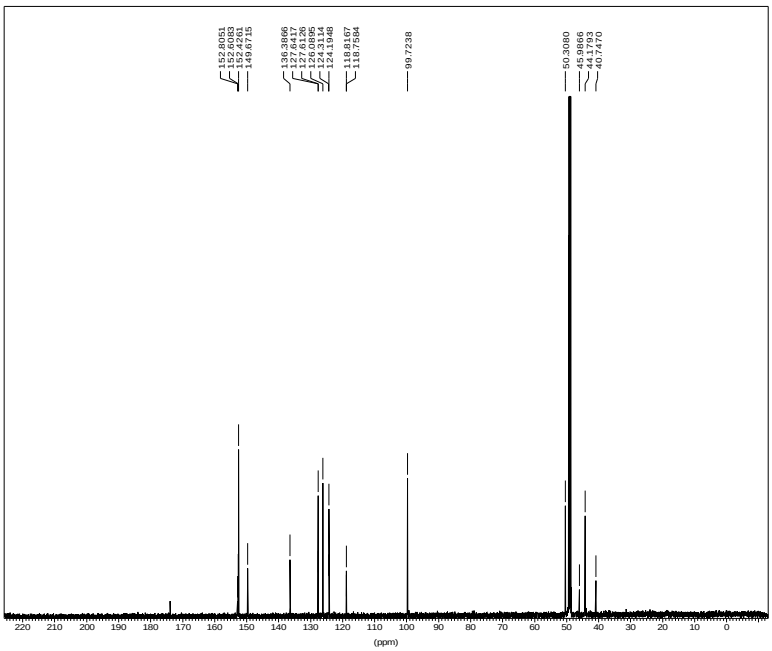


27a, n=1, 86%

1H normal range AC300 DCQA1



13C AMX500 DCQA1



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PROCNO : 1
*** Acquisition Parameters ***
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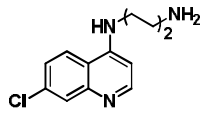
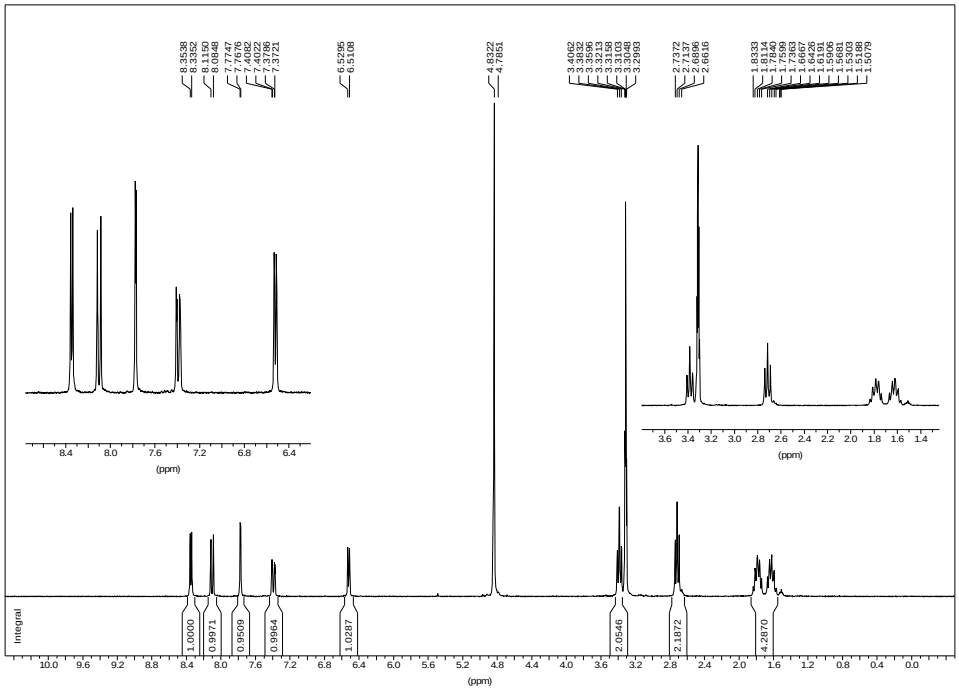
DATE_d : Oct 05 2009
DBPNAM0 :
INSTRUM : av500
LOCNUC : 2H
NS : 402
NUCLEUS : off
O1 : 13204.57 Hz
SFO1 : 125.7709936 MHz
SOLVENT : MeOD
*** 1D NMR Plot Parameters ***
NUCLEUS : off

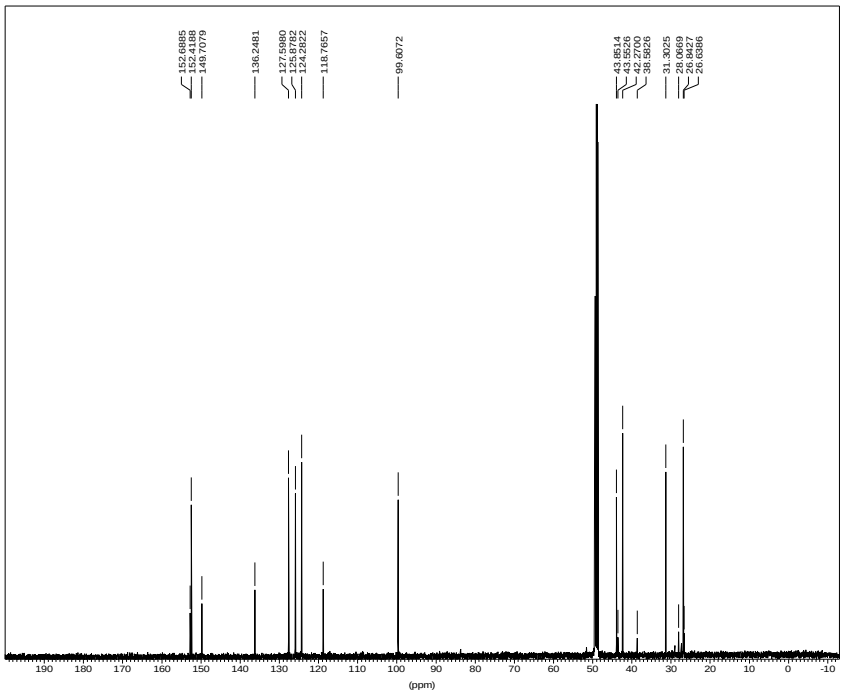


27b, n=2, 80%

1H normal range AC300 DCQA3 PURE



13C AMX500 DCQA3



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DATE_d : Oct 05 2009
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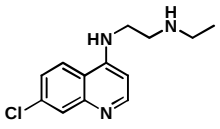
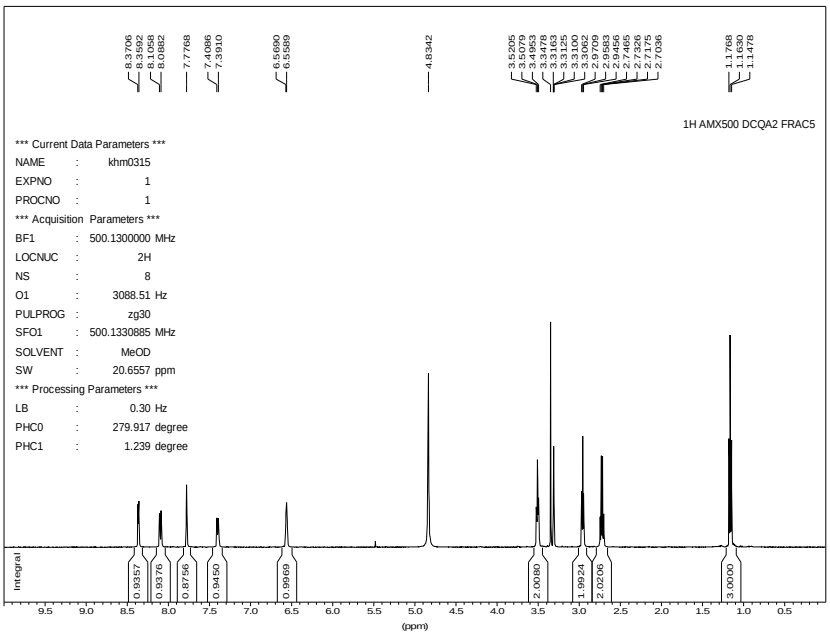
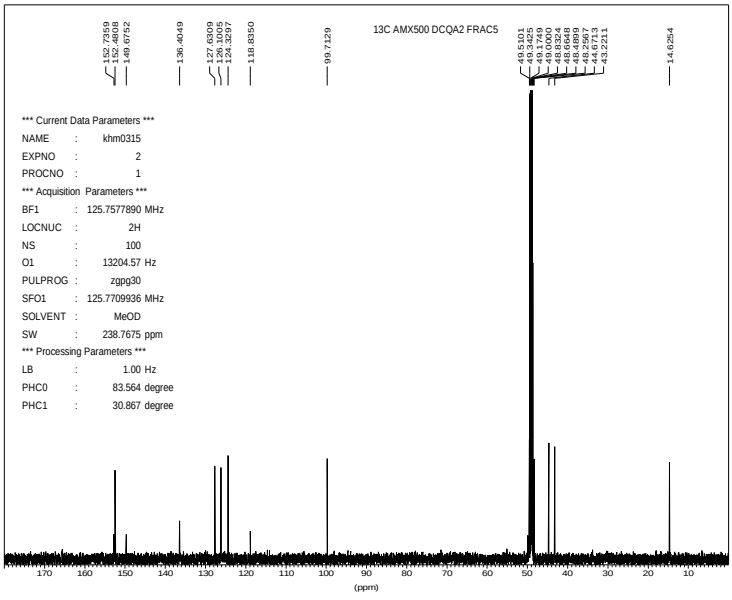
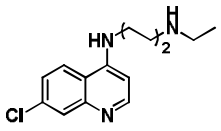
LOCNUC : 2H
NS : 300
NUCLEUS : off
O1 : 13204.57 Hz
SFO1 : 125.7709936 MHz
SOLVENT : MeOD
*** 1D NMR Plot Parameters ***
NUCLEUS : off



28a, n=1, 34%

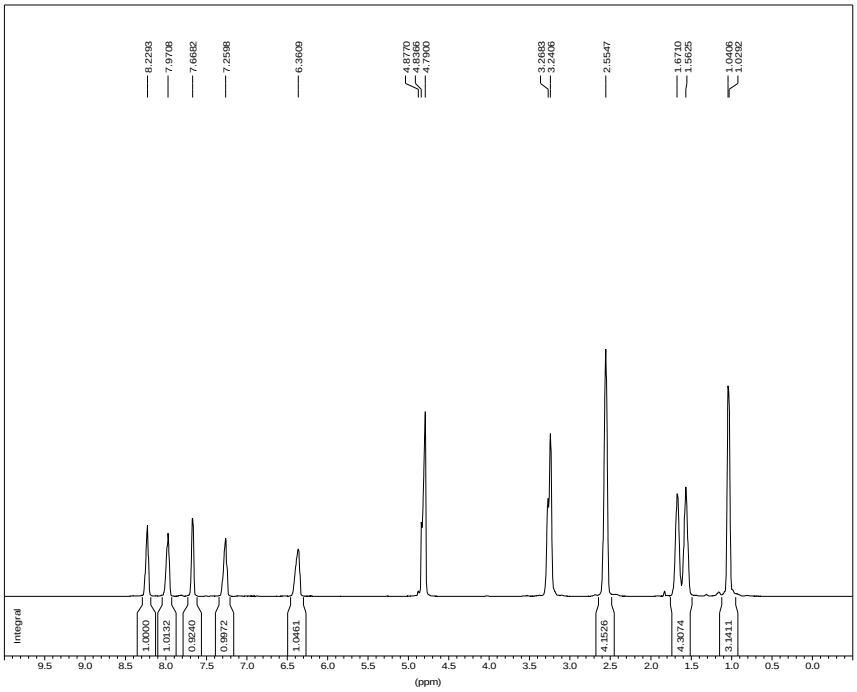

8.3706
8.3592
8.1498
8.0882
7.7768
7.4086
7.3910
6.5690
6.5589
4.8342
3.5205
3.5079
3.4953
3.4178
3.3455
3.3125
3.3100
3.2992
2.9709
2.9583
2.9456
2.7746
2.7326
2.7175
2.7036
1.1768
1.1630
1.1478
1H AMX500 DCQA2 FRAC5
*** Current Data Parameters ***
NAME : khm0315
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 500.1300000 MHz
LOCNUC : 2H
NS : 8
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : MeOD
SW : 20.6557 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 279.917 degree
PHC1 : 1.239 degree
Integral
0.9357
0.9376
0.8756
0.9450
0.9969
2.0080
1.9924
2.0206
3.0000
(ppm)


152.7359
152.4808
149.6752
136.4049
127.6309
126.1050
124.3297
118.8350
99.7129
13C AMX500 DCQA2 FRAC5
49.5101
49.4255
49.1749
49.0000
48.8324
48.6648
48.4899
48.3577
44.6713
43.2211
14.6254
*** Current Data Parameters ***
NAME : khm0315
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 125.7577890 MHz
LOCNUC : 2H
NS : 100
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : MeOD
SW : 238.7675 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 83.564 degree
PHC1 : 30.867 degree
(ppm)



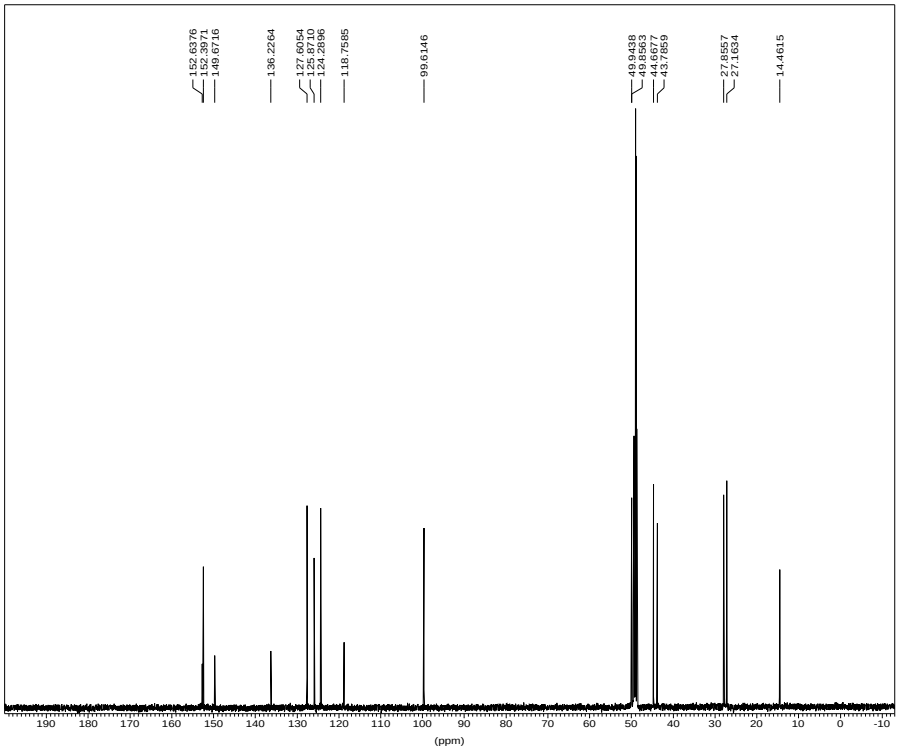
28b, n=2, 18%

1H AMX500 DCQA4 FRAC3

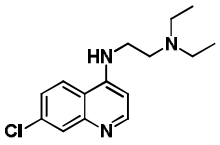



```
*** Current Data Parameters ***
NAME      :      khm1007
EXPNO     :            3
PROCNO    :            1
*** Acquisition  Parameters ***
DATE_t    :     06:03:47
DATE_d    :  Oct 07 2009
DBPNAM0   :
INSTRUM   :        av500
LOCNUC    :           2H
NS        :            8
NUCLEUS   :          off
O1        :    3088.51 Hz
SFO1      :  500.1330885 MH
SOLVENT   :         MeOD
*** 1D NMR Plot Parameters ***
NUCLEUS   :          off
```

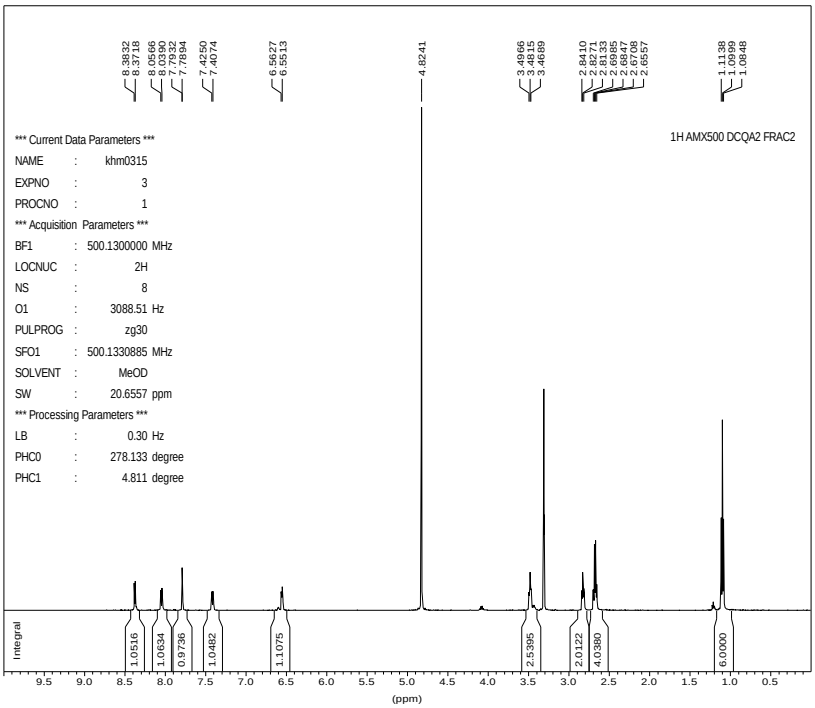
13C AMX500 DCQA4 FRAC3

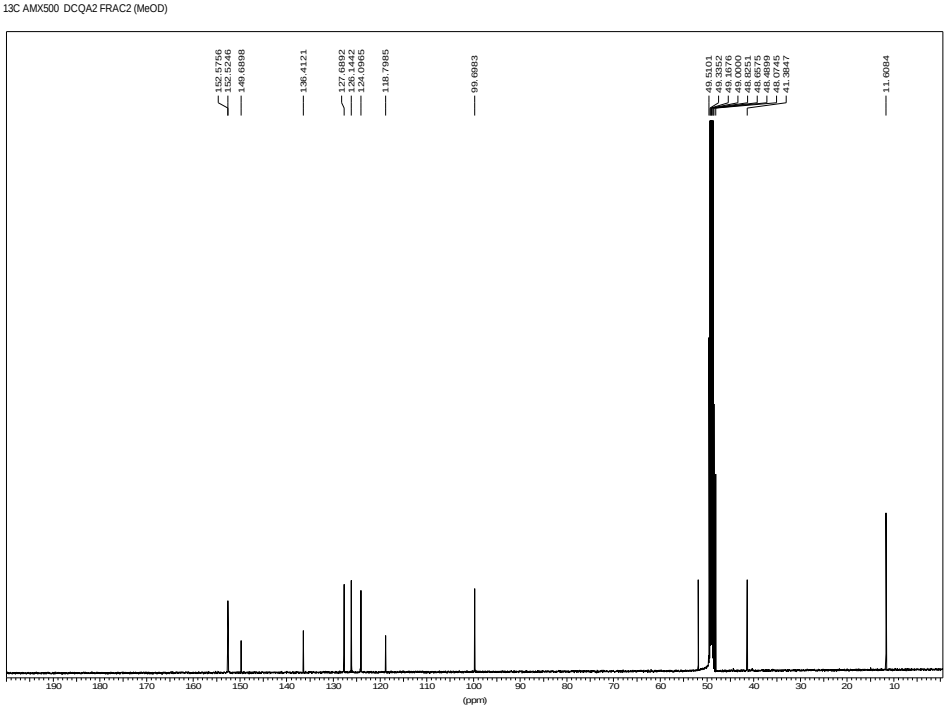


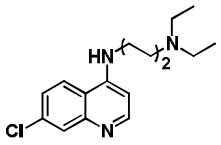

```
*** Current Data Parameters ***
NAME      :      khm1007
EXPNO     :            4
PROCNO    :            1
*** Acquisition  Parameters ***
DATE_t    :     06:14:24
DATE_d    :  Oct 07 2009
DBPNAM0   :
INSTRUM   :        av500
LOCNUC    :           2H
NS        :          361
NUCLEUS   :          off
O1        :   13204.57 Hz
SFO1      :  125.7709936 MH
SOLVENT   :         CDCl3
*** 1D NMR Plot Parameters ***
NUCLEUS   :          off
```


HN
N
Cl
N

29a, n=1, 17%

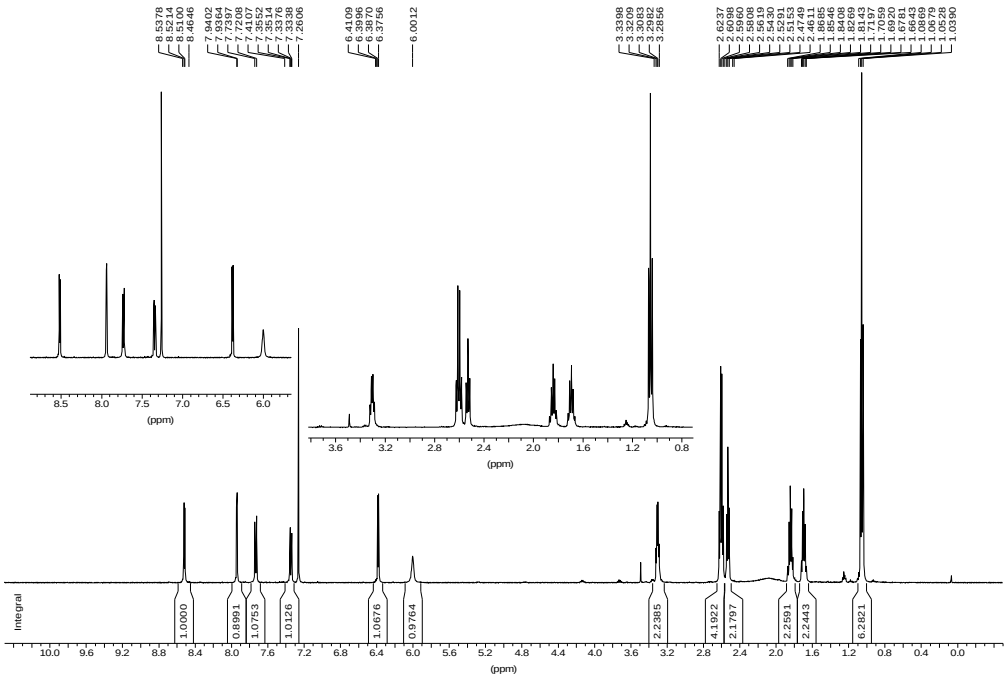

8.3832
8.3718
8.0566
8.0390
7.7932
7.7894
7.4250
7.4074
6.5627
6.5513
4.8241
3.4966
3.4815
3.4689
2.8410
2.8271
2.8133
2.6985
2.6847
2.6708
2.6557
1.1138
1.0999
1.0848
1H AMX500 DCQA2 FRAC2
*** Current Data Parameters ***
NAME : khm0315
EXPNO : 3
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 500.1300000 MHz
LOCNUC : 2H
NS : 8
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : MeOD
SW : 20.6557 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 278.133 degree
PHC1 : 4.811 degree
Integral
1.0516
1.0634
0.9736
1.0482
1.1075
2.5395
2.0122
4.0380
6.0000
(ppm)


13C AMX500 DCQA2 FRAC2 (MeOD)
152.5756
152.5246
149.6898
136.4121
127.6892
126.1442
124.4965
118.7985
99.6983
49.5101
49.3352
49.1676
49.0000
48.8251
48.6575
48.4899
48.0745
41.3847
11.6084
(ppm)

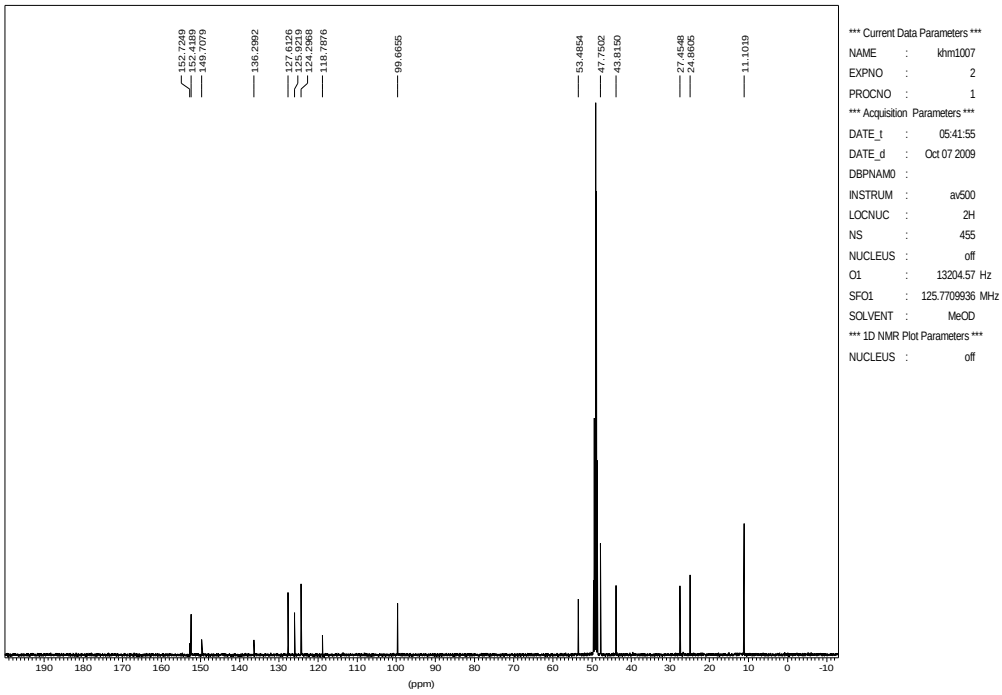


29b, n=2, 23%

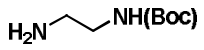
1H AMX500 DCQA4 FRAC2



13C AMX500 DCQA4 FRAC2

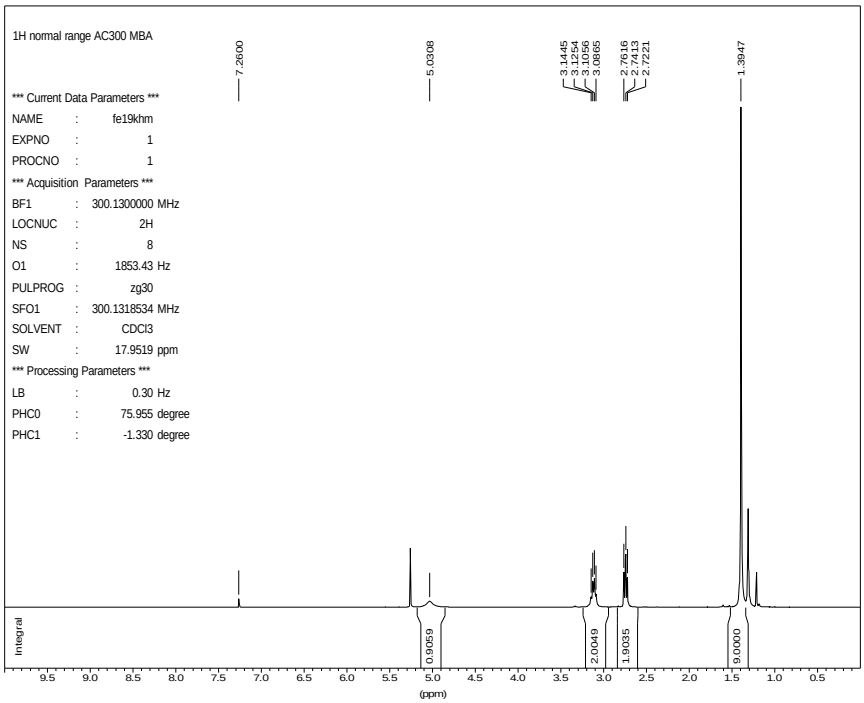


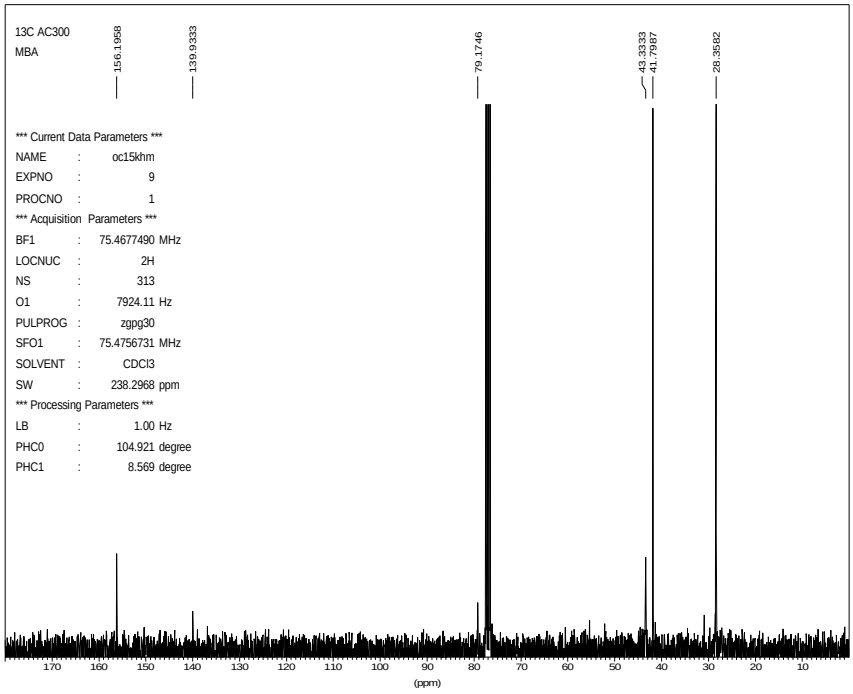
*** Current Data Parameters ***
NAME : khm1007
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
DATE_t : 05:41:55
DATE_d : Oct 07 2009
DBPNAM0 :
INSTRUM : av500
LOCNUC : 2H
NS : 455
NUCLEUS : off
O1 : 13204.57 Hz
SFO1 : 125.7709936 MHz
SOLVENT : MeOD
*** 1D NMR Plot Parameters ***
NUCLEUS : off

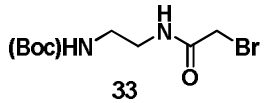

H2N
NH(Boc)

31

50%

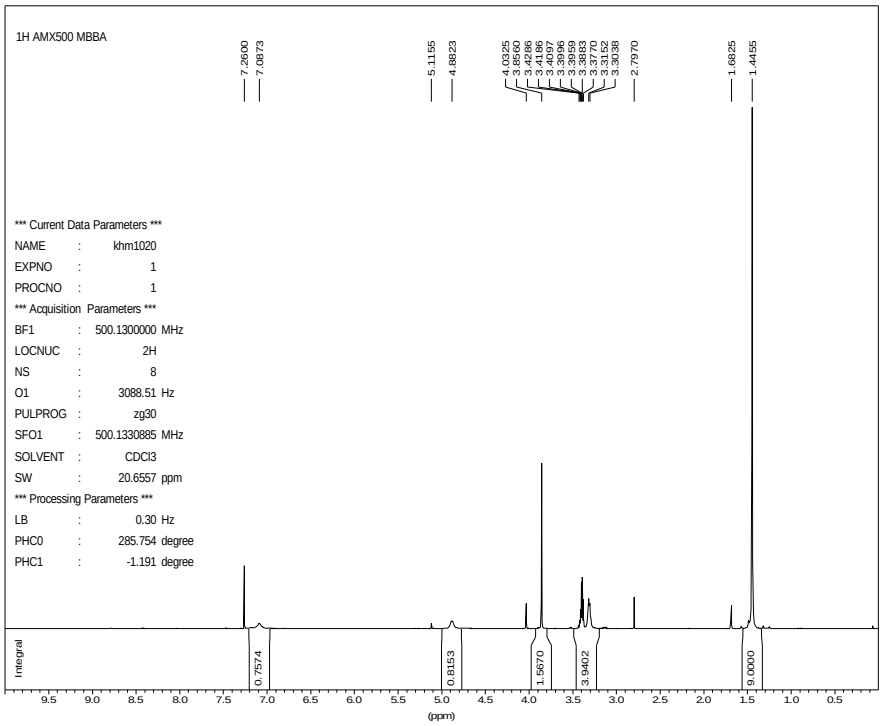

1H normal range AC300 MBA
7.2600
5.0308
3.1445
3.1254
3.1056
3.0865
2.7616
2.7413
2.7221
1.3947
*** Current Data Parameters ***
NAME : fe19khm
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 300.1300000 MHz
LOCNUC : 2H
NS : 8
O1 : 1853.43 Hz
PULPROG : zg30
SFO1 : 300.1318534 MHz
SOLVENT : CDCl3
SW : 17.9519 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 75.955 degree
PHC1 : -1.330 degree
Integral
0.9059
2.0049
1.9035
9.0000
(ppm)

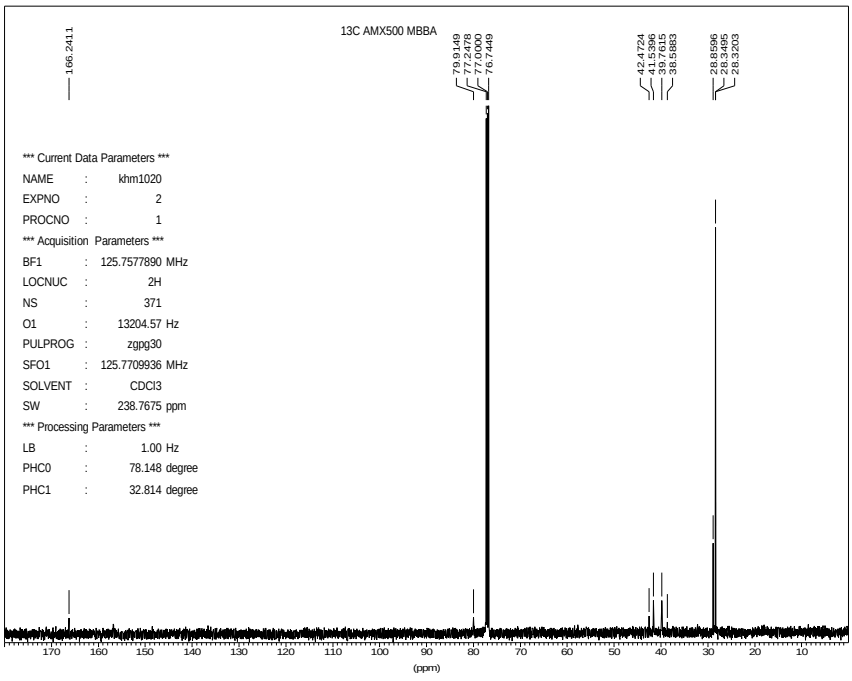

13C AC300
MBA
156.1958
139.9333
79.1746
43.3333
41.7987
28.3582
*** Current Data Parameters ***
NAME : oc15khm
EXPNO : 9
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 75.4677490 MHz
LOCNUC : 2H
NS : 313
O1 : 7924.11 Hz
PULPROG : zgpg30
SFO1 : 75.4756731 MHz
SOLVENT : CDCl3
SW : 238.2968 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 104.921 degree
PHC1 : 8.569 degree
(ppm)

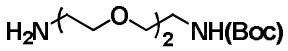

(Boc)HN
H
N
O
Br

33

82%

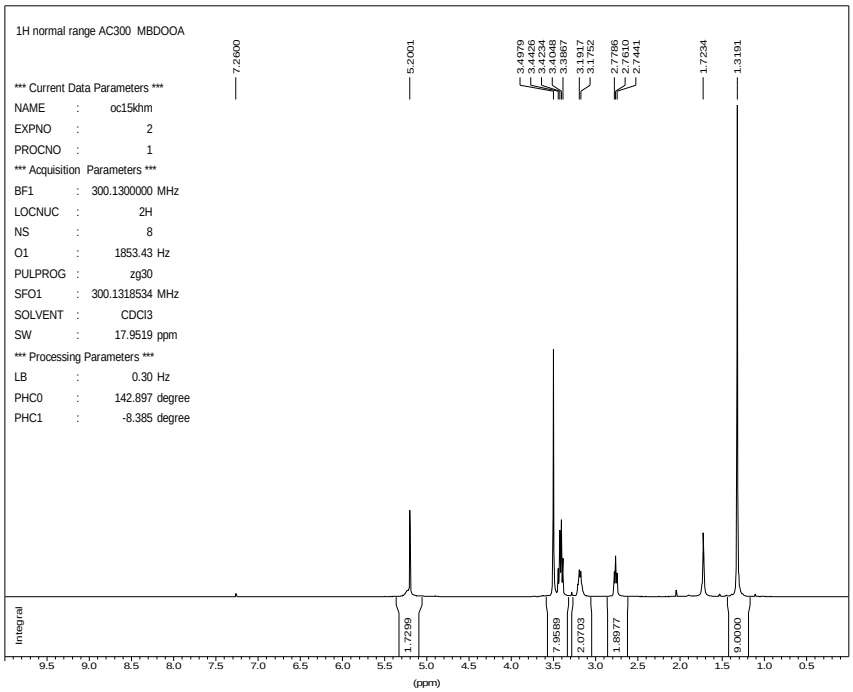

1H AMX500 MBBA
7.2600
7.0873
5.1155
4.8823
4.0325
3.9560
3.4286
3.4186
3.4097
3.3996
3.3950
3.3883
3.3770
3.3152
3.3038
2.7970
1.6825
1.4455
*** Current Data Parameters ***
NAME : khm1020
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 500.1300000 MHz
LOCNUC : 2H
NS : 8
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : CDCl3
SW : 20.6557 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 285.754 degree
PHC1 : -1.191 degree
Integral
0.7574
0.8153
1.5670
3.9402
9.0000
(ppm)

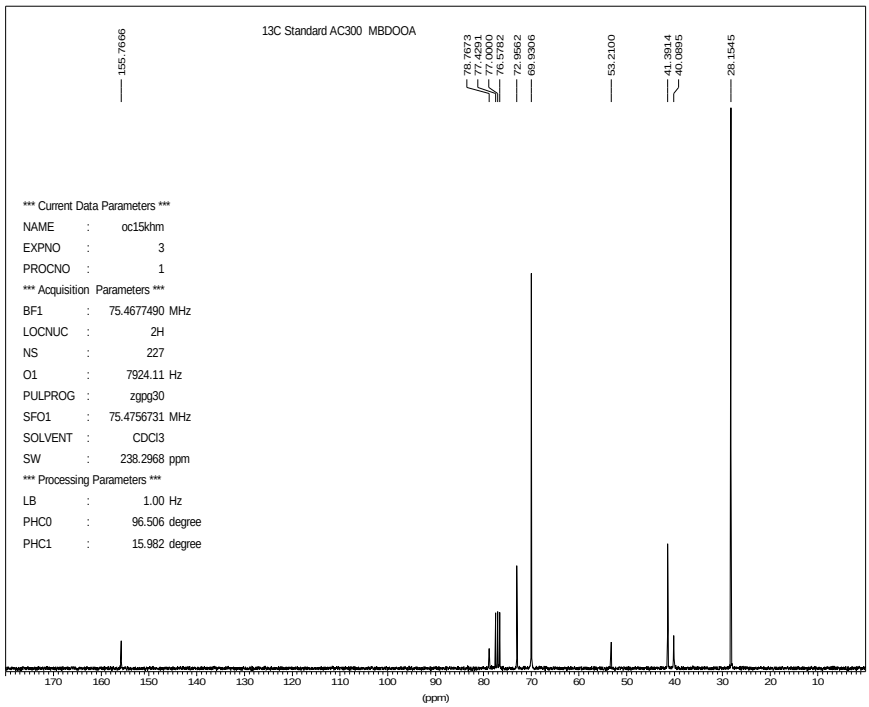

13C AMX500 MBBA
166.2411
79.9149
77.2478
77.0000
76.7448
42.4724
41.5396
39.7615
38.5983
28.8596
28.3495
28.3203
*** Current Data Parameters ***
NAME : khm1020
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 125.7577890 MHz
LOCNUC : 2H
NS : 371
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : CDCl3
SW : 238.7675 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 78.148 degree
PHC1 : 32.814 degree
(ppm)

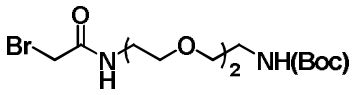

H2N
O
2
NH(Boc)

38

90%

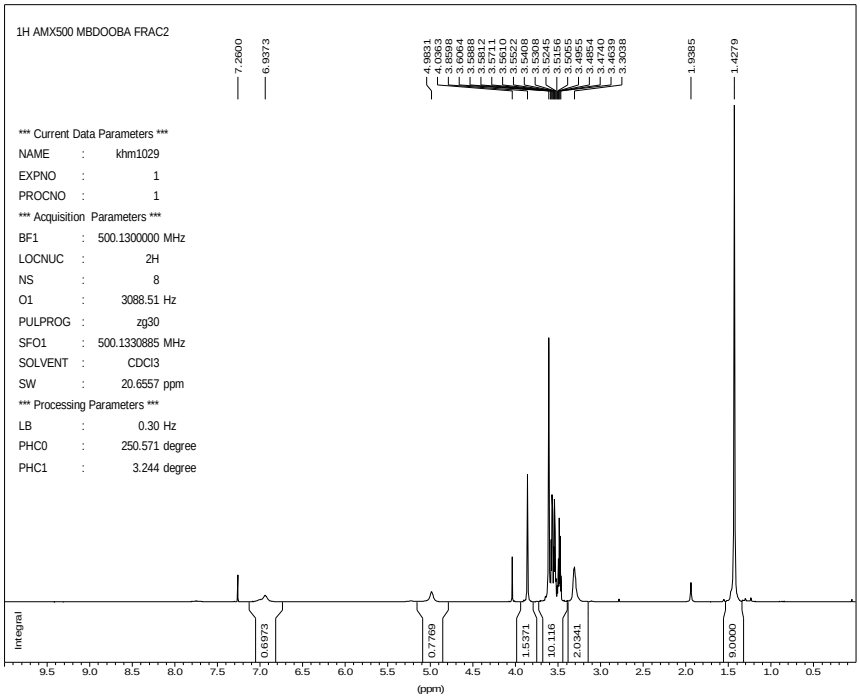

1H normal range AC300 MBDOOA
7.2600
5.2001
3.4979
3.4736
3.4254
3.4048
3.3867
3.1917
3.1752
2.7786
2.7610
2.7441
1.7234
1.3191
*** Current Data Parameters ***
NAME : oc15khm
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 300.1300000 MHz
LOCNUC : 2H
NS : 8
O1 : 1853.43 Hz
PULPROG : zg30
SFO1 : 300.1318534 MHz
SOLVENT : CDCl3
SW : 17.9519 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 142.897 degree
PHC1 : -8.385 degree
Integral
1.7299
7.9589
2.0703
1.8977
9.0000
(ppm)

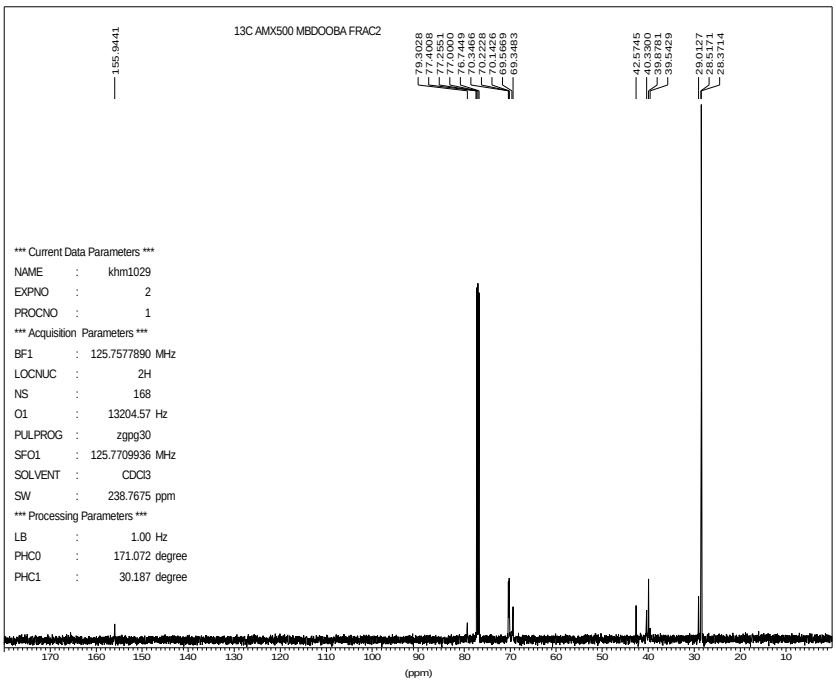

13C Standard AC300 MBDOOA
155.7666
78.7673
77.4251
77.0000
76.5782
72.9562
69.9306
53.2100
41.3914
40.0895
28.1545
*** Current Data Parameters ***
NAME : oc15khm
EXPNO : 3
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 75.4677490 MHz
LOCNUC : 2H
NS : 227
O1 : 7924.11 Hz
PULPROG : zgpg30
SFO1 : 75.4756731 MHz
SOLVENT : CDCl3
SW : 238.2968 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 96.506 degree
PHC1 : 15.982 degree
(ppm)

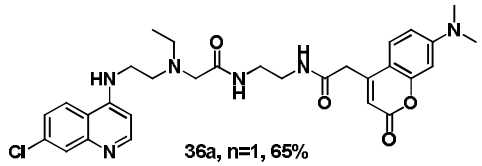


40

82%

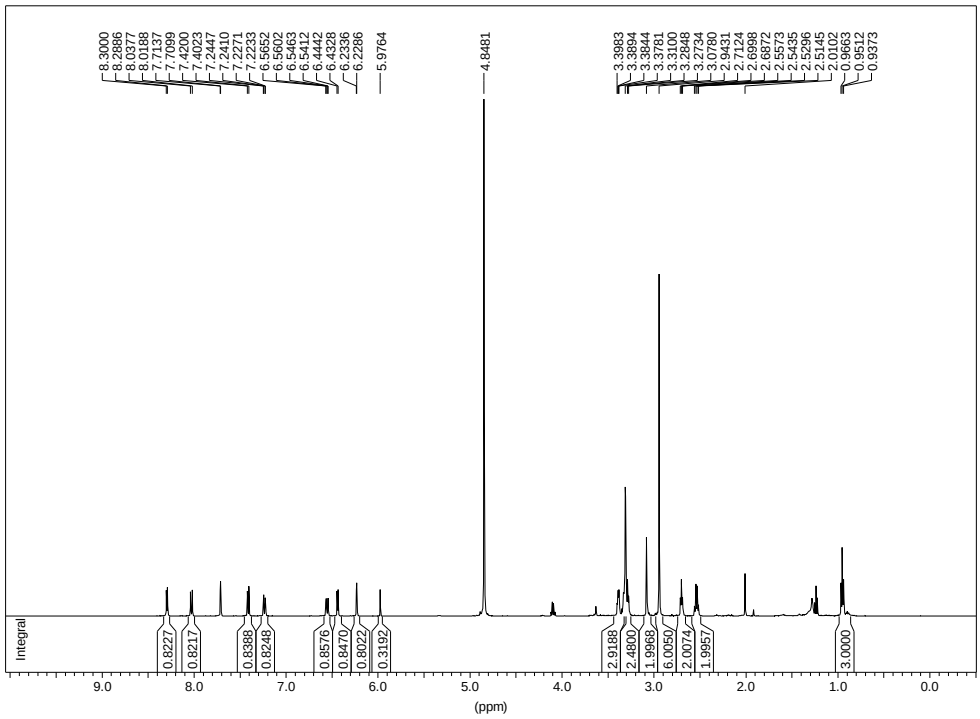

1H AMX500 MBDOOBA FRAC2
7.2600
6.9373
4.9831
4.0363
3.8598
3.6064
3.5688
3.5812
3.5711
3.5610
3.5522
3.5408
3.5308
3.5245
3.5156
3.5055
3.4955
3.4854
3.4740
3.4653
3.3038
1.9385
1.4279
*** Current Data Parameters ***
NAME : khm1029
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 500.1300000 MHz
LOCNUC : 2H
NS : 8
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : CDCl3
SW : 20.6557 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 250.571 degree
PHC1 : 3.244 degree
Integral
0.6973
0.7769
1.5371
10.116
2.0341
9.0000
(ppm)


13C AMX500 MBDOOBA FRAC2
155.9441
79.3028
77.4008
77.2551
77.0050
76.7449
70.3466
70.1228
70.1426
69.5669
69.3483
42.5745
42.3390
39.8781
39.5429
29.0127
28.5171
28.3714
*** Current Data Parameters ***
NAME : khm1029
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 125.7577890 MHz
LOCNUC : 2H
NS : 168
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : CDCl3
SW : 238.7675 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 171.072 degree
PHC1 : 30.187 degree
(ppm)

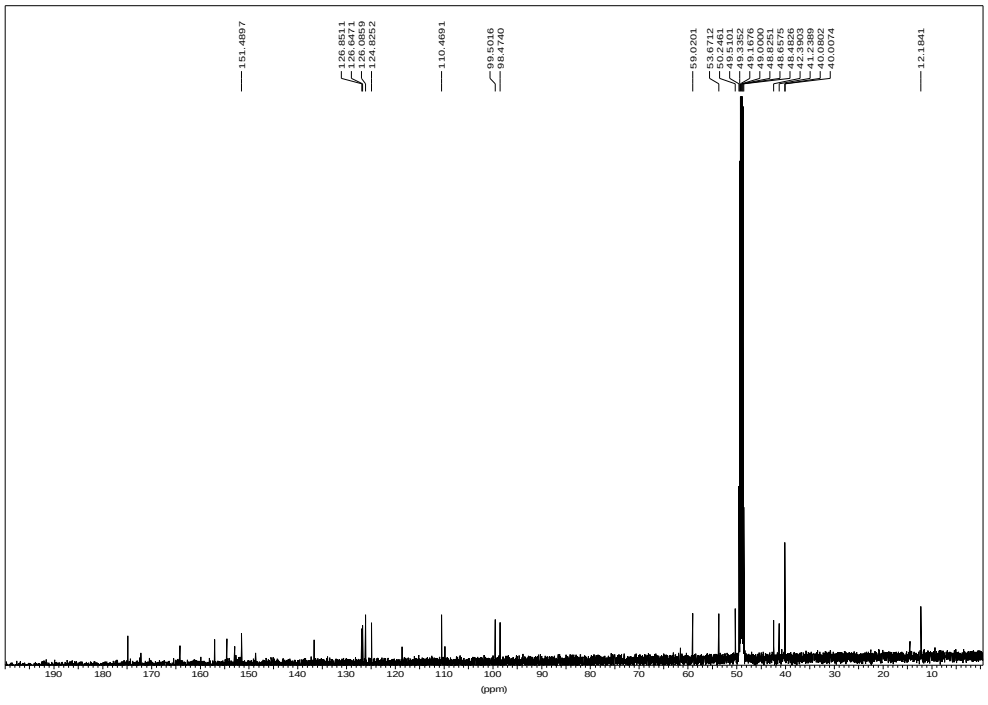


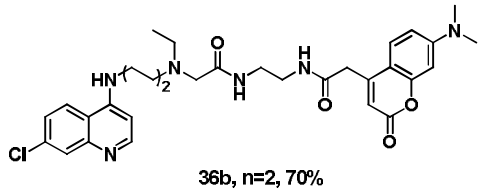
36a, n=1, 65%

1H AMX500 FDA1 after washing



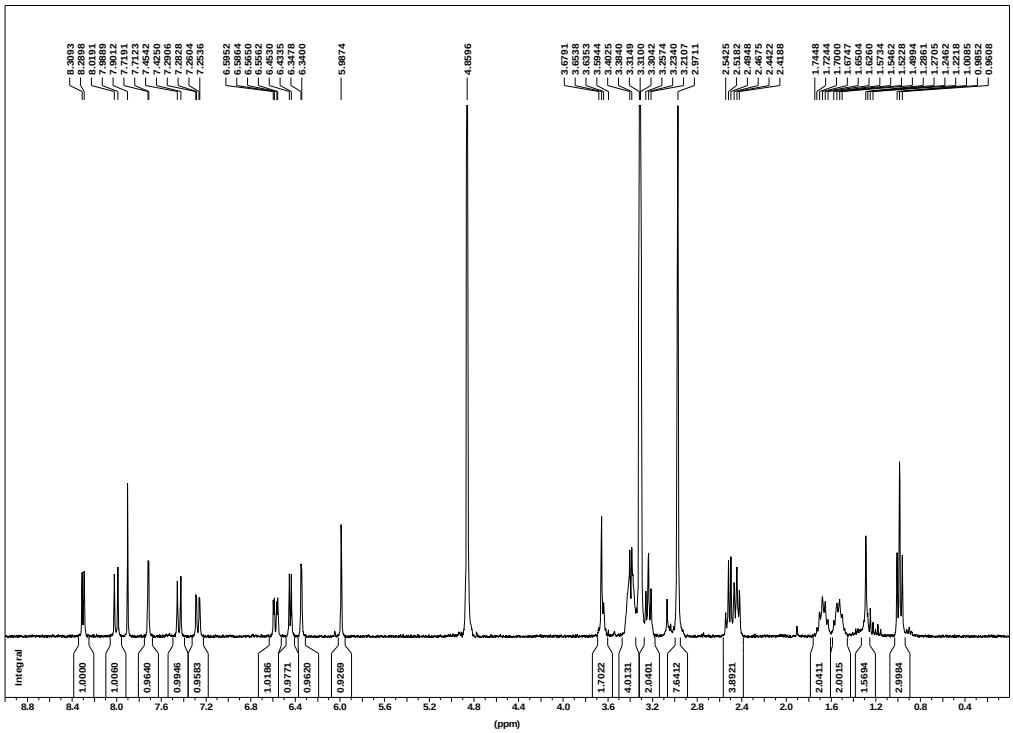
13C AMX500 FDA1 (MeOD)

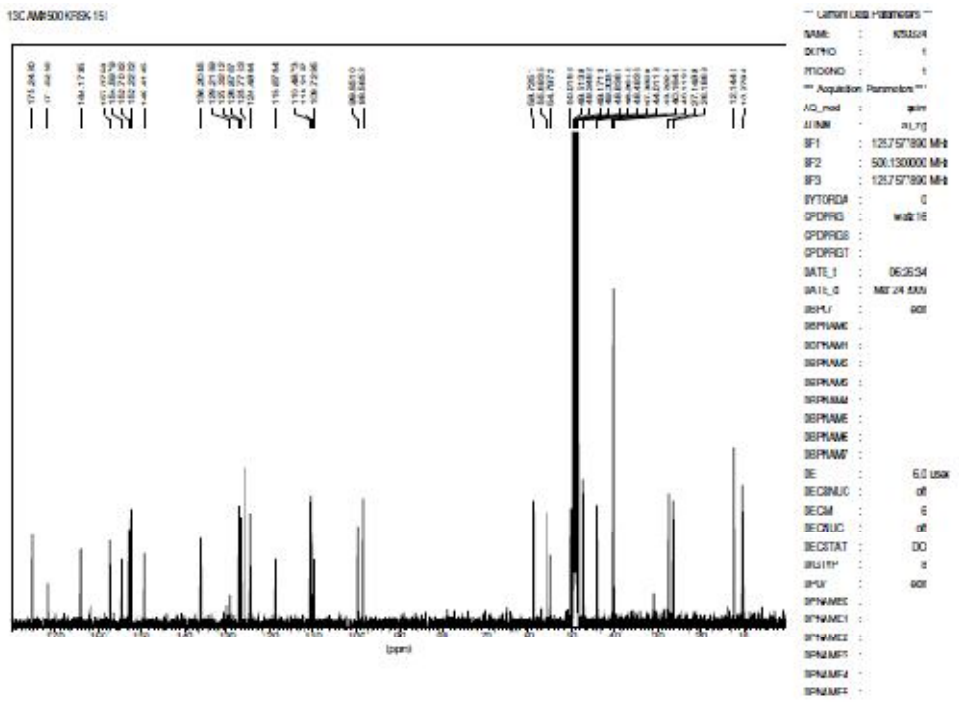


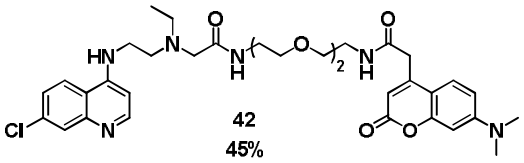


36b, n=2, 70%

krsk151

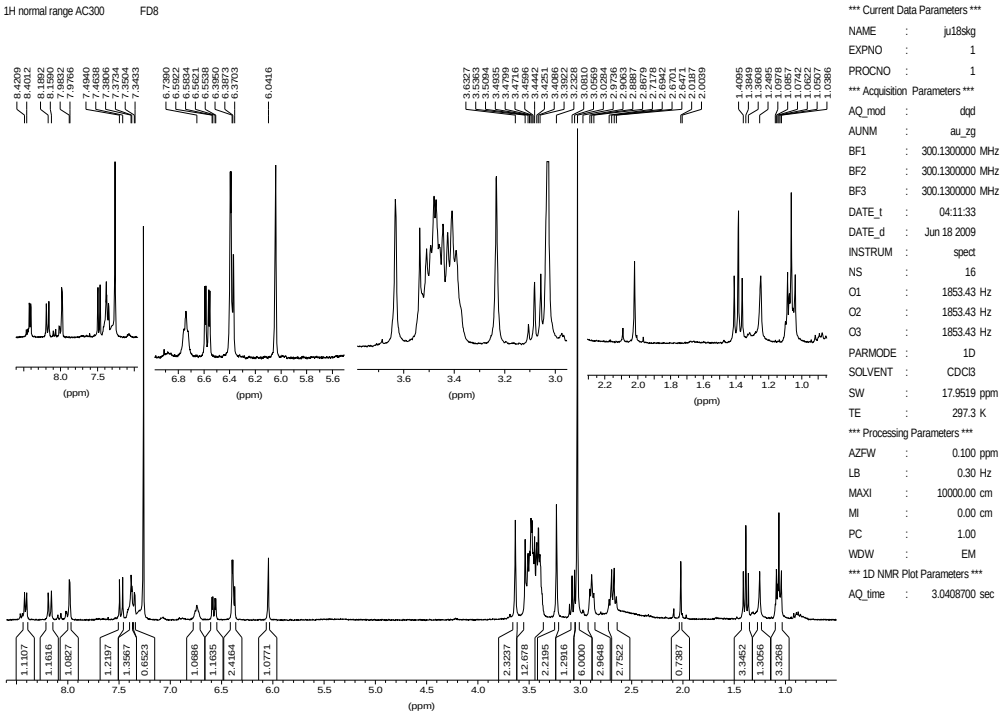


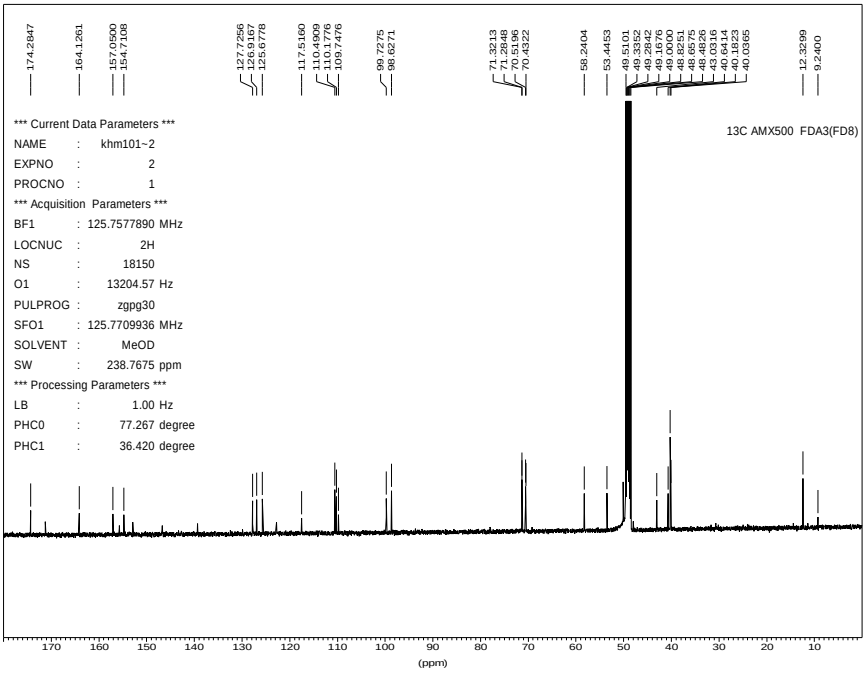


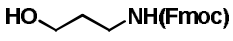


42

45%

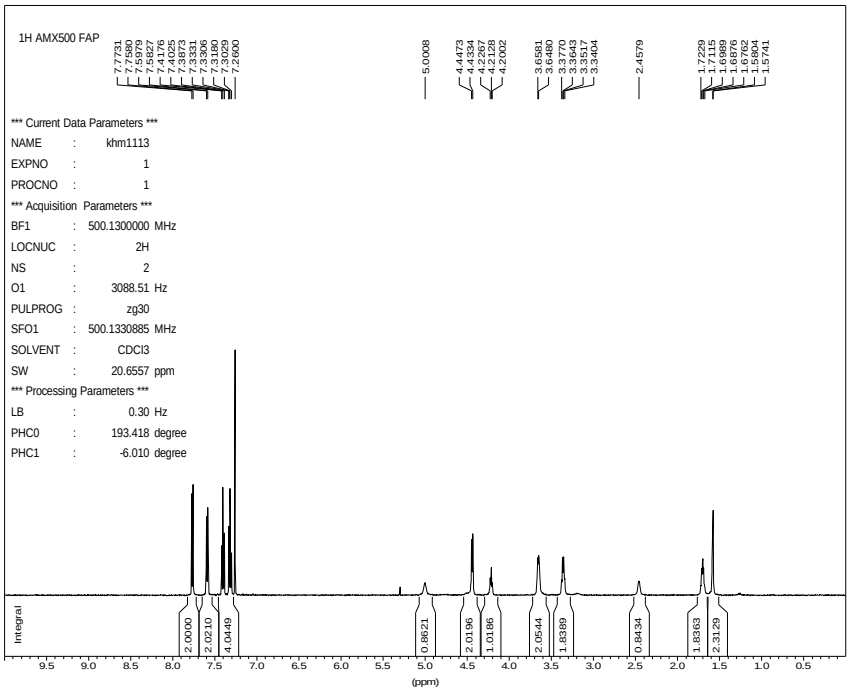

1H normal range AC300 FD8
*** Current Data Parameters ***
NAME : ju18skg
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
AQ_mod : dqd
AUNM : au_zg
BF1 : 300.1300000 MHz
BF2 : 300.1300000 MHz
BF3 : 300.1300000 MHz
DATE_t : 04:11:33
DATE_d : Jun 18 2009
INSTRUM : spect
NS : 16
O1 : 1853.43 Hz
O2 : 1853.43 Hz
O3 : 1853.43 Hz
PARMODE : 1D
SOLVENT : CDCl3
SW : 17.9519 ppm
TE : 297.3 K
*** Processing Parameters ***
AZFW : 0.100 ppm
LB : 0.30 Hz
MAXI : 10000.00 cm
MI : 0.00 cm
PC : 1.00
WDW : EM
*** 1D NMR Plot Parameters ***
AQ_time : 3.0408700 sec
(ppm)

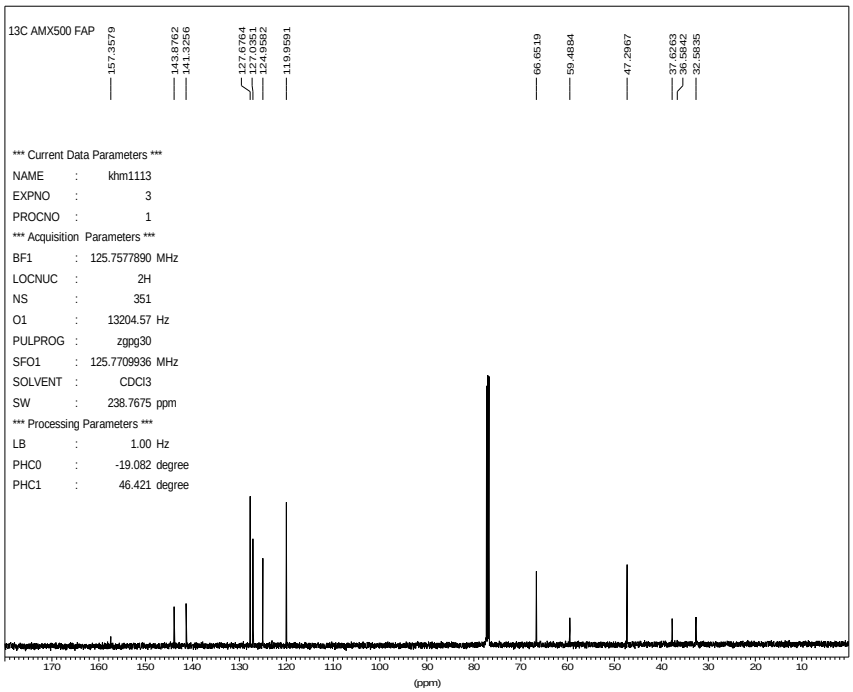

13C AMX500 FDA3(FD8)
*** Current Data Parameters ***
NAME : khm101~2
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 125.7577890 MHz
LOCNUC : 2H
NS : 18150
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : MeOD
SW : 238.7675 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 77.267 degree
PHC1 : 36.420 degree
(ppm)

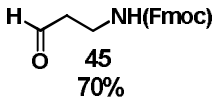

HO
NH(Fmoc)

44

90%

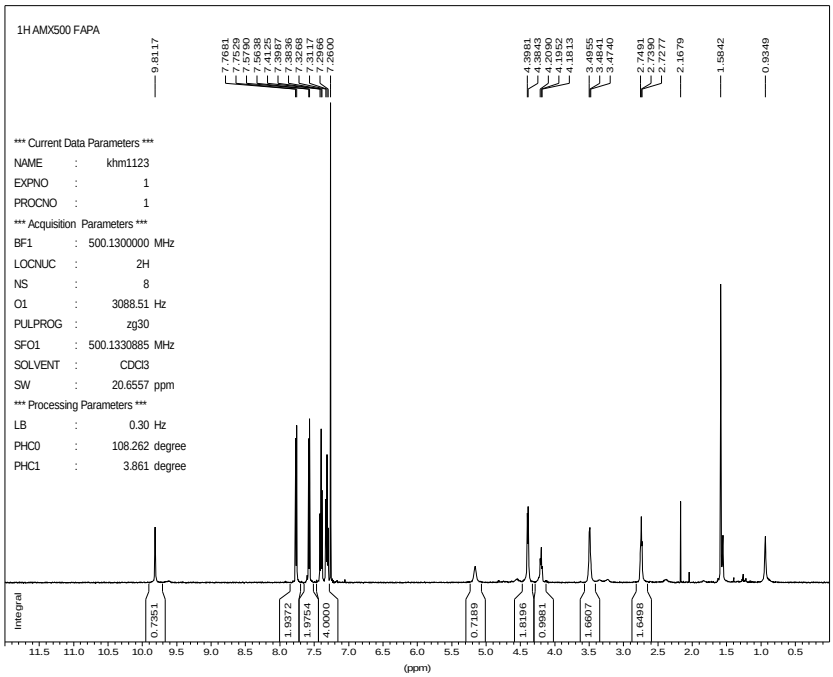

1H AMX500 FAP
7.7731
7.7580
7.5979
7.5827
7.4176
7.4025
7.3873
7.3331
7.3306
7.3180
7.3029
7.2600
5.0008
4.4473
4.4334
4.2297
4.2158
4.2002
3.6581
3.6460
3.3770
3.3643
3.3517
3.3404
2.4579
1.7229
1.7115
1.6989
1.6876
1.6762
1.5804
1.5741
*** Current Data Parameters ***
NAME : khm1113
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 500.1300000 MHz
LOCNUC : 2H
NS : 2
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : CDCl3
SW : 20.6557 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 193.418 degree
PHC1 : -6.010 degree
Integral
2.0000
2.0210
4.0449
0.8621
2.0196
1.0186
2.0544
1.8389
0.8434
1.8363
2.3129
(ppm)

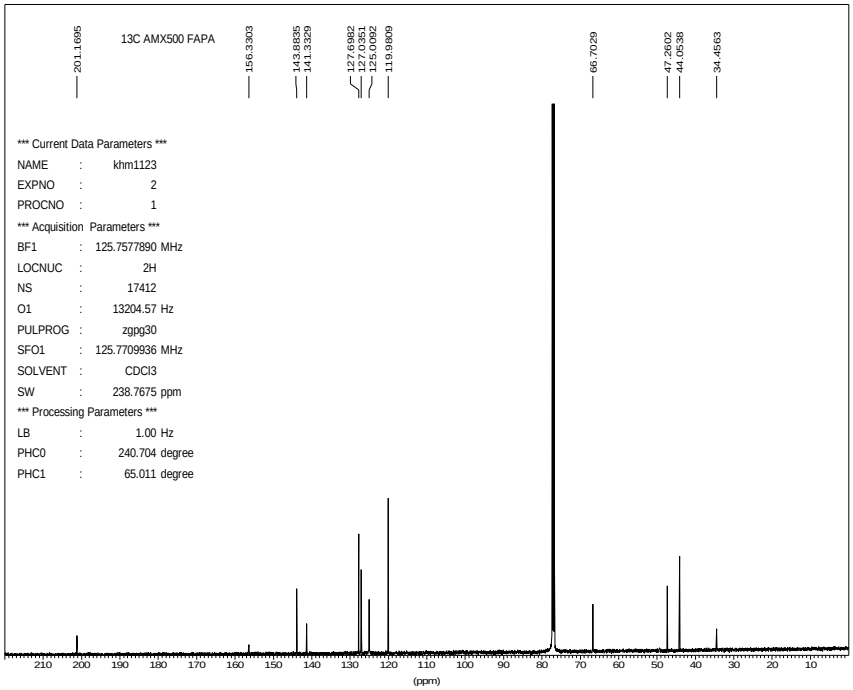

13C AMX500 FAP
157.3579
143.8762
141.3256
127.6764
127.0351
124.9382
119.9591
66.6519
59.4884
47.2967
37.6283
36.5842
32.5835
*** Current Data Parameters ***
NAME : khm1113
EXPNO : 3
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 125.7577890 MHz
LOCNUC : 2H
NS : 351
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : CDCl3
SW : 238.7675 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : -19.082 degree
PHC1 : 46.421 degree
(ppm)

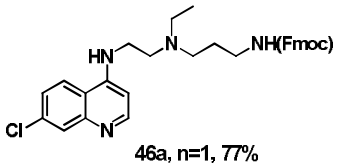

H
NH(Fmoc)
O

45

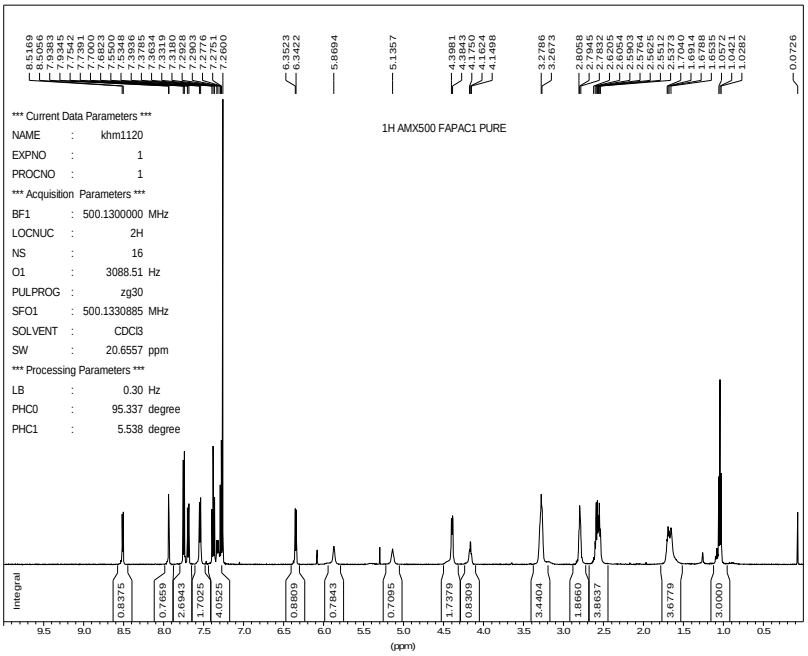
70%

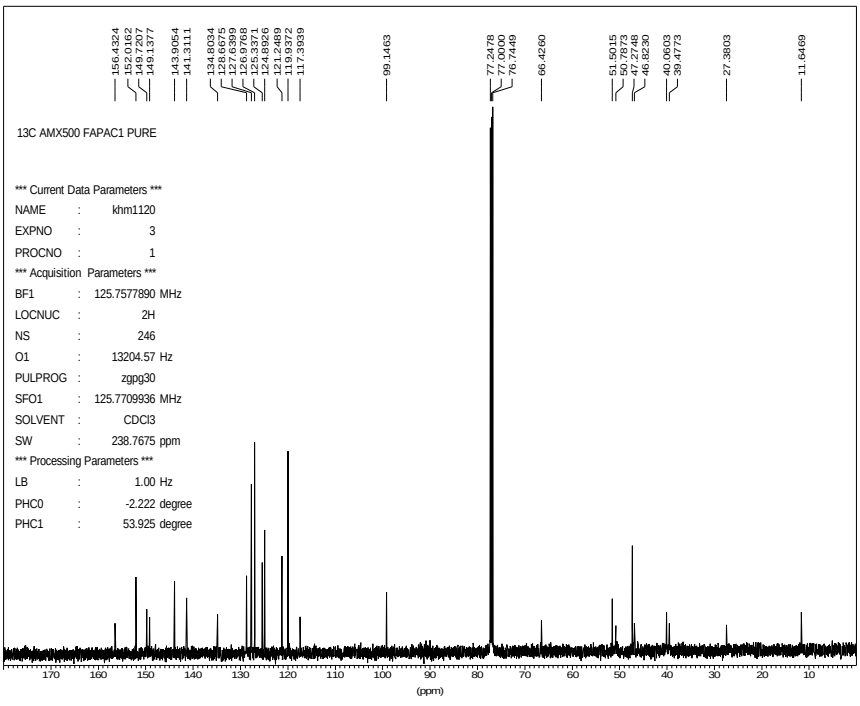

1H AMX500 FAPA
9.8117
7.7681
7.7529
7.5790
7.5638
7.4125
7.3967
7.3836
7.3268
7.3117
7.2966
7.2600
4.3981
4.3843
4.2090
4.1932
4.1813
3.4955
3.4841
3.4740
2.7491
2.7390
2.7277
2.1679
1.5842
0.9349
*** Current Data Parameters ***
NAME : khm1123
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 500.1300000 MHz
LOCNUC : 2H
NS : 8
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : CDCl3
SW : 20.6557 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 108.262 degree
PHC1 : 3.861 degree
Integral
0.7351
1.9372
1.9754
4.0000
0.7189
1.8196
0.9981
1.6607
1.6498
(ppm)

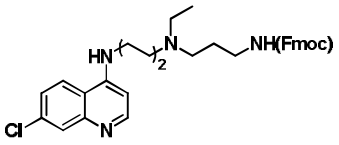

13C AMX500 FAPA
201.1695
156.3303
143.8835
141.3329
127.6982
127.0551
125.0092
119.9809
66.7029
47.2602
44.0538
34.4563
*** Current Data Parameters ***
NAME : khm1123
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 125.7577890 MHz
LOCNUC : 2H
NS : 17412
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : CDCl3
SW : 238.7675 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 240.704 degree
PHC1 : 65.011 degree
(ppm)


HN
N
NH(Fmoc)
Cl
N

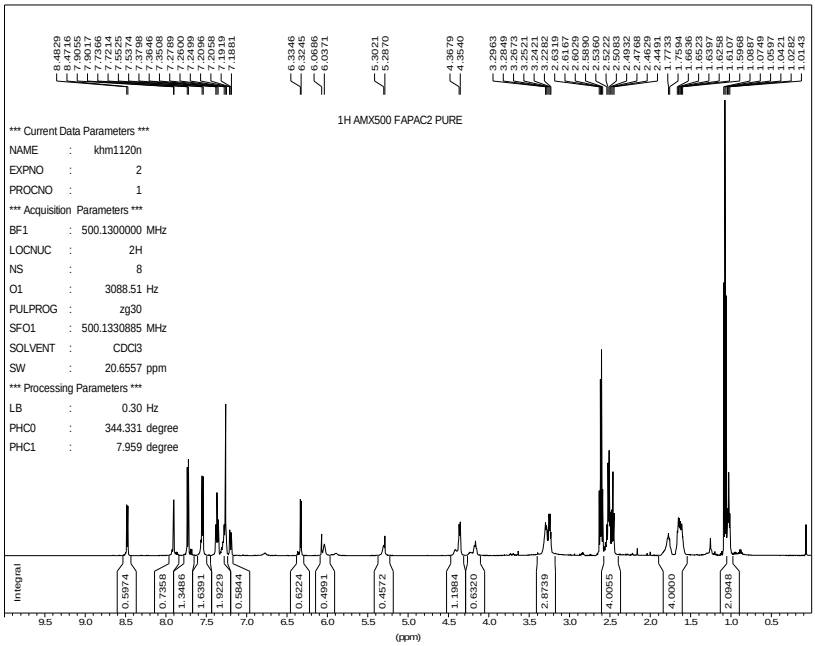
46a, n=1, 77%

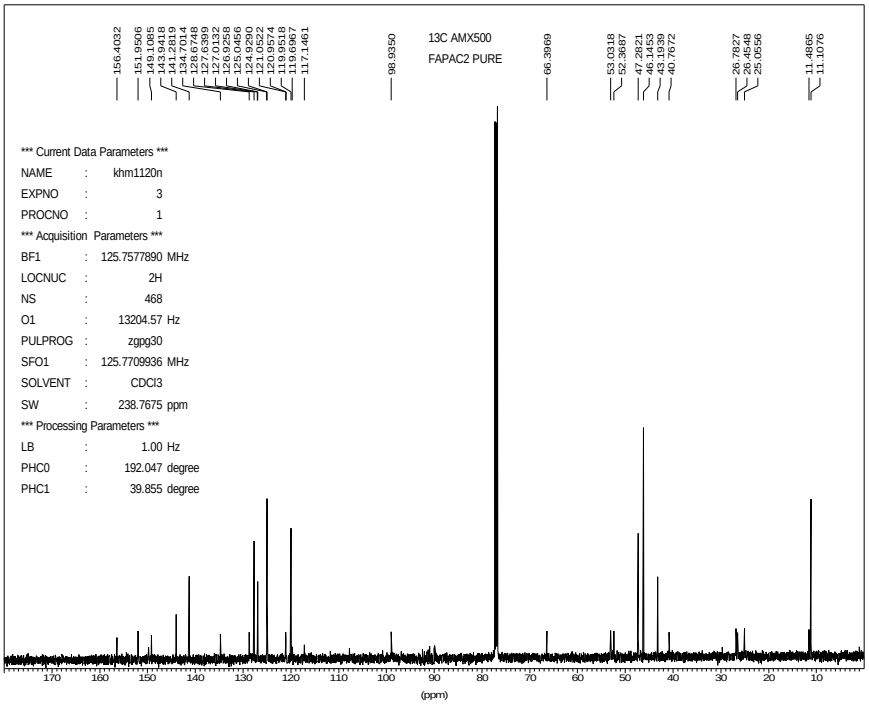

1H AMX500 FAPAC1 PURE
*** Current Data Parameters ***
NAME : khm1120
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 500.1300000 MHz
LOCNUC : 2H
NS : 16
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : CDCl3
SW : 20.6557 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 95.337 degree
PHC1 : 5.538 degree
Integral
(ppm)

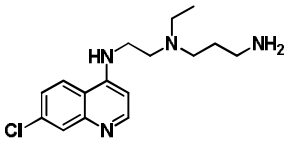

13C AMX500 FAPAC1 PURE
*** Current Data Parameters ***
NAME : khm1120
EXPNO : 3
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 125.7577890 MHz
LOCNUC : 2H
NS : 246
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : CDCl3
SW : 238.7675 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : -2.222 degree
PHC1 : 53.925 degree
(ppm)


HN
N
NH(Fmoc)
Cl
N
2

46b, n=2, 70%

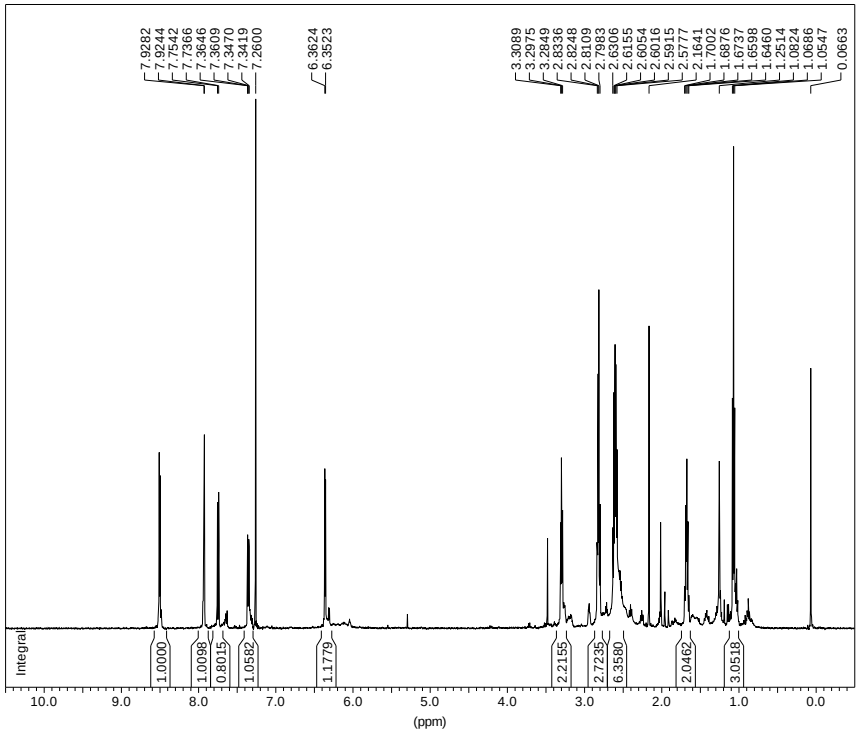

1H AMX500 FAPAC2 PURE
*** Current Data Parameters ***
NAME : khm1120n
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 500.1300000 MHz
LOCNUC : 2H
NS : 8
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : CDCl3
SW : 20.6557 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 344.331 degree
PHC1 : 7.959 degree
Integral
0.5974
0.7358
1.3486
1.6391
1.9229
0.5844
0.6224
0.4991
0.4572
1.1984
0.6320
2.8739
4.0055
4.0000
2.0548
(ppm)


13C AMX500
FAPAC2 PURE
*** Current Data Parameters ***
NAME : khm1120n
EXPNO : 3
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 125.7577890 MHz
LOCNUC : 2H
NS : 468
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : CDCl3
SW : 238.7675 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 192.047 degree
PHC1 : 39.855 degree
(ppm)



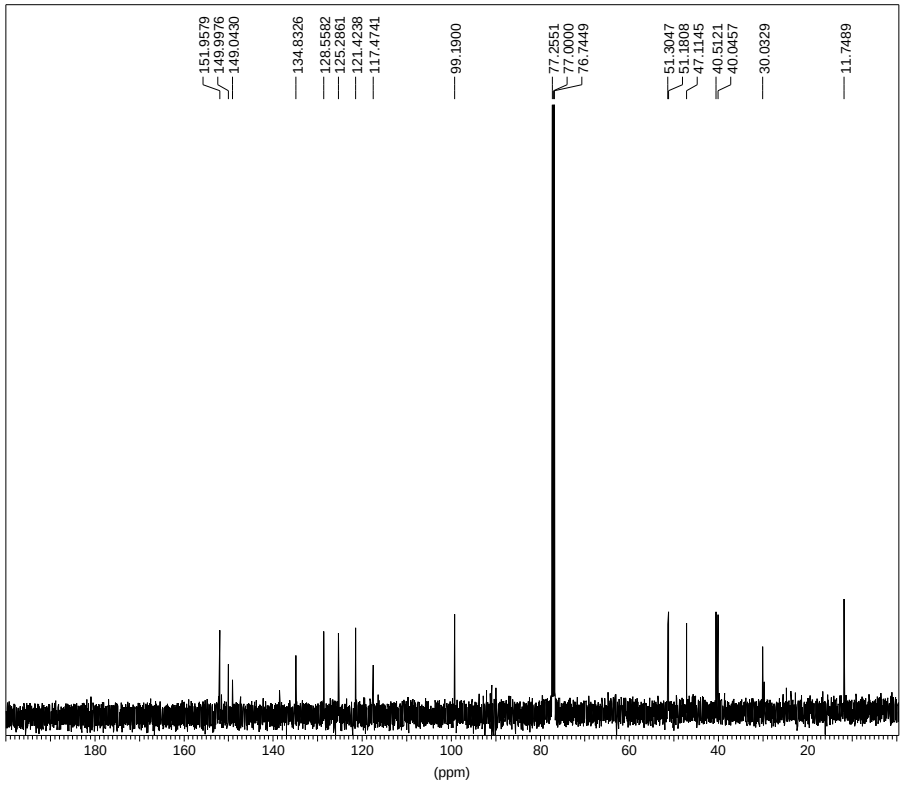
47a, n=1

1H AMX500 CAPA1

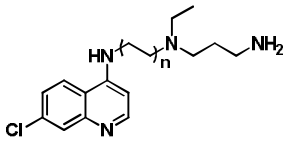


*** Current Data Parameters ***
NAME : khm1124
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
INSTRUM : av500
LOCNUC : 2H
NS : 2
NUCLEUS : off
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : CDCl3
SW : 20.6557 ppm
TD : 32768
TE : 300.0 K
*** Processing Parameters ***
LB : 0.30 Hz
*** 1D NMR Plot Parameters ***
NUCLEUS : off

13C AMX500 CAPA1

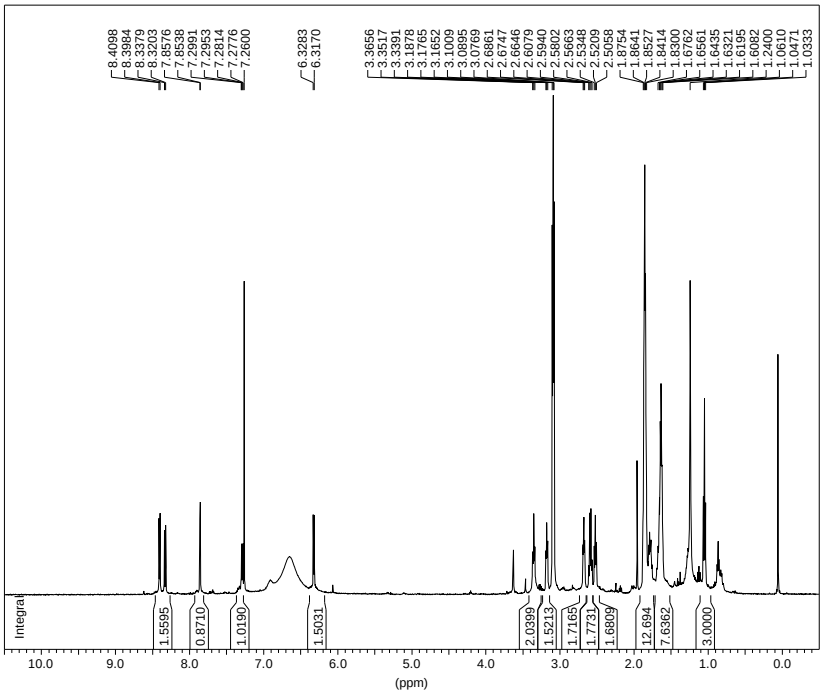


*** Current Data Parameters ***
NAME : khm1124
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
INSTRUM : av500
LOCNUC : 2H
NS : 257
NUCLEUS : off
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : CDCl3
SW : 238.7675 ppm
TD : 65536
TE : 300.1 K
*** Processing Parameters ***
LB : 1.00 Hz
*** 1D NMR Plot Parameters ***
NUCLEUS : off



47b, n=2

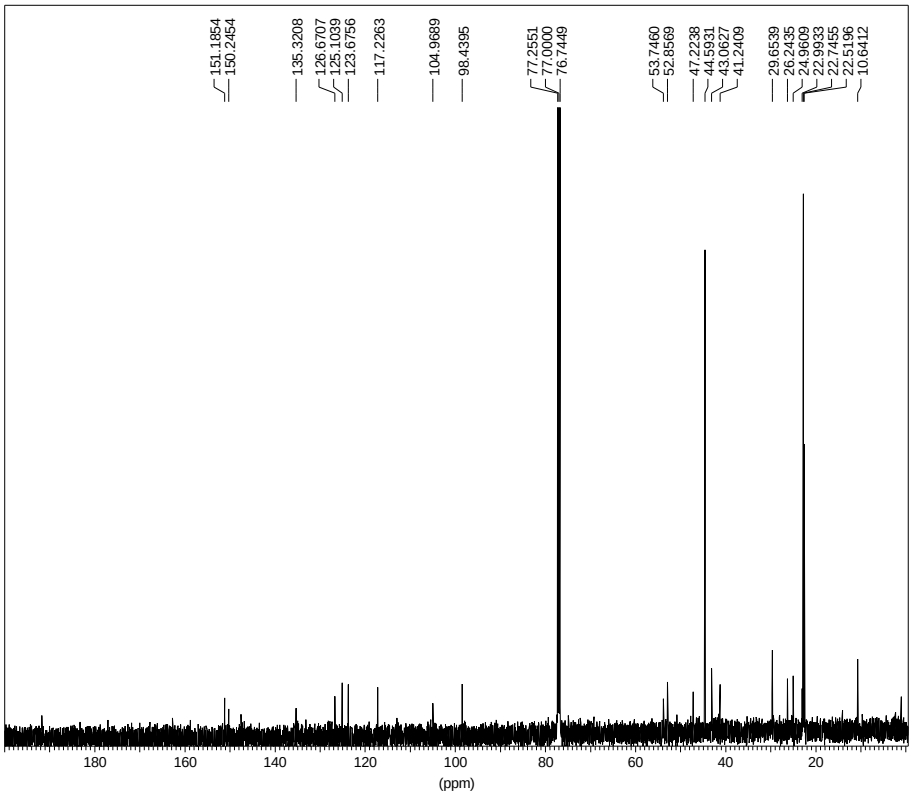
1H AMX500 CAPA2



*** Current Data Parameters ***

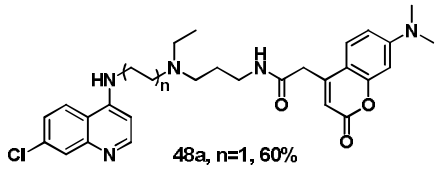
NAME	:	khm1207
EXPNO	:	1
PROCNO	:	1
*** Acquisition Parameters ***		
INSTRUM	:	av500
LOCNUC	:	2H
NS	:	8
NUCLEUS	:	off
O1	:	3088.51 Hz
PULPROG	:	zg30
SFO1	:	500.1330885 MHz
SOLVENT	:	CDCl3
SW	:	20.6557 ppm
TD	:	32768
TE	:	300.0 K
*** Processing Parameters ***		
LB	:	0.30 Hz
*** 1D NMR Plot Parameters ***		
NUCLEUS	:	off

13C AMX500 CAPA2



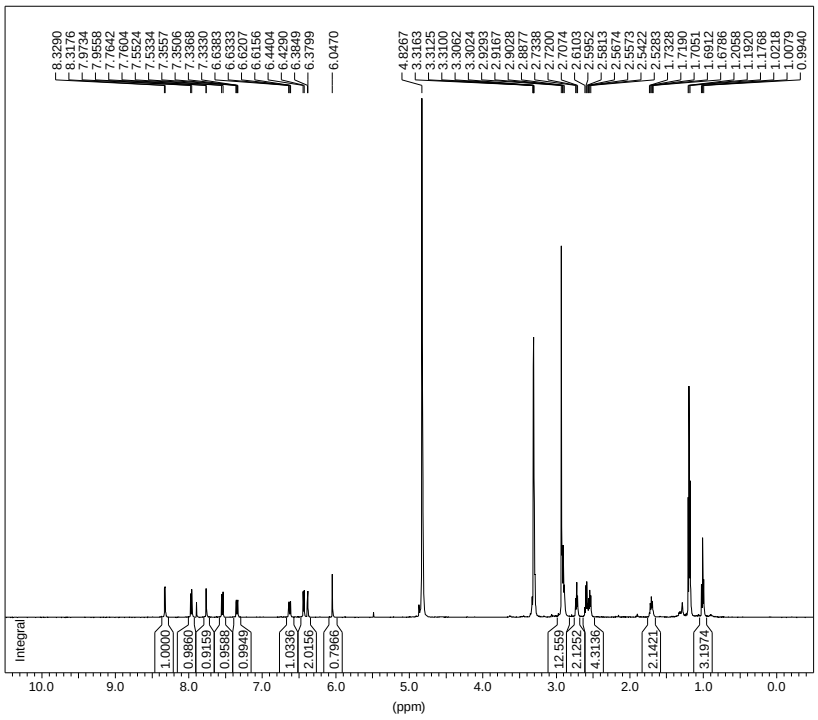
*** Current Data Parameters ***

NAME	:	khm1207
EXPNO	:	2
PROCNO	:	1
*** Acquisition Parameters ***		
INSTRUM	:	av500
LOCNUC	:	2H
NS	:	335
NUCLEUS	:	off
O1	:	13204.57 Hz
PULPROG	:	zgpg30
SFO1	:	125.7709936 MHz
SOLVENT	:	CDCl3
SW	:	238.7675 ppm
TD	:	65536
TE	:	300.0 K
*** Processing Parameters ***		
LB	:	1.00 Hz
*** 1D NMR Plot Parameters ***		
NUCLEUS	:	off



48a, n=1, 60%

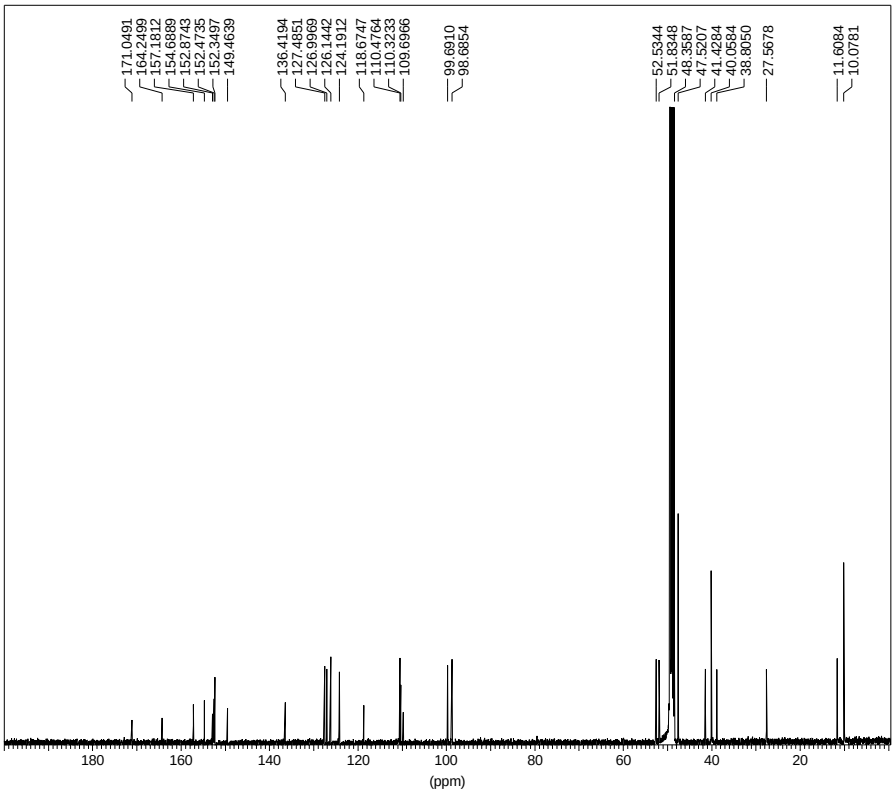
1H AMX500 FDA7 FRAC3



*** Current Data Parameters ***

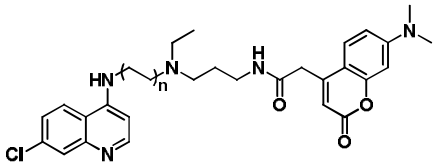
NAME	:	khm1127
EXPNO	:	3
PROCNO	:	1
*** Acquisition Parameters ***		
INSTRUM	:	av500
LOCNUC	:	2H
NS	:	16
NUCLEUS	:	off
O1	:	3088.51 Hz
PULPROG	:	zg30
SFO1	:	500.1330885 MHz
SOLVENT	:	MeOD
SW	:	20.6557 ppm
TD	:	32768
TE	:	300.0 K
*** Processing Parameters ***		
LB	:	0.30 Hz
*** 1D NMR Plot Parameters ***		
NUCLEUS	:	off

13C AMX500 FDA7 FRAC3

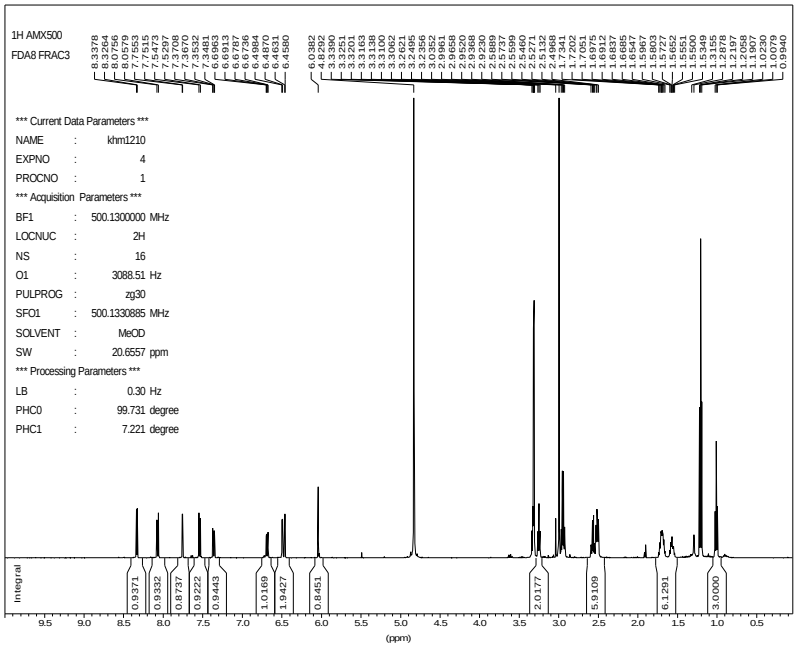


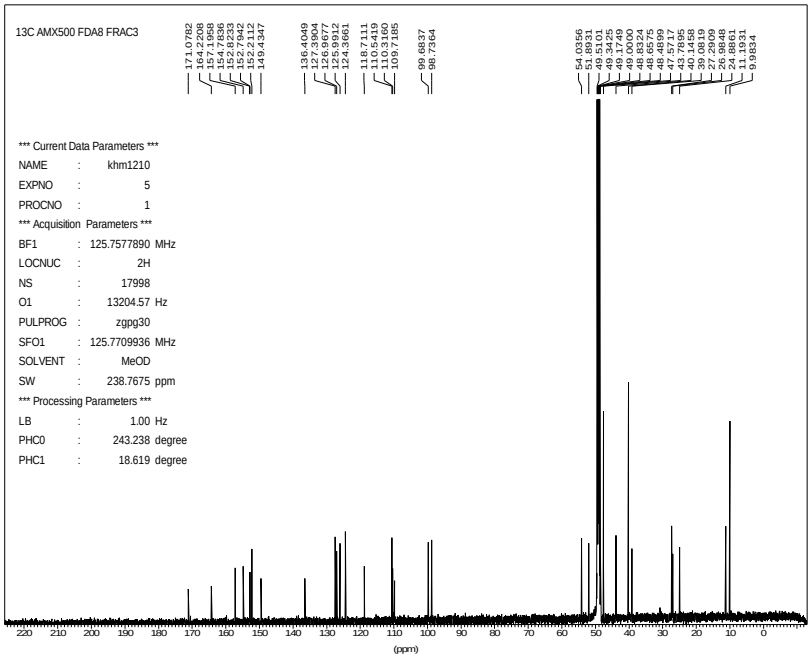
*** Current Data Parameters ***

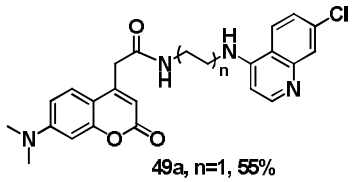
NAME	:	khm1127
EXPNO	:	4
PROCNO	:	1
*** Acquisition Parameters ***		
INSTRUM	:	av500
LOCNUC	:	2H
NS	:	19933
NUCLEUS	:	off
O1	:	13204.57 Hz
PULPROG	:	zgpg30
SFO1	:	125.7709936 MHz
SOLVENT	:	MeOD
SW	:	238.7675 ppm
TD	:	65536
TE	:	300.0 K
*** Processing Parameters ***		
LB	:	1.00 Hz
*** 1D NMR Plot Parameters ***		
NUCLEUS	:	off



48b, n=2, 65%

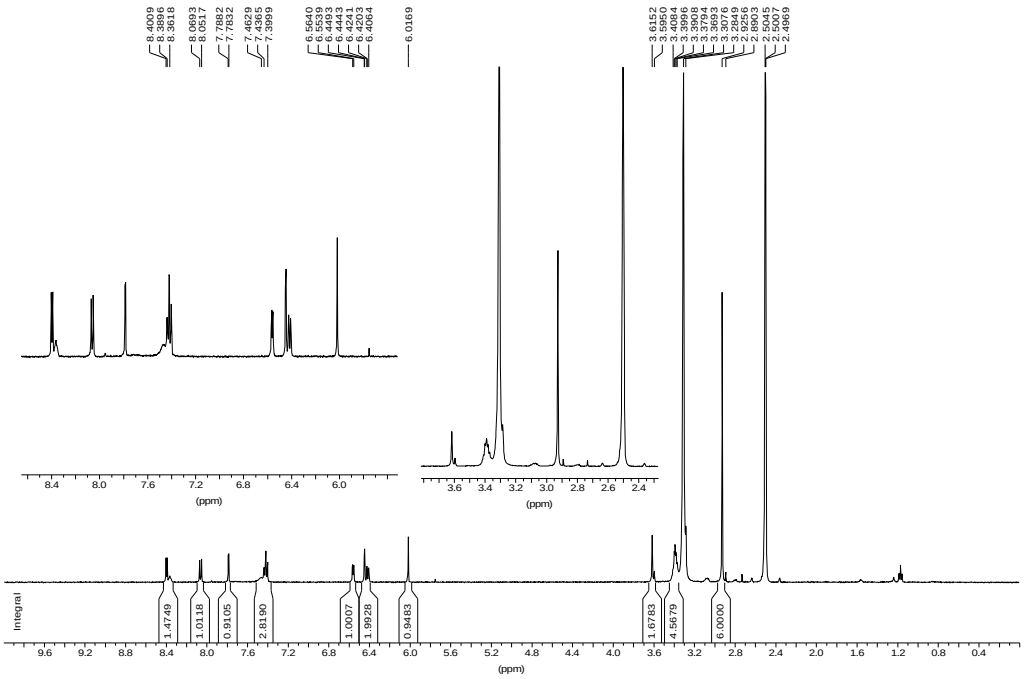

1H AMX500
FDA8 FRAC3
*** Current Data Parameters ***
NAME : khm1210
EXPNO : 4
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 500.1300000 MHz
LOCNUC : 2H
NS : 16
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : MeOD
SW : 20.6557 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 99.731 degree
PHC1 : 7.221 degree
integral
0.9371
0.9332
0.8737
0.9222
0.9443
1.0169
1.9427
0.8451
2.0177
5.9109
6.1291
3.0000
(ppm)

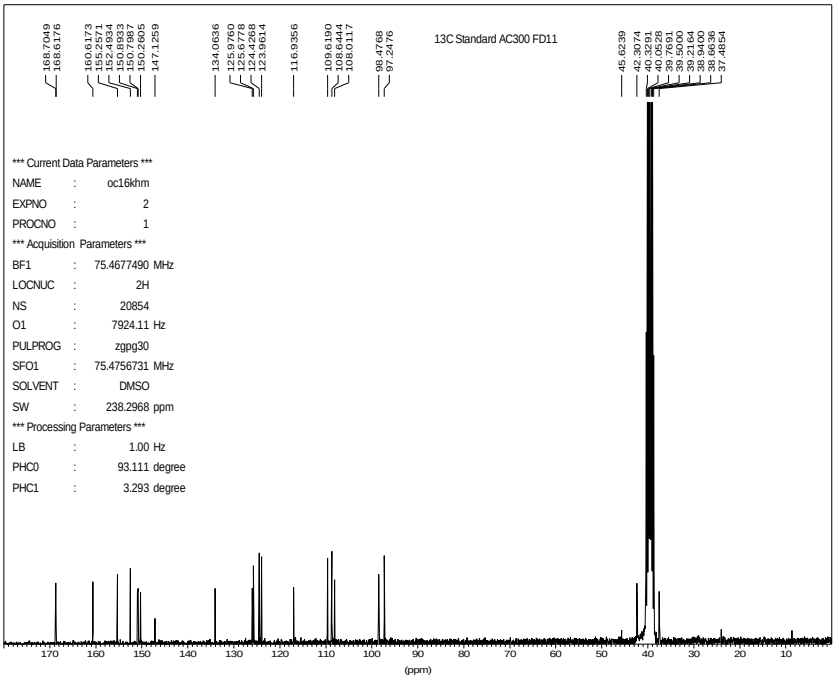

13C AMX500 FDA8 FRAC3
*** Current Data Parameters ***
NAME : khm1210
EXPNO : 5
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 125.7577890 MHz
LOCNUC : 2H
NS : 17998
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : MeOD
SW : 238.7675 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 243.238 degree
PHC1 : 18.619 degree
(ppm)

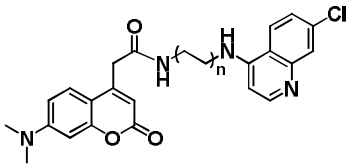


49a, n=1, 55%

1H AMX500 FD11

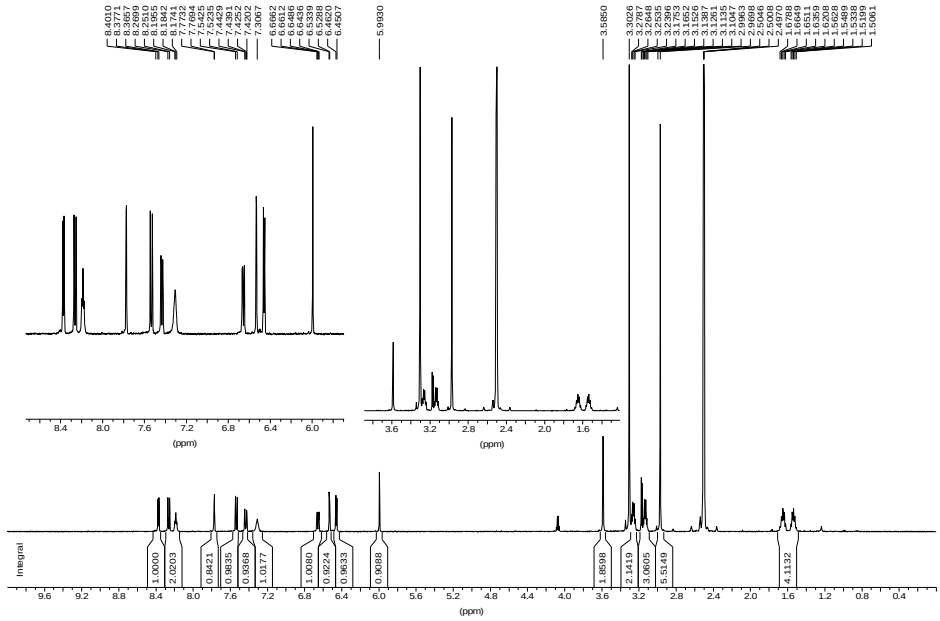




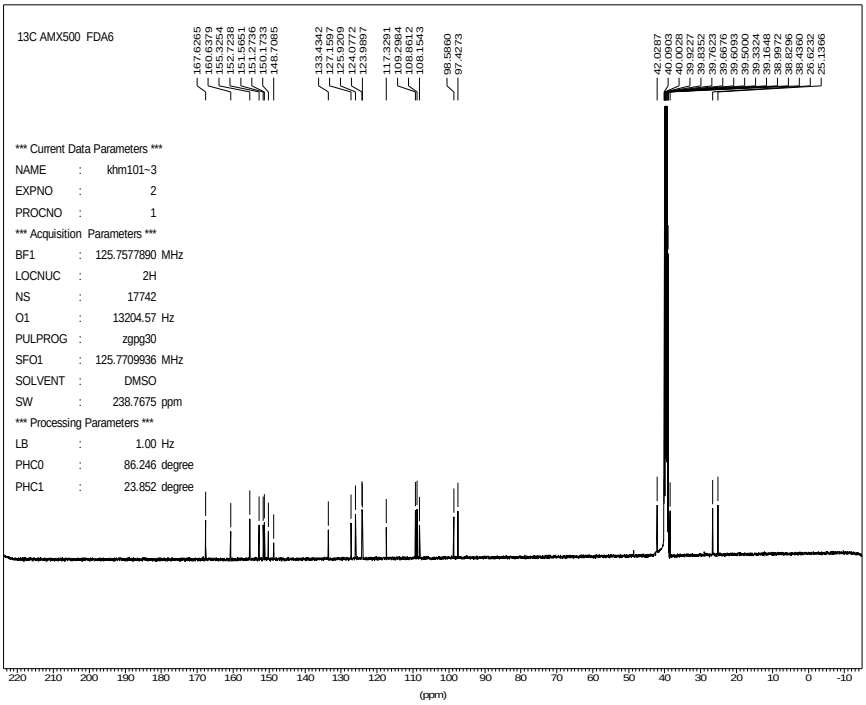


49b, n=2, 60%

1H AMX500 FD10 64 SCANS



13C AMX500 FDA6



*** Current Data Parameters ***

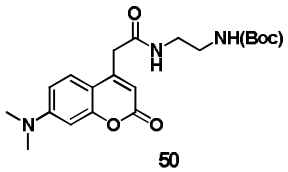
NAME	:	khm101~3
EXPNO	:	2
PROCNO	:	1

*** Acquisition Parameters ***

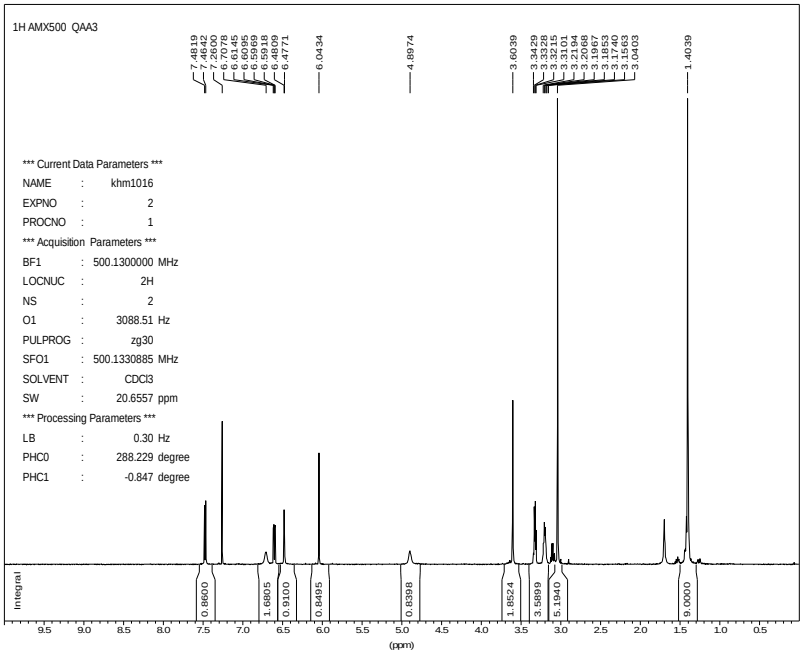
BF1	:	125.7577890 MHz
LOCNUC	:	2H
NS	:	17742
O1	:	13204.57 Hz
PULPROG	:	zgpg30
SFO1	:	125.7709936 MHz
SOLVENT	:	DMSO
SW	:	238.7675 ppm

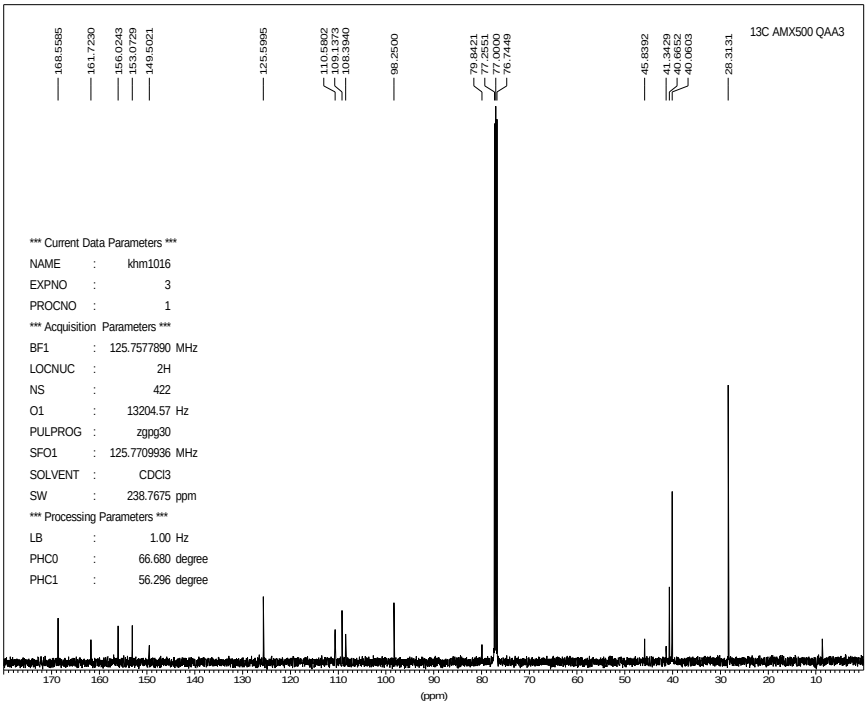
*** Processing Parameters ***

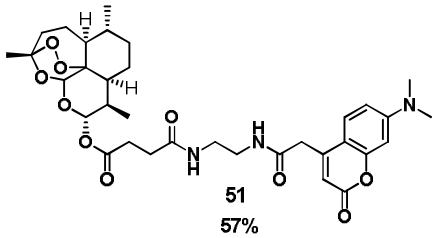
LB	:	1.00 Hz
PHC0	:	86.246 degree
PHC1	:	23.852 degree


O
NH(Boc)
N
H
N
O
O

50
60%


1H AMX500 QAA3
7.4819
7.4642
7.2600
6.7078
6.6145
6.6085
6.5969
6.5918
6.4899
6.4771
6.0434
4.8974
3.6039
3.3429
3.3328
3.3215
3.3101
3.2194
3.2068
3.1967
3.1853
3.1740
3.1563
3.0405
1.4039
*** Current Data Parameters ***
NAME : khm1016
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 500.1300000 MHz
LOCNUC : 2H
NS : 2
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : CDCl3
SW : 20.6557 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 288.229 degree
PHC1 : -0.847 degree
Integral
0.8600
1.6805
0.9100
0.8495
0.8398
1.8524
3.5899
5.1940
9.0000
(ppm)

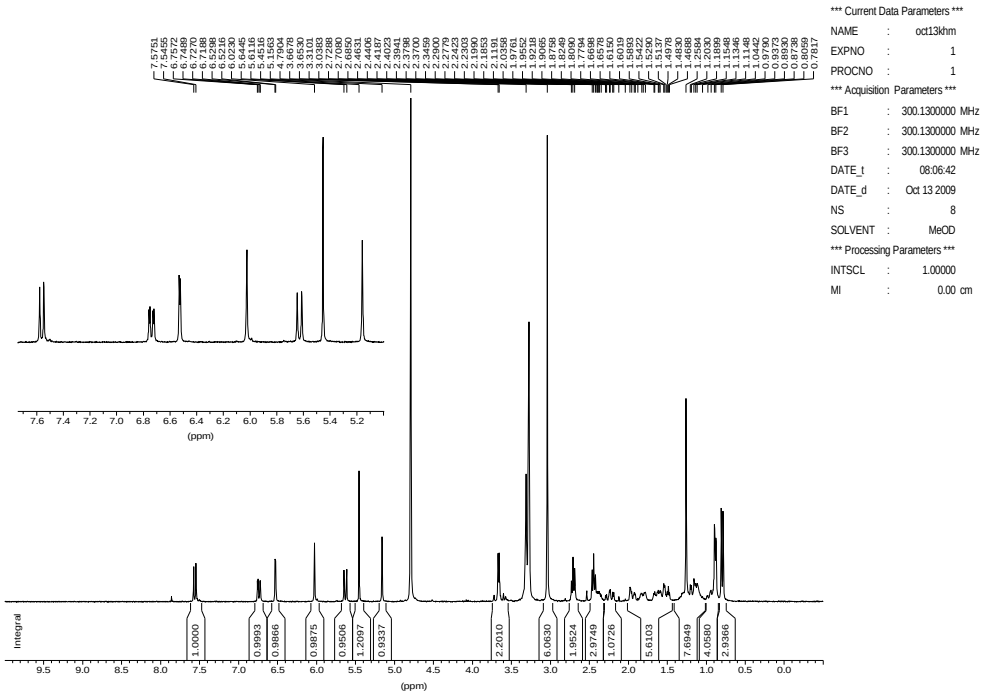

13C AMX500 QAA3
168.5585
161.7230
156.0243
153.0729
149.5021
125.5995
110.5802
109.1373
108.3940
98.2500
79.8421
77.2551
77.0000
76.7448
45.8392
41.3429
40.6652
40.0653
28.3131
*** Current Data Parameters ***
NAME : khm1016
EXPNO : 3
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 125.7577890 MHz
LOCNUC : 2H
NS : 422
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : CDCl3
SW : 238.7675 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 66.680 degree
PHC1 : 56.296 degree
(ppm)



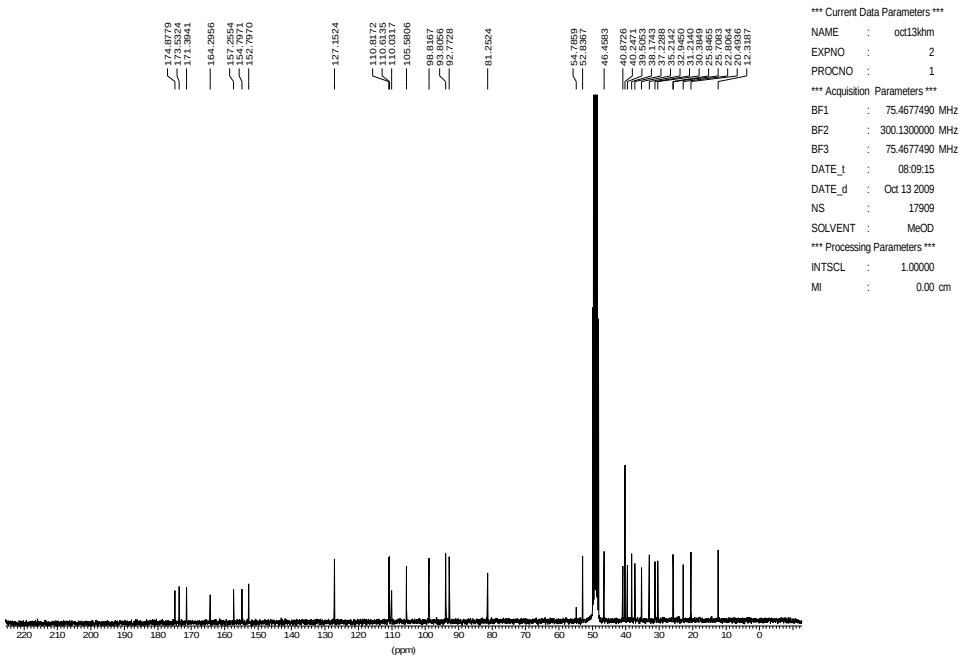
51

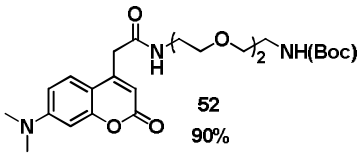
57%

1H normal range AC300 ASC1 FRAC2



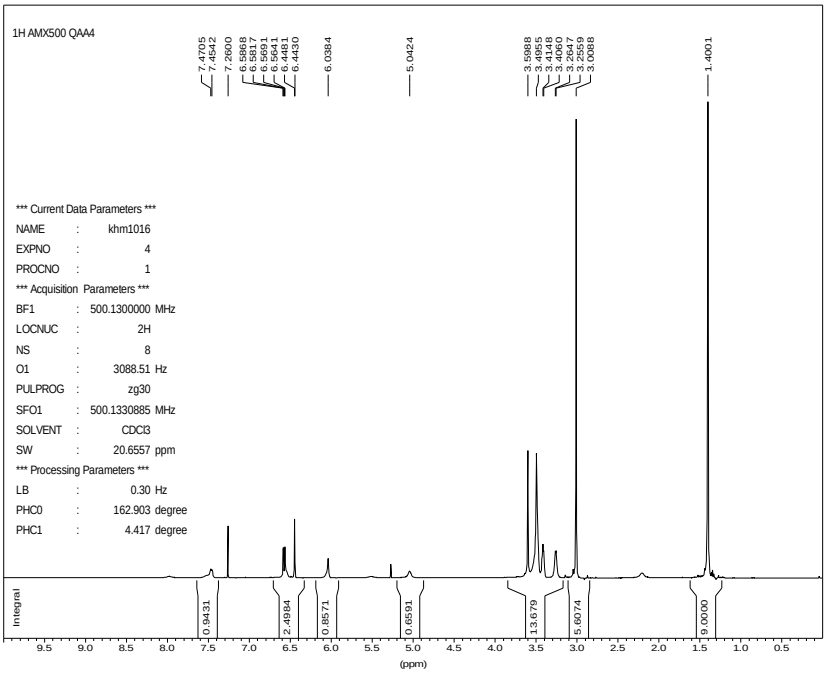
13C Standard AC300 ASC1 FRAC2

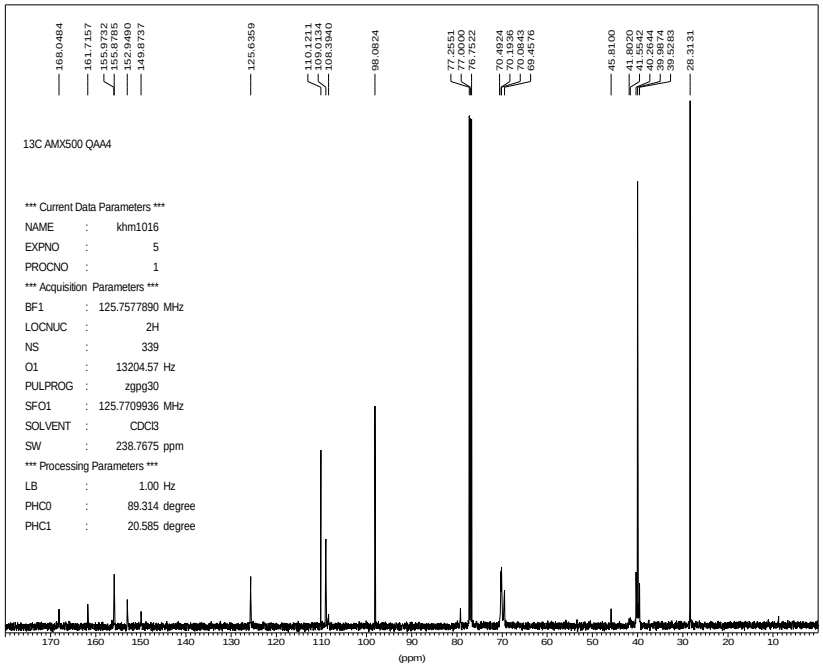


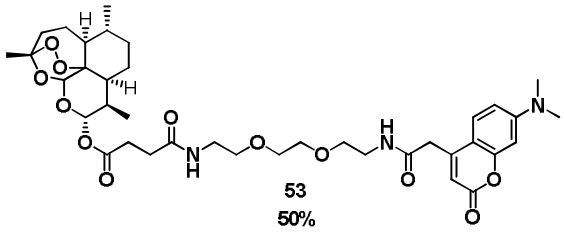

O
N
H
O
2
NH(Boc)
N
O
O

52

90%


1H AMX500 QAA4
7.4705
7.4542
7.2600
6.5868
6.5817
6.5691
6.5641
6.4481
6.4430
6.0384
5.0424
3.5988
3.4955
3.4148
3.4060
3.2547
3.2559
3.0088
1.4001
*** Current Data Parameters ***
NAME : khm1016
EXPNO : 4
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 500.1300000 MHz
LOCNUC : 2H
NS : 8
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : CDCl3
SW : 20.6557 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 162.903 degree
PHC1 : 4.417 degree
Integral
0.9431
2.4984
0.8571
0.6591
13.679
5.6074
9.0000
(ppm)

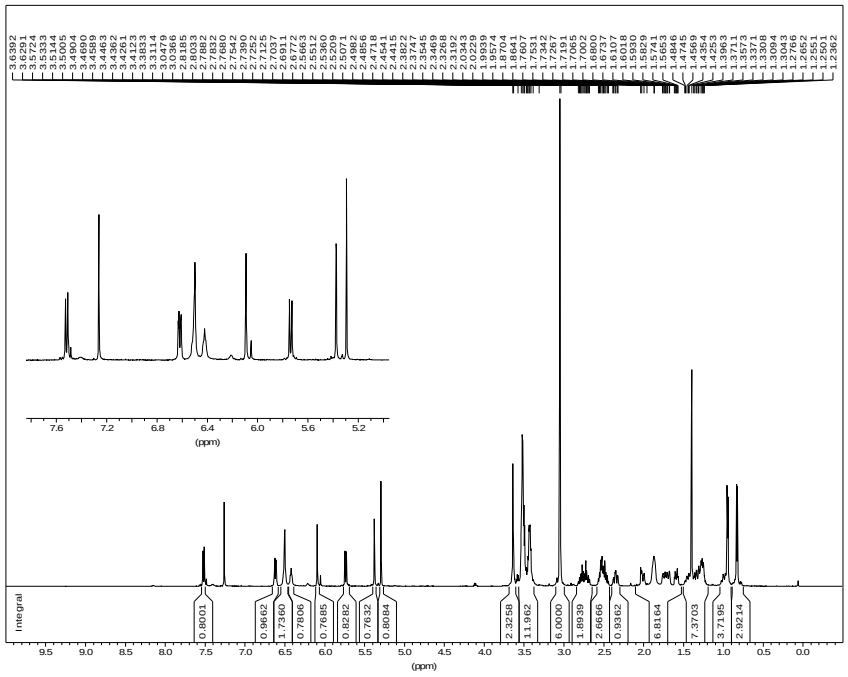

168.0484
161.7157
155.9732
155.8785
152.9490
149.8737
125.6359
110.1211
109.0134
108.3940
98.0824
77.2551
77.0000
76.7522
70.4924
70.1936
70.0843
69.4576
45.8100
41.8020
41.5542
40.2644
39.9874
39.5263
28.3131
13C AMX500 QAA4
*** Current Data Parameters ***
NAME : khm1016
EXPNO : 5
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 125.7577890 MHz
LOCNUC : 2H
NS : 339
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : CDCl3
SW : 238.7675 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 89.314 degree
PHC1 : 20.585 degree
(ppm)



53

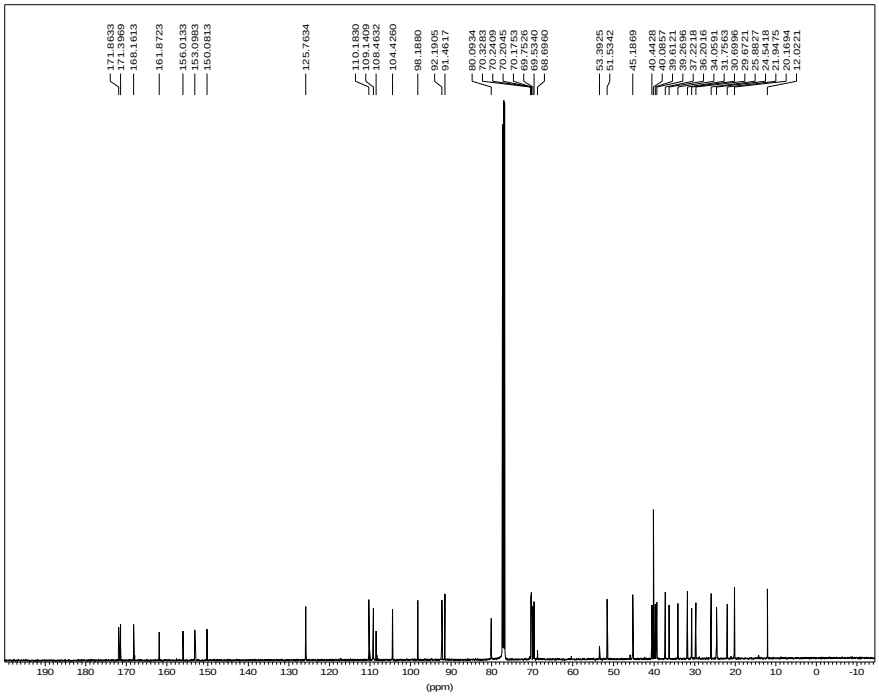
50%

1H AMX500 ASC2 FRAC2

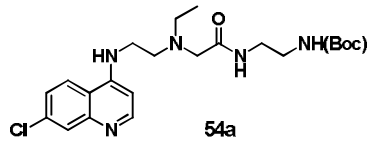


*** Current Data Parameters ***
NAME : khm1015
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
DATE_t : 07:27:48
DATE_d : Oct 15 2009
DBPNAM0 :
NS : 8
SFO1 : 500.1330885 MHz
SOLVENT : CDCl3
*** Processing Parameters ***
LB : 0.30 Hz
*** 1D NMR Plot Parameters ***
Start : 16.48 ppm
Stop : -4.18 ppm
SR : 13.06 Hz
AQ_time : 1.5859710 sec

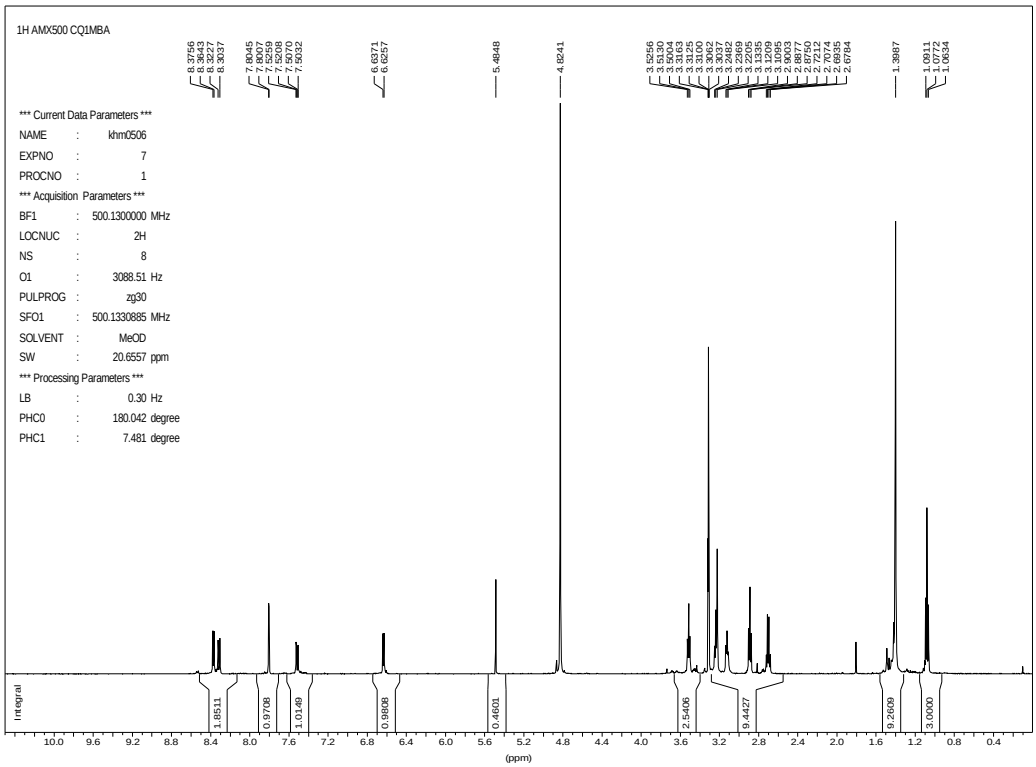
13C AMX500 ASC2 FRAC2

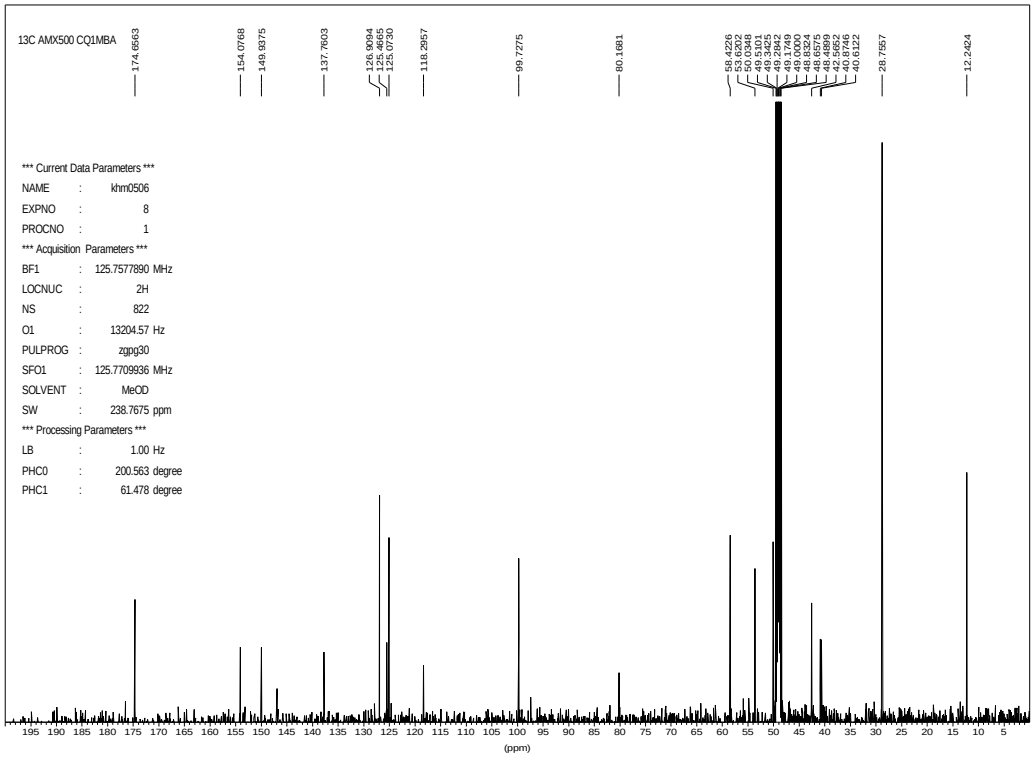


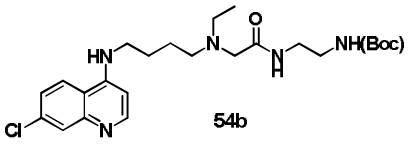
*** Current Data Parameters ***
NAME : khm1015
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
DATE_t : 07:30:23
DATE_d : Oct 15 2009
DBPNAM0 :
NS : 18665
SFO1 : 125.7709936 MHz
SOLVENT : CDCl3
*** Processing Parameters ***
LB : 1.00 Hz
*** 1D NMR Plot Parameters ***
Start : 224.37 ppm
Stop : -14.42 ppm
SR : 3.46 Hz
AQ_time : 1.0911740 sec


HN
N
O
N
H
NH(Boc)
Cl
N

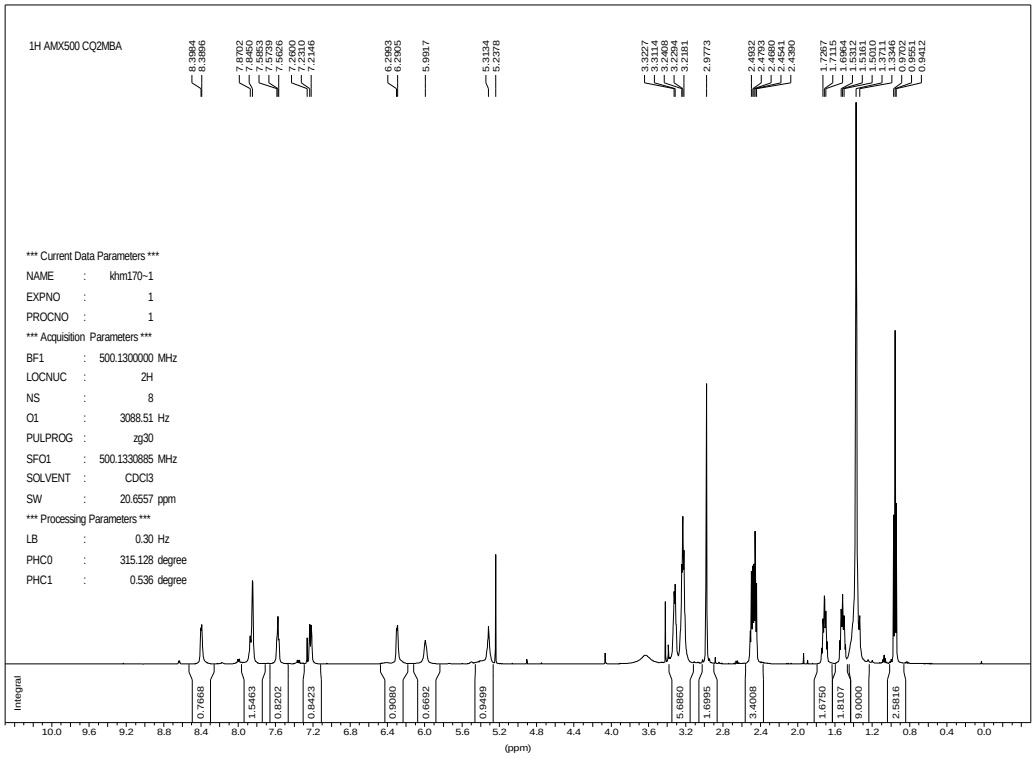
54a

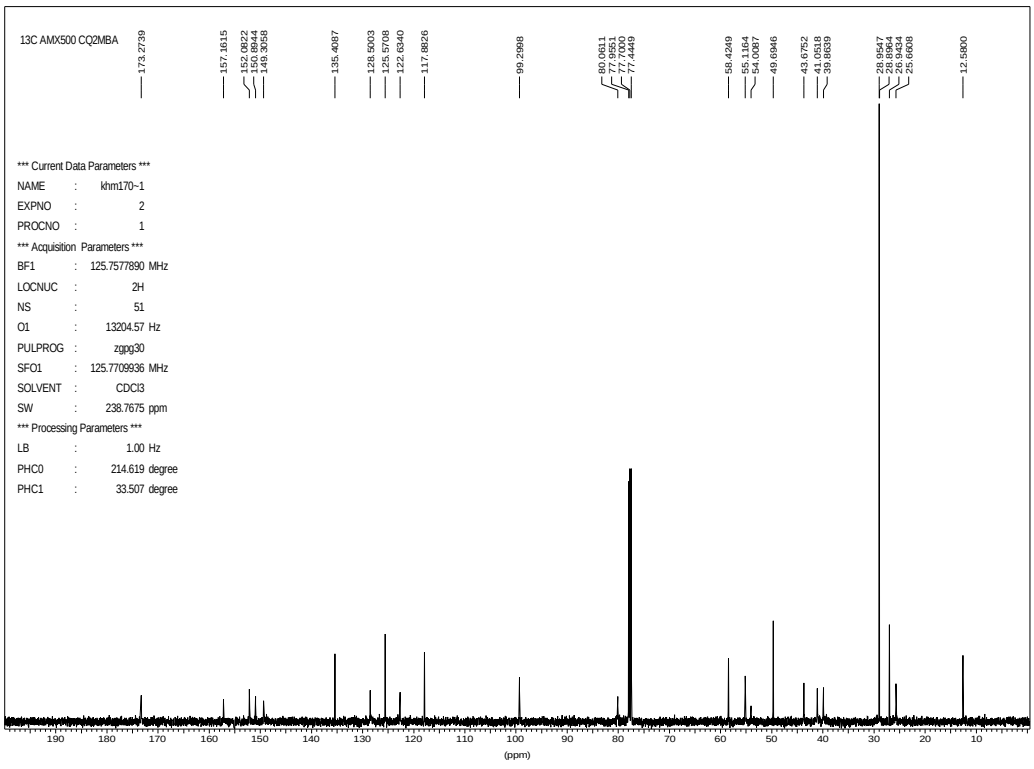

1H AMX500 CQ1MBA
*** Current Data Parameters ***
NAME : khm0506
EXPNO : 7
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 500.1300000 MHz
LOCNUC : 2H
NS : 8
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : MeOD
SW : 20.6557 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 180.042 degree
PHC1 : 7.481 degree
Integral
1.8511
0.9708
1.0149
0.9808
0.4601
2.5406
9.4427
9.2609
3.0000
(ppm)

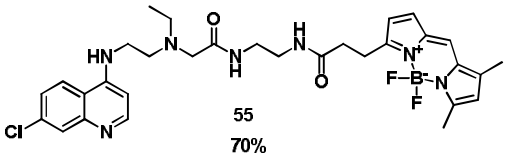

13C AMX500 CQ1MBA
*** Current Data Parameters ***
NAME : khm0506
EXPNO : 8
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 125.7577890 MHz
LOCNUC : 2H
NS : 822
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : MeOD
SW : 238.7675 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 200.563 degree
PHC1 : 61.478 degree
(ppm)


HN
N
O
N
H
NH(Boc)
Cl
N

54b

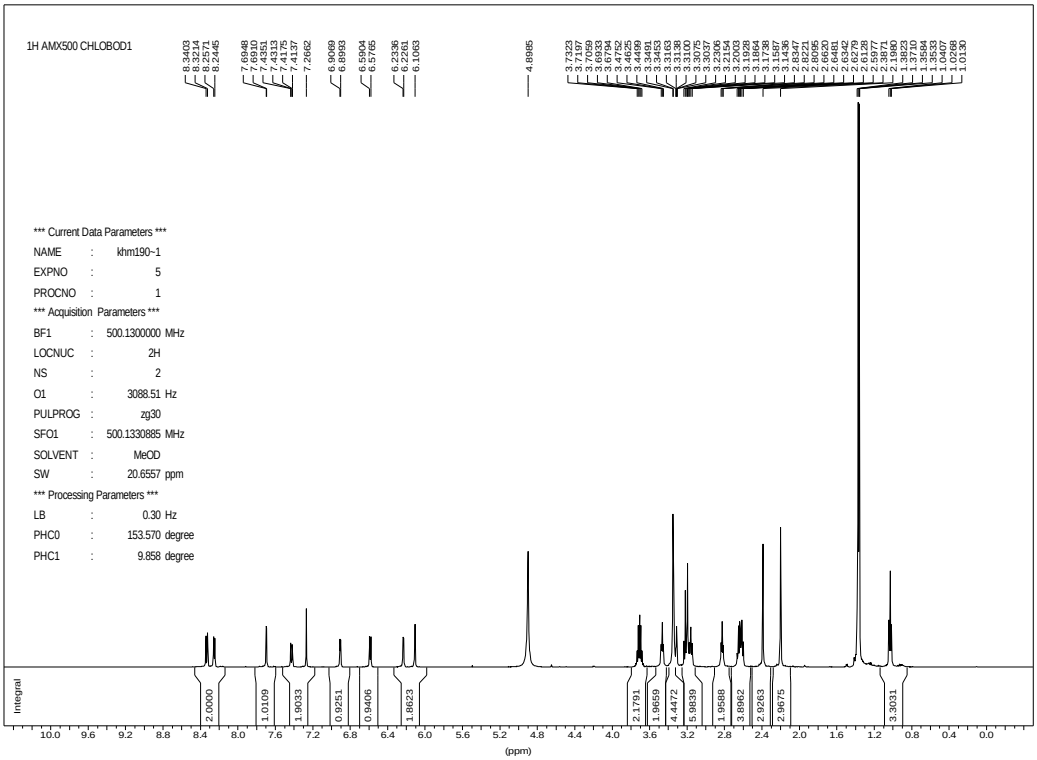

1H AMX500 CQ2MBA
*** Current Data Parameters ***
NAME : khm170~1
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 500.1300000 MHz
LOCNUC : 2H
NS : 8
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : CDCl3
SW : 20.6557 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 315.128 degree
PHC1 : 0.536 degree
Integral
(ppm)

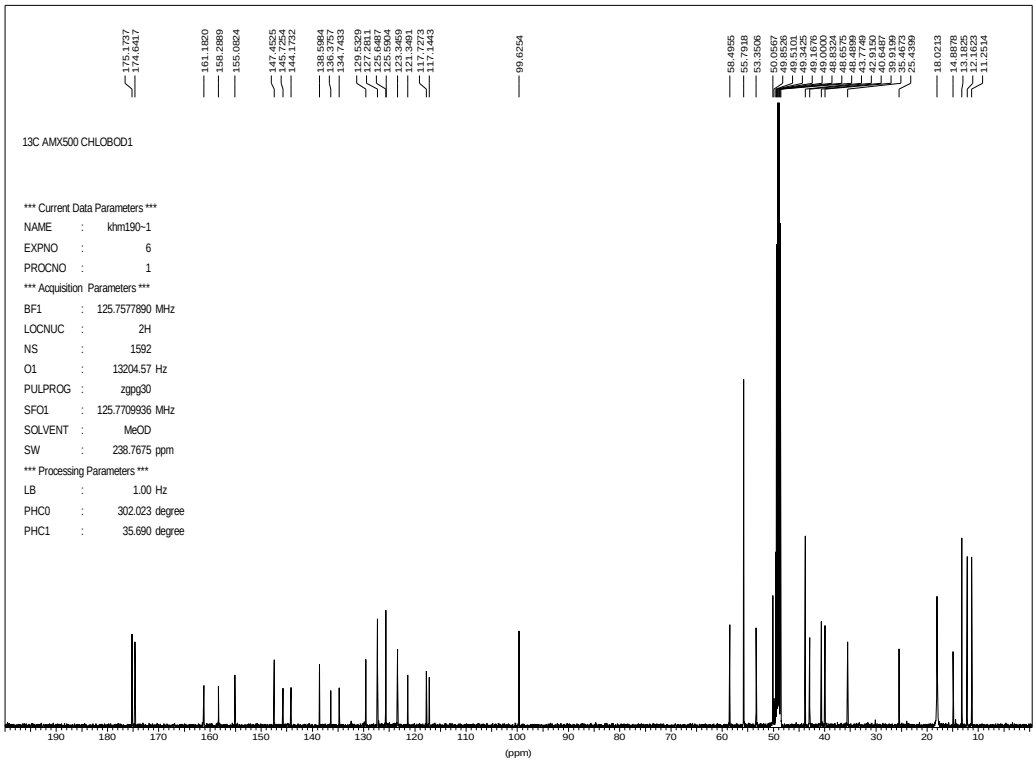

13C AMX500 CQ2MBA
*** Current Data Parameters ***
NAME : khm170~1
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 125.7577890 MHz
LOCNUC : 2H
NS : 51
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : CDCl3
SW : 238.7675 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 214.619 degree
PHC1 : 33.507 degree
(ppm)

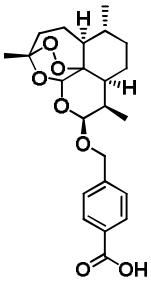


55

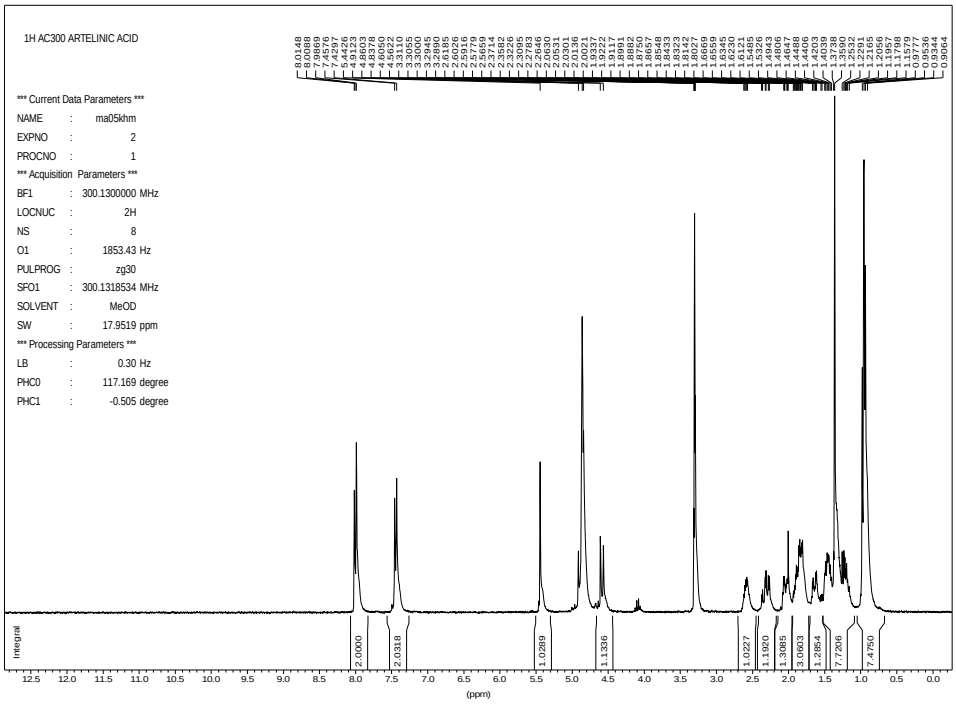
70%

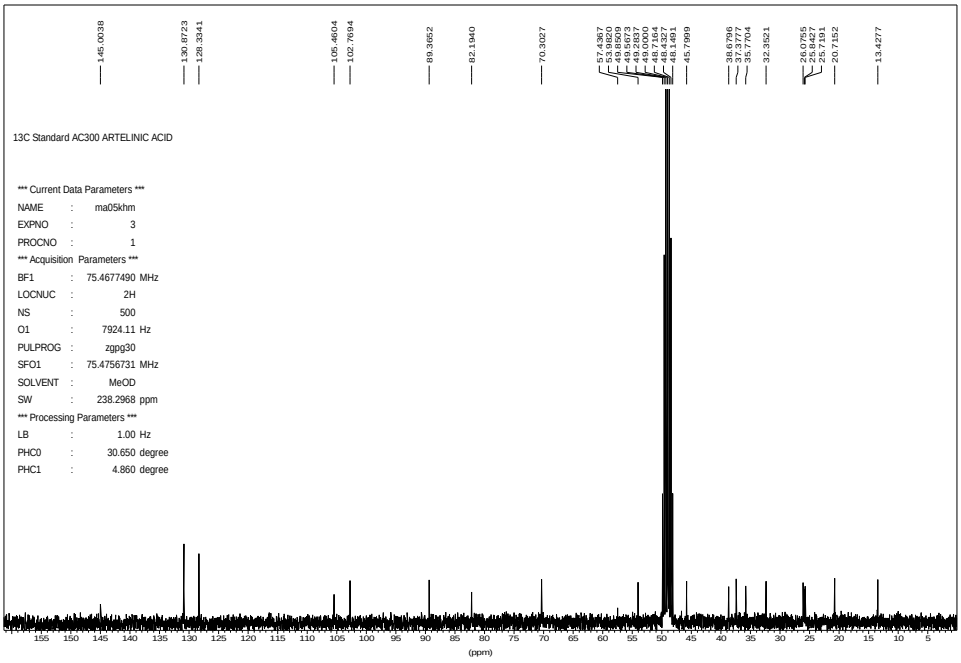

1H AMX500 CHLOBOD1
*** Current Data Parameters ***
NAME : khm190~1
EXPNO : 5
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 500.1300000 MHz
LOCNUC : 2H
NS : 2
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : MeOD
SW : 20.6557 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 153.570 degree
PHC1 : 9.858 degree
Integral
(ppm)

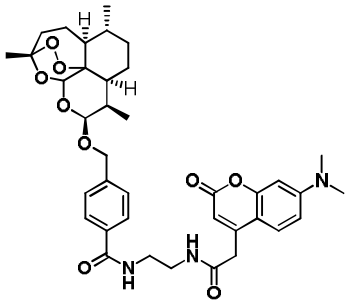

13C AMX500 CHLOBOD1
*** Current Data Parameters ***
NAME : khm190~1
EXPNO : 6
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 125.7577890 MHz
LOCNUC : 2H
NS : 1592
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : MeOD
SW : 238.7675 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 302.023 degree
PHC1 : 35.690 degree
(ppm)



56


1H AC300 ARTELINIC ACID
*** Current Data Parameters ***
NAME : ma05khm
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 300.1300000 MHz
LOCNUC : 2H
NS : 8
O1 : 1853.43 Hz
PULPROG : zg30
SFO1 : 300.1318534 MHz
SOLVENT : MeOD
SW : 17.9519 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 117.169 degree
PHC1 : -0.505 degree
Integral
2.0000
2.0318
1.0289
1.1336
1.0227
1.1920
1.3085
3.0613
1.2854
7.7206
7.4750
(ppm)


13C Standard AC300 ARTELINIC ACID
*** Current Data Parameters ***
NAME : ma05khm
EXPNO : 3
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 75.4677490 MHz
LOCNUC : 2H
NS : 500
O1 : 7924.11 Hz
PULPROG : zgpg30
SFO1 : 75.4756731 MHz
SOLVENT : MeOD
SW : 238.2968 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 30.650 degree
PHC1 : 4.860 degree
145.0038
130.8723
129.3341
105.4604
102.7694
89.3652
82.1940
70.3027
57.4367
53.9820
49.8093
49.5673
49.2837
49.0000
48.7164
48.4327
48.1491
45.7999
38.6796
37.3777
35.7704
32.3821
26.0755
25.8427
25.7191
20.7152
13.4277
(ppm)



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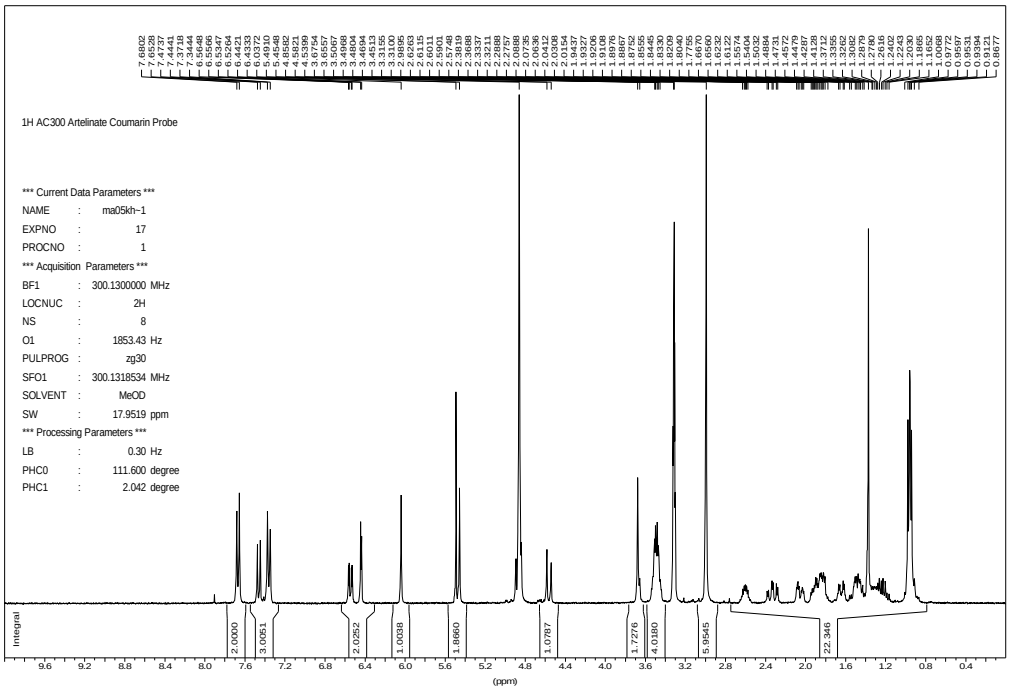
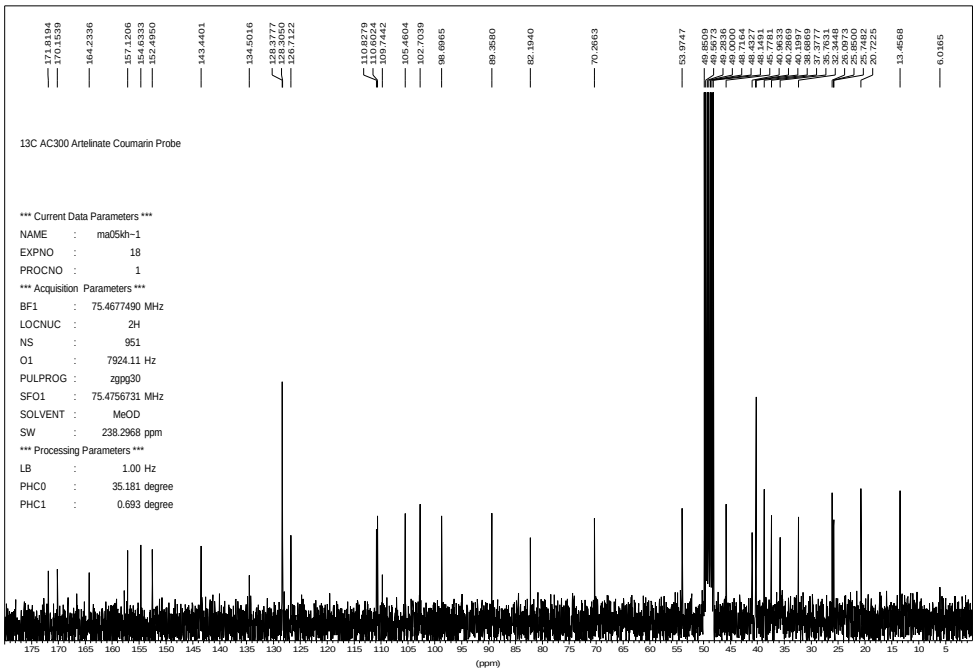
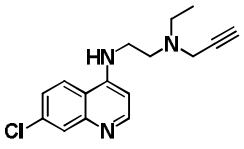

1H AC300 Artelinate Coumarin Probe
*** Current Data Parameters ***
NAME : ma05kh~1
EXPNO : 17
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 300.1300000 MHz
LOCNUC : 2H
NS : 8
O1 : 1853.43 Hz
PULPROG : zg30
SFO1 : 300.1318534 MHz
SOLVENT : MeOD
SW : 17.9519 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 111.600 degree
PHC1 : 2.042 degree
Integral
2.0000
3.0051
2.0252
1.0038
1.8660
1.0787
1.7276
4.0180
5.9545
22.346
(ppm)


13C AC300 Artelinate Coumarin Probe
*** Current Data Parameters ***
NAME : ma05kh~1
EXPNO : 18
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 75.4677490 MHz
LOCNUC : 2H
NS : 951
O1 : 7924.11 Hz
PULPROG : zgpg30
SFO1 : 75.4756731 MHz
SOLVENT : MeOD
SW : 238.2968 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 35.181 degree
PHC1 : 0.693 degree
(ppm)



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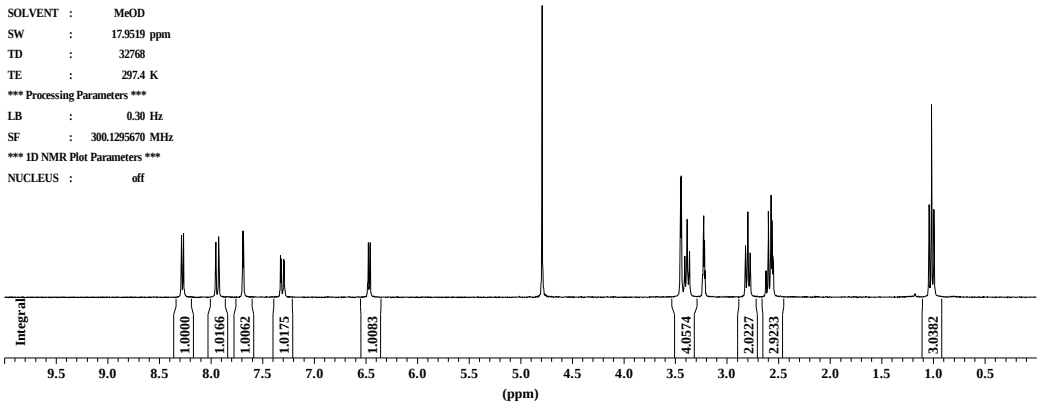
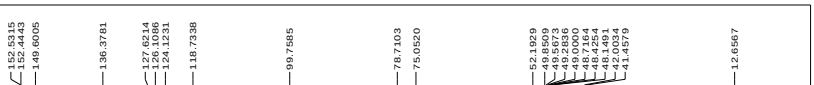
1H AC300 DCQA2 Alkyne

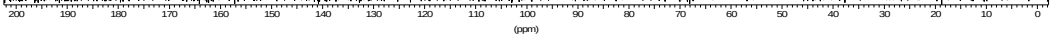
*** Current Data Parameters ***
NAME : ma05kh~1
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
LOCNUC : 2H
NS : 2
NUCLEUS : off
O1 : 1853.43 Hz
PULPROG : zg30
SFO1 : 300.1318534 MHz
SOLVENT : MeOD
SW : 17.9519 ppm
TD : 32768
TE : 297.4 K
*** Processing Parameters ***
LB : 0.30 Hz
SF : 300.1295670 MHz
*** 1D NMR Plot Parameters ***
NUCLEUS : off

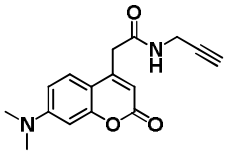


13C AC300 DCQA2 Alkyne



*** Current Data Parameters ***
NAME : ma05kh~1
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 75.4677490 MHz
LOCNUC : 2H
NS : 301
O1 : 7924.11 Hz
PULPROG : zgpg30
SFO1 : 75.4756731 MHz
SOLVENT : MeOD
SW : 238.2968 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 46.099 degree
PHC1 : -13.736 degree





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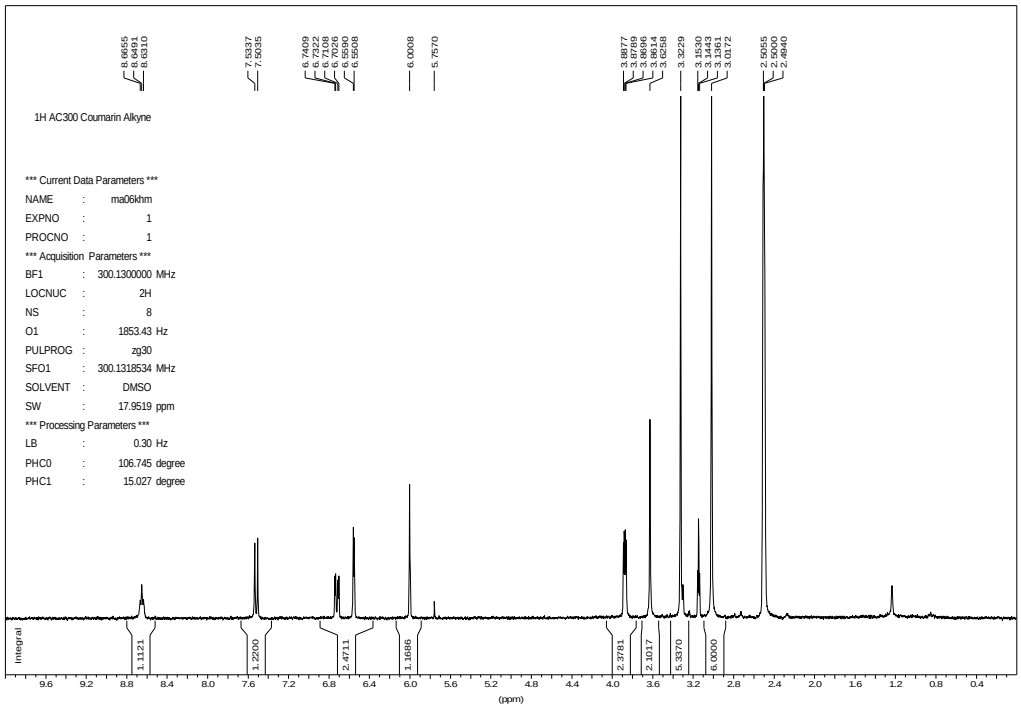
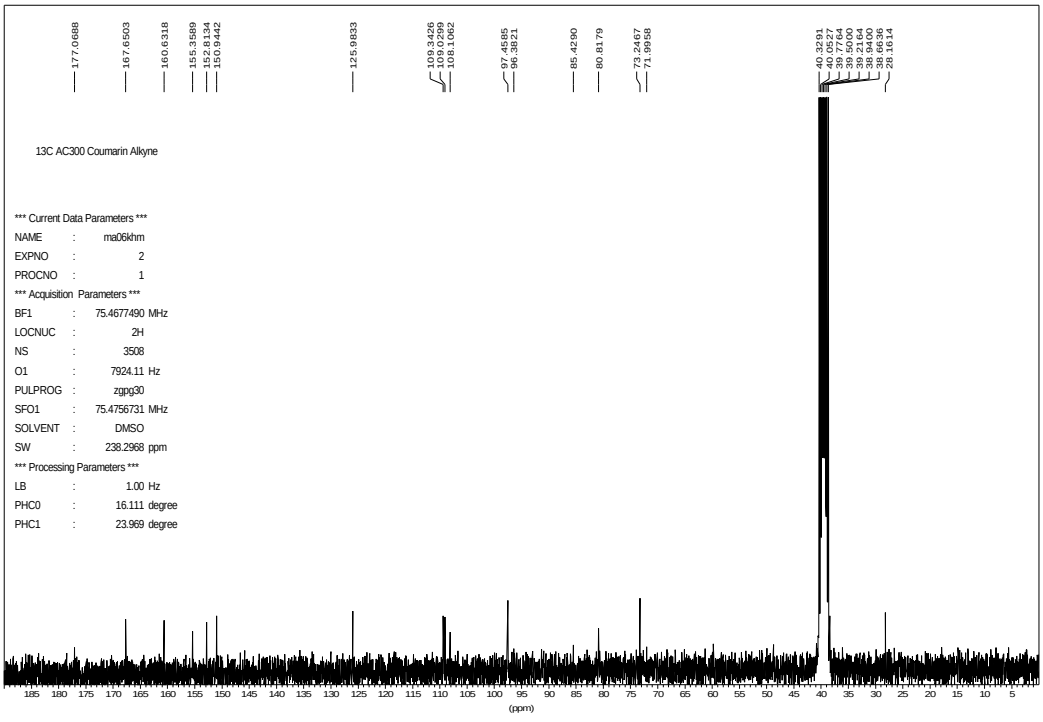
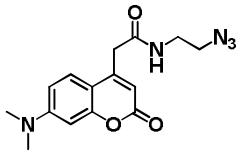

8.6655
8.6491
8.6310
7.5337
7.5035
6.7409
6.7322
6.7198
6.7026
6.5590
6.5508
6.0008
5.7570
3.8877
3.8759
3.8696
3.8614
3.8258
3.3229
3.1530
3.1443
3.1361
3.0172
2.9055
2.5000
2.4940
1H AC300 Coumarin Alkyne
*** Current Data Parameters ***
NAME : ma06khm
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 300.1300000 MHz
LOCNUC : 2H
NS : 8
O1 : 1853.43 Hz
PULPROG : zg30
SFO1 : 300.1318534 MHz
SOLVENT : DMSO
SW : 17.9519 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 106.745 degree
PHC1 : 15.027 degree
Integral
1.1121
1.2200
2.4711
1.1686
2.3781
2.1017
5.3370
6.0000
(ppm)


177.0688
167.6503
160.6318
155.3589
152.8134
150.9442
125.9833
109.3426
109.0299
108.1062
97.4585
96.3821
85.4290
80.8179
73.2467
71.9958
40.3291
40.0527
39.7764
39.5000
39.2164
38.9400
38.6636
28.1614
13C AC300 Coumarin Alkyne
*** Current Data Parameters ***
NAME : ma06khm
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 75.4677490 MHz
LOCNUC : 2H
NS : 3508
O1 : 7924.11 Hz
PULPROG : zgpg30
SFO1 : 75.4756731 MHz
SOLVENT : DMSO
SW : 238.2968 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 16.111 degree
PHC1 : 23.969 degree
(ppm)



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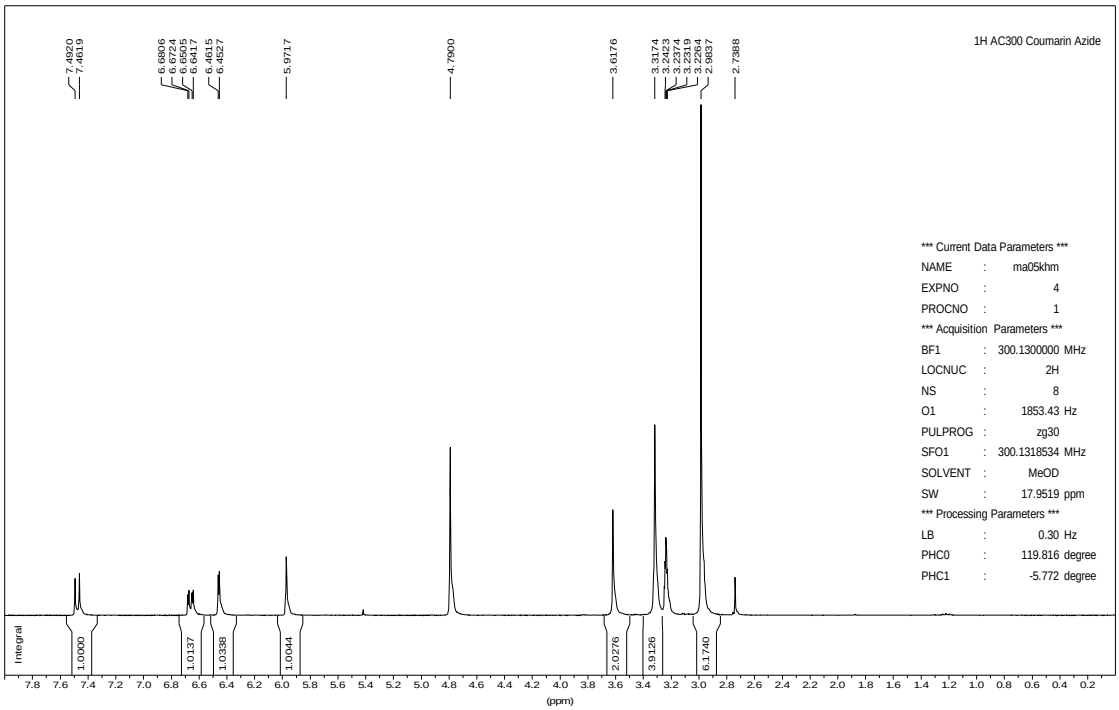
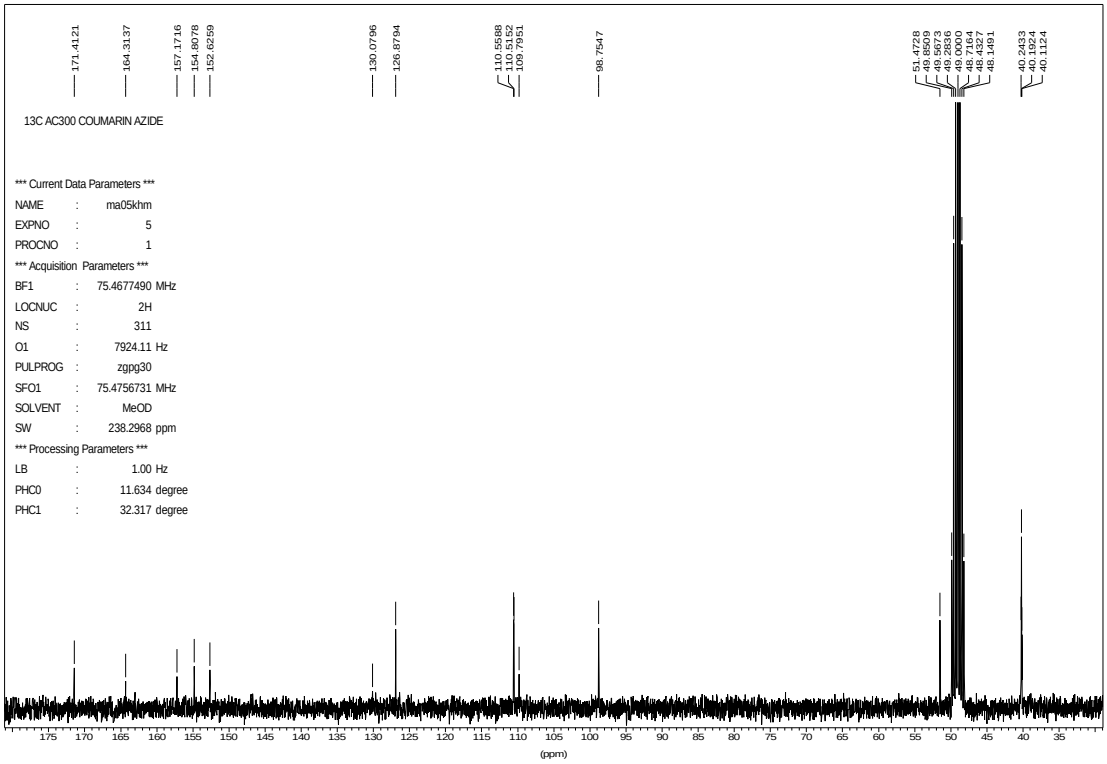
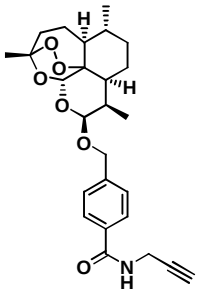

1H AC300 Coumarin Azide
7.4920
7.4619
6.6806
6.6724
6.6605
6.6417
6.4515
6.4527
5.9717
4.7900
3.6176
3.3174
3.2423
3.2374
3.2219
3.2064
2.9837
2.7388
*** Current Data Parameters ***
NAME : ma05khm
EXPNO : 4
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 300.1300000 MHz
LOCNUC : 2H
NS : 8
O1 : 1853.43 Hz
PULPROG : zg30
SFO1 : 300.1318534 MHz
SOLVENT : MeOD
SW : 17.9519 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 119.816 degree
PHC1 : -5.772 degree
Integral
1.0000
1.0137
1.0338
1.0044
2.0276
3.9126
6.1740
(ppm)


171.4121
164.3137
157.1716
154.8078
152.6259
130.0796
126.8794
110.5588
110.5152
109.7951
98.7547
51.4728
49.8509
49.5673
49.2836
49.0000
48.7164
48.4327
48.1491
40.2433
40.1924
40.1124
13C AC300 COUMARIN AZIDE
*** Current Data Parameters ***
NAME : ma05khm
EXPNO : 5
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 75.4677490 MHz
LOCNUC : 2H
NS : 311
O1 : 7924.11 Hz
PULPROG : zgpg30
SFO1 : 75.4756731 MHz
SOLVENT : MeOD
SW : 238.2968 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 11.634 degree
PHC1 : 32.317 degree
(ppm)



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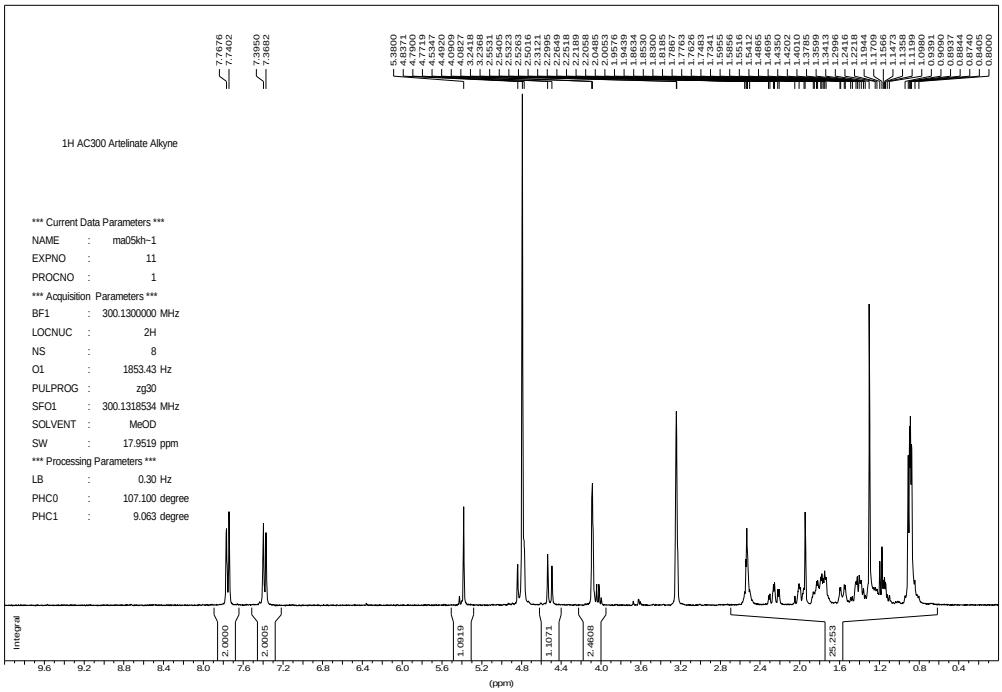
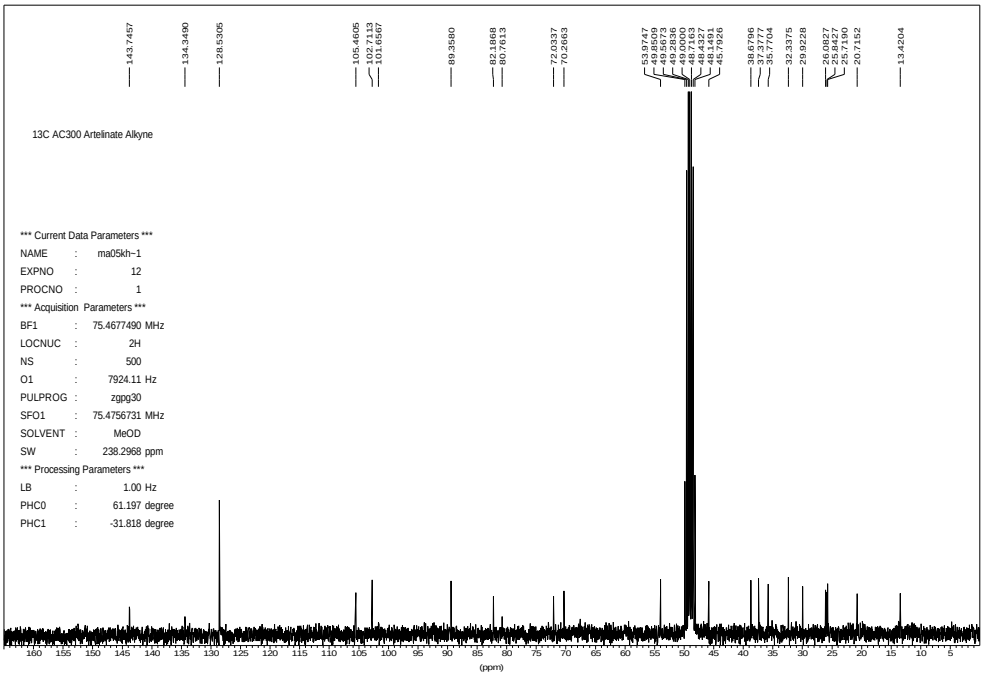
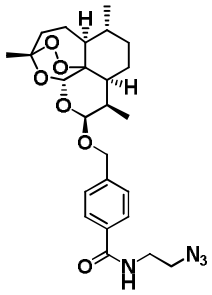
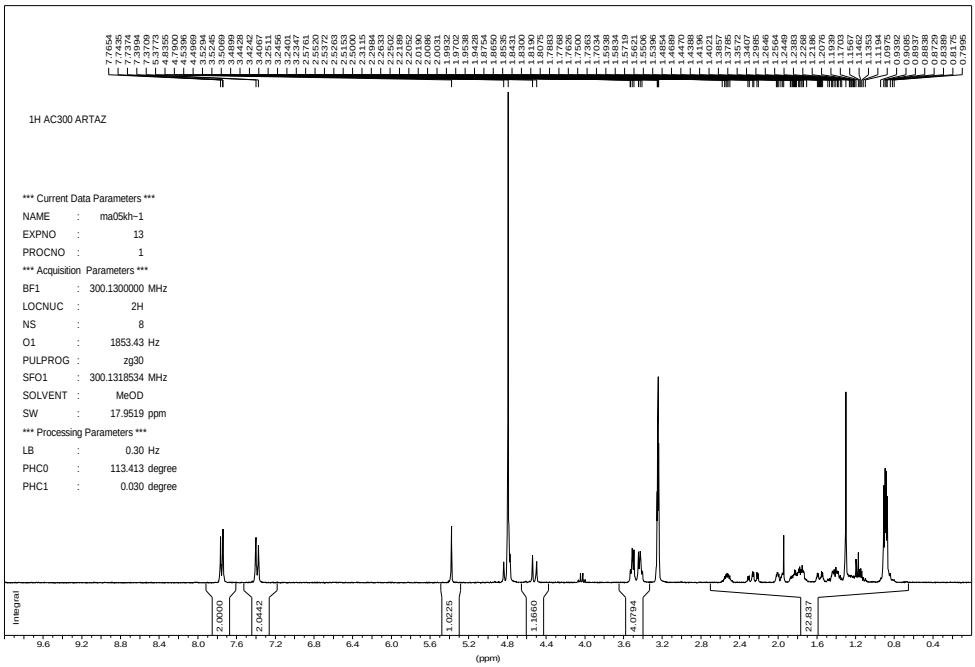
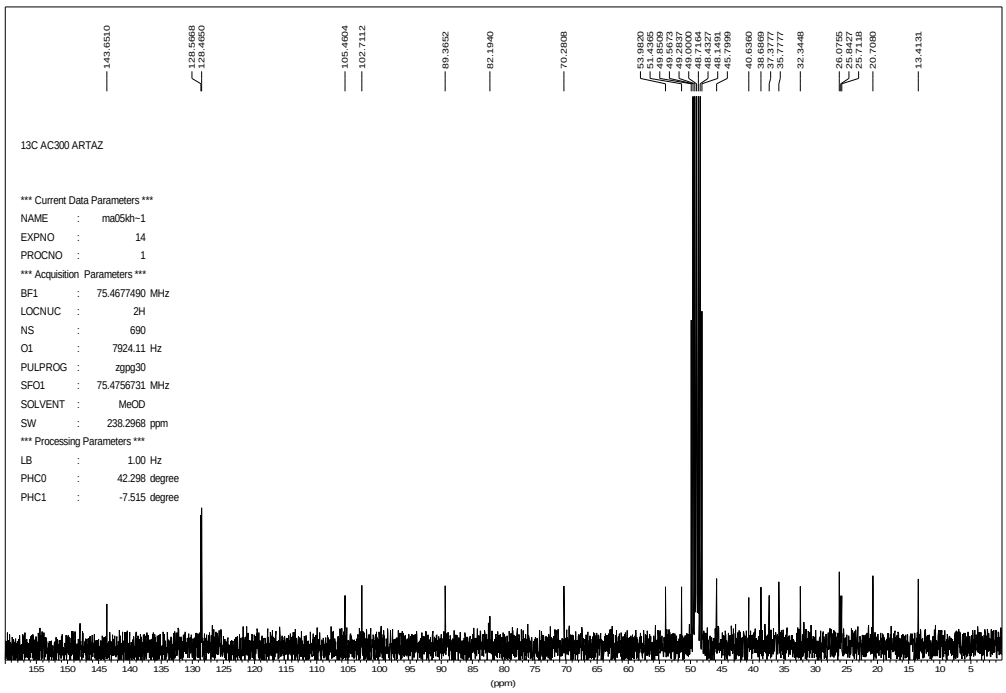

1H AC300 Artelinate Alkyne
*** Current Data Parameters ***
NAME : ma05kh−1
EXPNO : 11
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 300.1300000 MHz
LOCNUC : 2H
NS : 8
O1 : 1853.43 Hz
PULPROG : zg30
SFO1 : 300.1318534 MHz
SOLVENT : MeOD
SW : 17.9519 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 107.100 degree
PHC1 : 9.063 degree
Integral
2.0000
2.0005
1.0919
1.1071
2.4608
25.253
(ppm)

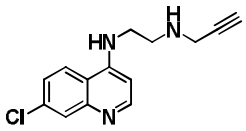

13C AC300 Artelinate Alkyne
*** Current Data Parameters ***
NAME : ma05kh−1
EXPNO : 12
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 75.4677490 MHz
LOCNUC : 2H
NS : 500
O1 : 7924.11 Hz
PULPROG : zgpg30
SFO1 : 75.4756731 MHz
SOLVENT : MeOD
SW : 238.2968 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 61.197 degree
PHC1 : -31.818 degree
(ppm)



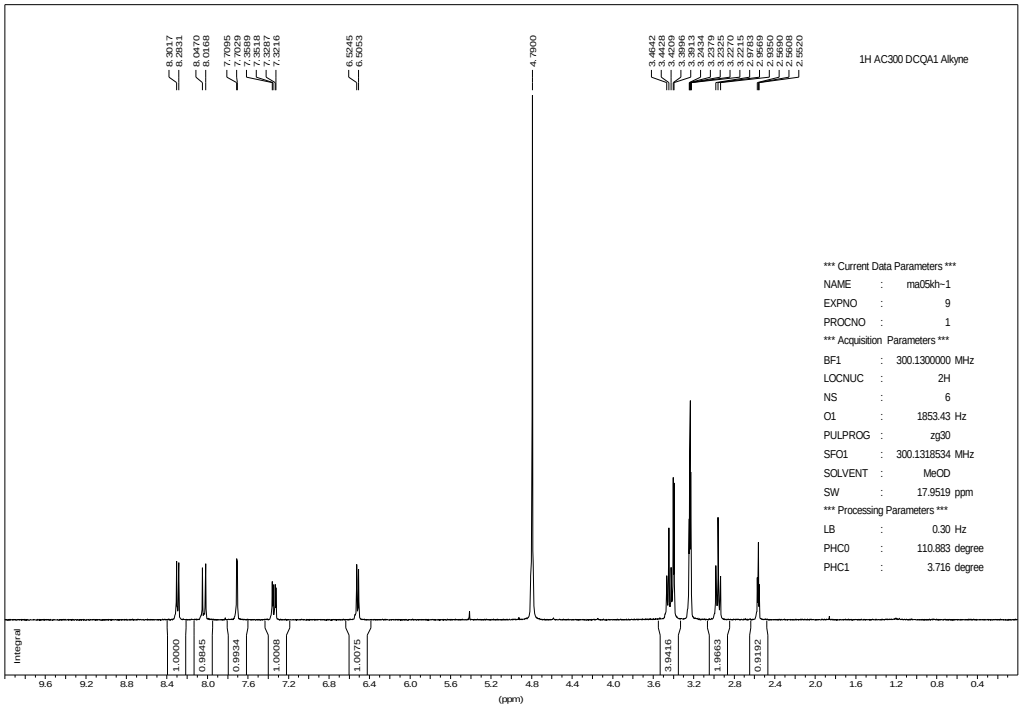
62

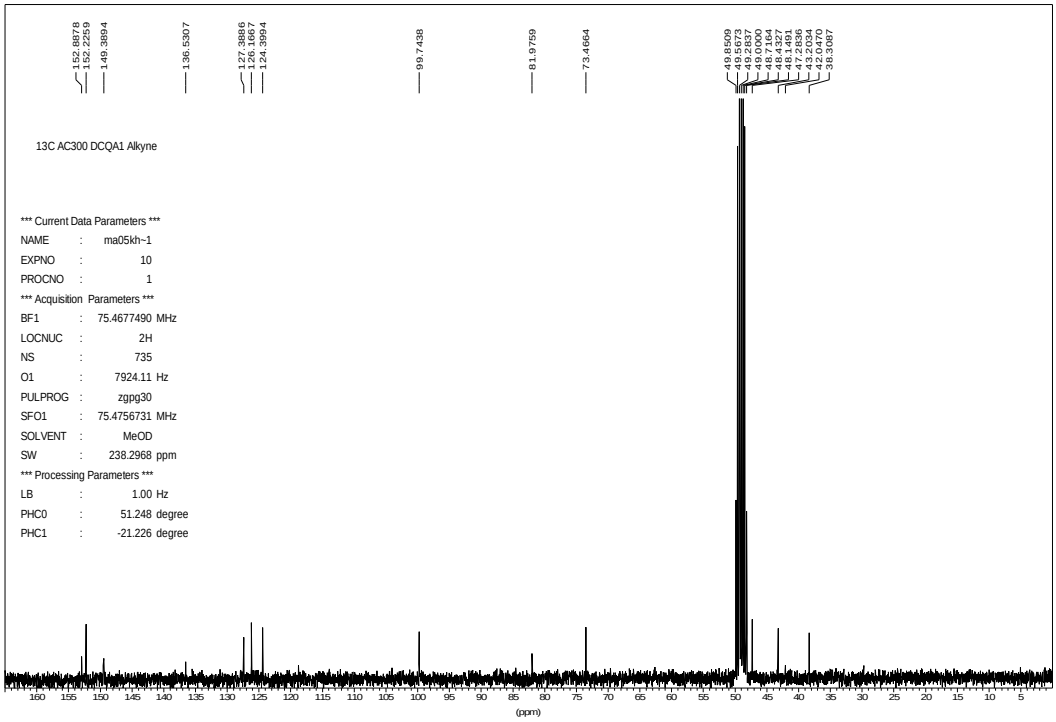

1H AC300 ARTAZ
*** Current Data Parameters ***
NAME : ma05kh-1
EXPNO : 13
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 300.1300000 MHz
LOCNUC : 2H
NS : 8
O1 : 1853.43 Hz
PULPROG : zg30
SFO1 : 300.1318534 MHz
SOLVENT : MeOD
SW : 17.9519 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 113.413 degree
PHC1 : 0.030 degree
integral
2.0000
2.0442
1.0225
1.1660
4.0794
22.837
(ppm)

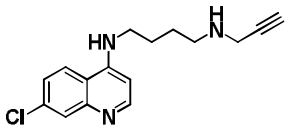

13C AC300 ARTAZ
*** Current Data Parameters ***
NAME : ma05kh-1
EXPNO : 14
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 75.4677490 MHz
LOCNUC : 2H
NS : 690
O1 : 7924.11 Hz
PULPROG : zgpg30
SFO1 : 75.4756731 MHz
SOLVENT : MeOD
SW : 238.2968 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 42.298 degree
PHC1 : -7.515 degree
(ppm)



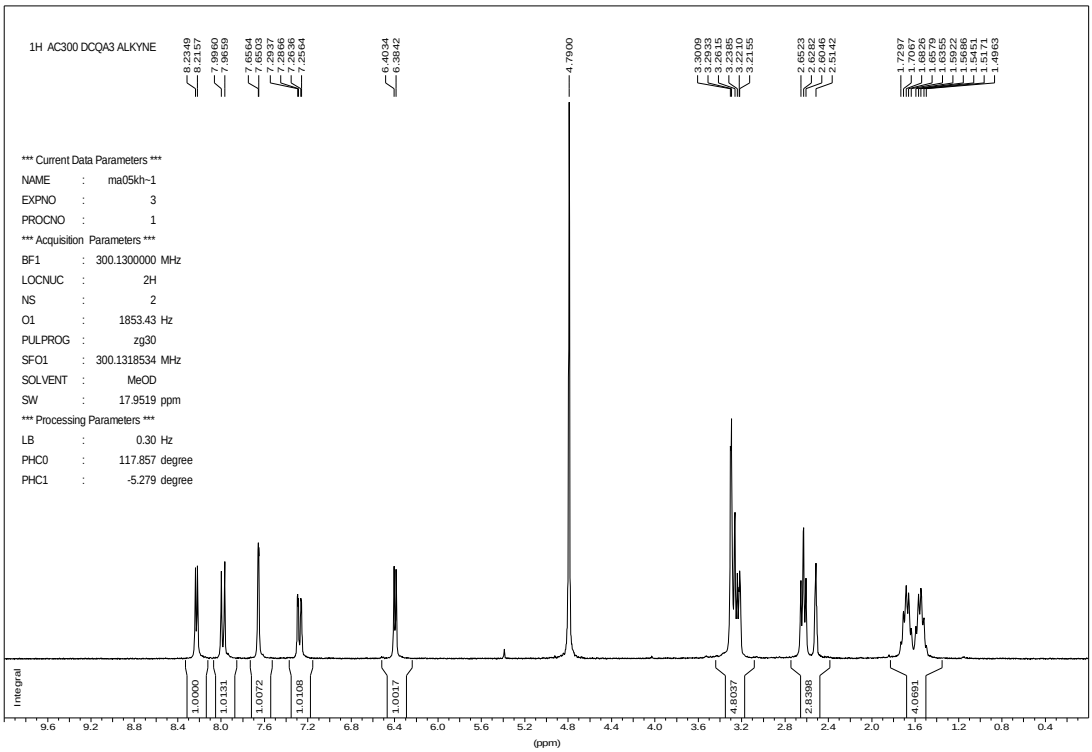
63a

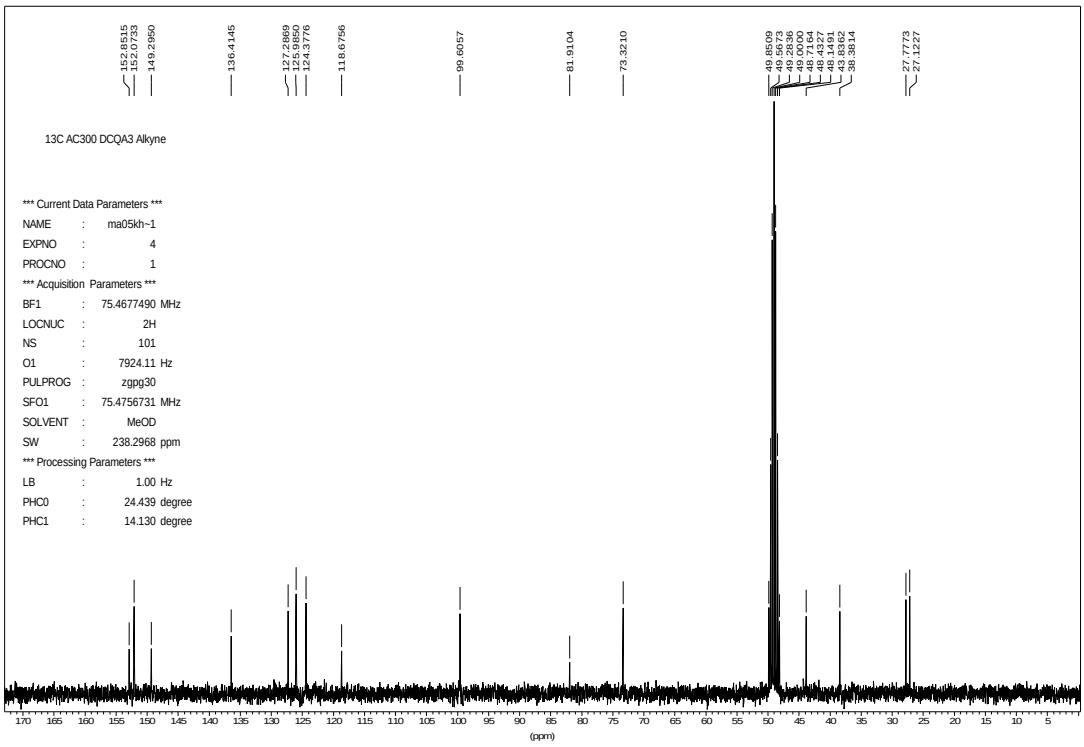

8.3017
8.2831
8.0470
8.0168
7.7095
7.7029
7.3659
7.3518
7.3287
7.3216
6.6245
6.5953
4.7900
3.4642
3.4428
3.4209
3.3966
3.3913
3.2434
3.2379
3.2335
3.2215
3.2153
2.9569
2.9350
2.9050
2.5608
2.5520
1H AC300 DCQA1 Alkyne
*** Current Data Parameters ***
NAME : ma05kh~1
EXPNO : 9
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 300.1300000 MHz
LOCNUC : 2H
NS : 6
O1 : 1853.43 Hz
PULPROG : zg30
SFO1 : 300.1318534 MHz
SOLVENT : MeOD
SW : 17.9519 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 110.883 degree
PHC1 : 3.716 degree
integral
1.0000
0.9845
0.9934
1.0008
1.0075
3.9416
1.9663
0.9192
(ppm)

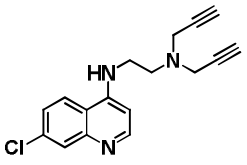

152.8878
152.2259
149.3894
136.5307
127.3886
126.1667
124.3994
99.7438
81.9759
73.4664
49.8509
49.6573
49.2837
49.0000
48.7164
48.4327
48.1491
47.2836
42.0794
42.0470
38.3087
13C AC300 DCQA1 Alkyne
*** Current Data Parameters ***
NAME : ma05kh~1
EXPNO : 10
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 75.4677490 MHz
LOCNUC : 2H
NS : 735
O1 : 7924.11 Hz
PULPROG : zgpg30
SFO1 : 75.4756731 MHz
SOLVENT : MeOD
SW : 238.2968 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 51.248 degree
PHC1 : -21.226 degree
(ppm)



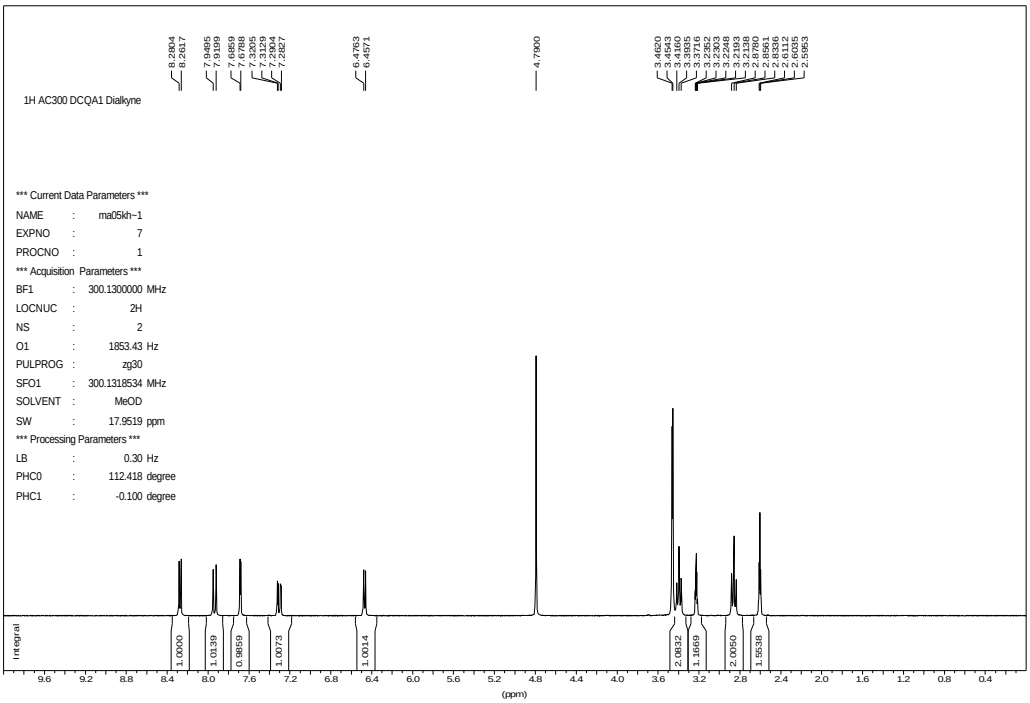
63b

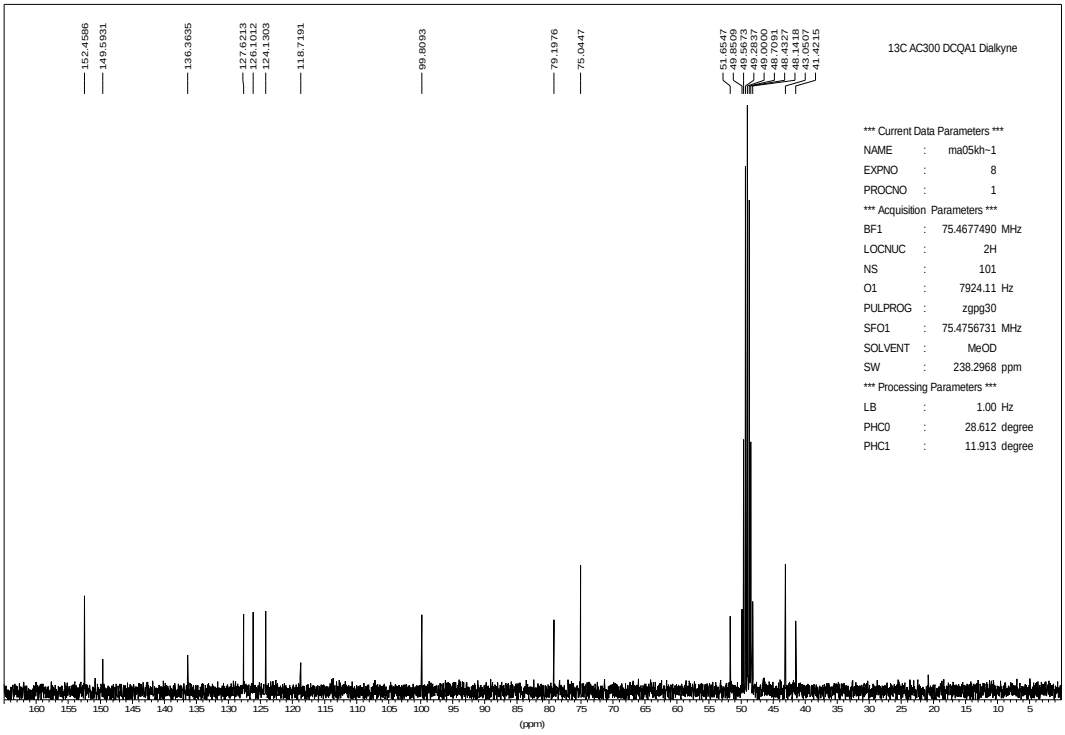

1H AC300 DCQA3 ALKYNE
*** Current Data Parameters ***
NAME : ma05kh~1
EXPNO : 3
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 300.1300000 MHz
LOCNUC : 2H
NS : 2
O1 : 1853.43 Hz
PULPROG : zg30
SFO1 : 300.1318534 MHz
SOLVENT : MeOD
SW : 17.9519 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 117.857 degree
PHC1 : -5.279 degree
Integral
(ppm)

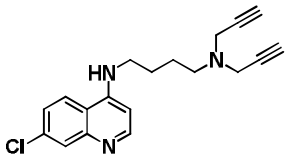

13C AC300 DCQA3 Alkyne
*** Current Data Parameters ***
NAME : ma05kh~1
EXPNO : 4
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 75.4677490 MHz
LOCNUC : 2H
NS : 101
O1 : 7924.11 Hz
PULPROG : zgpg30
SFO1 : 75.4756731 MHz
SOLVENT : MeOD
SW : 238.2968 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 24.439 degree
PHC1 : 14.130 degree
(ppm)


HN
N
Cl
N

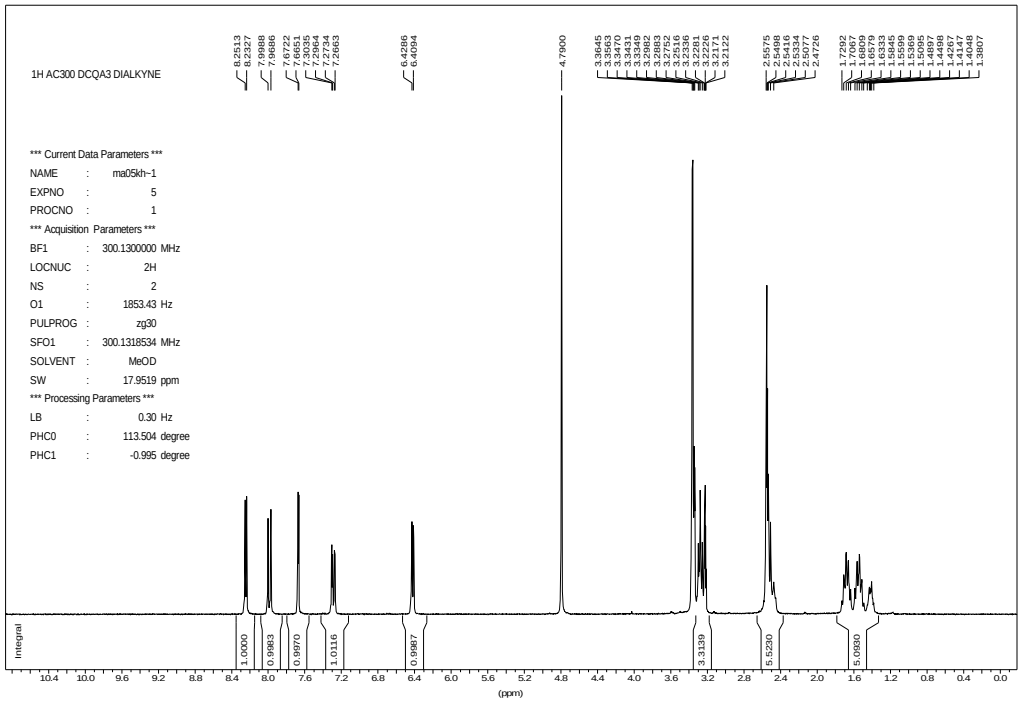
64a

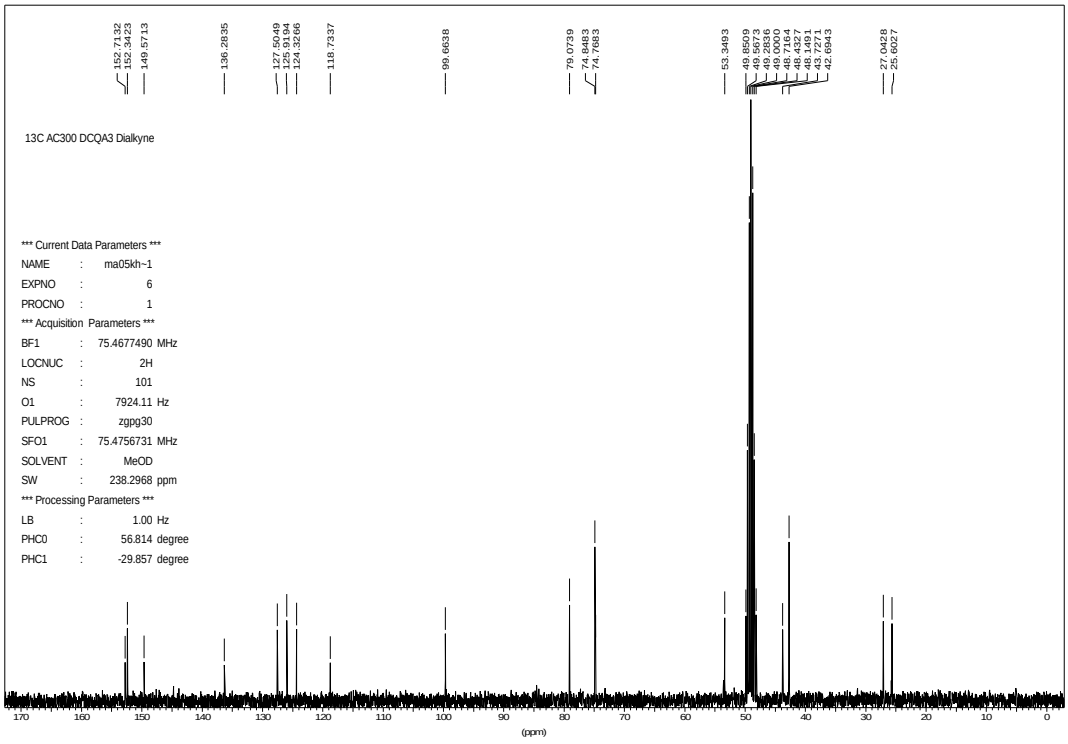

1H AC300 DCQA1 Dialkyne
*** Current Data Parameters ***
NAME : ma05kh~1
EXPNO : 7
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 300.1300000 MHz
LOCNUC : 2H
NS : 2
O1 : 1853.43 Hz
PULPROG : zg30
SFO1 : 300.1318534 MHz
SOLVENT : MeOD
SW : 17.9519 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 112.418 degree
PHC1 : -0.100 degree
Integral
(ppm)

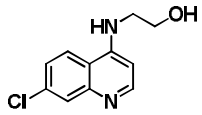

13C AC300 DCQA1 Dialkyne
*** Current Data Parameters ***
NAME : ma05kh~1
EXPNO : 8
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 75.4677490 MHz
LOCNUC : 2H
NS : 101
O1 : 7924.11 Hz
PULPROG : zgpg30
SFO1 : 75.4756731 MHz
SOLVENT : MeOD
SW : 238.2968 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 28.612 degree
PHC1 : 11.913 degree
(ppm)


HN
N
Cl
N

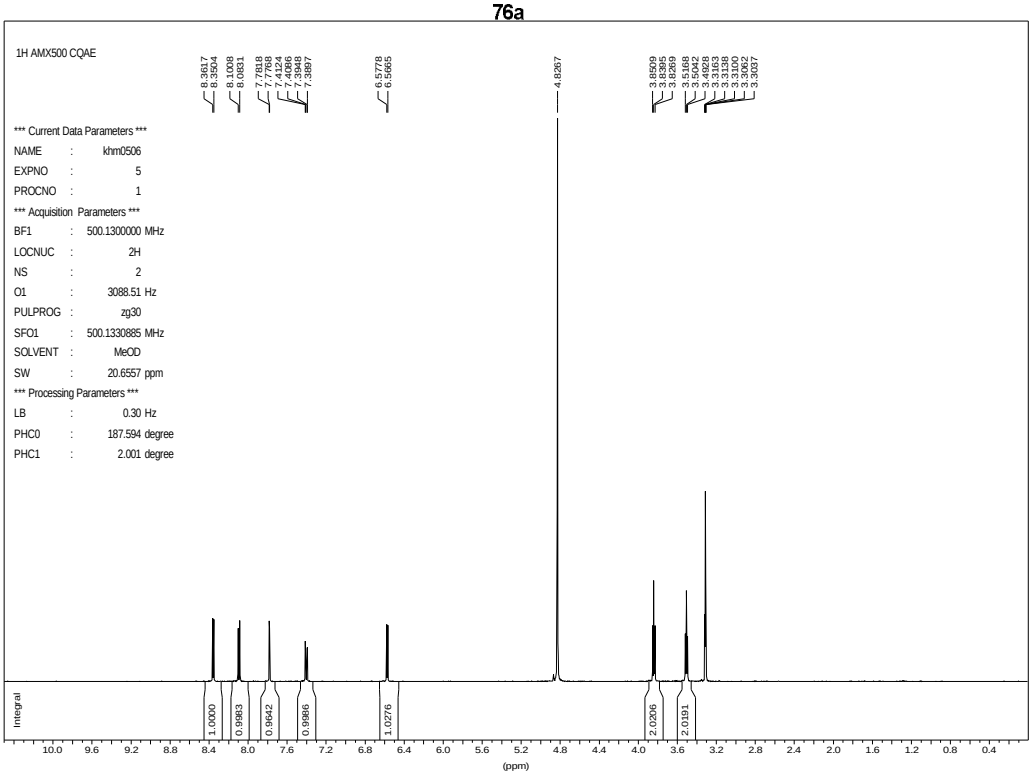
64b

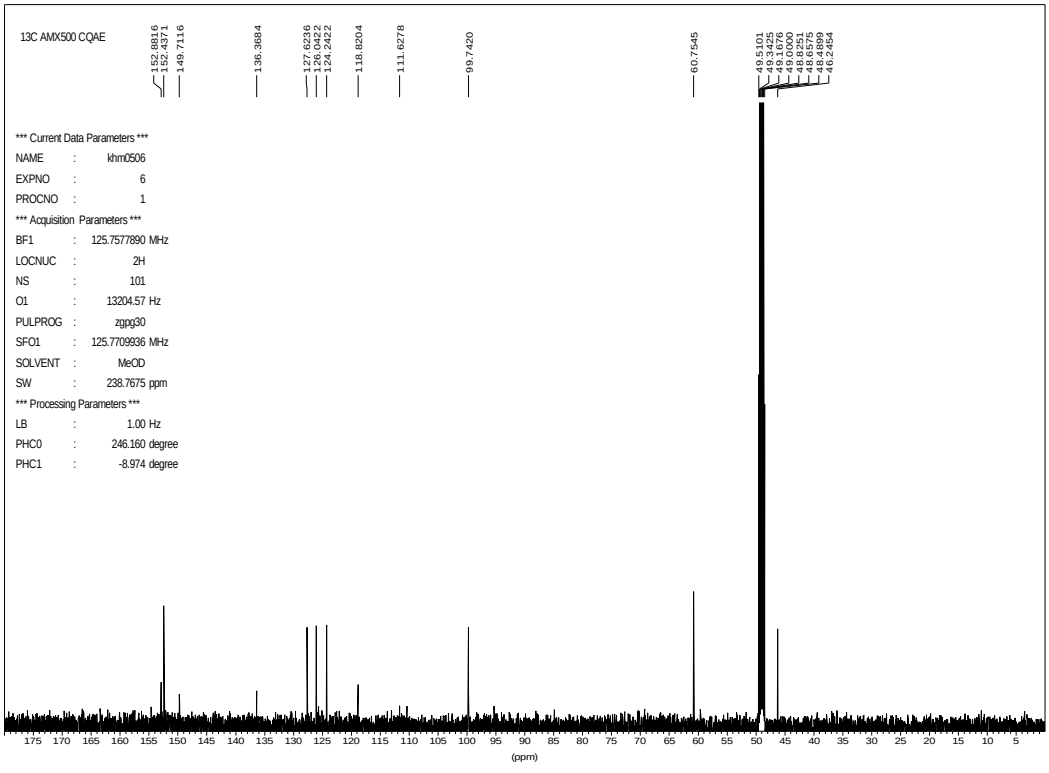

1H AC300 DCQA3 DIALKYNE
*** Current Data Parameters ***
NAME : ma05kh~1
EXPNO : 5
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 300.1300000 MHz
LOCNUC : 2H
NS : 2
O1 : 1853.43 Hz
PULPROG : zg30
SFO1 : 300.1318534 MHz
SOLVENT : MeOD
SW : 17.9519 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 113.504 degree
PHC1 : -0.995 degree
Integral
1.0000
0.9983
0.9970
1.0116
0.9987
3.3139
5.5230
5.0930
(ppm)

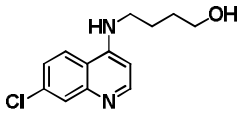

13C AC300 DCQA3 Dialkyne
*** Current Data Parameters ***
NAME : ma05kh~1
EXPNO : 6
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 75.4677490 MHz
LOCNUC : 2H
NS : 101
O1 : 7924.11 Hz
PULPROG : zgpg30
SFO1 : 75.4756731 MHz
SOLVENT : MeOD
SW : 238.2968 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 56.814 degree
PHC1 : -29.857 degree
(ppm)


HN
OH
Cl
N

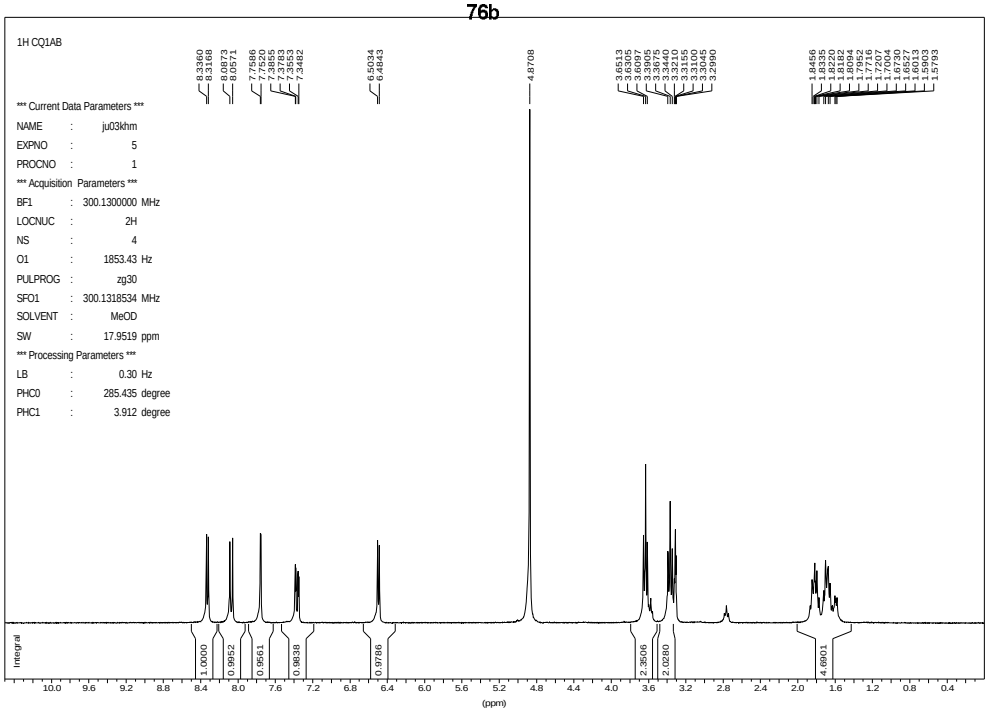
76a

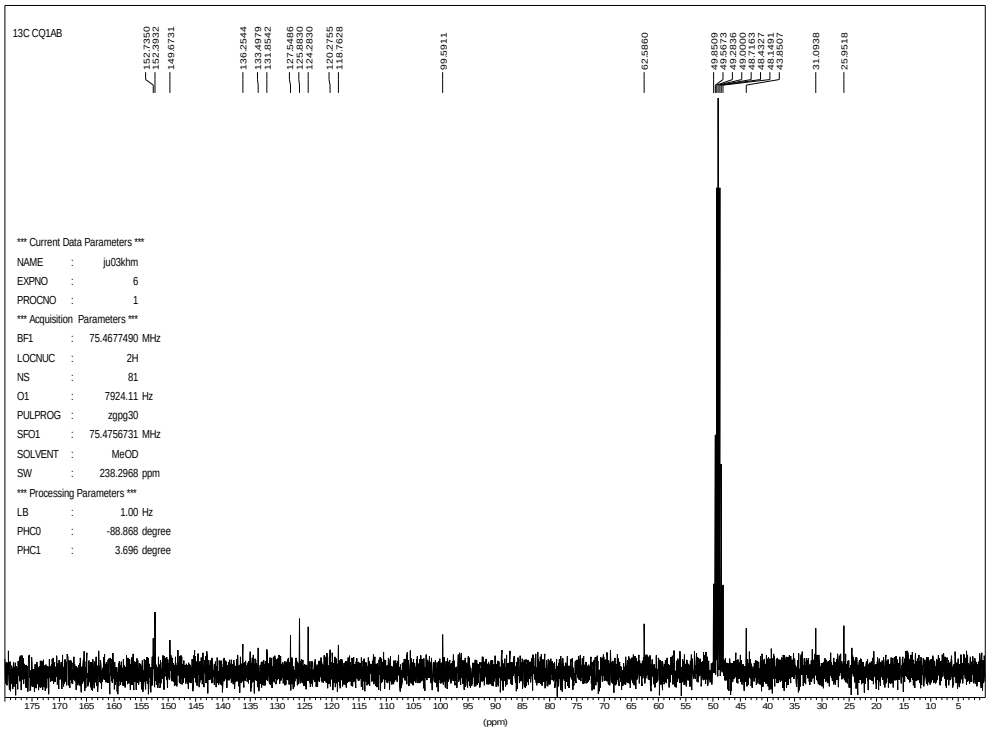

1H AMX500 CQAE
8.3617
8.3504
8.1008
8.0831
7.7818
7.7768
7.4254
7.4066
7.3948
7.3897
6.5778
6.5665
4.8267
3.8509
3.8395
3.8269
3.5188
3.5042
3.4928
3.3183
3.3138
3.3100
3.3062
3.3037
*** Current Data Parameters ***
NAME : khm0506
EXPNO : 5
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 500.1300000 MHz
LOCNUC : 2H
NS : 2
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : MeOD
SW : 20.6557 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 187.594 degree
PHC1 : 2.001 degree
Integral
1.0000
0.9983
0.9642
0.9986
1.0276
2.0206
2.0191
(ppm)

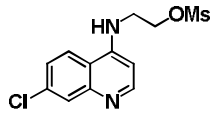

13C AMX500 CQAE
152.8816
152.4371
149.7116
136.3684
127.6236
126.0422
124.2422
118.8204
111.6278
99.7420
60.7545
49.5101
49.3425
49.1676
49.0000
48.8251
48.6575
48.4689
46.2454
*** Current Data Parameters ***
NAME : khm0506
EXPNO : 6
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 125.7577890 MHz
LOCNUC : 2H
NS : 101
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : MeOD
SW : 238.7675 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 246.160 degree
PHC1 : -8.974 degree
(ppm)


HN
OH
Cl
N

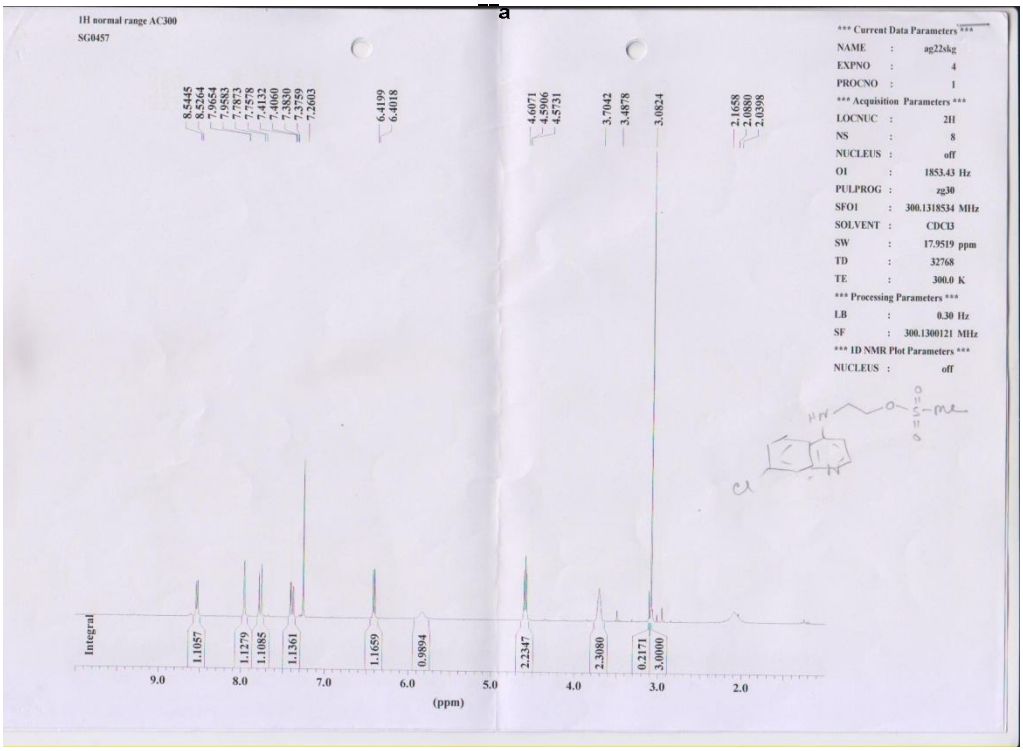
76b

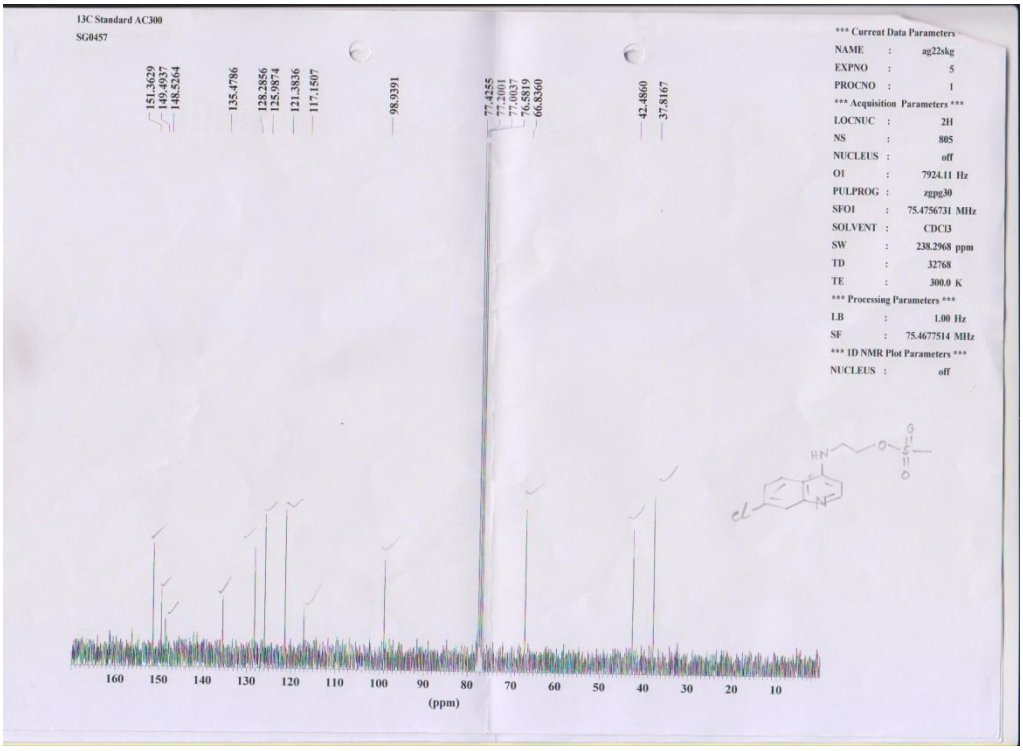

1H CQ1AB
8.3360
8.3168
8.0873
8.0571
7.7586
7.7520
7.3965
7.3783
7.3553
7.3462
6.5034
6.4843
4.8708
3.6513
3.6305
3.6097
3.3965
3.3875
3.3440
3.3310
3.3155
3.3100
3.2946
3.2990
1.8456
1.8335
1.8220
1.8182
1.8094
1.7952
1.7716
1.7207
1.7004
1.6730
1.6527
1.6403
1.5903
1.5793
*** Current Data Parameters ***
NAME : ju03khm
EXPNO : 5
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 300.1300000 MHz
LOCNUC : 2H
NS : 4
O1 : 1853.43 Hz
PULPROG : zg30
SFO1 : 300.1318534 MHz
SOLVENT : MeOD
SW : 17.9519 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 285.435 degree
PHC1 : 3.912 degree
Integral
1.0000
0.9952
0.9561
0.9838
0.9786
2.3506
2.0280
4.6901
(ppm)

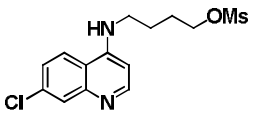

13C CQ1AB
152.7350
152.3932
149.6731
136.2544
133.4979
131.8542
127.5486
125.8830
124.2830
120.2755
118.7628
99.5911
62.5860
49.8509
49.5673
49.2836
49.0000
48.7163
48.4327
48.1491
43.8507
31.0938
25.9518
*** Current Data Parameters ***
NAME : ju03khm
EXPNO : 6
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 75.4677490 MHz
LOCNUC : 2H
NS : 81
O1 : 7924.11 Hz
PULPROG : zgpg30
SFO1 : 75.4756731 MHz
SOLVENT : MeOD
SW : 238.2968 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : -88.868 degree
PHC1 : 3.696 degree
(ppm)


HN
OMs
Cl
N

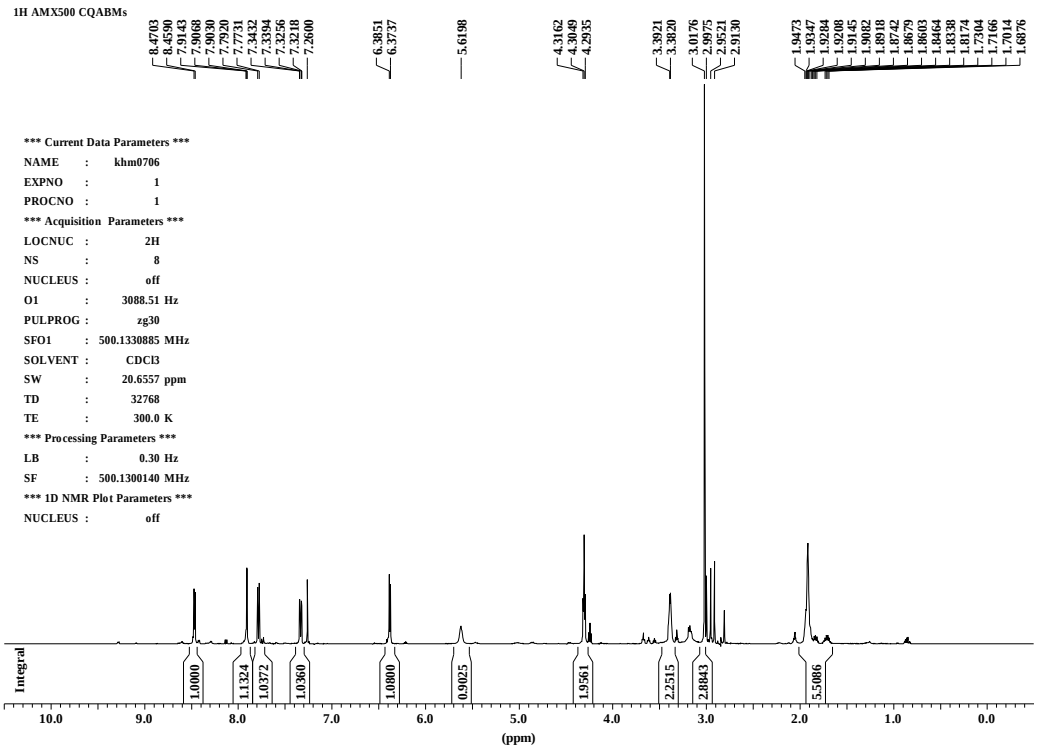
"a"

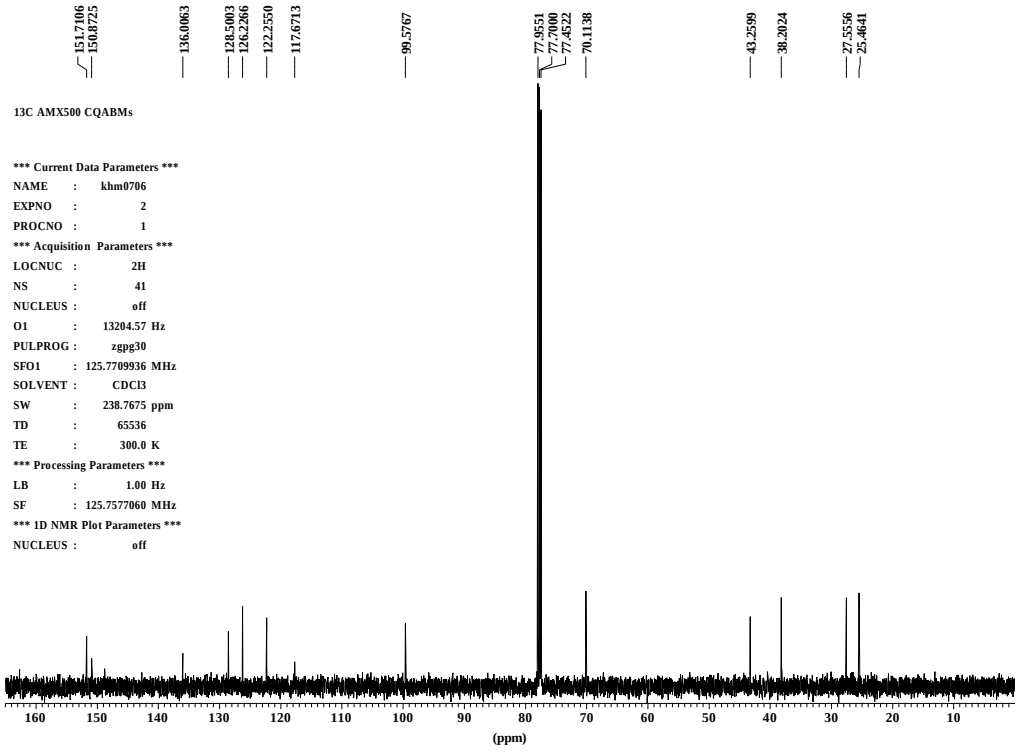

1H normal range AC300
SG0457
8.5445
8.5264
7.9664
7.9583
7.7873
7.7578
7.4132
7.4060
7.3830
7.3759
7.2603
6.4199
6.4018
4.6071
4.5906
4.5731
3.7042
3.4878
3.0824
2.1658
2.0880
2.0398
*** Current Data Parameters ***
NAME : ag22skg
EXPNO : 4
PROCNO : 1
*** Acquisition Parameters ***
LOCNUC : 2H
NS : 8
NUCLEUS : off
OI : 1853.43 Hz
PULPROG : zg30
SFOI : 300.1318534 MHz
SOLVENT : CDCl3
SW : 17.9519 ppm
TD : 32768
TE : 300.0 K
*** Processing Parameters ***
LB : 0.30 Hz
SF : 300.1300121 MHz
*** 1D NMR Plot Parameters ***
NUCLEUS : off
Integral
1.1057
1.1279
1.1085
1.1361
1.1659
0.9894
2.2347
2.3080
0.2171
3.0000
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
(ppm)

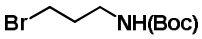

13C Standard AC300
SG0457
151.3629
149.4937
148.5264
135.4786
128.2856
125.9874
121.3836
117.1507
98.9391
77.4255
77.2001
77.0037
76.5819
66.8360
42.4860
37.8167
*** Current Data Parameters ***
NAME : ag22skg
EXPNO : 5
PROCNO : 1
*** Acquisition Parameters ***
LOCNUC : 2H
NS : 805
NUCLEUS : off
OI : 7924.11 Hz
PULPROG : zgpg30
SFOI : 75.4756731 MHz
SOLVENT : CDCl3
SW : 238.2968 ppm
TD : 32768
TE : 300.0 K
*** Processing Parameters ***
LB : 1.00 Hz
SF : 75.4677514 MHz
*** 1D NMR Plot Parameters ***
NUCLEUS : off
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)


HN
OMs
Cl
N

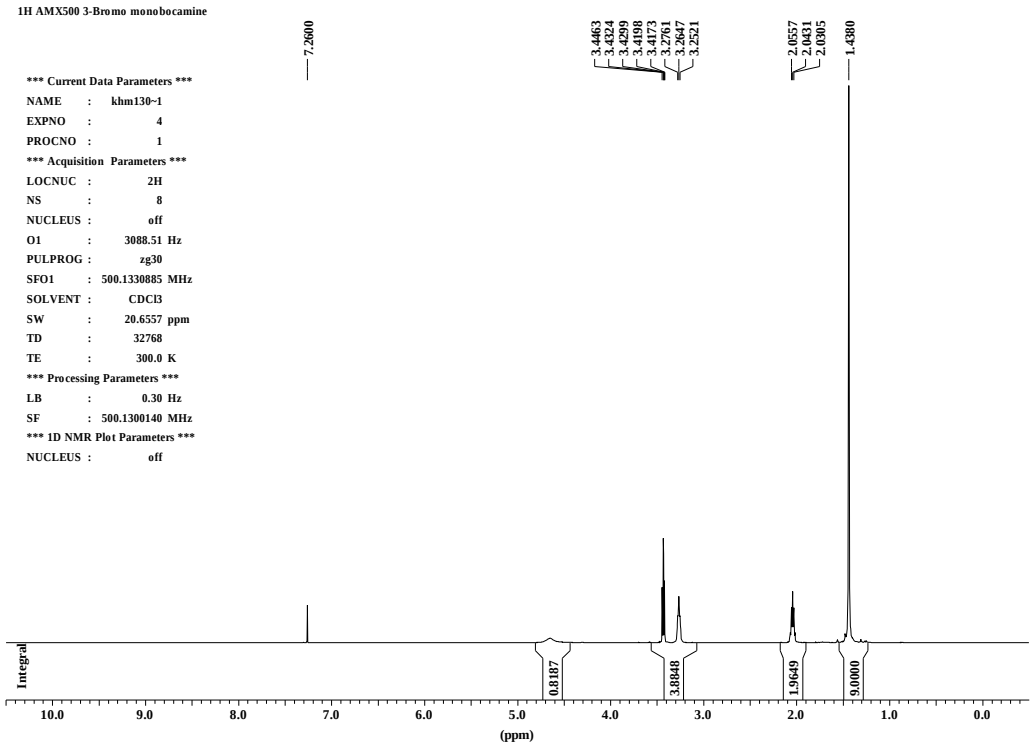
77b

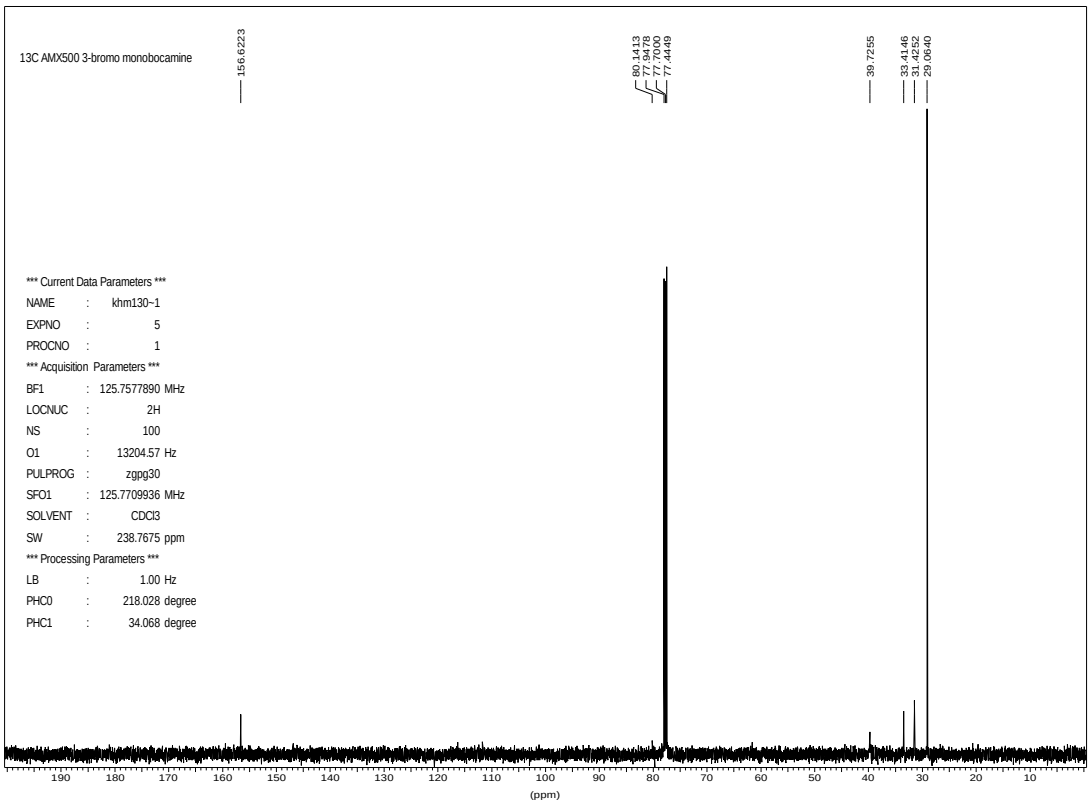

1H AMX500 CQABMs
8.4703
8.4590
7.9143
7.9068
7.9030
7.7920
7.7731
7.3432
7.3394
7.3256
7.3218
7.2600
6.3851
6.3737
5.6198
4.3162
4.3049
4.2935
3.3921
3.3820
3.0176
2.9975
2.9521
2.9130
1.9473
1.9347
1.9284
1.9208
1.9145
1.9082
1.8918
1.8742
1.8679
1.8603
1.8464
1.8338
1.8174
1.7304
1.7166
1.7014
1.6876
*** Current Data Parameters ***
NAME : khm0706
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
LOCNUC : 2H
NS : 8
NUCLEUS : off
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : CDCl3
SW : 20.6557 ppm
TD : 32768
TE : 300.0 K
*** Processing Parameters ***
LB : 0.30 Hz
SF : 500.1300140 MHz
*** 1D NMR Plot Parameters ***
NUCLEUS : off
Integral
1.0000
1.1324
1.0372
1.0360
1.0800
0.9025
1.9561
2.2515
2.8843
5.5086
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)

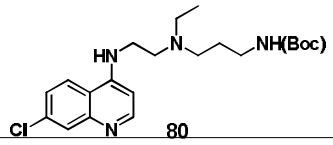

151.7106
150.8725
136.0063
128.5003
126.2266
122.2550
117.6713
99.5767
77.9551
77.7000
77.4522
70.1138
43.2599
38.2024
27.5556
25.4641
13C AMX500 CQABMs
*** Current Data Parameters ***
NAME : khm0706
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
LOCNUC : 2H
NS : 41
NUCLEUS : off
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : CDCl3
SW : 238.7675 ppm
TD : 65536
TE : 300.0 K
*** Processing Parameters ***
LB : 1.00 Hz
SF : 125.7577060 MHz
*** 1D NMR Plot Parameters ***
NUCLEUS : off
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)


Br
NH(Boc)

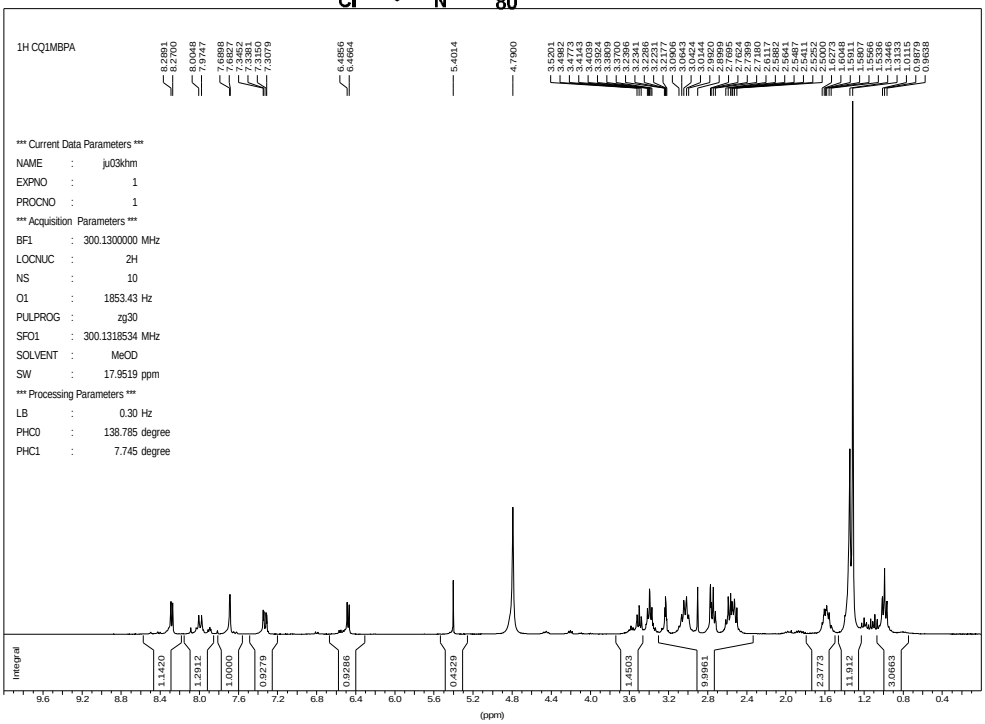
79

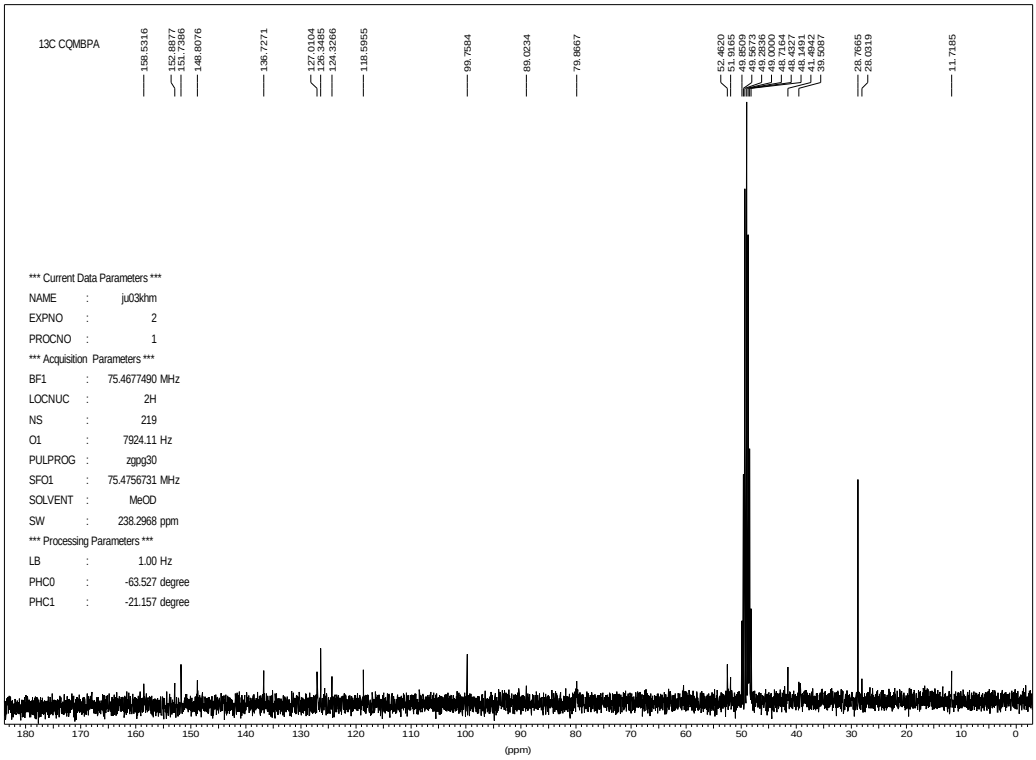

1H AMX500 3-Bromo monobocamine
7.2600
3.4463
3.4324
3.4299
3.4198
3.4173
3.2761
3.2647
3.2521
2.0557
2.0431
2.0305
1.4380
*** Current Data Parameters ***
NAME : khm130~1
EXPNO : 4
PROCNO : 1
*** Acquisition Parameters ***
LOCNUC : 2H
NS : 8
NUCLEUS : off
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : CDCl3
SW : 20.6557 ppm
TD : 32768
TE : 300.0 K
*** Processing Parameters ***
LB : 0.30 Hz
SF : 500.1300140 MHz
*** 1D NMR Plot Parameters ***
NUCLEUS : off
Integral
0.8187
3.8848
1.9649
9.0000
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)

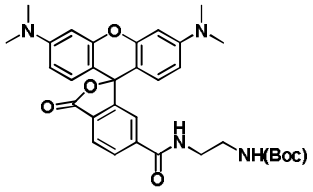

13C AMX500 3-bromo monobocamine
156.6223
80.1413
77.9478
77.7009
77.4449
39.7255
33.4146
31.4252
29.0640
*** Current Data Parameters ***
NAME : khm130~1
EXPNO : 5
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 125.7577890 MHz
LOCNUC : 2H
NS : 100
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : CDCl3
SW : 238.7675 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 218.028 degree
PHC1 : 34.068 degree
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)


HN
N
NH(Boc)
Cl
N

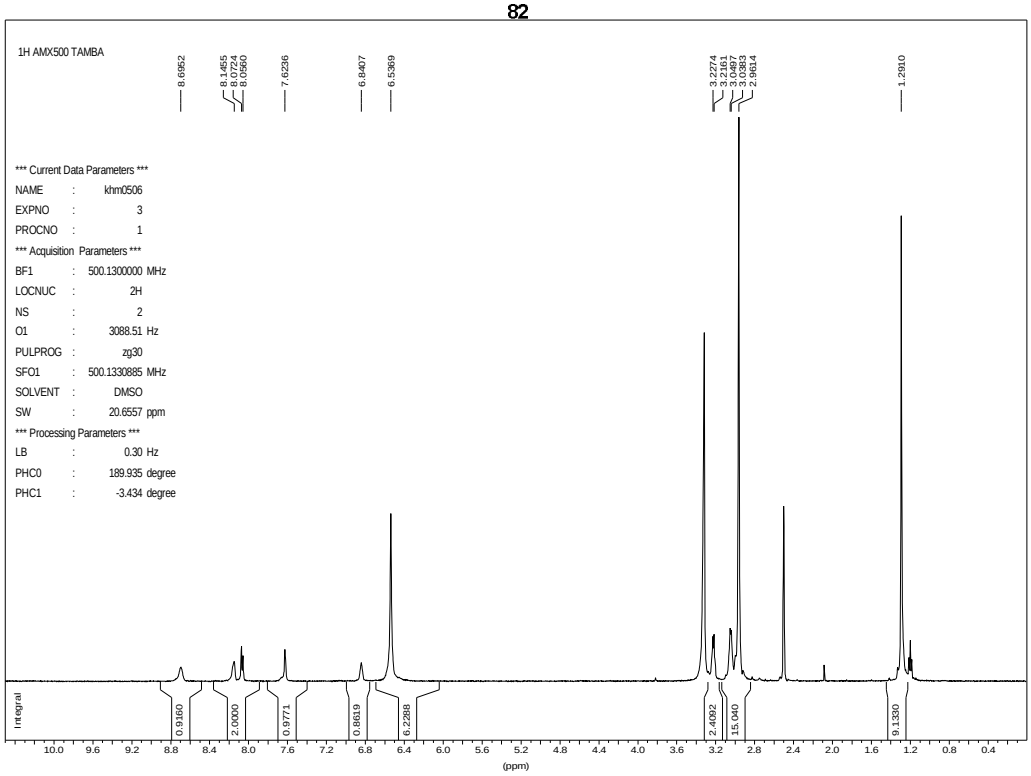
80

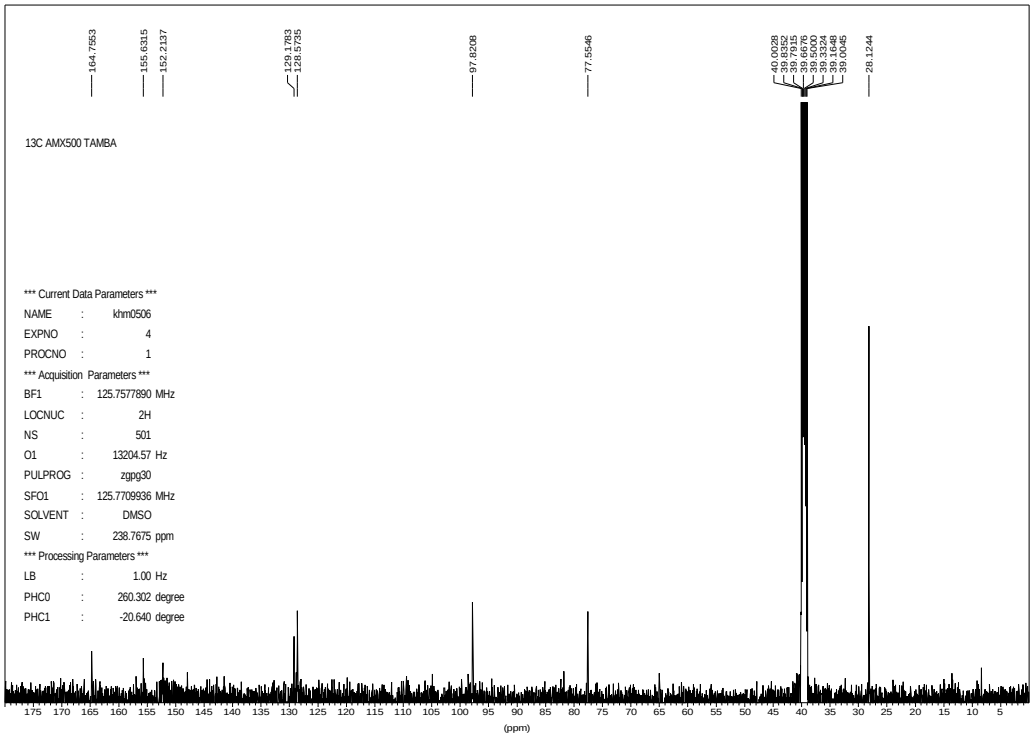

1H CQ1MBPA
*** Current Data Parameters ***
NAME : ju03khm
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 300.1300000 MHz
LOCNUC : 2H
NS : 10
O1 : 1853.43 Hz
PULPROG : zg30
SFO1 : 300.1318534 MHz
SOLVENT : MeOD
SW : 17.9519 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 138.785 degree
PHC1 : 7.745 degree
Integral
(ppm)

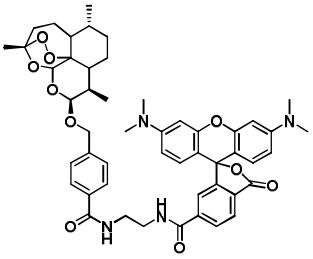

13C CQMBPA
*** Current Data Parameters ***
NAME : ju03khm
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 75.4677490 MHz
LOCNUC : 2H
NS : 219
O1 : 7924.11 Hz
PULPROG : zgpg30
SFO1 : 75.4756731 MHz
SOLVENT : MeOD
SW : 238.2968 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : -63.527 degree
PHC1 : -21.157 degree
(ppm)


N
O
N
O
O
H
N
NH(Boc)
O

82


1H AMX500 TAMBA
8.6952
8.1455
8.0724
8.0560
7.6236
6.8407
6.5369
3.2274
3.2161
3.0497
3.0383
2.9614
1.2910
*** Current Data Parameters ***
NAME : khm0506
EXPNO : 3
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 500.1300000 MHz
LOCNUC : 2H
NS : 2
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : DMSO
SW : 20.6557 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 189.935 degree
PHC1 : -3.434 degree
Integral
0.9160
2.0000
0.9771
0.8619
6.2288
2.4892
15.040
9.1330
(ppm)


164.7553
155.6315
152.2137
129.1783
128.5735
97.8208
77.5546
40.0028
39.9192
39.7915
39.6676
39.5000
39.3324
39.1648
39.0045
28.1244
13C AMX500 TAMBA
*** Current Data Parameters ***
NAME : khm0506
EXPNO : 4
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 125.7577890 MHz
LOCNUC : 2H
NS : 501
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : DMSO
SW : 238.7675 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 260.302 degree
PHC1 : -20.640 degree
(ppm)



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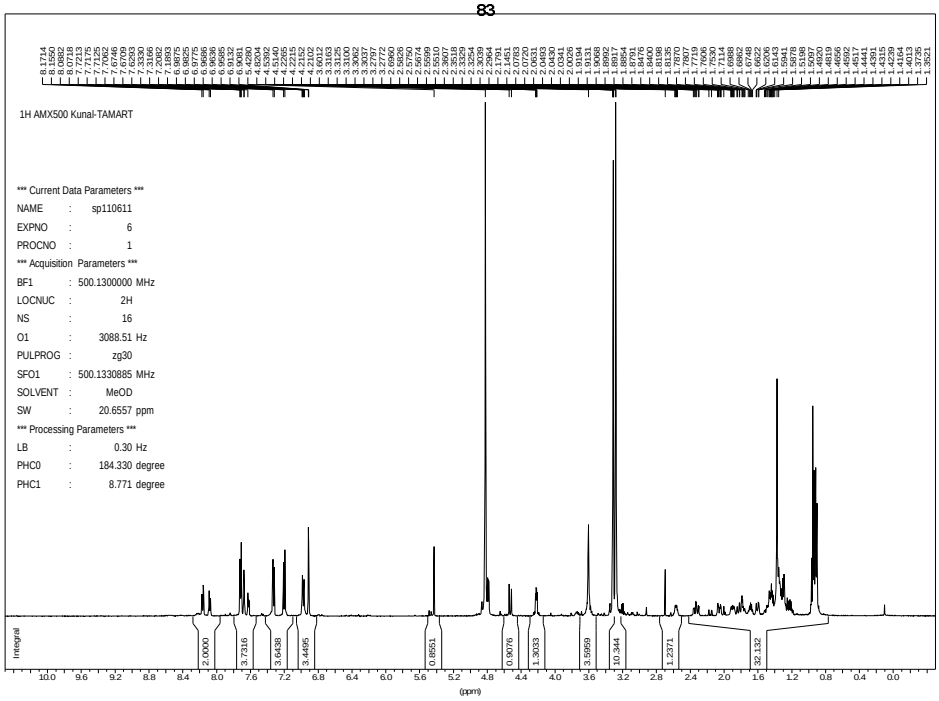
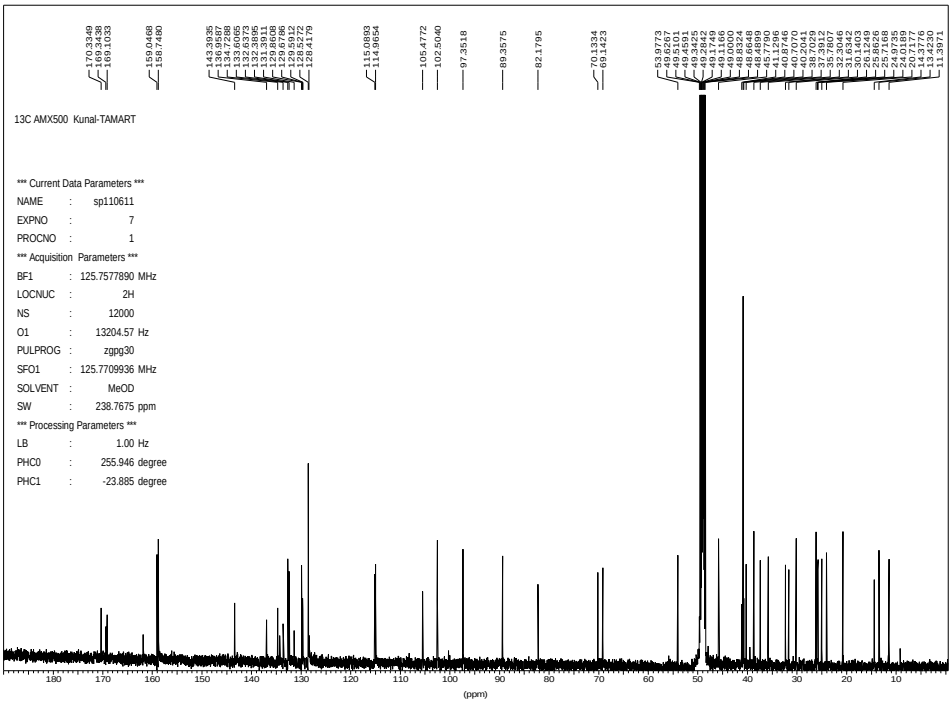
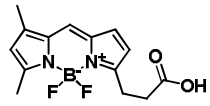
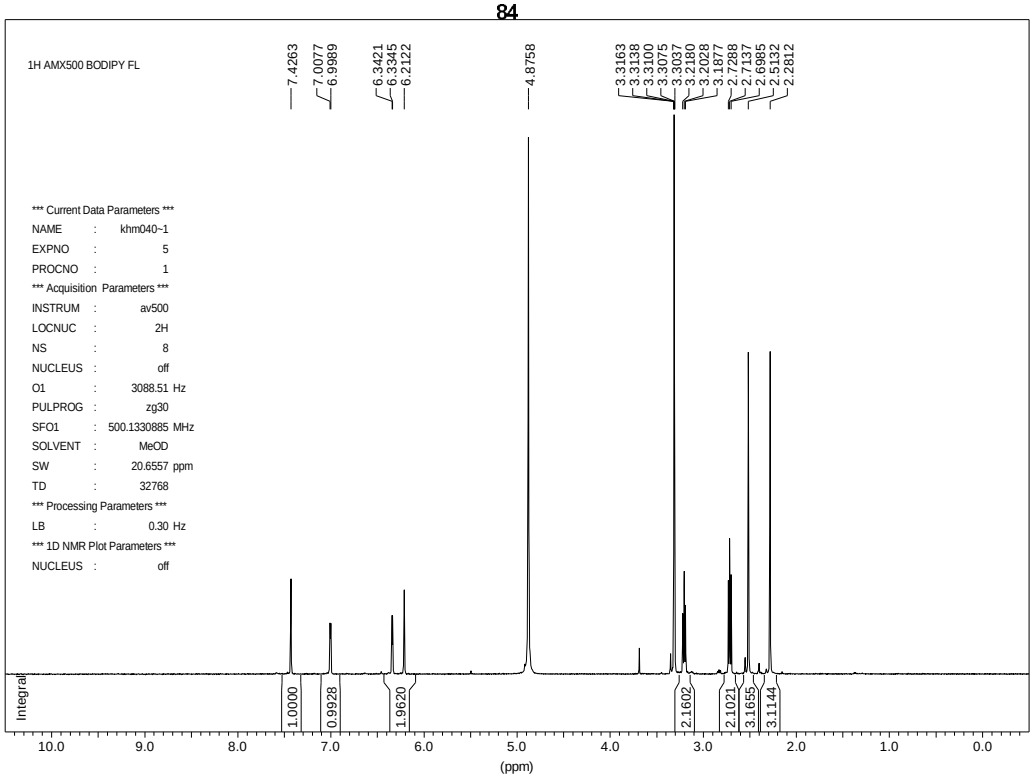
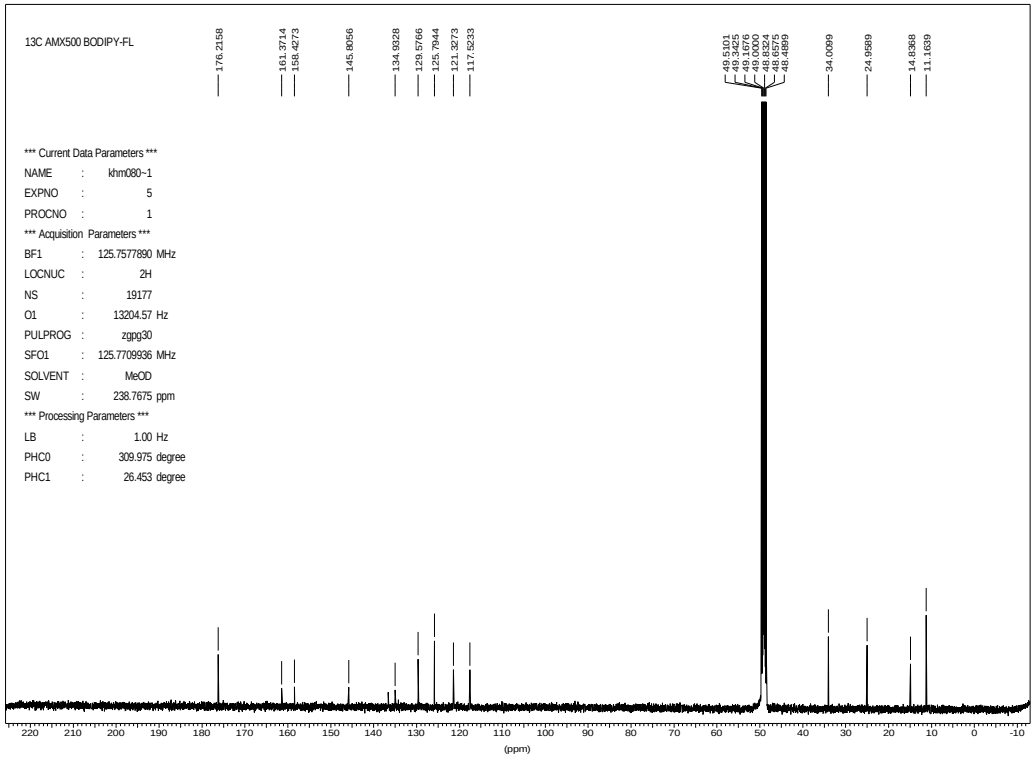

1H AMX500 Kunal-TAMART
*** Current Data Parameters ***
NAME : sp110611
EXPNO : 6
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 500.1300000 MHz
LOCNUC : 2H
NS : 16
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : MeOD
SW : 20.6557 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 184.330 degree
PHC1 : 8.771 degree
Integral
2.0000
3.7316
3.6438
3.4495
0.8651
0.9676
1.3033
3.5959
10.344
1.2371
32.132
(ppm)

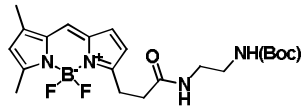

13C AMX500 Kunal-TAMART
*** Current Data Parameters ***
NAME : sp110611
EXPNO : 7
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 125.7577890 MHz
LOCNUC : 2H
NS : 12000
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : MeOD
SW : 238.7675 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 255.946 degree
PHC1 : -23.885 degree
(ppm)


N
B
N+
F
F
O
OH

84


1H AMX500 BODIPY FL
7.4263
7.0077
6.9989
6.3421
6.3345
6.2122
4.8758
3.3163
3.3138
3.3100
3.3075
3.3037
3.2180
3.2028
3.1877
2.7288
2.7137
2.6985
2.5132
2.2812
*** Current Data Parameters ***
NAME : khm040~1
EXPNO : 5
PROCNO : 1
*** Acquisition Parameters ***
INSTRUM : av500
LOCNUC : 2H
NS : 8
NUCLEUS : off
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : MeOD
SW : 20.6557 ppm
TD : 32768
*** Processing Parameters ***
LB : 0.30 Hz
*** 1D NMR Plot Parameters ***
NUCLEUS : off
Integral
1.0000
0.9928
1.9620
2.1602
2.1021
3.1655
3.1144
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)


13C AMX500 BODIPY-FL
176.2158
161.9714
158.4273
145.8056
134.9328
129.5766
125.7944
121.3273
117.5233
49.5101
49.3425
49.1676
49.0000
48.8324
48.6575
48.4899
34.0099
24.9589
14.8368
11.1639
*** Current Data Parameters ***
NAME : khm080~1
EXPNO : 5
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 125.7577890 MHz
LOCNUC : 2H
NS : 19177
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : MeOD
SW : 238.7675 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 309.975 degree
PHC1 : 26.453 degree
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
(ppm)


NH(Boc)
O
N
H
N
B
F
F
N+

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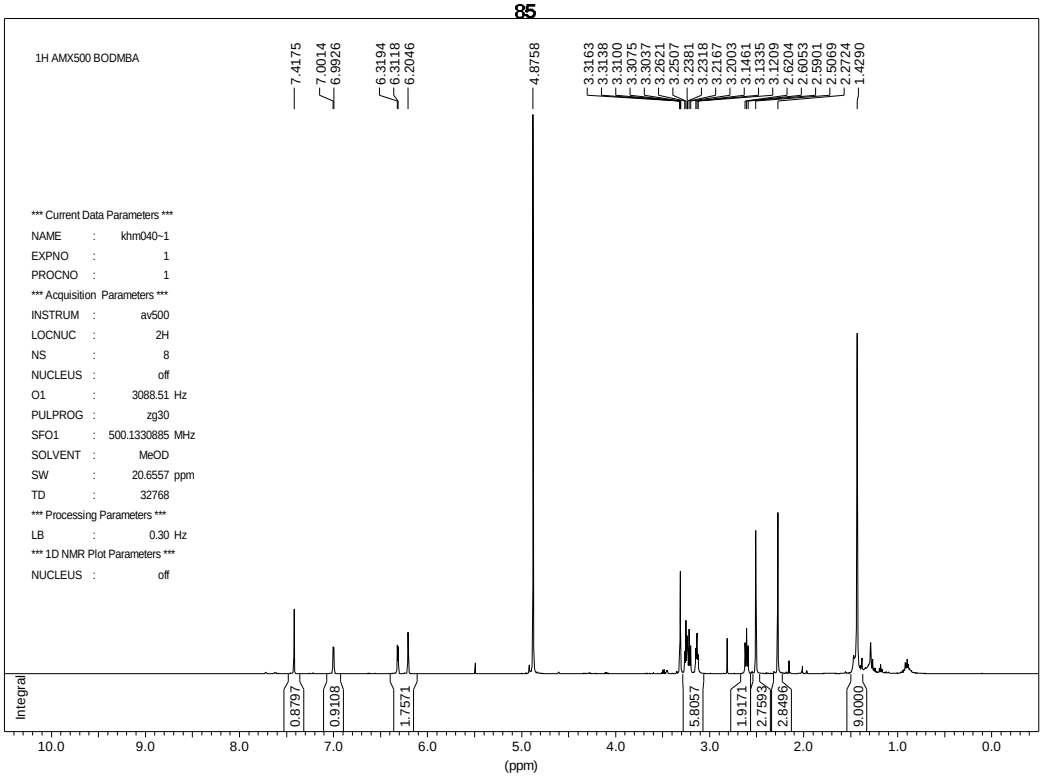
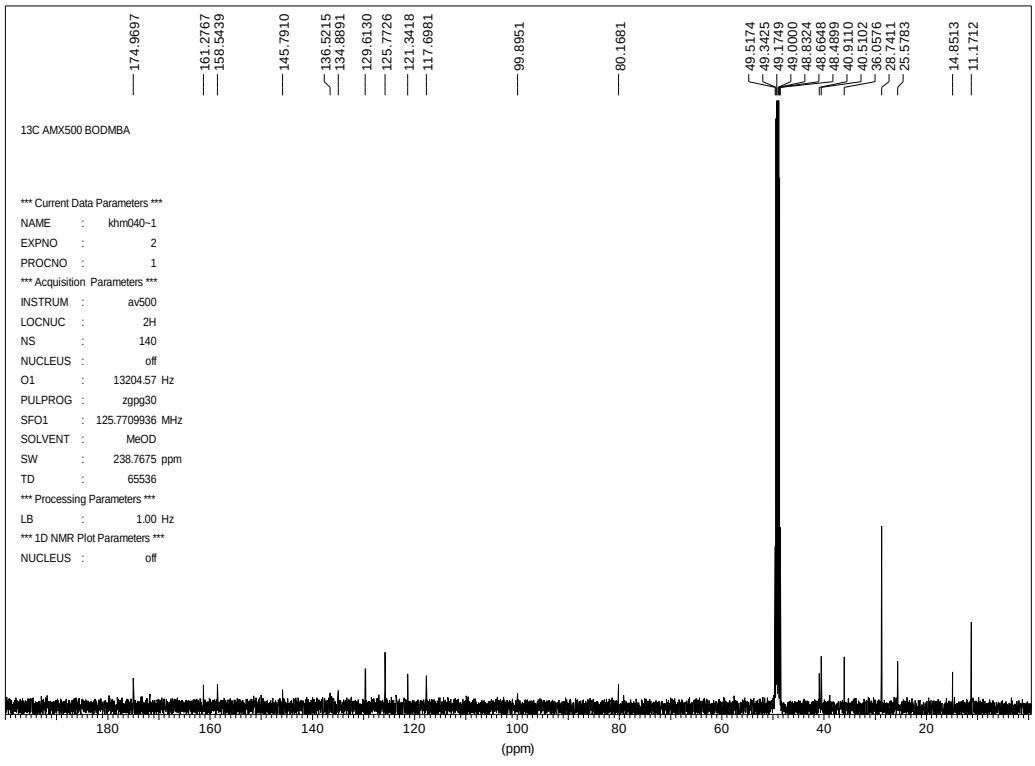
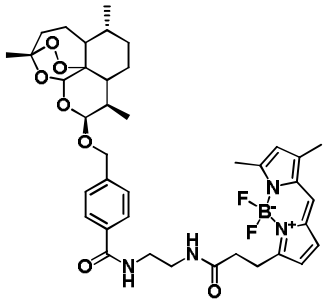

1H AMX500 BODMBA
7.4175
7.0014
6.9926
6.3194
6.3118
6.2046
4.8758
3.3163
3.3138
3.3100
3.3075
3.3037
3.2621
3.2507
3.2381
3.2318
3.2167
3.2003
3.1461
3.1335
3.1209
2.6204
2.6053
2.5901
2.5069
2.2724
1.4290
*** Current Data Parameters ***
NAME : khm040~1
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
INSTRUM : av500
LOCNUC : 2H
NS : 8
NUCLEUS : off
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : MeOD
SW : 20.6557 ppm
TD : 32768
*** Processing Parameters ***
LB : 0.30 Hz
*** 1D NMR Plot Parameters ***
NUCLEUS : off
Integral
0.8797
0.9108
1.7571
5.8057
1.9171
2.7593
2.8496
9.0000
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)


13C AMX500 BODMBA
174.9697
161.2767
158.5439
145.7910
136.5215
134.8891
129.6130
125.7726
121.3418
117.6981
99.8951
80.1681
49.5174
49.3425
49.1749
49.0000
48.8324
48.6648
48.4899
40.9110
40.5102
36.0576
28.7411
25.5783
14.8513
11.1712
*** Current Data Parameters ***
NAME : khm040~1
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
INSTRUM : av500
LOCNUC : 2H
NS : 140
NUCLEUS : off
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : MeOD
SW : 238.7675 ppm
TD : 65536
*** Processing Parameters ***
LB : 1.00 Hz
*** 1D NMR Plot Parameters ***
NUCLEUS : off
180
160
140
120
100
80
60
40
20
(ppm)



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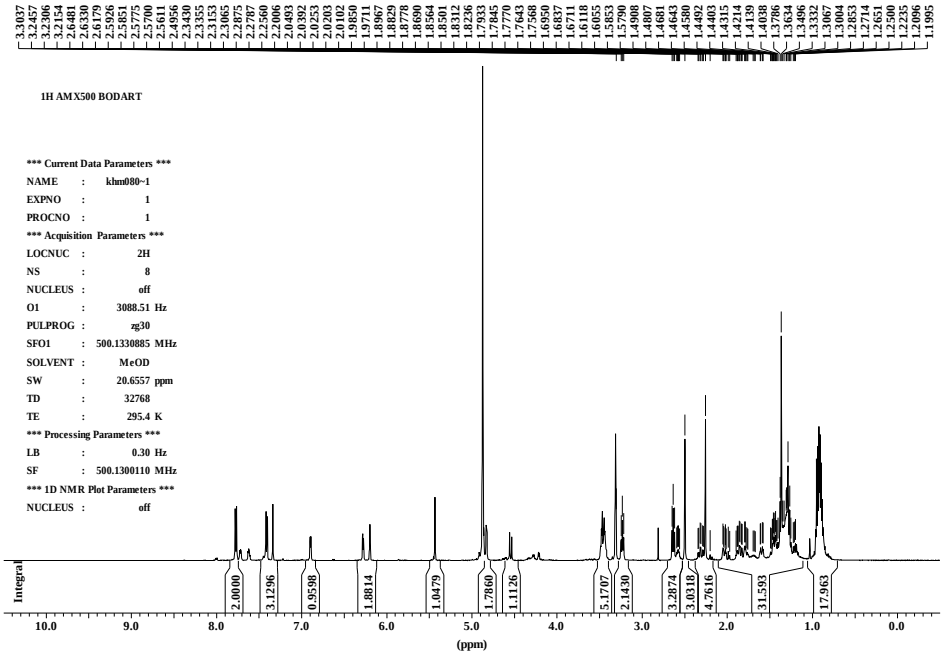
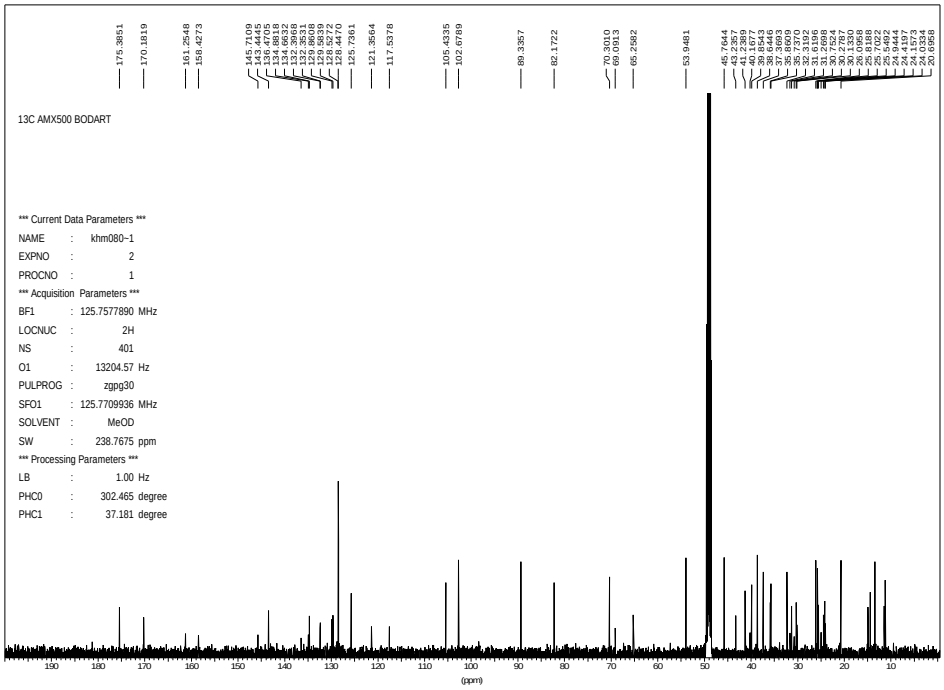
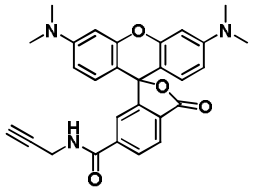

1H AMX500 BODART
*** Current Data Parameters ***
NAME : khm080~1
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
LOCNUC : 2H
NS : 8
NUCLEUS : off
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : MeOD
SW : 20.6557 ppm
TD : 32768
TE : 295.4 K
*** Processing Parameters ***
LB : 0.30 Hz
SF : 500.1300110 MHz
*** 1D NMR Plot Parameters ***
NUCLEUS : off
Integral
2.0000
3.1296
0.9598
1.8814
1.0479
1.7860
1.1126
5.1707
2.1430
3.2874
3.0318
4.7616
31.593
17.963
(ppm)


13C AMX500 BODART
*** Current Data Parameters ***
NAME : khm080~1
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 125.7577890 MHz
LOCNUC : 2H
NS : 401
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : MeOD
SW : 238.7675 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 302.465 degree
PHC1 : 37.181 degree
(ppm)



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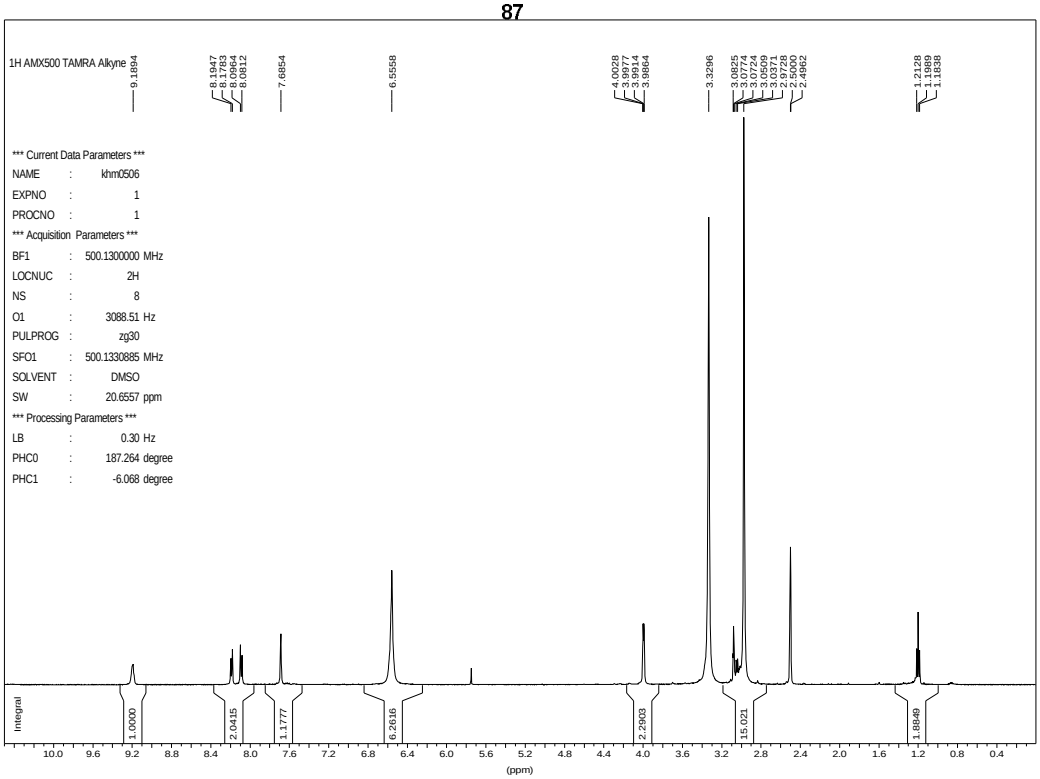
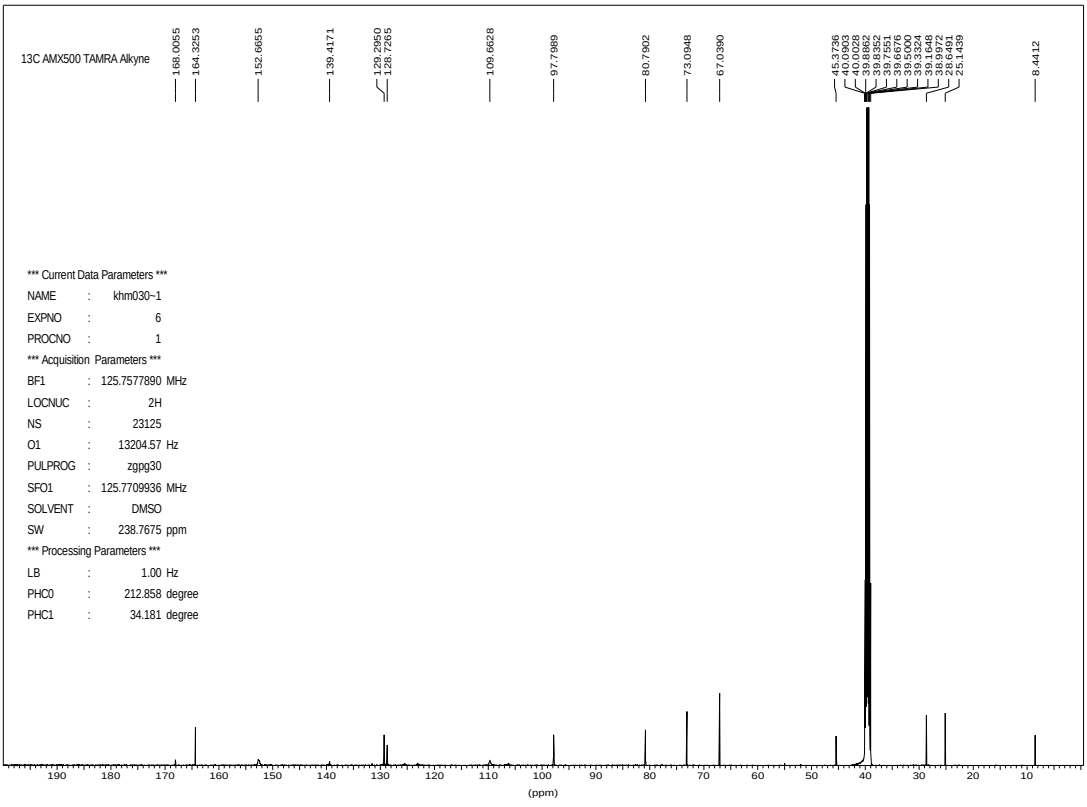
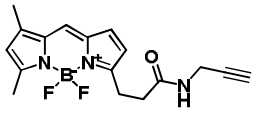

1H AMX500 TAMRA Alkyne
*** Current Data Parameters ***
NAME : khm0506
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 500.1300000 MHz
LOCNUC : 2H
NS : 8
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : DMSO
SW : 20.6557 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 187.264 degree
PHC1 : -6.068 degree
Integral
(ppm)


13C AMX500 TAMRA Alkyne
*** Current Data Parameters ***
NAME : khm030~1
EXPNO : 6
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 125.7577890 MHz
LOCNUC : 2H
NS : 23125
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : DMSO
SW : 238.7675 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 212.858 degree
PHC1 : 34.181 degree
(ppm)



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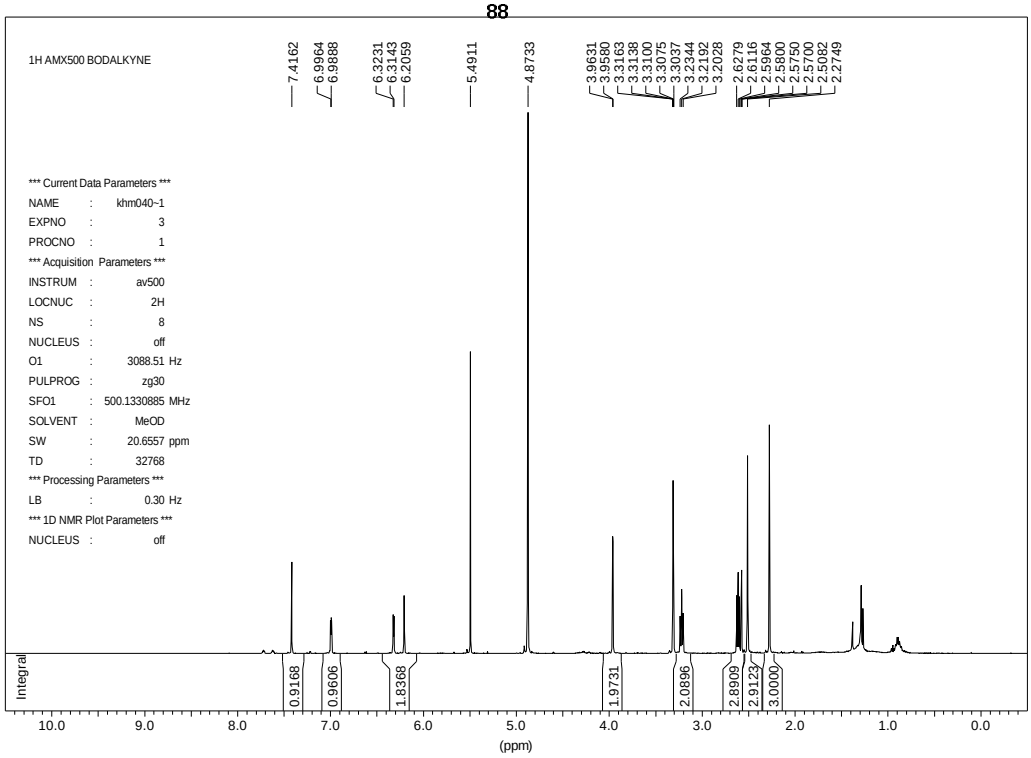
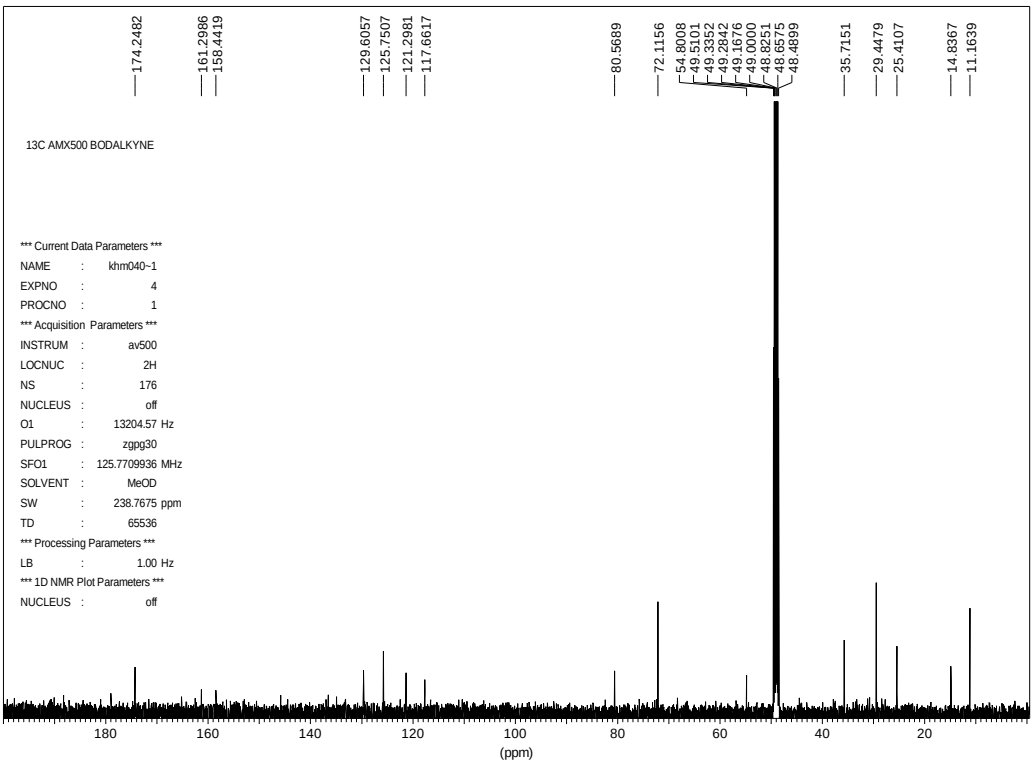
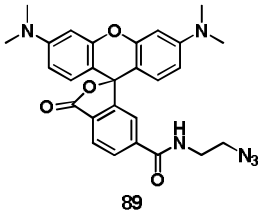

1H AMX500 BODALKYNE
7.4162
6.9964
6.9888
6.3231
6.3143
6.2059
5.4911
4.8733
3.9631
3.9580
3.3163
3.3138
3.3100
3.3075
3.3037
3.2344
3.2192
3.2028
2.6279
2.6116
2.5964
2.5800
2.5750
2.5700
2.5092
2.2749
*** Current Data Parameters ***
NAME : khm040~1
EXPNO : 3
PROCNO : 1
*** Acquisition Parameters ***
INSTRUM : av500
LOCNUC : 2H
NS : 8
NUCLEUS : off
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : MeOD
SW : 20.6557 ppm
TD : 32768
*** Processing Parameters ***
LB : 0.30 Hz
*** 1D NMR Plot Parameters ***
NUCLEUS : off
Integral
0.9168
0.9606
1.8368
1.9731
2.0896
2.8909
2.9123
3.0000
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)


13C AMX500 BODALKYNE
174.2482
161.2986
158.4419
129.6057
125.7507
121.2981
117.6617
80.5689
72.1156
54.8008
49.5101
49.3352
49.2842
49.1676
49.0000
48.8251
48.6575
48.4899
35.7151
29.4479
25.4107
14.8367
11.1639
*** Current Data Parameters ***
NAME : khm040~1
EXPNO : 4
PROCNO : 1
*** Acquisition Parameters ***
INSTRUM : av500
LOCNUC : 2H
NS : 176
NUCLEUS : off
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : MeOD
SW : 238.7675 ppm
TD : 65536
*** Processing Parameters ***
LB : 1.00 Hz
*** 1D NMR Plot Parameters ***
NUCLEUS : off
180
160
140
120
100
80
60
40
20
(ppm)



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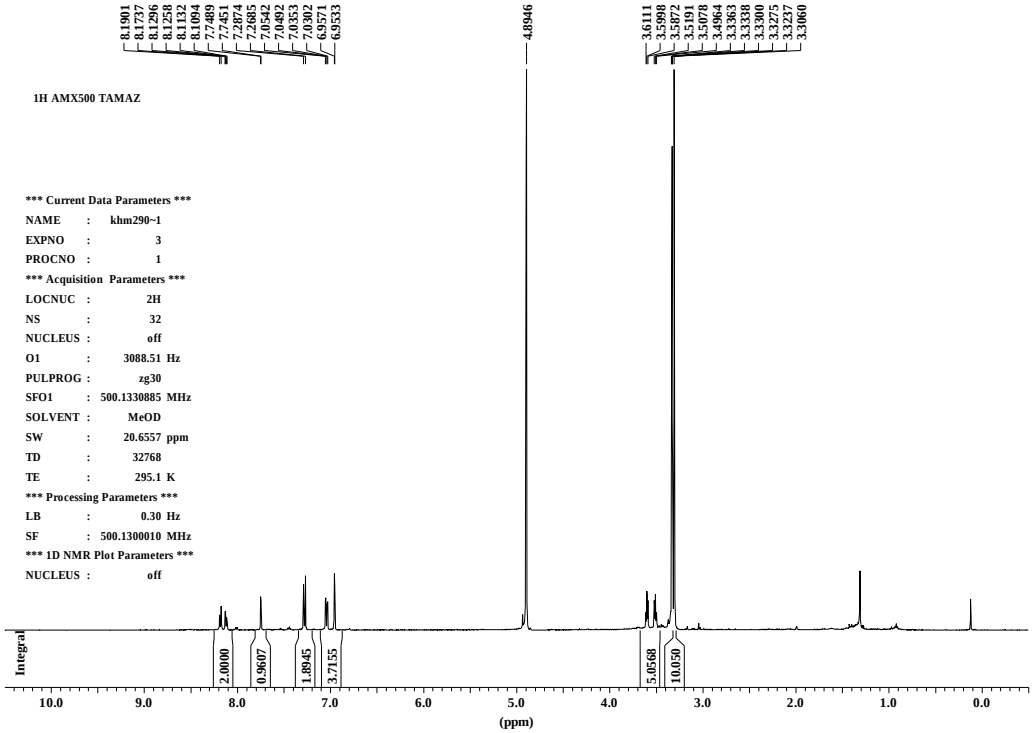
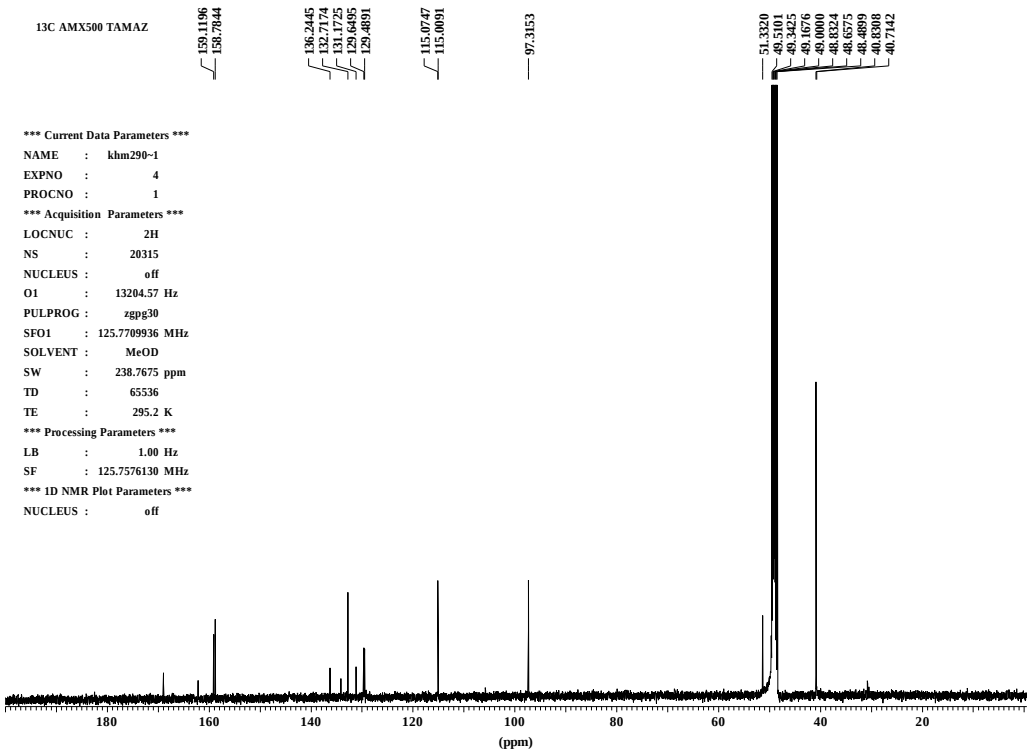
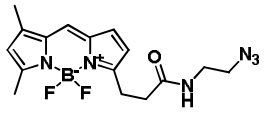

8.1901
8.1737
8.1296
8.1258
8.1132
8.1094
7.7489
7.7451
7.2874
7.2685
7.0542
7.0492
7.0353
7.0302
6.9571
6.9533
4.8946
3.6111
3.5998
3.5872
3.5191
3.5078
3.4964
3.3363
3.3338
3.3300
3.3275
3.3237
3.3060
1H AMX500 TAMAZ
*** Current Data Parameters ***
NAME : khm290~1
EXPNO : 3
PROCNO : 1
*** Acquisition Parameters ***
LOCNUC : 2H
NS : 32
NUCLEUS : off
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : MeOD
SW : 20.6557 ppm
TD : 32768
TE : 295.1 K
*** Processing Parameters ***
LB : 0.30 Hz
SF : 500.1300010 MHz
*** 1D NMR Plot Parameters ***
NUCLEUS : off
Integral
2.0000
0.9607
1.8945
3.7155
5.0568
10.050
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)


13C AMX500 TAMAZ
159.1196
158.7844
136.2445
132.7174
131.1725
129.6495
129.4891
115.0747
115.0091
97.3153
51.3320
49.5101
49.3425
49.1676
49.0000
48.8324
48.6575
48.4899
40.8308
40.7142
*** Current Data Parameters ***
NAME : khm290~1
EXPNO : 4
PROCNO : 1
*** Acquisition Parameters ***
LOCNUC : 2H
NS : 20315
NUCLEUS : off
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : MeOD
SW : 238.7675 ppm
TD : 65536
TE : 295.2 K
*** Processing Parameters ***
LB : 1.00 Hz
SF : 125.7576130 MHz
*** 1D NMR Plot Parameters ***
NUCLEUS : off
180
160
140
120
100
80
60
40
20
(ppm)



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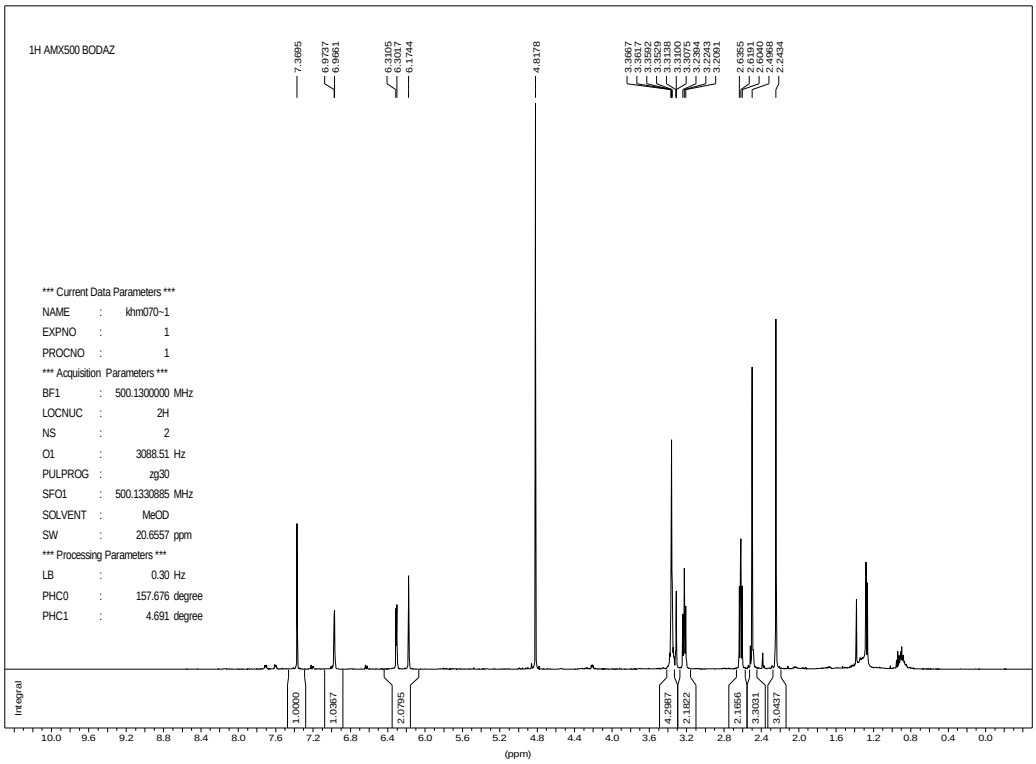
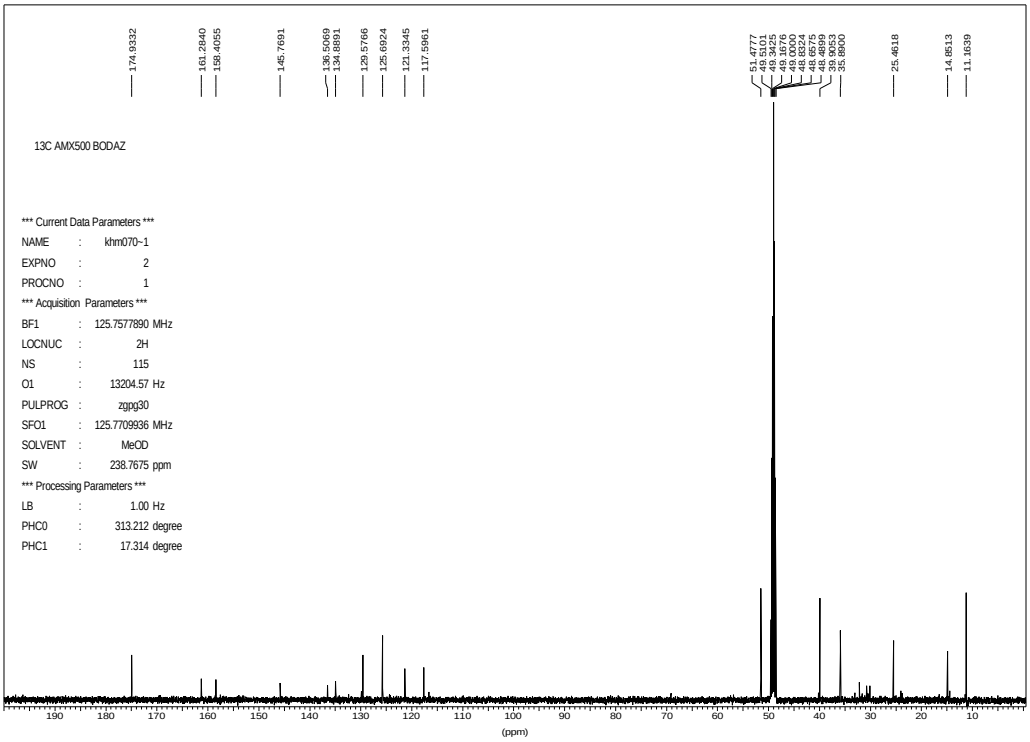
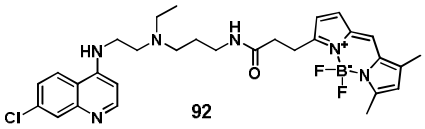

1H AMX500 BODAZ
*** Current Data Parameters ***
NAME : khm070~1
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 500.1300000 MHz
LOCNUC : 2H
NS : 2
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : MeOD
SW : 20.6557 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 157.676 degree
PHC1 : 4.691 degree
integral
(ppm)


13C AMX500 BODAZ
*** Current Data Parameters ***
NAME : khm070~1
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 125.7577890 MHz
LOCNUC : 2H
NS : 115
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : MeOD
SW : 238.7675 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 313.212 degree
PHC1 : 17.314 degree
(ppm)



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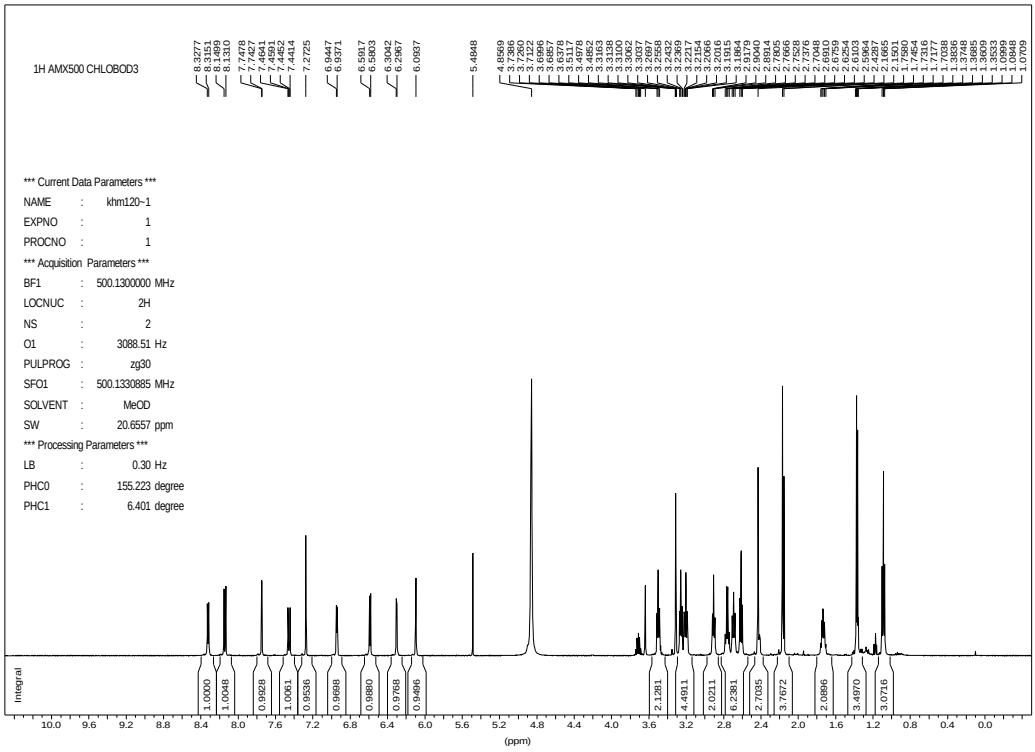
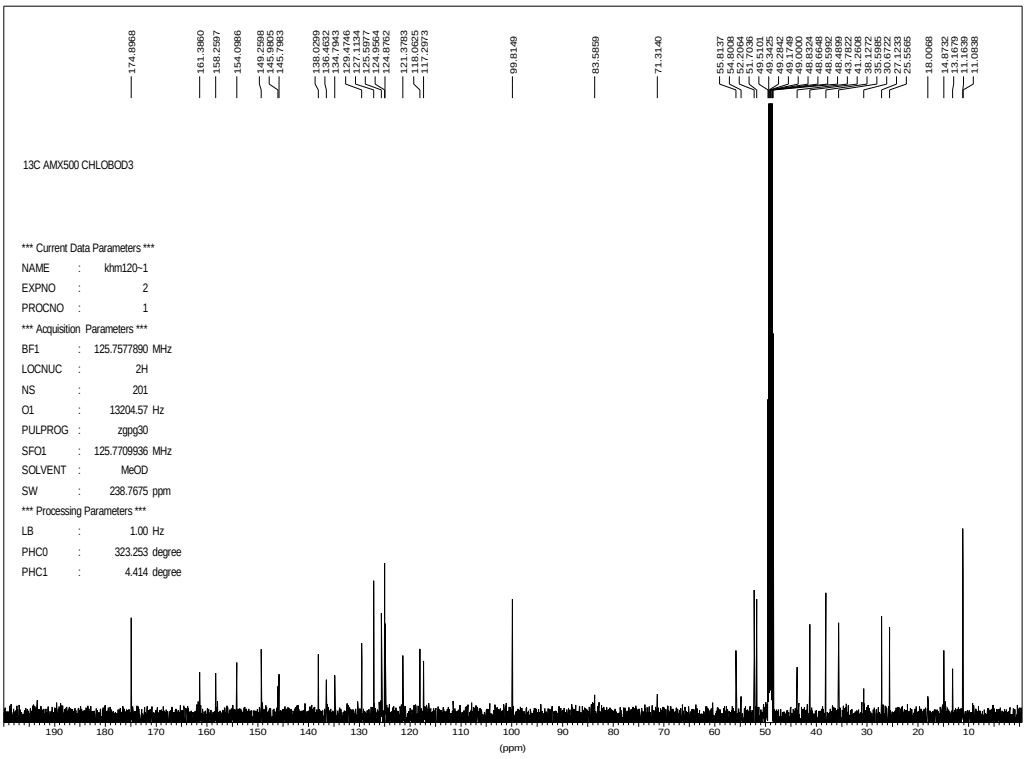
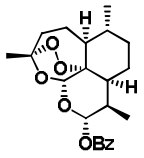

1H AMX500 CHLOBOD3
*** Current Data Parameters ***
NAME : khm120~1
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 500.1300000 MHz
LOCNUC : 2H
NS : 2
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : MeOD
SW : 20.6557 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 155.223 degree
PHC1 : 6.401 degree
Integral
(ppm)


13C AMX500 CHLOBOD3
*** Current Data Parameters ***
NAME : khm120~1
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 125.7577890 MHz
LOCNUC : 2H
NS : 201
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : MeOD
SW : 238.7675 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 323.253 degree
PHC1 : 4.414 degree
(ppm)


H
O
O
O
H
O
OBz

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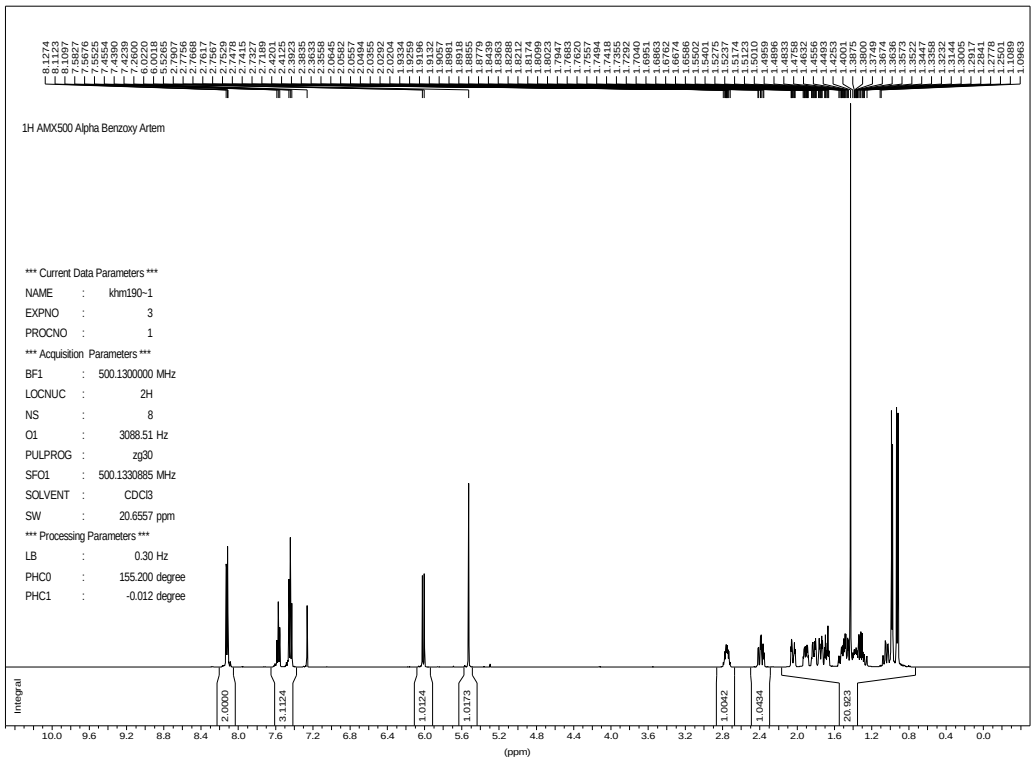
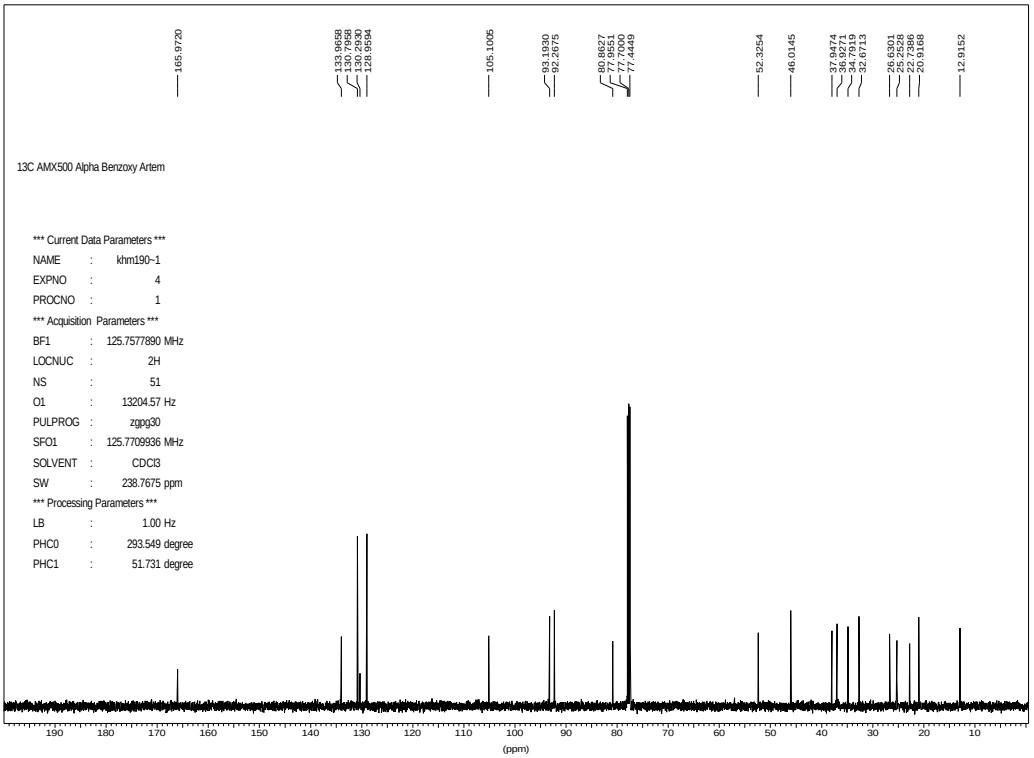
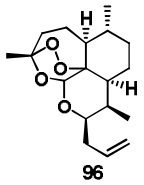

1H AMX500 Alpha Benzoxy Artem
*** Current Data Parameters ***
NAME : khm190~1
EXPNO : 3
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 500.1300000 MHz
LOCNUC : 2H
NS : 8
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : CDCl3
SW : 20.6557 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 155.200 degree
PHC1 : -0.012 degree
Integral
2.0000
3.1124
1.0124
1.0173
1.0042
1.0434
20.923
(ppm)


165.9720
133.9658
131.2955
130.2930
128.9594
105.1065
92.1930
92.2875
89.8627
77.9551
77.7000
77.4449
52.3254
46.0145
37.9474
36.9271
34.7919
32.6713
26.6301
25.2528
22.7386
20.9166
12.9152
13C AMX500 Alpha Benzoxy Artem
*** Current Data Parameters ***
NAME : khm190~1
EXPNO : 4
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 125.7577890 MHz
LOCNUC : 2H
NS : 51
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : CDCl3
SW : 238.7675 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 293.549 degree
PHC1 : 51.731 degree
(ppm)



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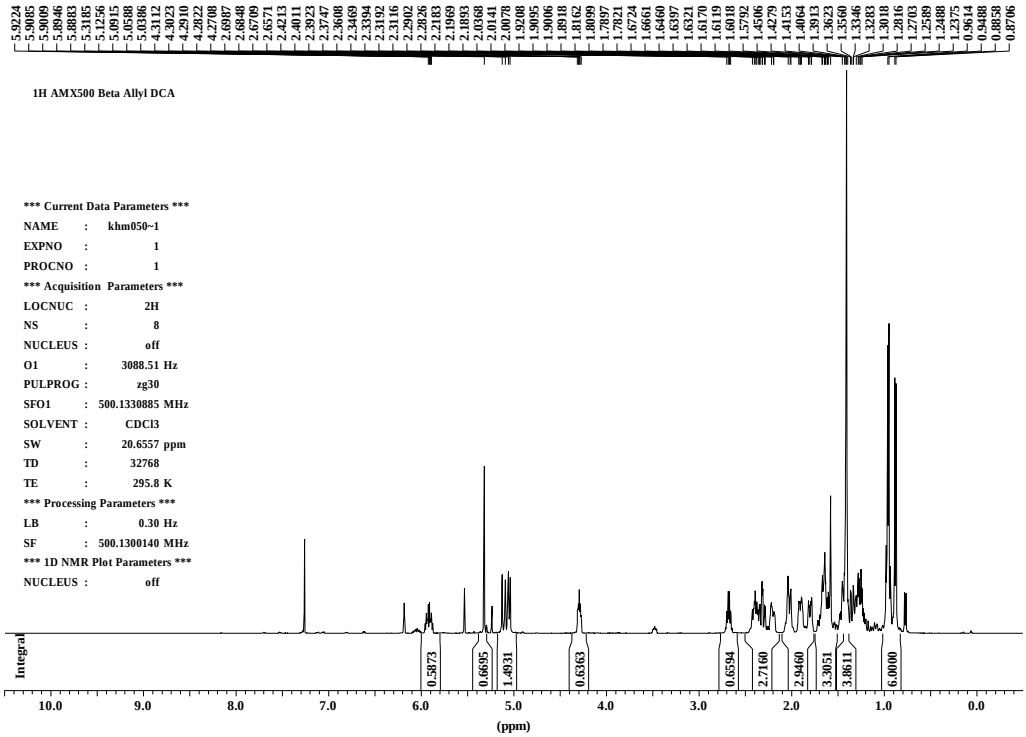
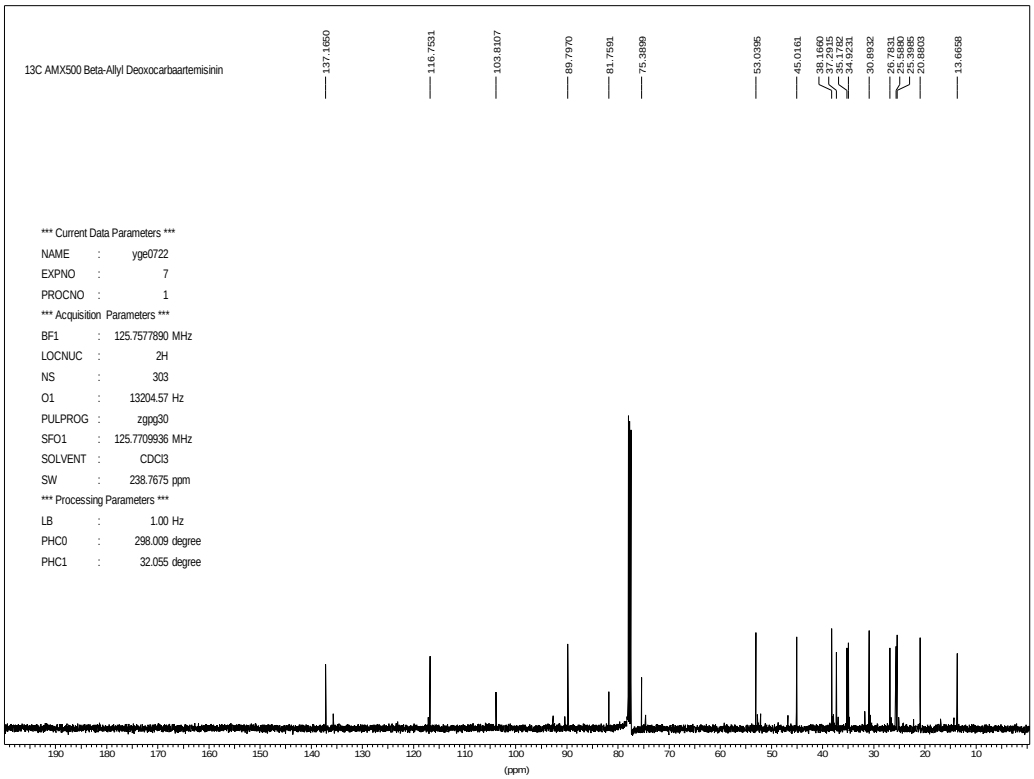
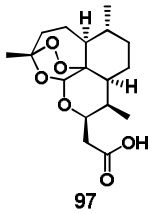

1H AMX500 Beta Allyl DCA
*** Current Data Parameters ***
NAME : khm050~1
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
LOCNUC : 2H
NS : 8
NUCLEUS : off
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : CDCl3
SW : 20.6557 ppm
TD : 32768
TE : 295.8 K
*** Processing Parameters ***
LB : 0.30 Hz
SF : 500.1300140 MHz
*** 1D NMR Plot Parameters ***
NUCLEUS : off
Integral
0.5873
0.6695
1.4931
0.6363
0.6594
2.7160
2.9460
3.3051
3.8611
6.0000
(ppm)


13C AMX500 Beta-Allyl Deoxocarbaartemisinin
137.1650
116.7531
103.8107
89.7970
81.7591
75.3899
53.0395
45.0161
38.1660
37.2915
36.1782
34.9231
30.8932
26.7831
25.5980
25.3095
20.8803
13.6658
*** Current Data Parameters ***
NAME : yge0722
EXPNO : 7
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 125.7577890 MHz
LOCNUC : 2H
NS : 303
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : CDCl3
SW : 238.7675 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 298.009 degree
PHC1 : 32.055 degree
(ppm)



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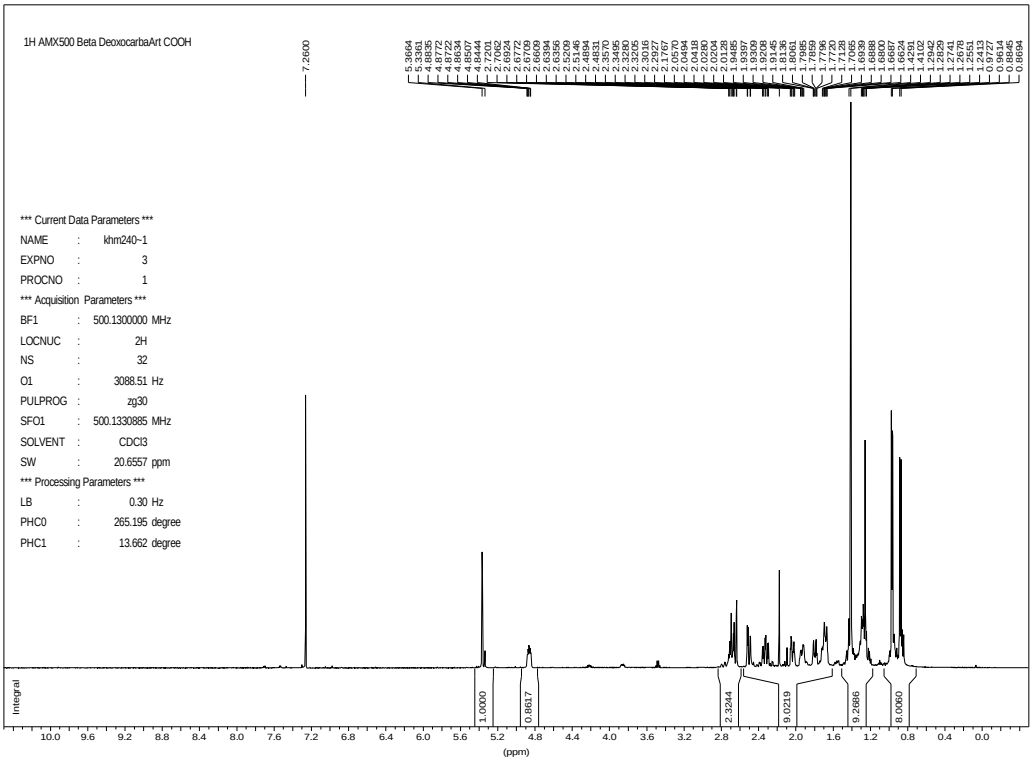
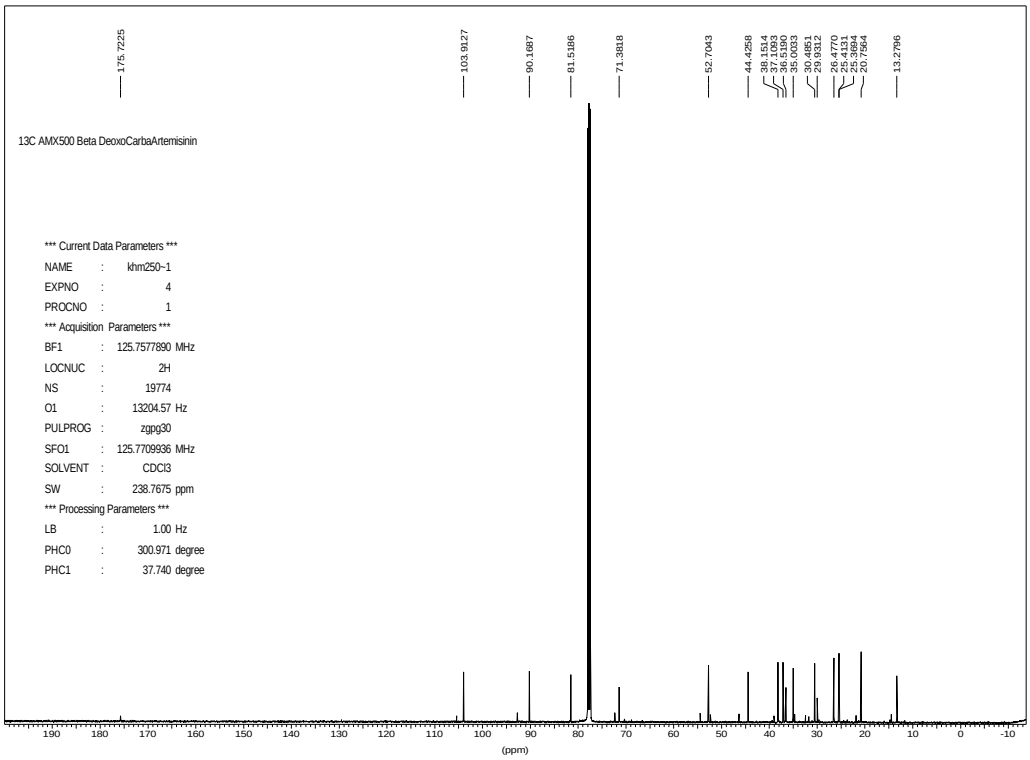
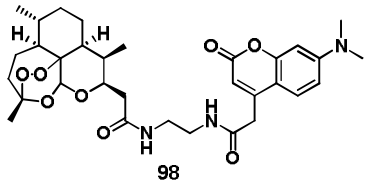

1H AMX500 Beta DeoxocarbaArt COOH
*** Current Data Parameters ***
NAME : khm240~1
EXPNO : 3
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 500.1300000 MHz
LOCNUC : 2H
NS : 32
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : CDCl3
SW : 20.6557 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 265.195 degree
PHC1 : 13.662 degree
7.2600
Integral
1.0000
0.8617
2.3244
9.0219
9.2686
8.0060
(ppm)


13C AMX500 Beta DeoxoCarbaArtemisinin
*** Current Data Parameters ***
NAME : khm250~1
EXPNO : 4
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 125.7577890 MHz
LOCNUC : 2H
NS : 19774
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : CDCl3
SW : 238.7675 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 300.971 degree
PHC1 : 37.740 degree
175.7225
103.9127
90.1867
81.5186
71.3818
52.7043
44.4258
38.1514
37.1093
34.1550
35.0033
30.4851
29.9312
26.4770
25.4131
23.3984
20.7994
13.2796
(ppm)



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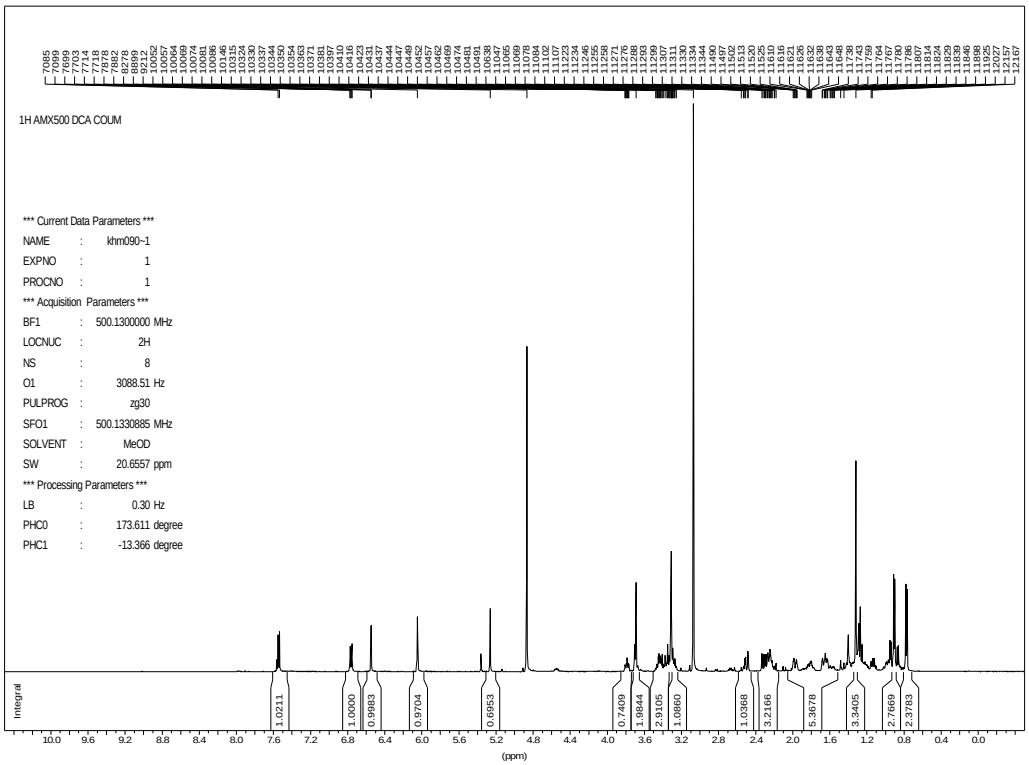
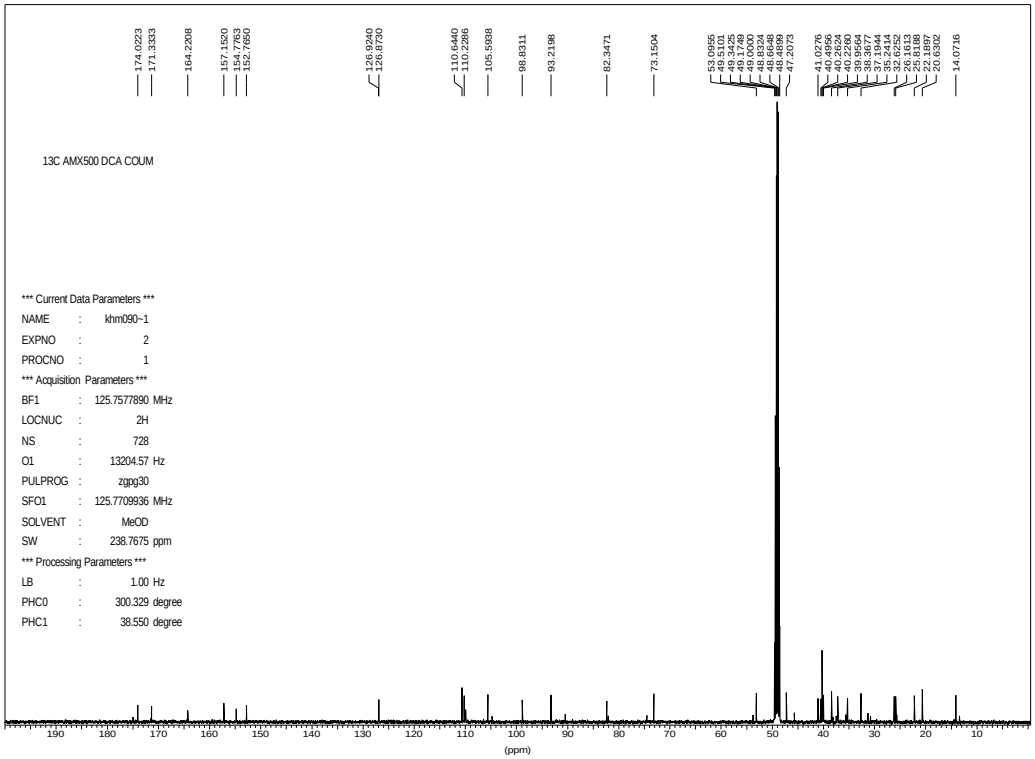
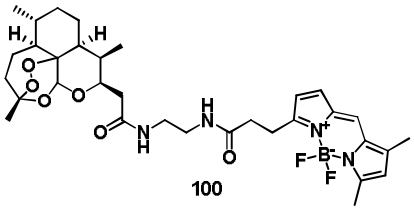

1H AMX500 DCA COUM
*** Current Data Parameters ***
NAME : khm090~1
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 500.1300000 MHz
LOCNUC : 2H
NS : 8
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : MeOD
SW : 20.6557 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 173.611 degree
PHC1 : -13.366 degree
Integral
1.0211
1.0000
0.9983
0.9704
0.6953
0.7409
1.9844
2.9105
1.0860
1.0368
3.2166
5.3678
3.3405
2.7669
2.3783
(ppm)


13C AMX500 DCA COUM
*** Current Data Parameters ***
NAME : khm090~1
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 125.7577890 MHz
LOCNUC : 2H
NS : 728
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : MeOD
SW : 238.7675 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 300.329 degree
PHC1 : 38.550 degree
174.0223
171.3333
164.2208
157.1520
154.7765
152.7660
126.9240
126.8730
110.6440
110.2286
105.5938
98.8311
93.2198
82.3471
73.1504
53.0955
49.5101
49.3425
49.1749
49.0000
48.8324
48.6648
48.4898
47.2073
41.0276
40.4956
40.2624
40.2260
39.9564
39.7677
37.1944
35.2414
32.6252
29.1615
25.8188
22.1897
20.6302
14.0716
(ppm)



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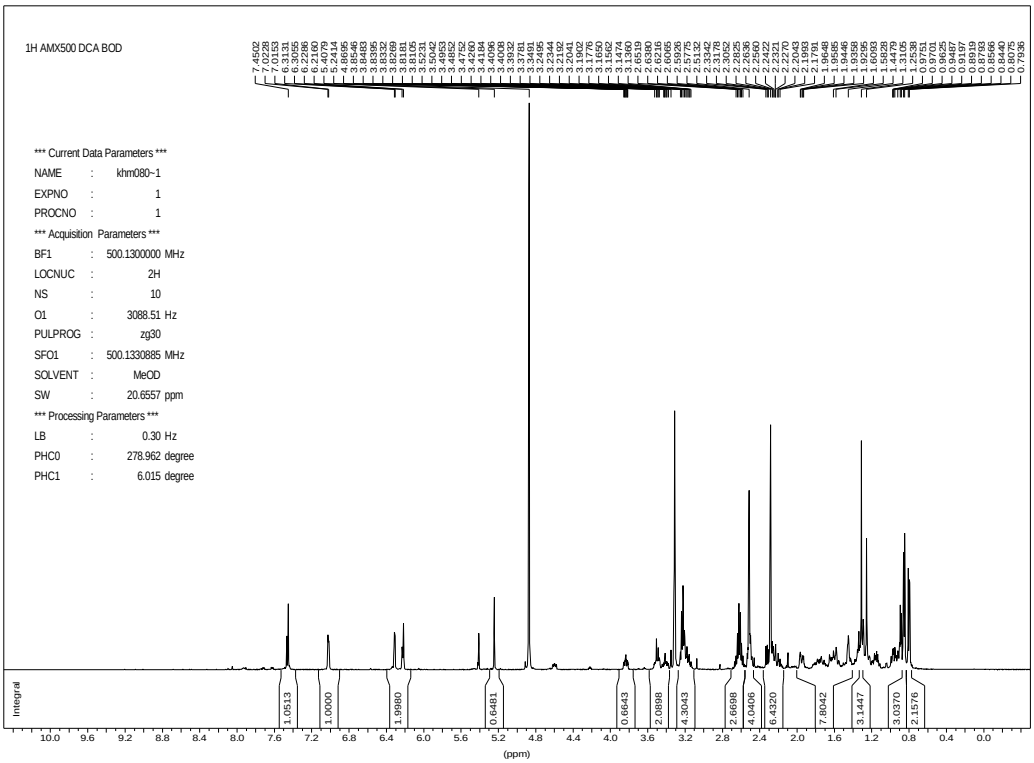
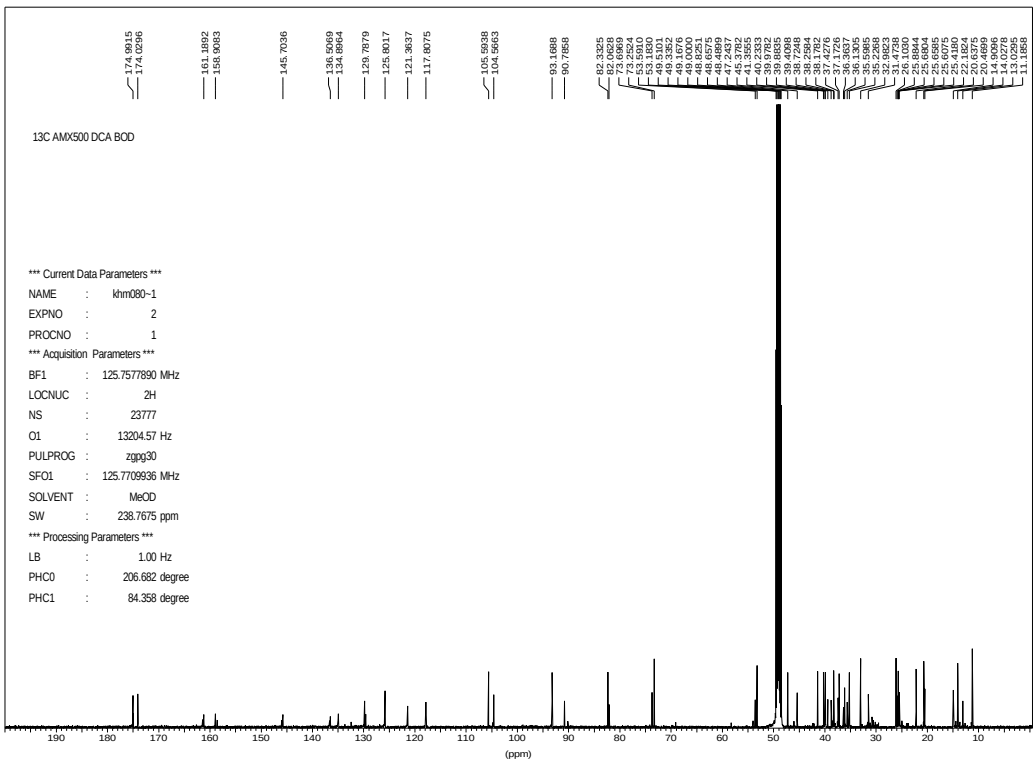
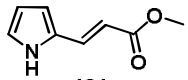

1H AMX500 DCA BOD
*** Current Data Parameters ***
NAME : khm080~1
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 500.1300000 MHz
LOCNUC : 2H
NS : 10
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : MeOD
SW : 20.6557 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 278.962 degree
PHC1 : 6.015 degree
Integral
1.0513
1.0000
1.9980
0.6481
0.6643
2.0898
4.3043
2.6698
4.0406
6.4320
7.8042
3.1447
3.0370
2.1576
(ppm)


13C AMX500 DCA BOD
*** Current Data Parameters ***
NAME : khm080~1
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 125.7577890 MHz
LOCNUC : 2H
NS : 23777
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : MeOD
SW : 238.7675 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 206.682 degree
PHC1 : 84.358 degree
(ppm)



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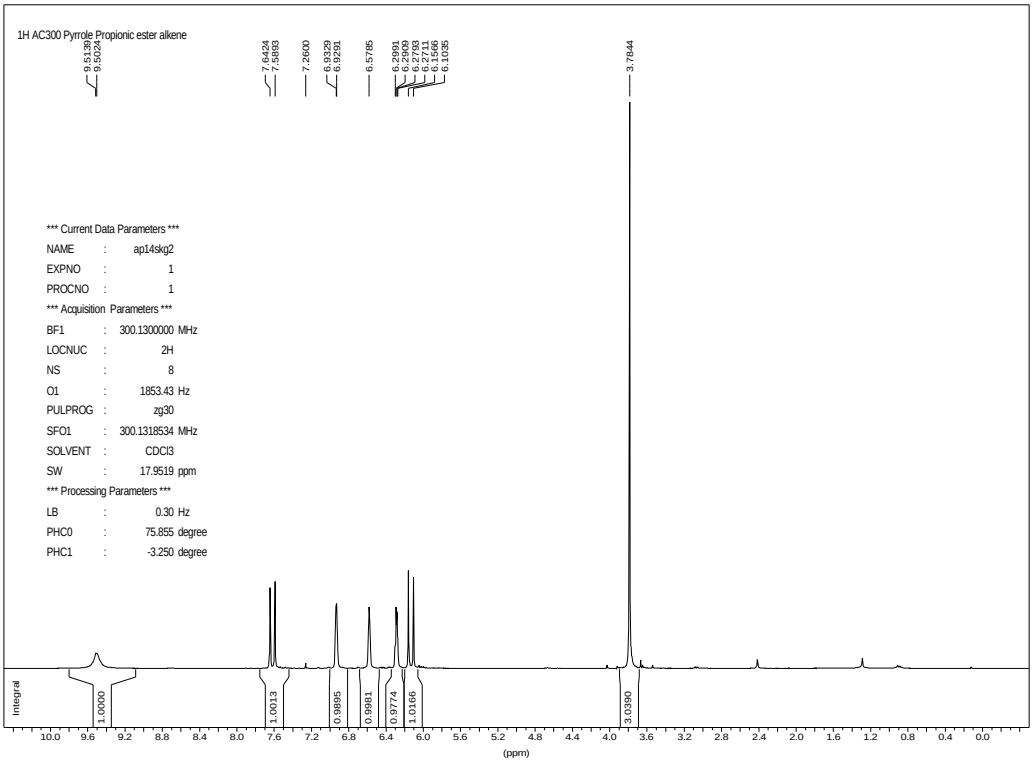
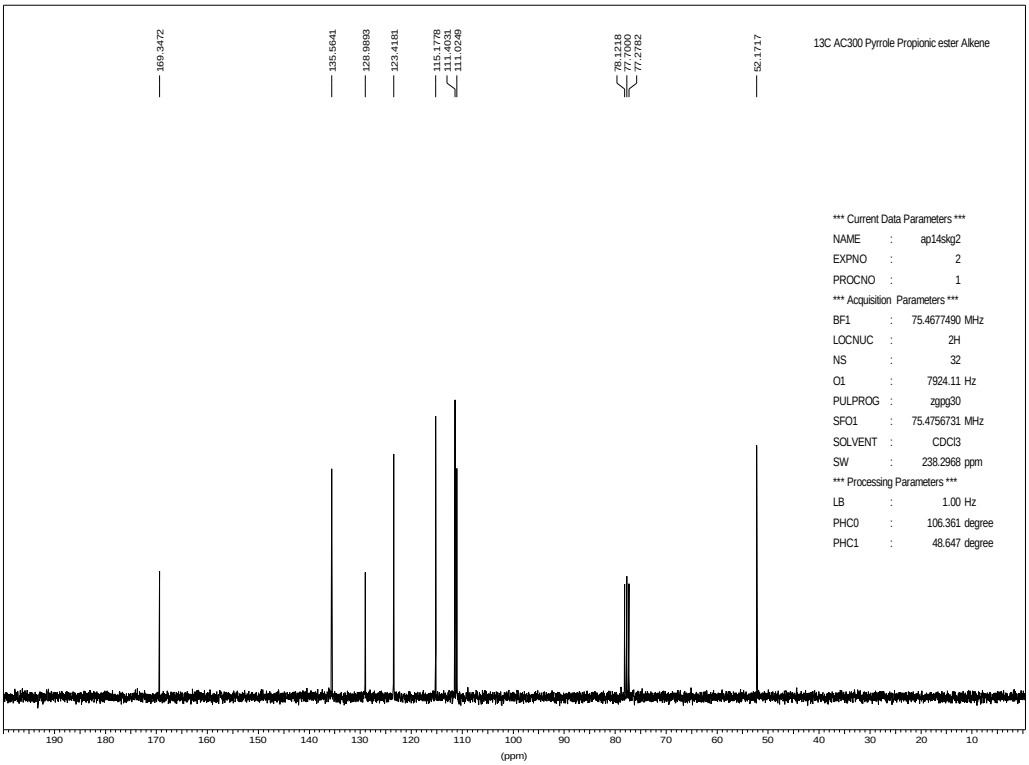
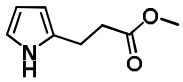

1H AC300 Pyrrole Propionic ester alkene
9.5139
9.5028
7.6424
7.5893
7.2600
6.9329
6.9291
6.5785
6.2991
6.2809
6.2793
6.2711
6.1566
6.1055
3.7844
*** Current Data Parameters ***
NAME : ap14skg2
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 300.1300000 MHz
LOCNUC : 2H
NS : 8
O1 : 1853.43 Hz
PULPROG : zg30
SFO1 : 300.1318534 MHz
SOLVENT : CDCl3
SW : 17.9519 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 75.855 degree
PHC1 : -3.250 degree
Integral
1.0000
1.0013
0.9895
0.9981
0.9774
1.0166
3.0390
(ppm)


13C AC300 Pyrrole Propionic ester Alkene
169.3472
135.5641
128.9883
123.4181
115.1778
111.4031
111.0249
79.1218
77.7080
77.2762
52.1717
*** Current Data Parameters ***
NAME : ap14skg2
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 75.4677490 MHz
LOCNUC : 2H
NS : 32
O1 : 7924.11 Hz
PULPROG : zgpg30
SFO1 : 75.4756731 MHz
SOLVENT : CDCl3
SW : 238.2968 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 106.361 degree
PHC1 : 48.647 degree
(ppm)



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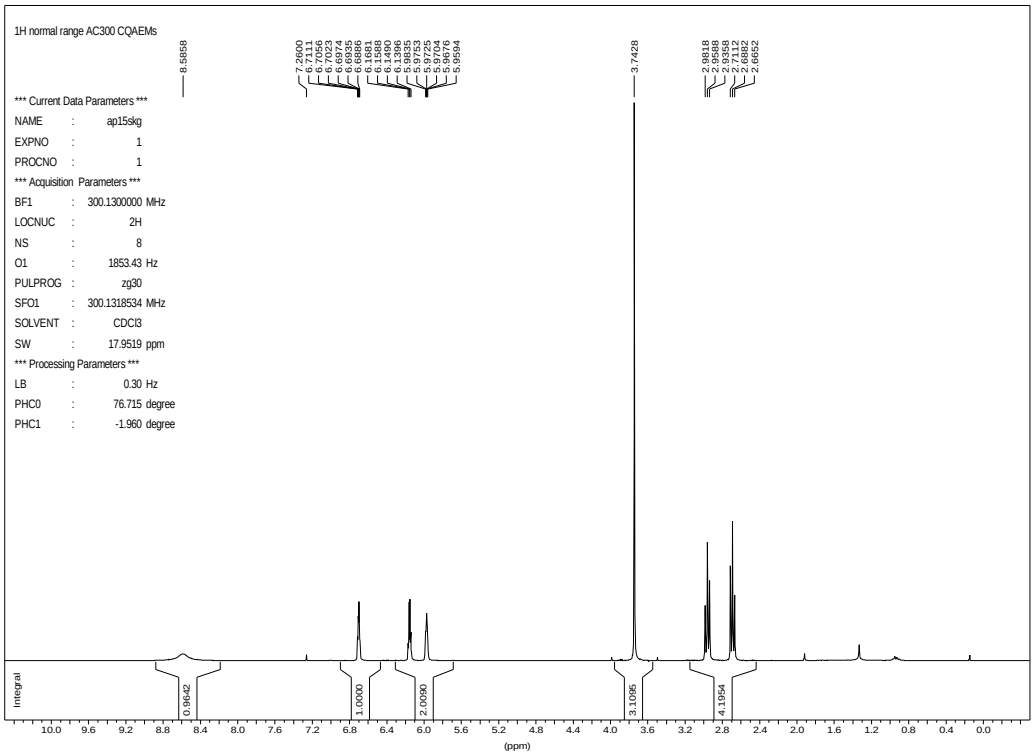
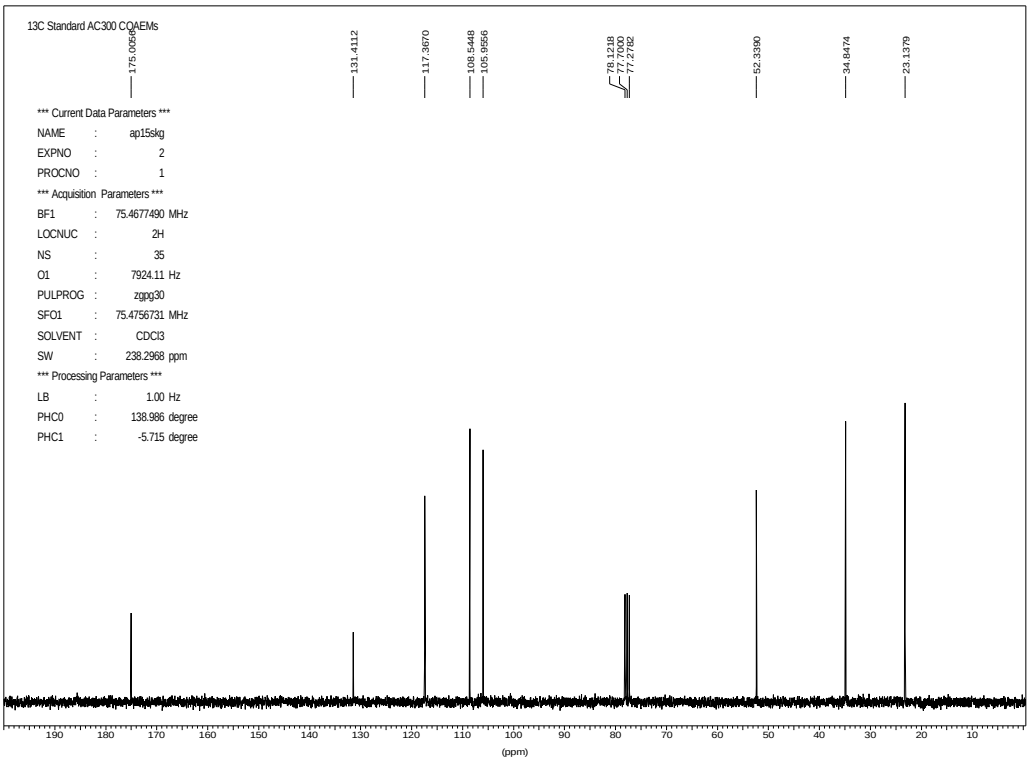
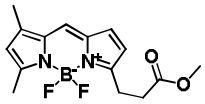

1H normal range AC300 CQAEMs
*** Current Data Parameters ***
NAME : ap15skg
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 300.1300000 MHz
LOCNUC : 2H
NS : 8
O1 : 1853.43 Hz
PULPROG : zg30
SFO1 : 300.1318534 MHz
SOLVENT : CDCl3
SW : 17.9519 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 76.715 degree
PHC1 : -1.960 degree
8.5858
7.2600
6.7211
6.7056
6.7023
6.6974
6.6835
6.6886
6.1681
6.1598
6.1480
6.1396
5.9935
5.9753
5.9725
5.9704
5.9676
5.9594
3.7428
2.9818
2.9588
2.9358
2.7112
2.6882
2.6652
Integral
0.9642
1.0000
2.0090
3.1095
4.1954
(ppm)


13C Standard AC300 CQAEMs
*** Current Data Parameters ***
NAME : ap15skg
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 75.4677490 MHz
LOCNUC : 2H
NS : 35
O1 : 7924.11 Hz
PULPROG : zgpg30
SFO1 : 75.4756731 MHz
SOLVENT : CDCl3
SW : 238.2968 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 138.986 degree
PHC1 : -5.715 degree
175.0566
131.4112
117.3670
108.5448
105.9556
78.1218
77.7000
77.2782
52.3390
34.8474
23.1379
(ppm)



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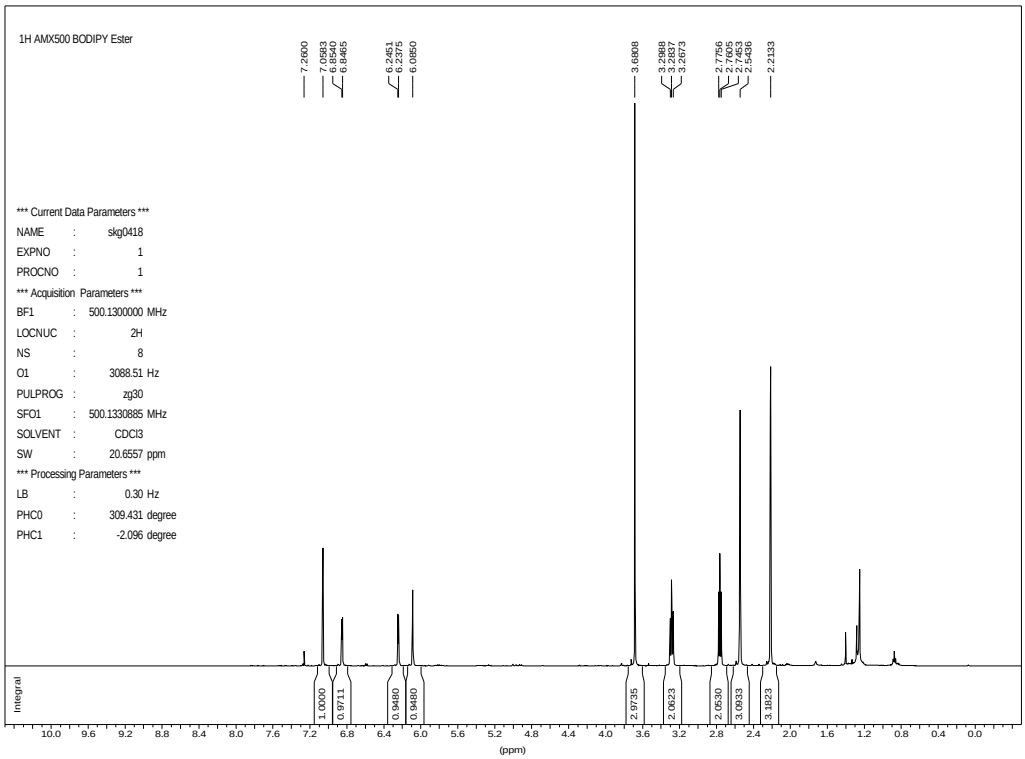
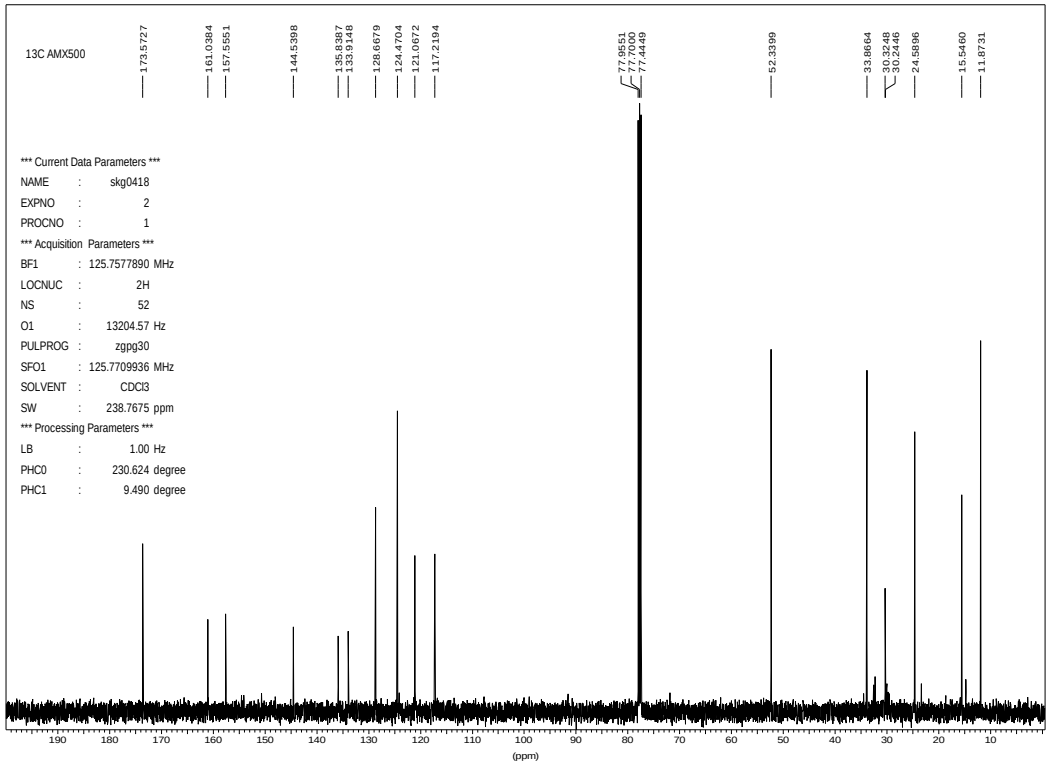
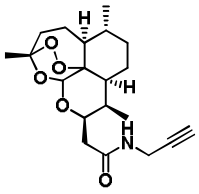

1H AMX500 BODIPY Ester
7.2600
7.0583
6.8640
6.8465
6.2451
6.2375
6.0850
3.6808
3.2988
3.2837
3.2673
2.7756
2.7605
2.7453
2.5436
2.2133
*** Current Data Parameters ***
NAME : skg0418
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 500.1300000 MHz
LOCNUC : 2H
NS : 8
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : CDCl3
SW : 20.6557 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 309.431 degree
PHC1 : -2.096 degree
Integral
1.0000
0.9711
0.9480
0.9480
2.9735
2.0623
2.0530
3.0033
3.1823
(ppm)


13C AMX500
173.5727
161.0384
157.5551
144.5398
135.8387
133.9148
128.6679
124.4704
121.0672
117.2194
77.9551
77.7000
77.4449
52.3399
33.8664
30.3248
30.2446
24.5896
15.5460
11.8731
*** Current Data Parameters ***
NAME : skg0418
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 125.7577890 MHz
LOCNUC : 2H
NS : 52
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : CDCl3
SW : 238.7675 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 230.624 degree
PHC1 : 9.490 degree
(ppm)



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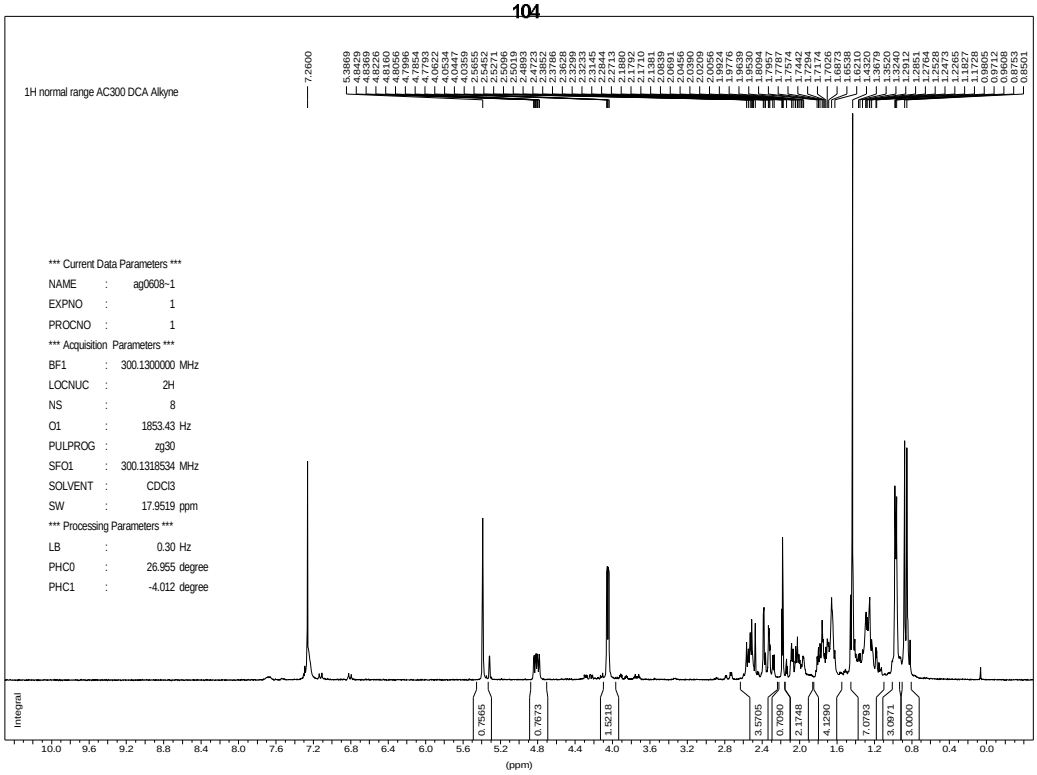
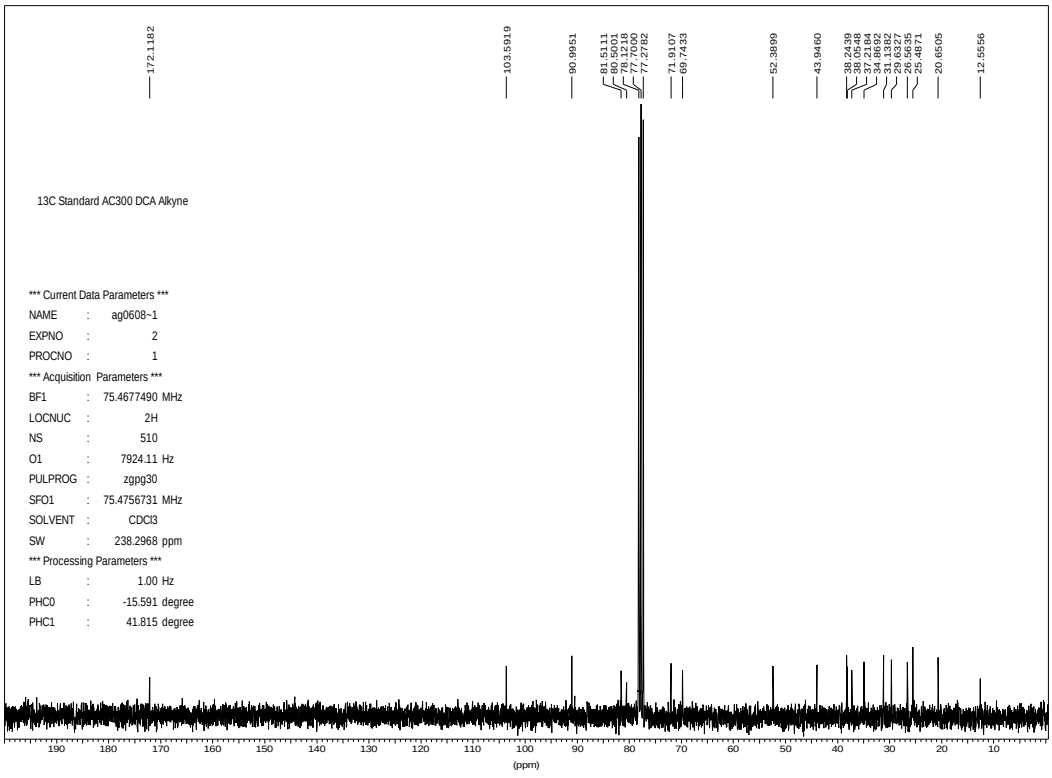
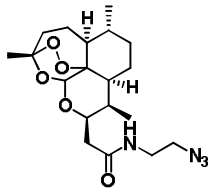

1H normal range AC300 DCA Alkyne
*** Current Data Parameters ***
NAME : ag0608~1
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 300.1300000 MHz
LOCNUC : 2H
NS : 8
O1 : 1853.43 Hz
PULPROG : zg30
SFO1 : 300.1318534 MHz
SOLVENT : CDCl3
SW : 17.9519 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 26.955 degree
PHC1 : -4.012 degree
Integral
0.7565
0.7673
1.5218
3.5705
0.7090
2.1748
4.1290
7.0793
3.0971
3.0000
(ppm)


13C Standard AC300 DCA Alkyne
*** Current Data Parameters ***
NAME : ag0608~1
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 75.4677490 MHz
LOCNUC : 2H
NS : 510
O1 : 7924.11 Hz
PULPROG : zgpg30
SFO1 : 75.4756731 MHz
SOLVENT : CDCl3
SW : 238.2968 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : -15.591 degree
PHC1 : 41.815 degree
(ppm)



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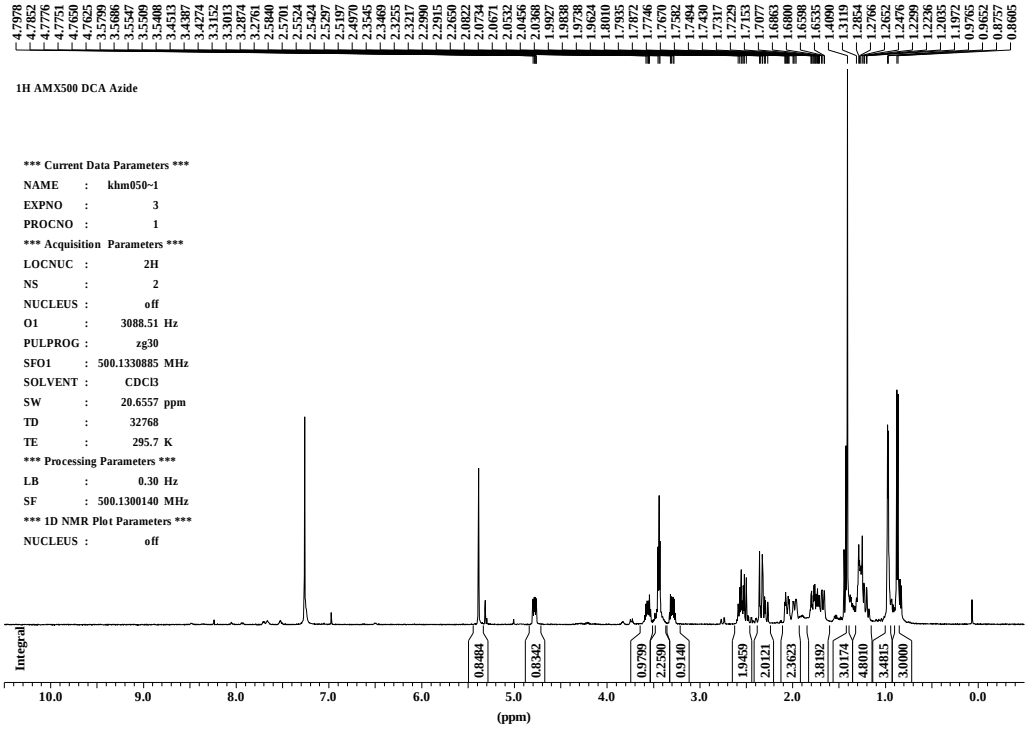
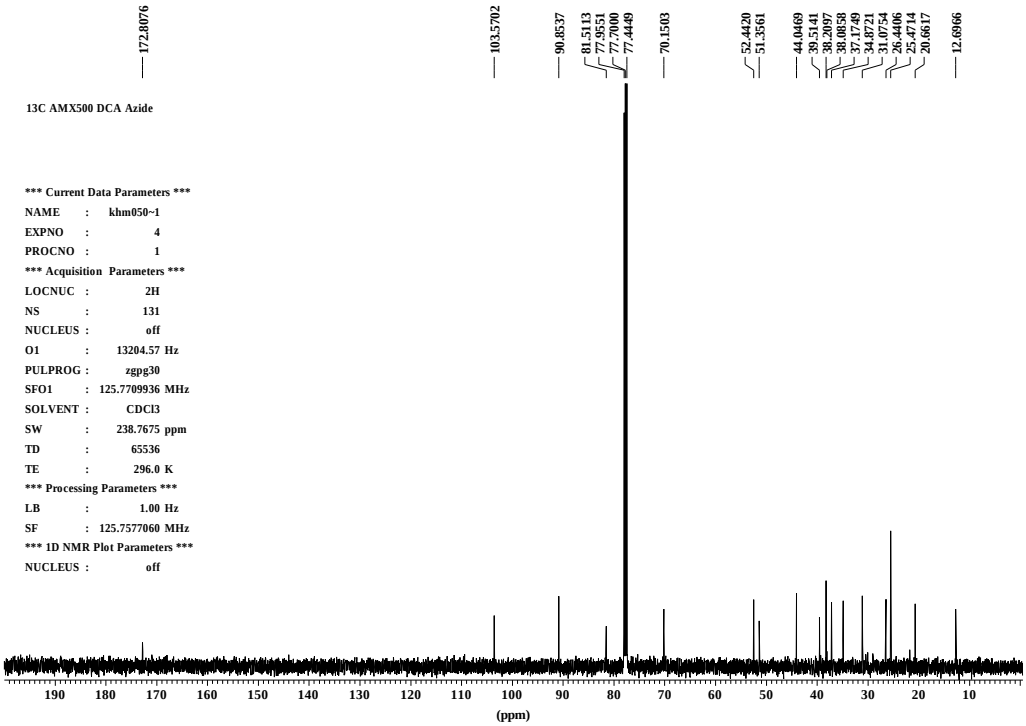
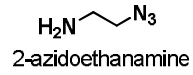

1H AMX500 DCA Azide
*** Current Data Parameters ***
NAME : khm050~1
EXPNO : 3
PROCNO : 1
*** Acquisition Parameters ***
LOCNUC : 2H
NS : 2
NUCLEUS : off
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : CDCl3
SW : 20.6557 ppm
TD : 32768
TE : 295.7 K
*** Processing Parameters ***
LB : 0.30 Hz
SF : 500.1300140 MHz
*** 1D NMR Plot Parameters ***
NUCLEUS : off
Integral
0.8484
0.8342
0.9799
2.2590
0.9140
1.9459
2.0121
2.3623
3.8192
3.0174
4.8010
3.4815
3.0000
(ppm)


172.8076
103.5702
90.8537
81.5113
77.9551
77.7000
77.4449
70.1503
52.4420
51.3561
44.0469
39.5141
38.2097
38.0858
37.1749
34.8721
31.0754
26.4406
25.4714
20.6617
12.6966
13C AMX500 DCA Azide
*** Current Data Parameters ***
NAME : khm050~1
EXPNO : 4
PROCNO : 1
*** Acquisition Parameters ***
LOCNUC : 2H
NS : 131
NUCLEUS : off
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : CDCl3
SW : 238.7675 ppm
TD : 65536
TE : 296.0 K
*** Processing Parameters ***
LB : 1.00 Hz
SF : 125.7577060 MHz
*** 1D NMR Plot Parameters ***
NUCLEUS : off
(ppm)

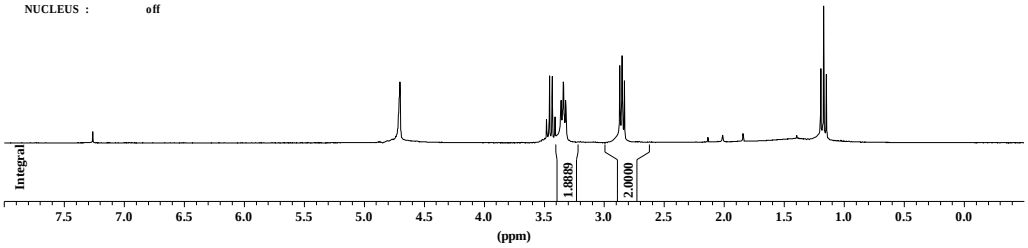


2-azidoethanamine

1H AC300 Azidoethylamine

4.7015
3.4793
3.4557
3.4322
3.4092
3.3588
3.3401
3.3209
2.8684
2.8492
2.8306
1.1942
1.1712
1.1476

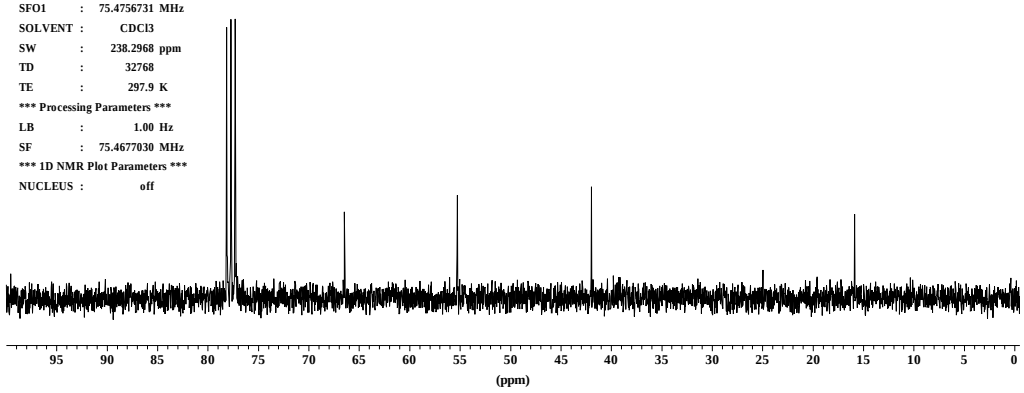
*** Current Data Parameters ***
NAME : ju22khm
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
LOCNUC : 2H
NS : 2
NUCLEUS : off
O1 : 1853.43 Hz
PULPROG : zg30
SFO1 : 300.1318534 MHz
SOLVENT : CDCl3
SW : 17.9519 ppm
TD : 32768
TE : 297.9 K
*** Processing Parameters ***
LB : 0.30 Hz
SF : 300.1300130 MHz
*** 1D NMR Plot Parameters ***
NUCLEUS : off



13C AC300 Azido ethylamine

78.1291
77.7000
77.2782
66.4487
55.2700
41.9604
24.9416
15.8576

*** Current Data Parameters ***
NAME : ju22khm
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
LOCNUC : 2H
NS : 57
NUCLEUS : off
O1 : 7924.11 Hz
PULPROG : zgpg30
SFO1 : 75.4756731 MHz
SOLVENT : CDCl3
SW : 238.2968 ppm
TD : 32768
TE : 297.9 K
*** Processing Parameters ***
LB : 1.00 Hz
SF : 75.4677030 MHz
*** 1D NMR Plot Parameters ***
NUCLEUS : off



Appendix 3

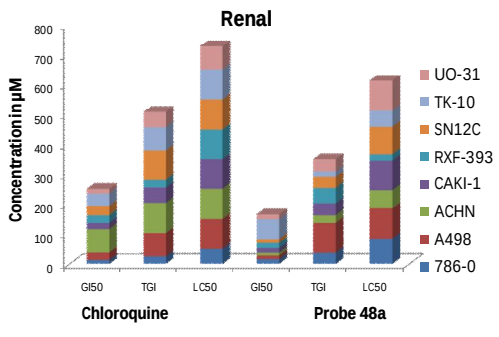
A.3 NCI 60 mean graph data –

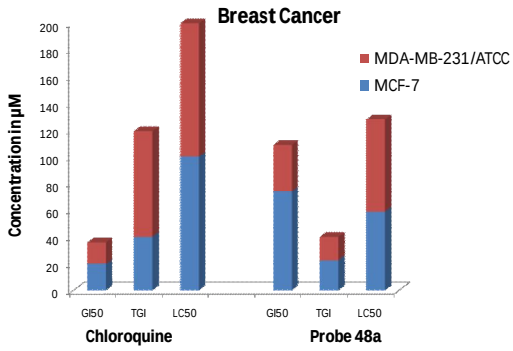
A3.1 Chloroquine diphosphate parent molecule

GI_{50} Mean Graph for Compound 14050
NCI Cancer Screen Current Data
Average GI_{50} over all cell lines is 2.47E-5

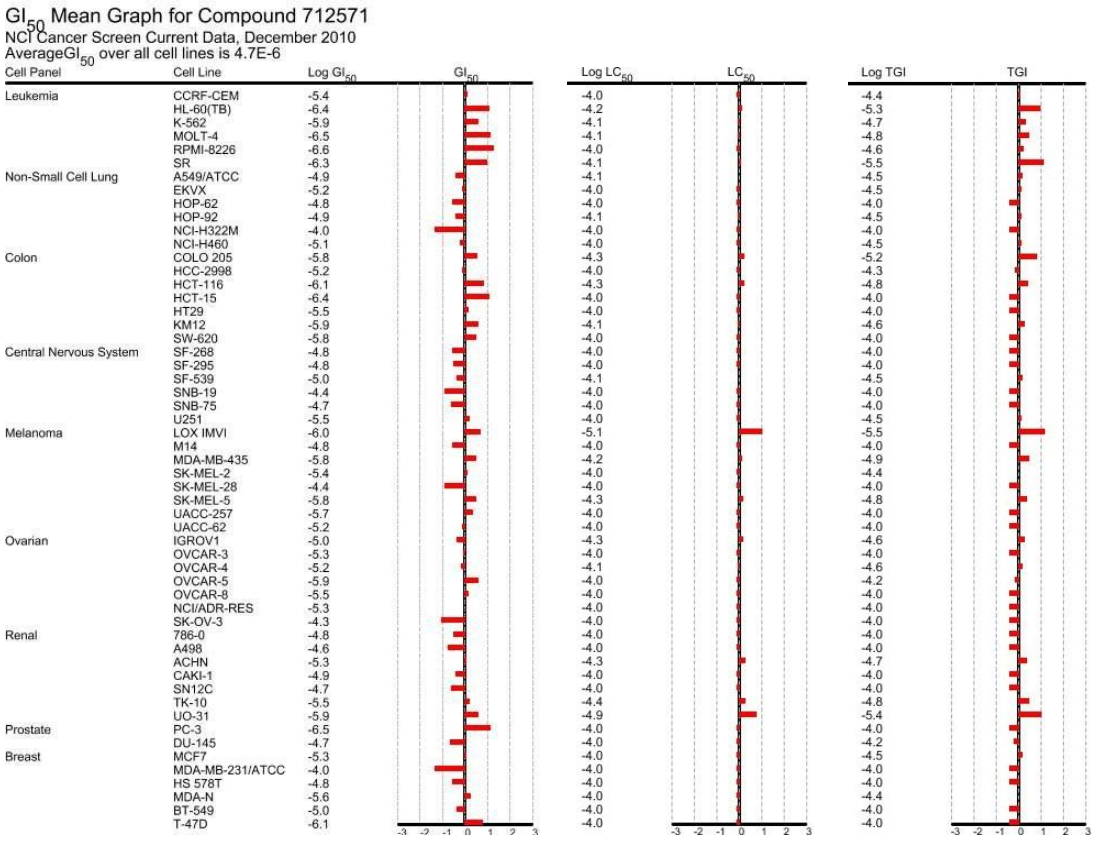
Cell Panel	Cell Line	Log GI_{50}	GI_{50}	Log TGI	TGI	Log LC_{50}	LC_{50}
Leukemia	CCRF-CEM	-4.7		-4.0		-4.0	
	HL-60(TB)	-4.9		-4.5		-4.1	
	K-562	-4.9		-4.5		-4.1	
	MOLT-4	-4.8		-4.3		-4.0	
	RPMI-8226	-4.7		-4.2		-4.0	
	SR	-4.9		-4.2		-4.0	
Non-Small Cell Lung	A549/ATCC	-4.4		-4.0		-4.0	
	EKVX	-4.2		-4.0		-4.0	
	HOP-62	-4.5		-4.1		-4.0	
	HOP-92	-4.8		-4.3		-4.0	
	NCI-H23	-4.1		-4.0		-4.0	
	NCI-H322M	-4.5		-4.0		-4.0	
	NCI-H460	-4.7		-4.3		-4.1	
	NCI-H522	-4.6		-4.2		-4.1	
	LXFL 529	-4.2		-4.0		-4.0	
Small Cell Lung	DMS 114	-4.6		-4.2		-4.0	
	DMS 273	-4.6		-4.1		-4.0	
Colon	COLO 205	-5.0		-4.4		-4.0	
	DLD-1	-4.0		-4.0		-4.0	
	HCC-2998	-4.7		-4.3		-4.1	
	HCT-116	-4.9		-4.4		-4.0	
	HCT-15	-4.6		-4.1		-4.0	
	HT29	-4.8		-4.2		-4.0	
	KM12	-4.5		-4.0		-4.0	
	KM20L2	-4.8		-4.4		-4.0	
	SW-620	-4.2		-4.0		-4.0	
Central Nervous System	SF-268	-4.2		-4.0		-4.0	
	SF-295	-4.7		-4.2		-4.1	
	SF-539	-4.8		-4.5		-4.2	
	SNB-19	-4.1		-4.0		-4.0	
	SNB-75	-4.2		-4.0		-4.0	
	SNB-78	-4.5		-4.0		-4.0	
	U251	-4.7		-4.2		-4.0	
	XF 498	-4.7		-4.5		-4.2	
Melanoma	LOX IMVI	-4.7		-4.3		-4.1	
	MALME-3M	-4.7		-4.4		-4.2	
	M14	-4.8		-4.5		-4.3	
	MDA-MB-435	-4.8		-4.4		-4.1	
	SK-MEL-2	-5.0		-4.7		-4.3	
	SK-MEL-28	-4.7		-4.4		-4.1	
	SK-MEL-5	-4.6		-4.2		-4.0	
	UACC-257	-4.6		-4.2		-4.0	
	UACC-62	-4.5		-4.2		-4.1	
Ovarian	IGROV1	-4.6		-4.2		-4.0	
	OVCAR-3	-4.0		-4.0		-4.0	
	OVCAR-4	-4.3		-4.0		-4.0	
	OVCAR-5	-4.6		-4.0		-4.0	
	OVCAR-8	-4.5		-4.0		-4.0	
	NCI/ADR-RES	-4.4		-4.0		-4.0	
	SK-OV-3	-4.1		-4.0		-4.0	
Renal	786-0	-4.9		-4.6		-4.3	
	A498	-4.6		-4.1		-4.0	
	ACHN	-4.1		-4.0		-4.0	
	CAKI-1	-4.7		-4.3		-4.0	
	RXF 393	-5.6		-4.6		-4.0	
	RXF-631	-4.6		-4.3		-4.0	
	SN12C	-4.5		-4.0		-4.0	
	TK-10	-4.4		-4.1		-4.0	
	UO-31	-4.8		-4.3		-4.1	
Prostate	PC-3	-4.7		-4.0		-4.0	
	DU-145	-5.1		-4.0		-4.0	
Breast	MCF7	-4.7		-4.4		-4.0	
	MDA-MB-231/ATCC	-4.8		-4.1		-4.0	
	MDA-N	-4.8		-4.5		-4.2	
	T-47D	-4.6		-4.2		-4.0	

A3.2 Chloroquine probe **(48 a)** vs Chloroquine remaining NCI 60 comparision data –





A3.3 Artesunate parent molecule



A3.4 Artesunate probe **(51)** vs Artesunate remaining NCI 60 comparision data –

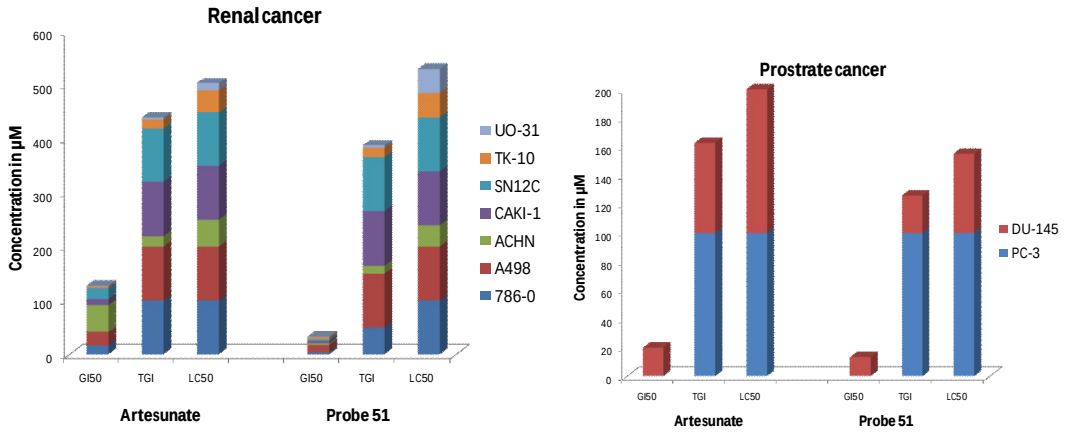
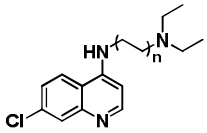


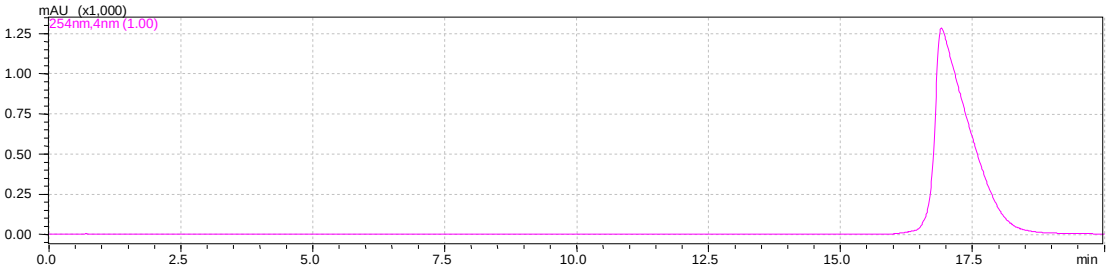
Fig. 42 – Mean graph data for parent molecule Artesunate (A3.3) and Comparision of Mean graph data of Probe 51 vs Artesunate (A3.4)

Appendix 4

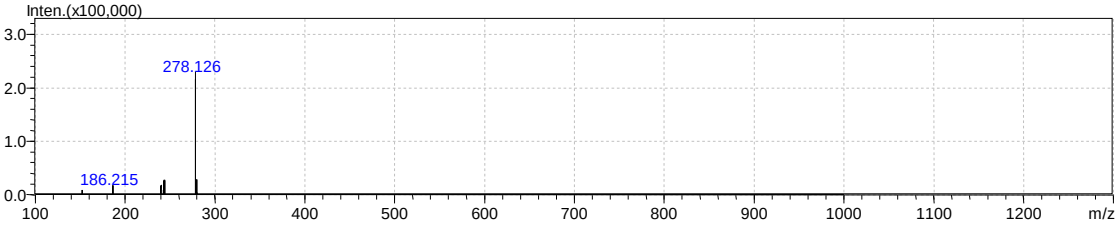
A.4 LCMS data of compounds showing purity profile for Biotesting submission – All compounds submitted for bio-testing listed in this appendix have purity of 96% as observed on LCMS.

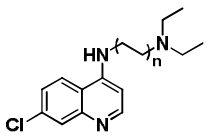


29a, n=1

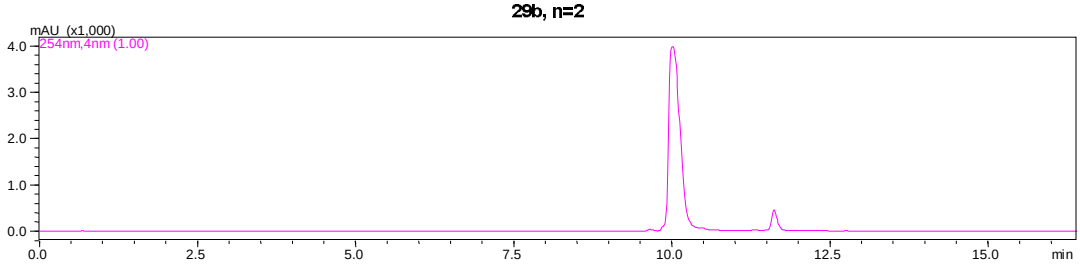


Event#: 1 MS(E+) Ret. Time : 16.880 -> 16.893 - 16.840 <-> 16.913 Scan# : 5065 -> 5069 - 5053 <-> 5075

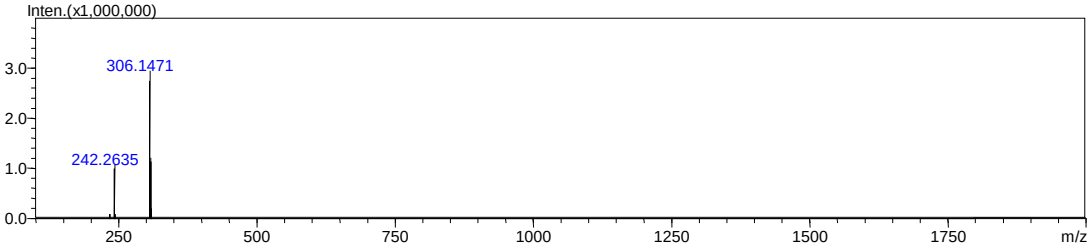


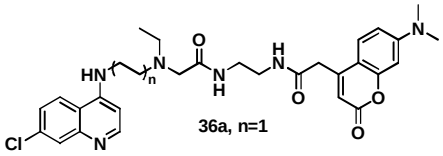


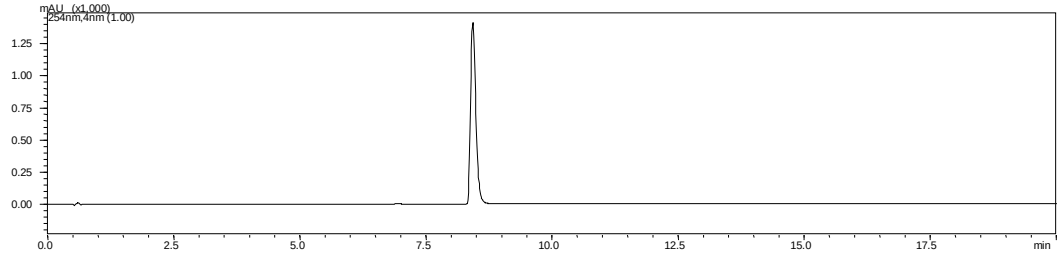
29b, n=2



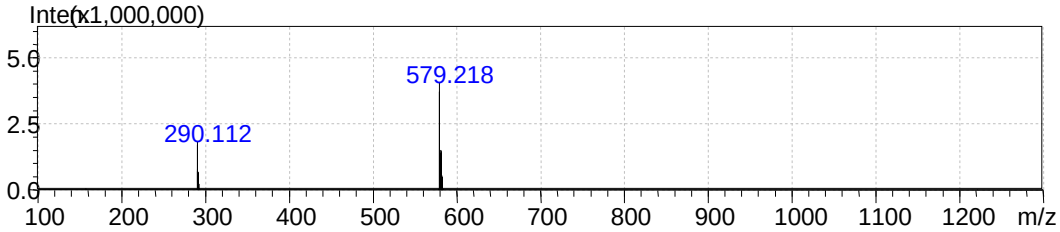
Event#: 1 MS(E+) Ret. Time : 10.060 Scan# : 3019

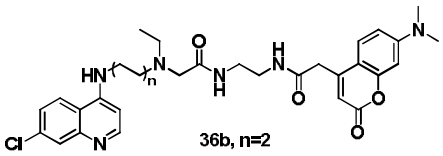


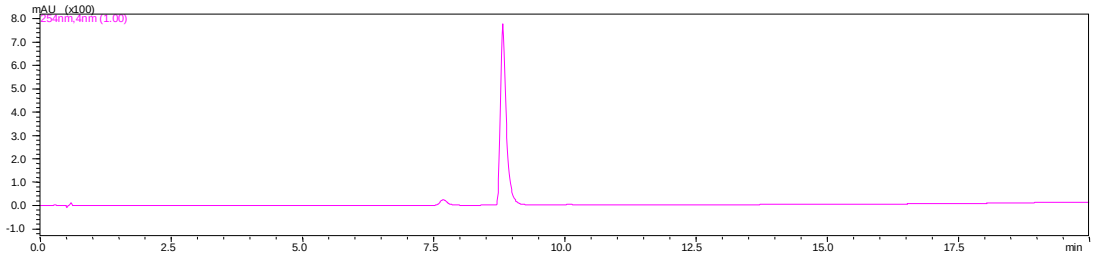




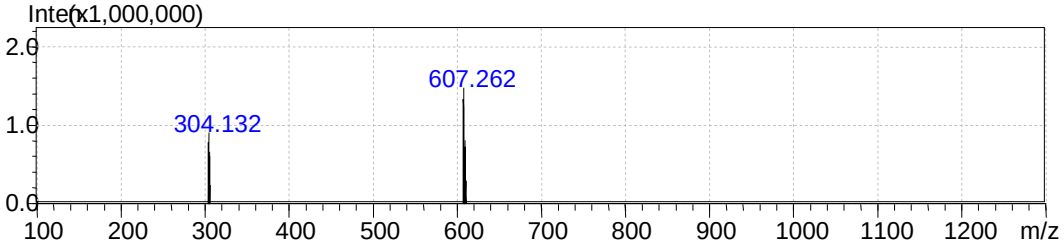
Event#: 1 MS(E+) Ret. Time : 8.460 -> 8.473 - 8.333 <-> 8.753 Scan# : 2539 -> 2543 - 2501 <-> 2627

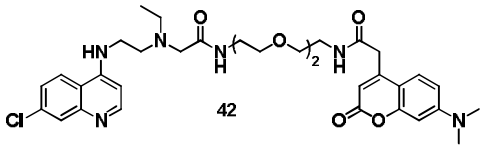




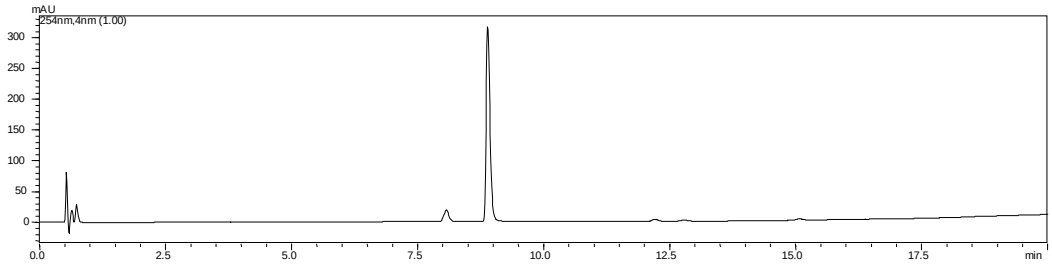


Event#: 1 MS(E+) Ret. Time : 8.720 -> 8.733 - 8.553 <-> 8.980 Scan# : 2617 -> 2621 - 2567 <-> 2695

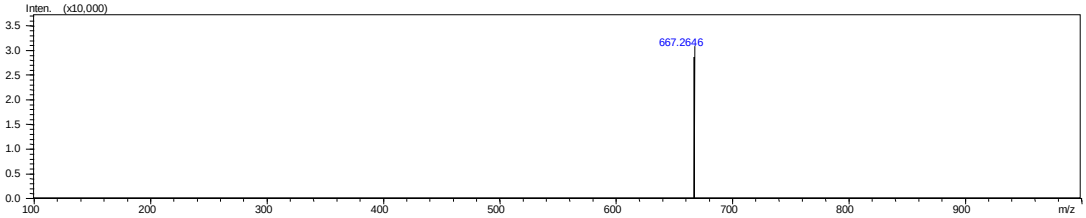


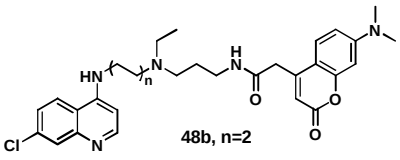


42

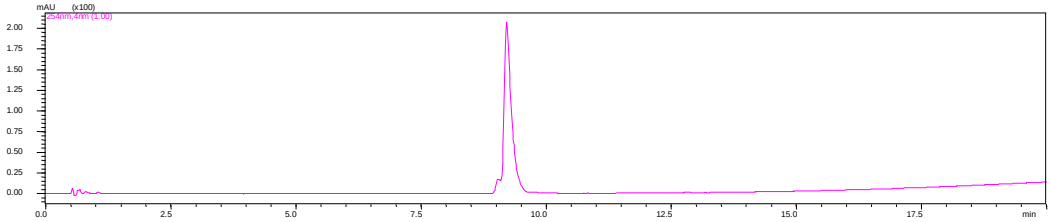


Event#: 1 MS(E+) Ret. Time : 8.860 -> 8.873 - 8.833 <-> 8.893

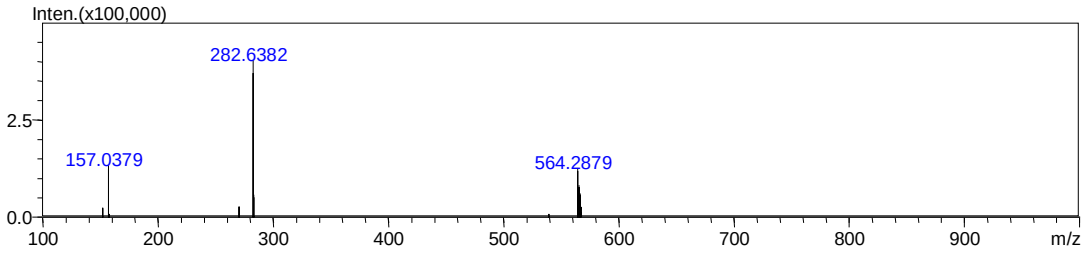


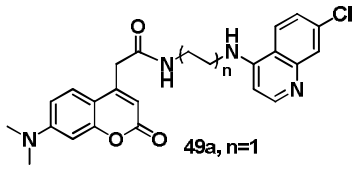


48b, n=2

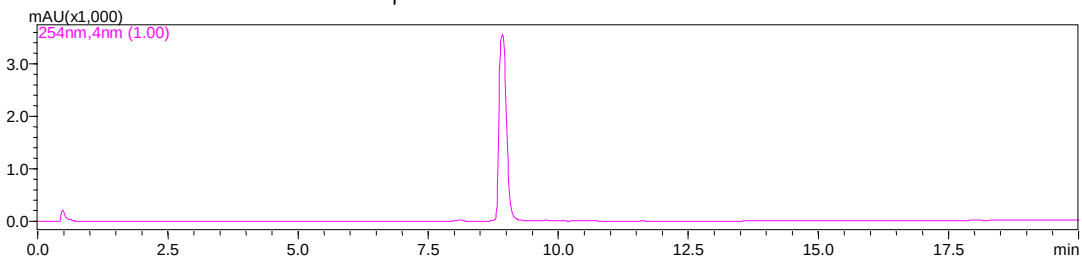


Event#: 1 MS(E+) Ret. Time : 9.213 -> 9.227 - 9.200 <-> 9.247

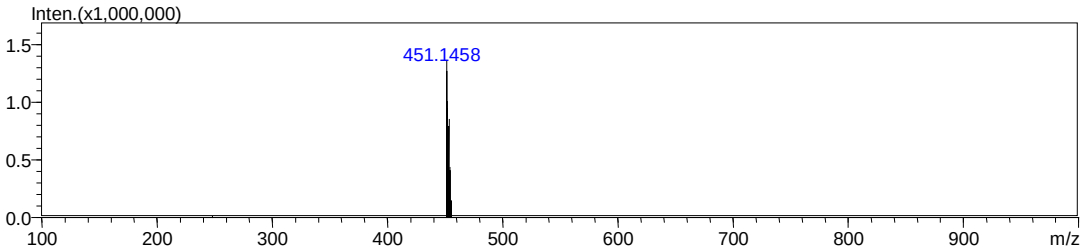


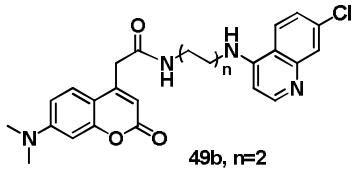


49a, n=1

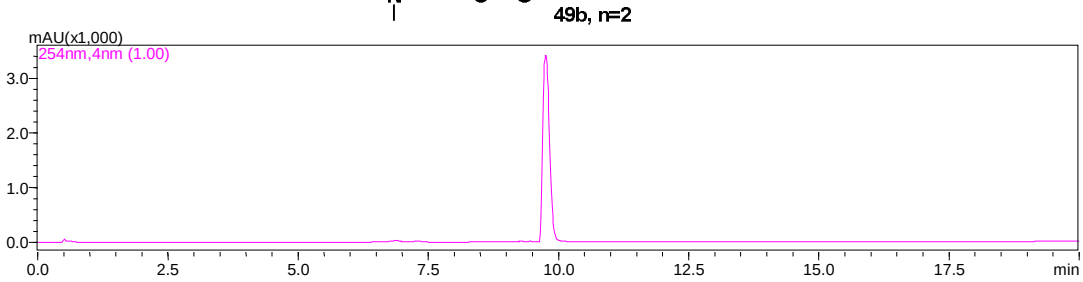


Event#: 1 MS(E+) Ret. Time : 8.980 -> 8.993 - 8.800 <-> 9.187

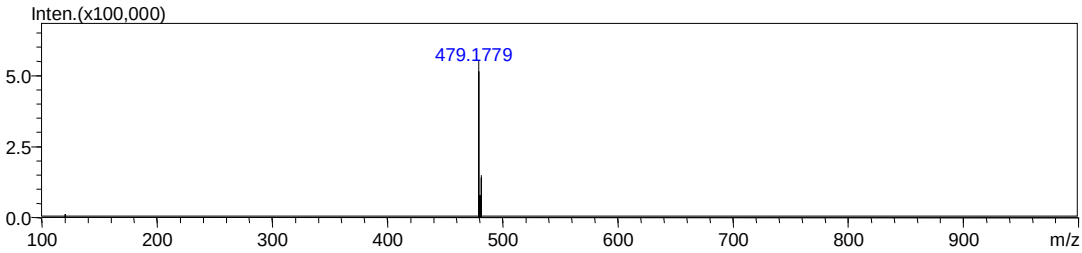


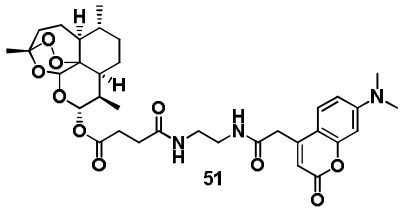


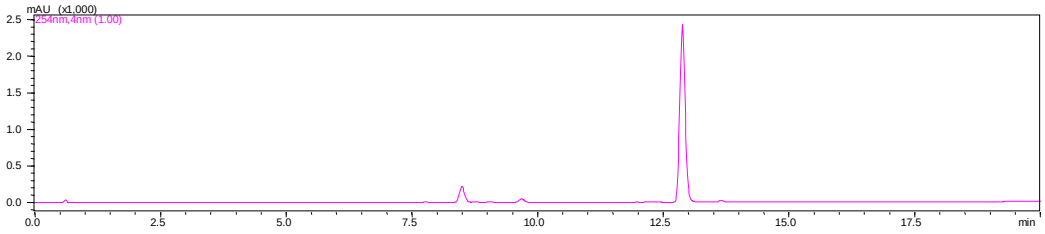
49b, n=2



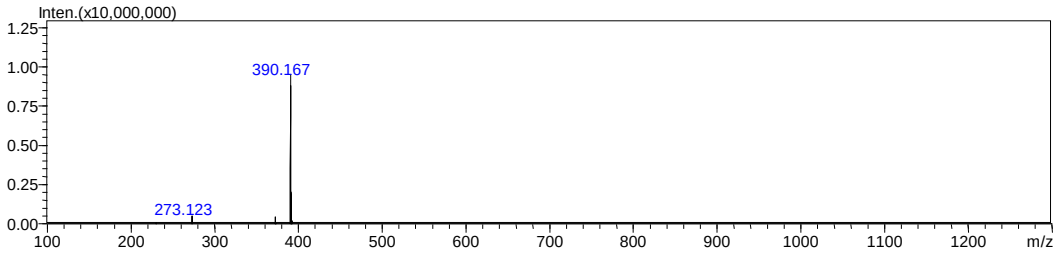
Event#: 1 MS(E+) Ret. Time : 9.720 -> 9.733 - 9.687 <-> 9.753

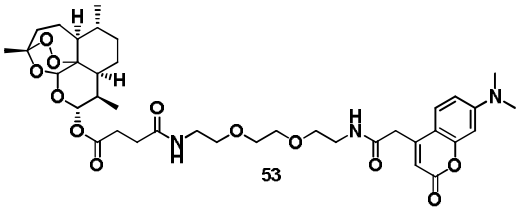


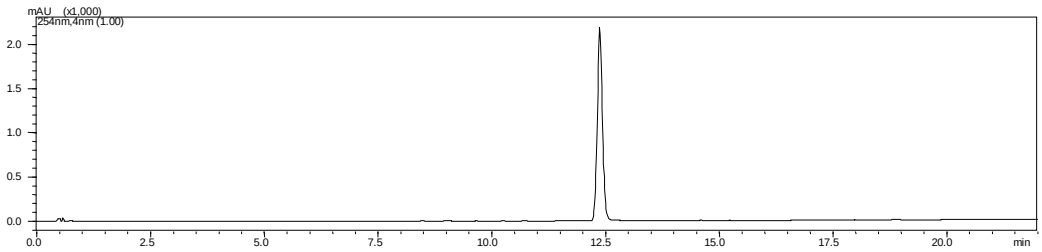




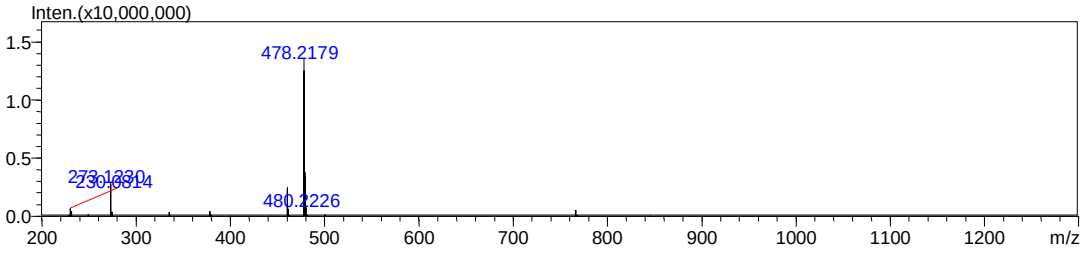
Event#: 1 MS(E+) Ret. Time : 8.533 -> 8.547 - 8.413 <-> 8.773

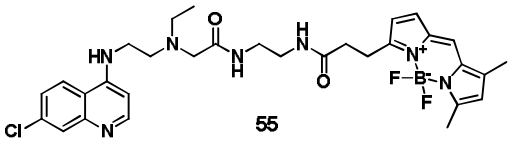




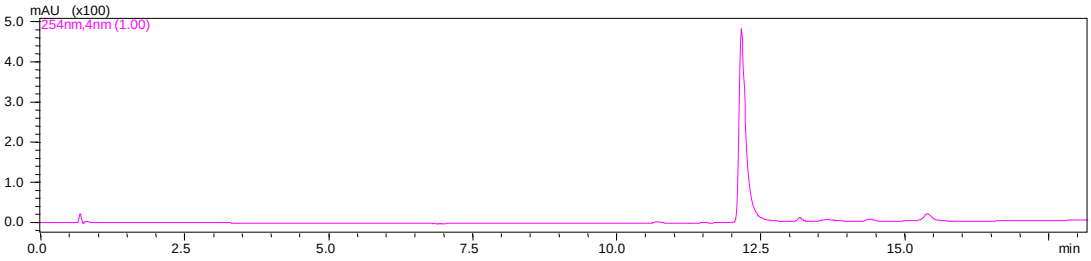


Event#: 1 MS(E+) Ret. Time : 12.423 -> 12.437 - 12.230 <-> 12.757

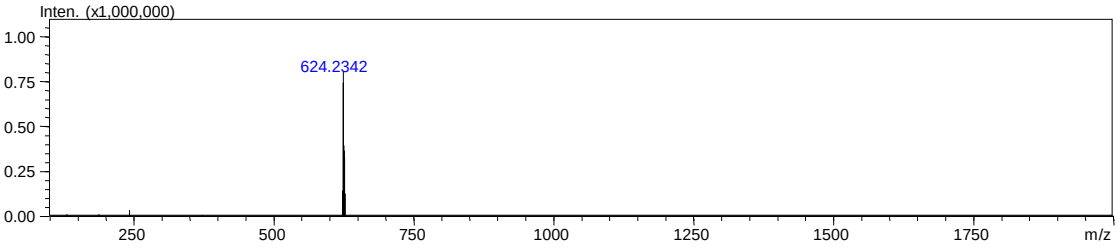


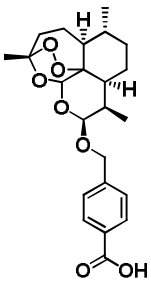


55

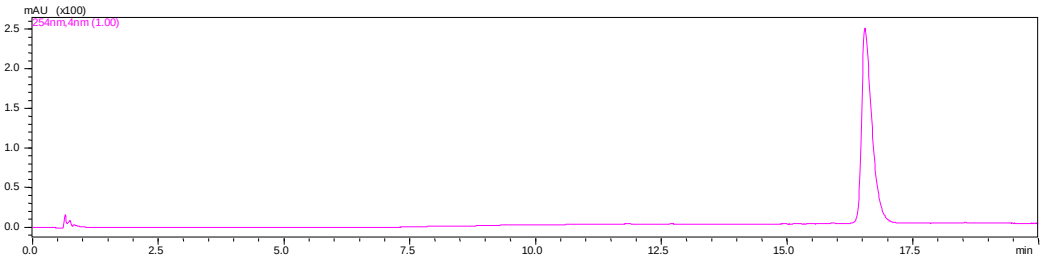


Event#: 1 MS(E+) Ret. Time : 12.240 -> 12.253 - 12.107 <-> 12.447

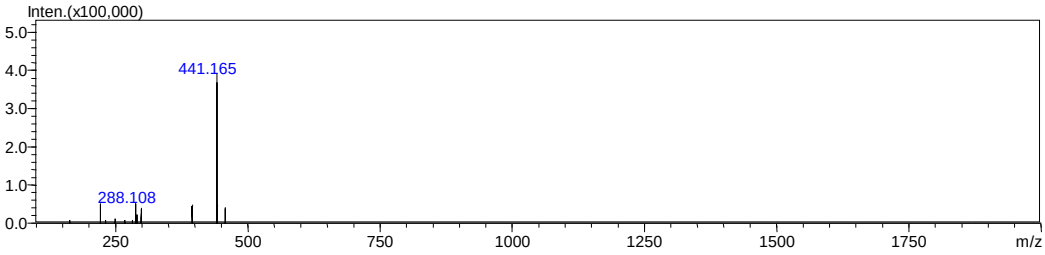


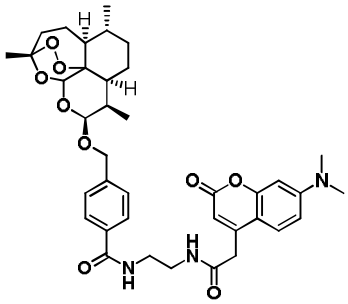


56

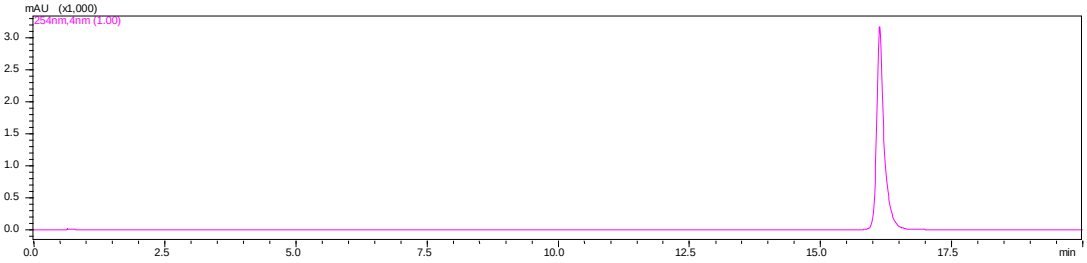


Event#: 1 MS(E+) Ret. Time : 16.593 -> 16.607 - 16.407 <-> 16.707

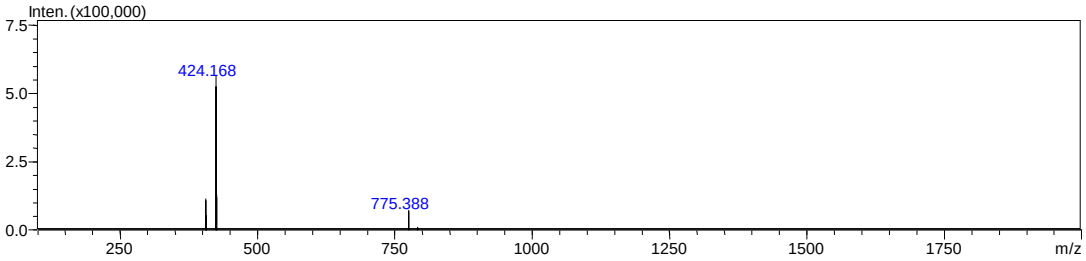


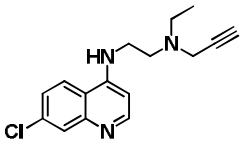


57

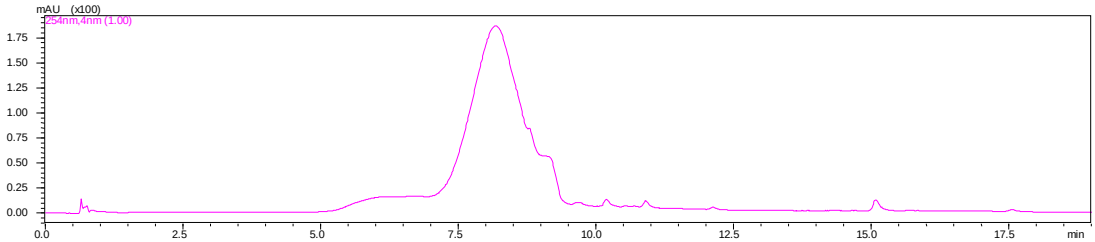


MS(E+) Ret. Time : 16.193 -> 16.207 - 15.940 <-> 16.553

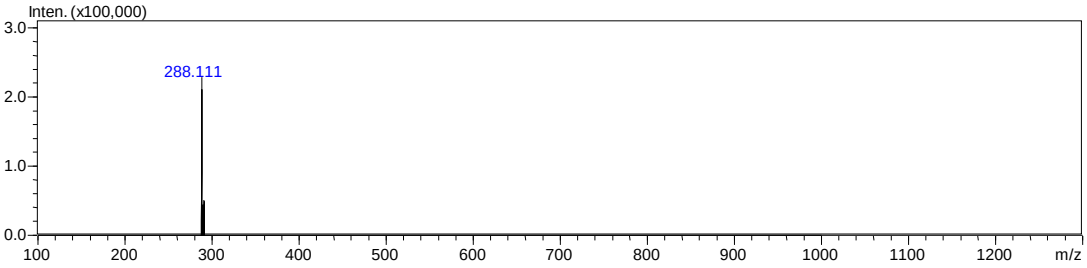


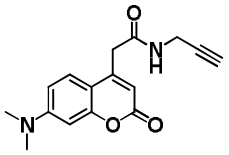


58

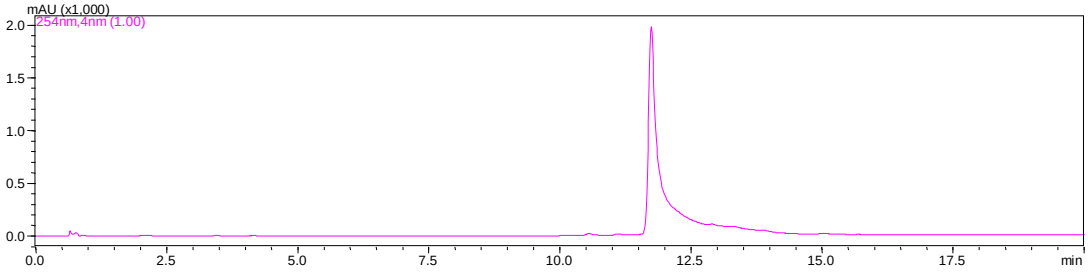


MS(E+) Ret. Time : 8.200 -> 8.213 - 8.153 <-> 8.280

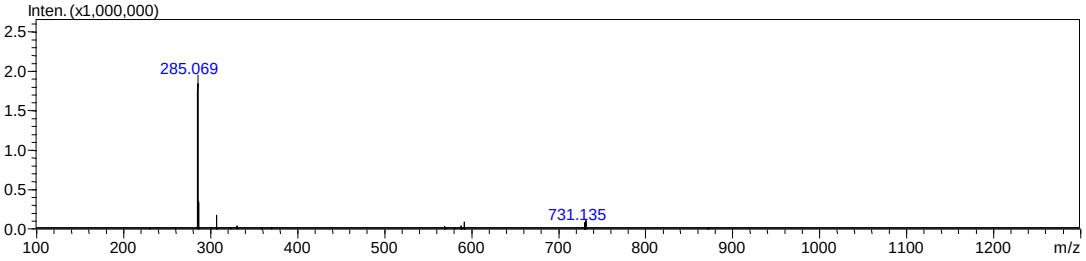


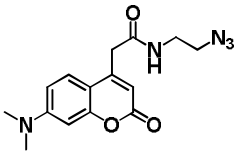


59

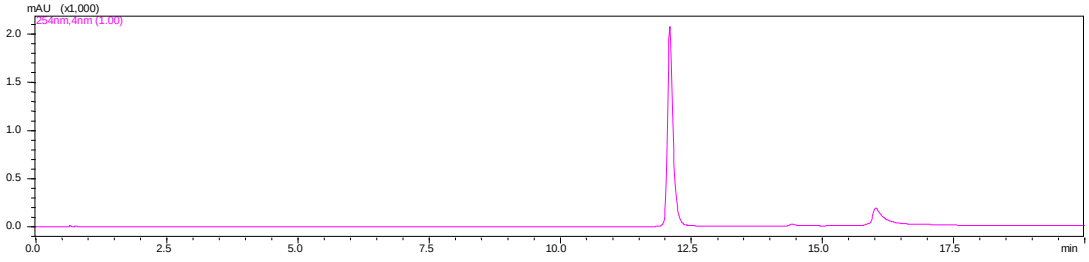


MS(E+) Ret. Time : 11.753 -> 11.767 - 11.600 <-> 11.840

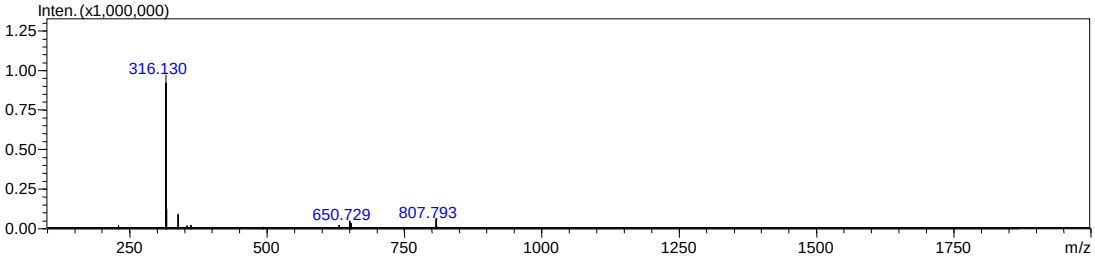


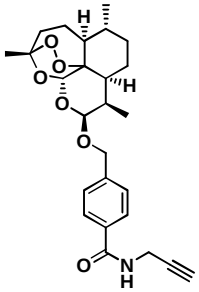


60

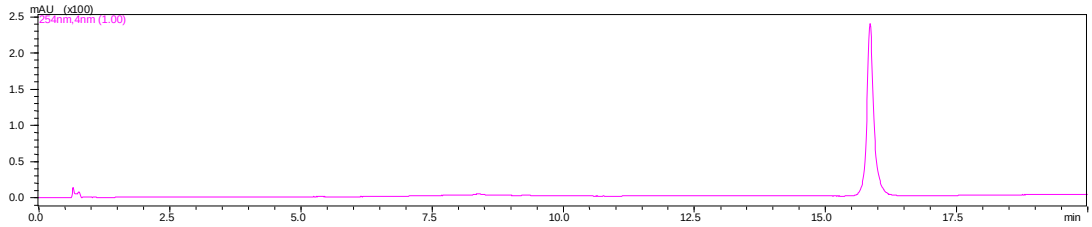


MS(E+) Ret. Time : 12.087 -> 12.100 - 11.960 <-> 12.120

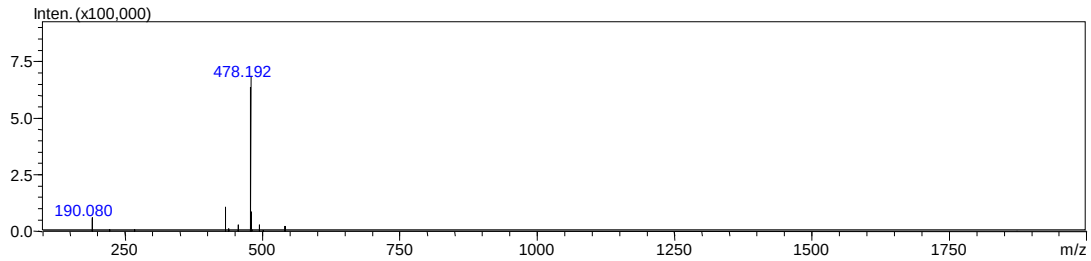


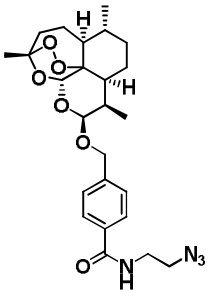


61

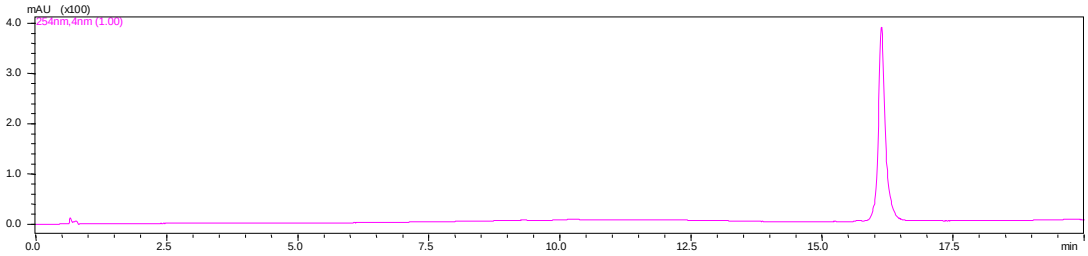


MS(E+) Ret. Time : 16.293 -> 16.307 - 16.120 <-> 16.367

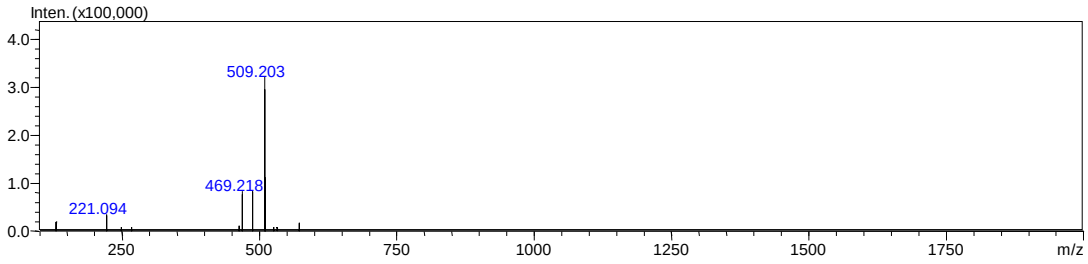


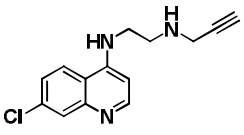


62

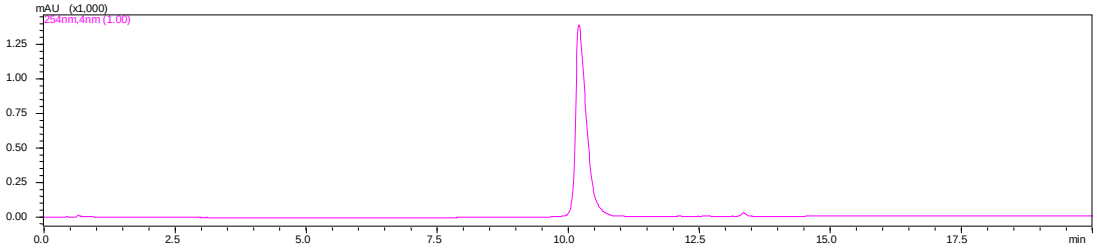


MS(E+) Ret. Time : 16.567 -> 16.580 - 16.413 <-> 16.660

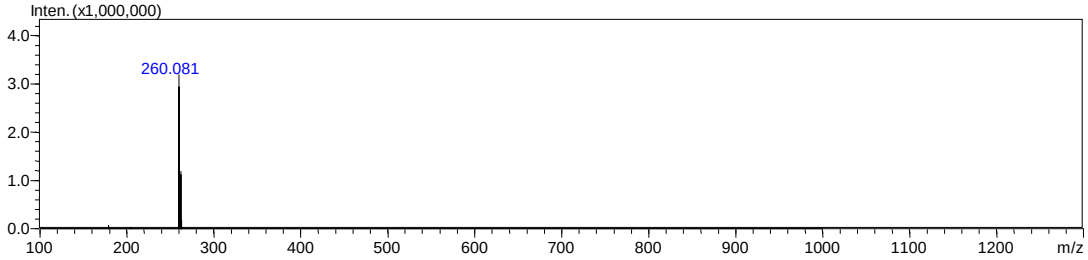


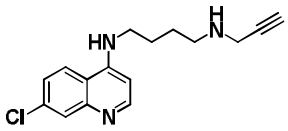


63a

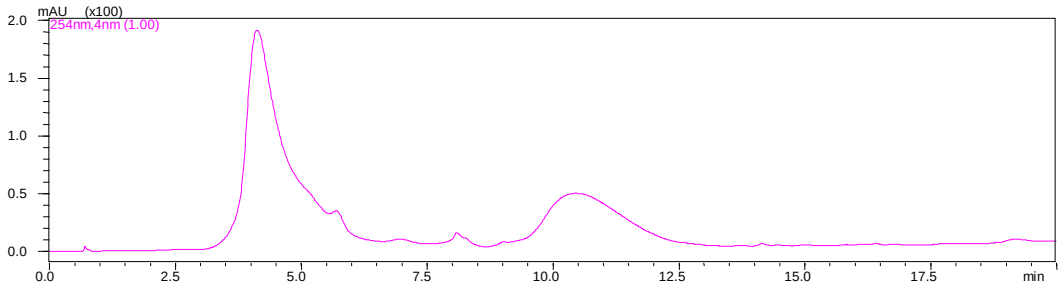


MS(E+) Ret. Time : 10.267 -> 10.280 - 10.027

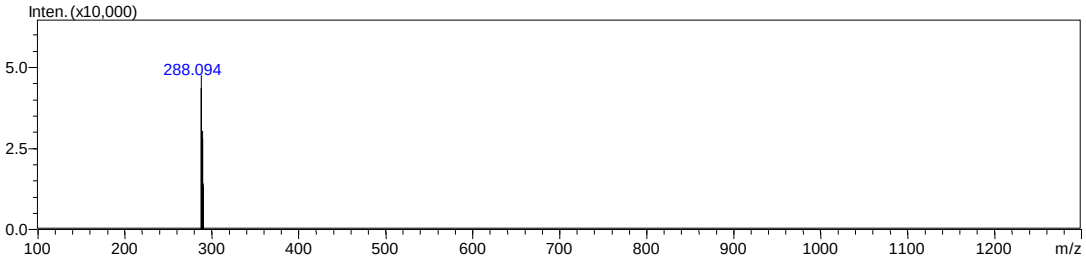


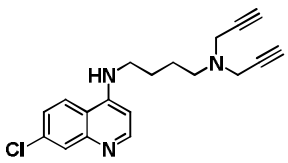


63b

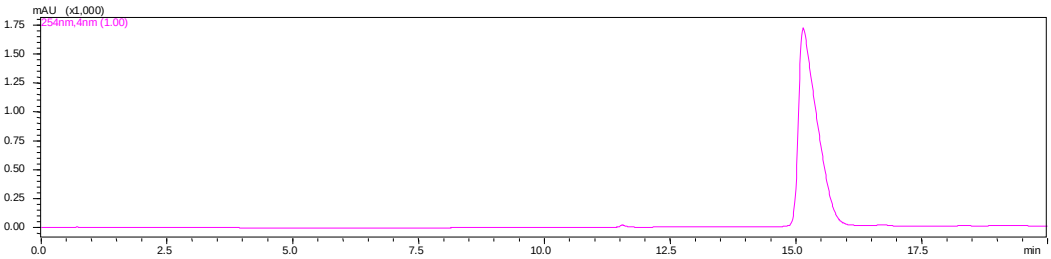


MS(E+) Ret. Time : 4.353 -> 4.367 - 4.313 <-> 4.413

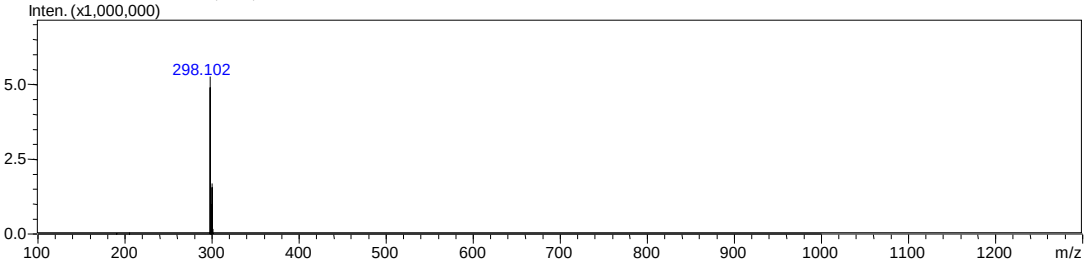


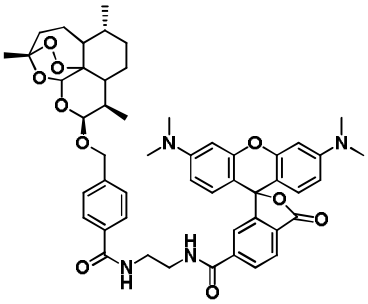


64b



Event#: 1 MS(E+) Ret. Time : 15.213 -> 15.227 - 14.893 <-> 15.253





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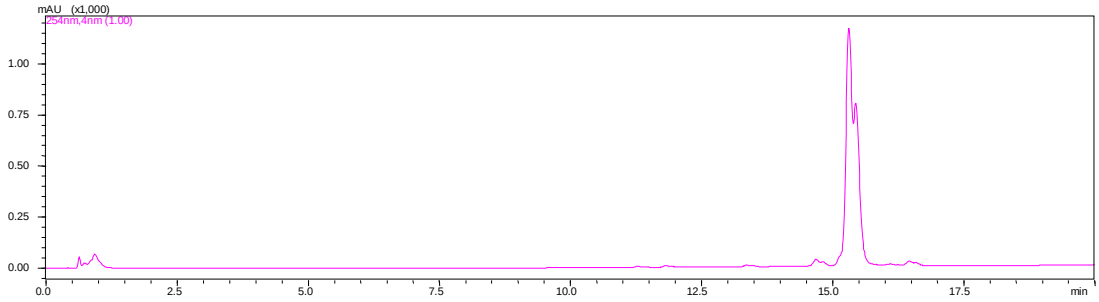
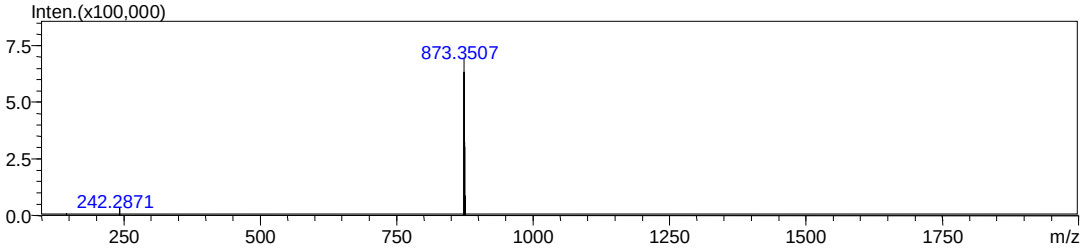
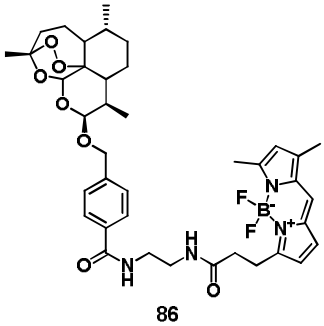
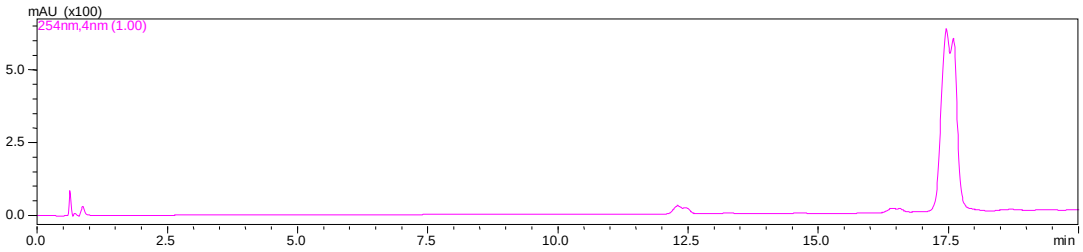


Event#: 1 MS(E+) Ret. Time : 15.393 -> 15.407 - 15.140 <-> 15.500

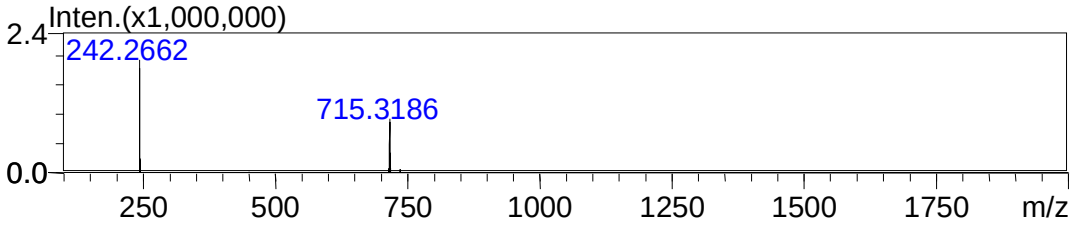


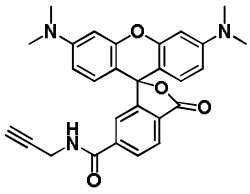


86

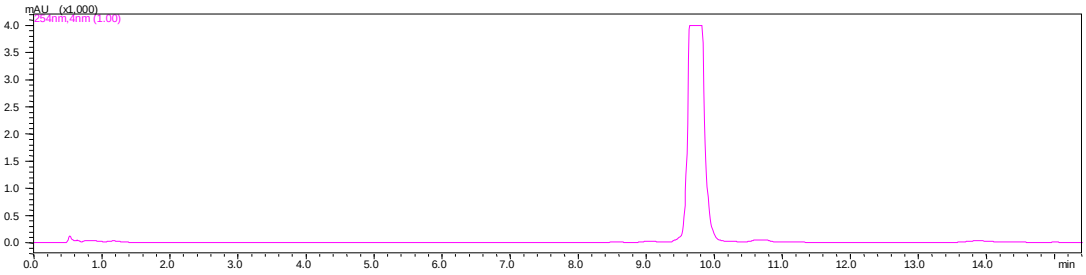


Event#: 1 MS(E+) Ret. Time : 17.533 -> 17.547 - 17.253 <-> 17.613

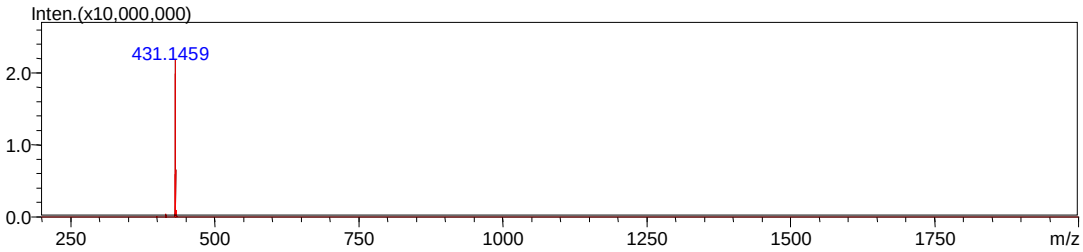


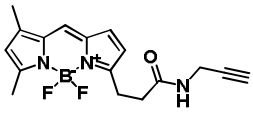


87

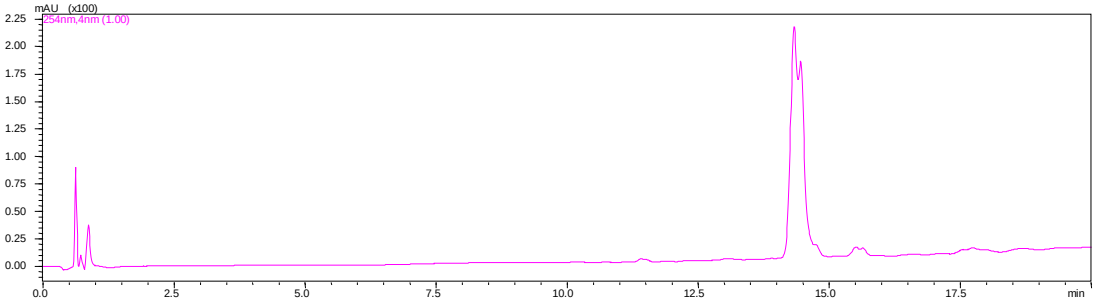


Event#: 1 MS(E+) Ret. Time : 9.760 -> 9.773 - 9.400

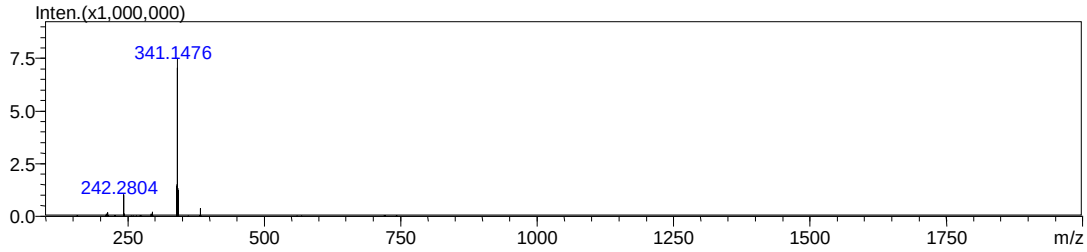


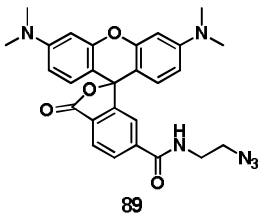


88

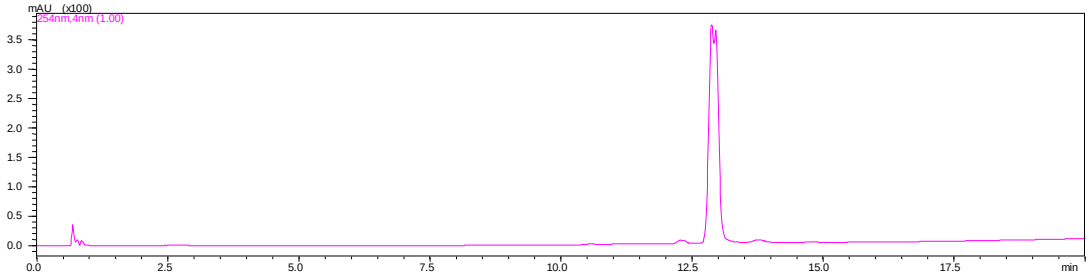


Event#: 1 MS(E+) Ret. Time : 14.367 -> 14.380 - 14.033 <-> 14.400 Scan# : 4311 -> 4315 - 4211 <-> 4321

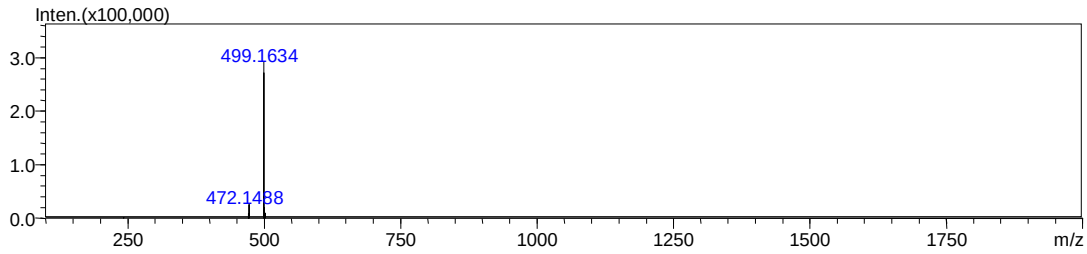


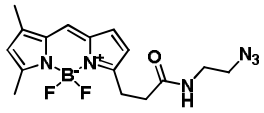


89



Event#: 1 MS(E+) Ret. Time : 12.913 -> 12.927 - 12.893 <-> 12.947 Scan# : 3875 -> 3879 - 3869 <-> 3885





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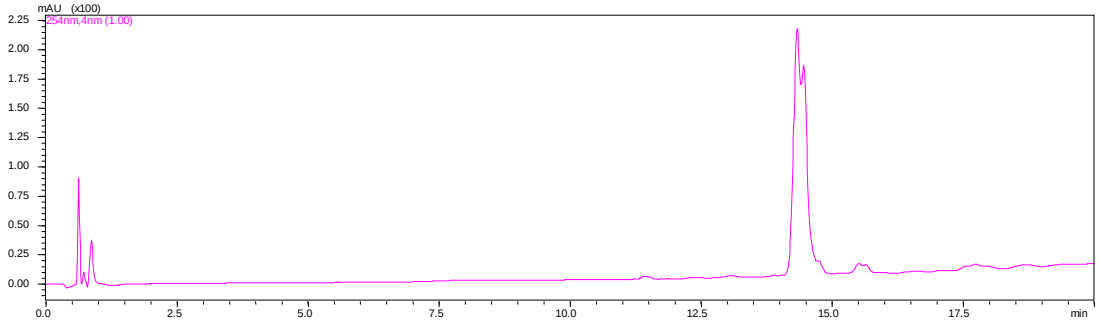
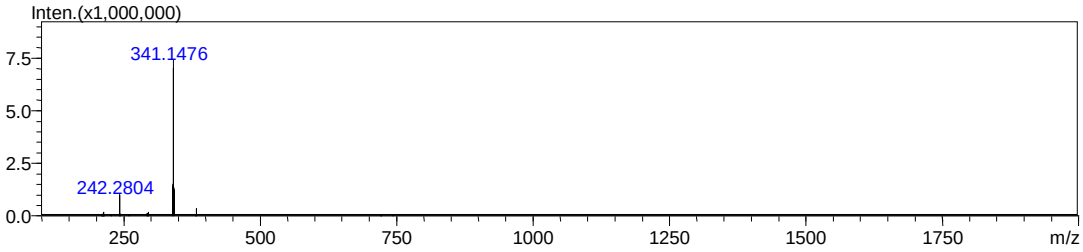
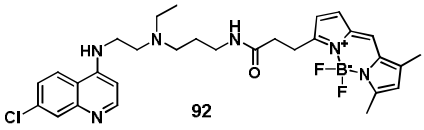


Event#: 1 MS(E+) Ret. Time : 14.367 -> 14.380 - 14.033 <-> 14.400 Scan# : 4311 -> 4315 - 4211 <-> 4321





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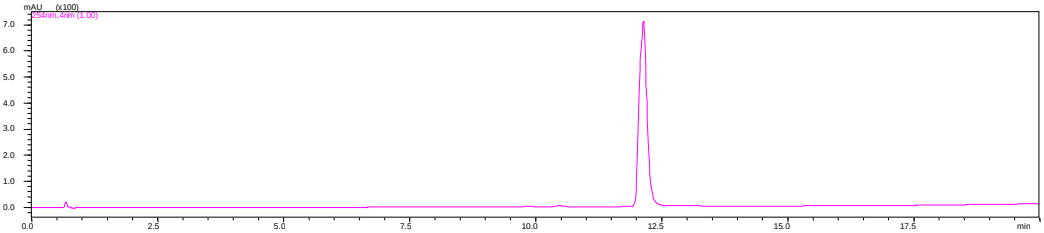
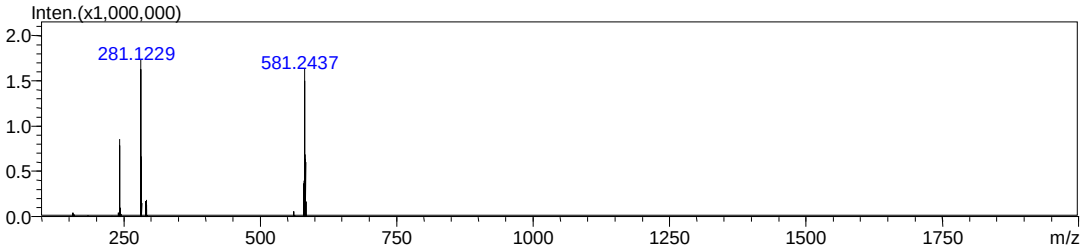
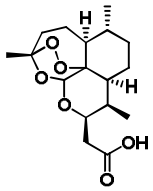
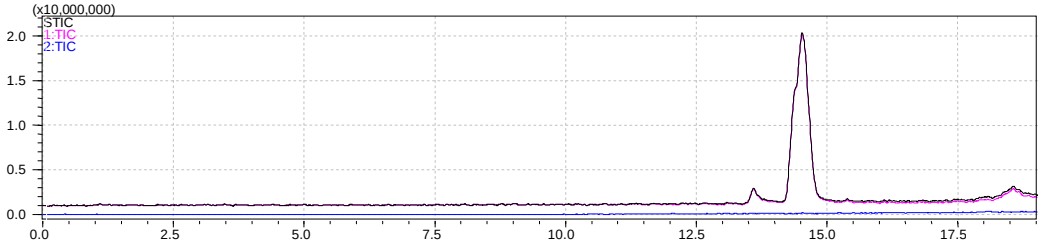


Event#: 1 MS(E+) Ret. Time : 12.193 -> 12.207 - 11.940 <-> 12.460 Scan# : 3659 -> 3663 - 3583 <-> 3739

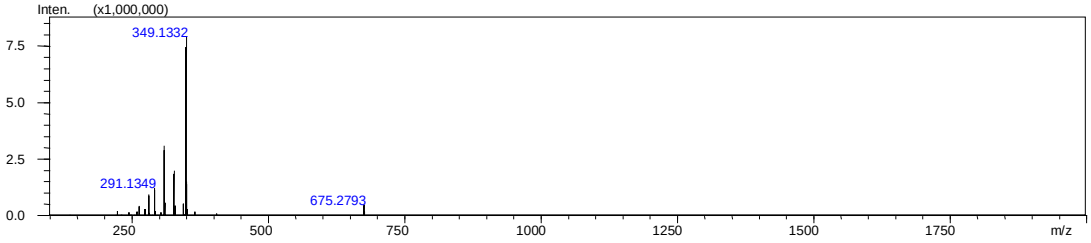


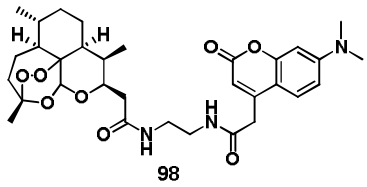


97

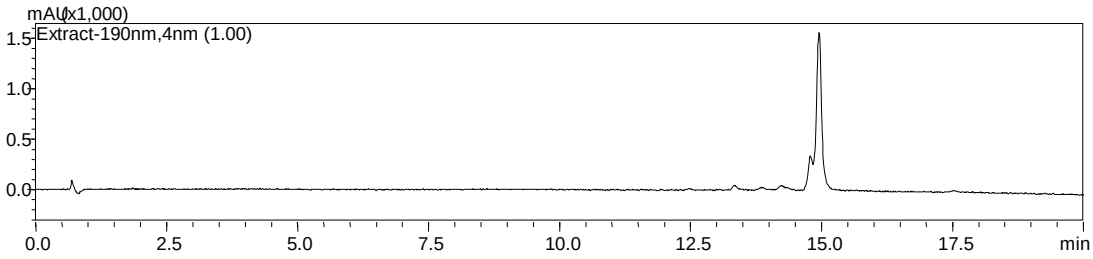


Event#: 1 MS(E+) Ret. Time : 14.520 -> 14.533 - 14.140 <-> 15.253 Scan# : 4357 -> 4361 - 4243 <-> 4577

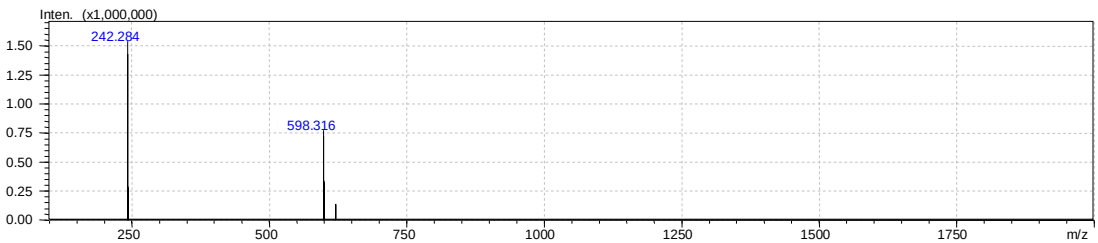


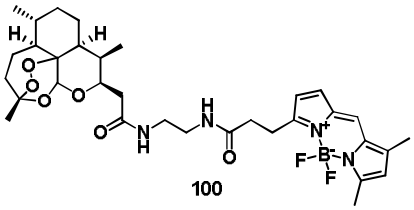


98

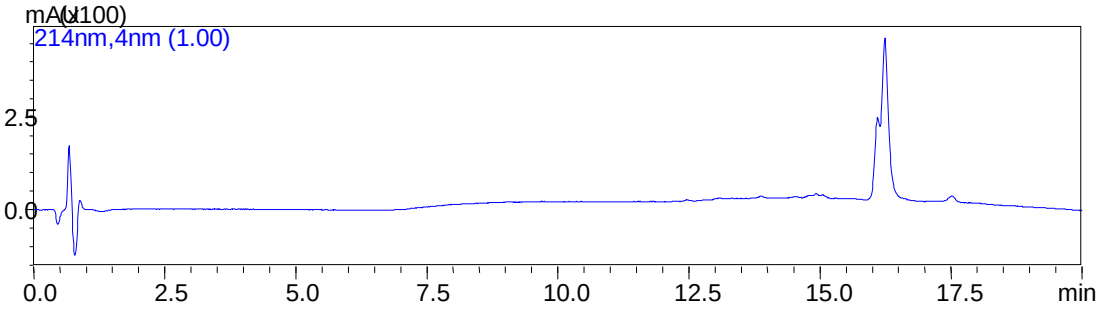


Event#: 1 MS(E+) Ret. Time : 14.947 Scan# : 4485

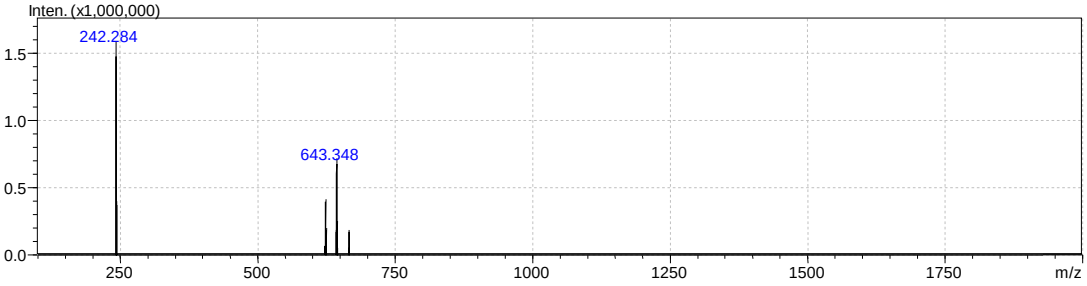


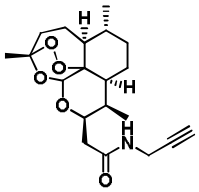


100

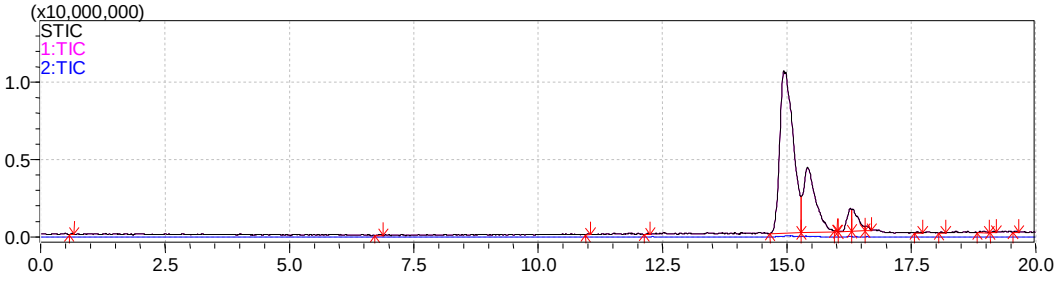


Event#: 1 MS(E+) Ret. Time : 16.327 Scan# : 4899

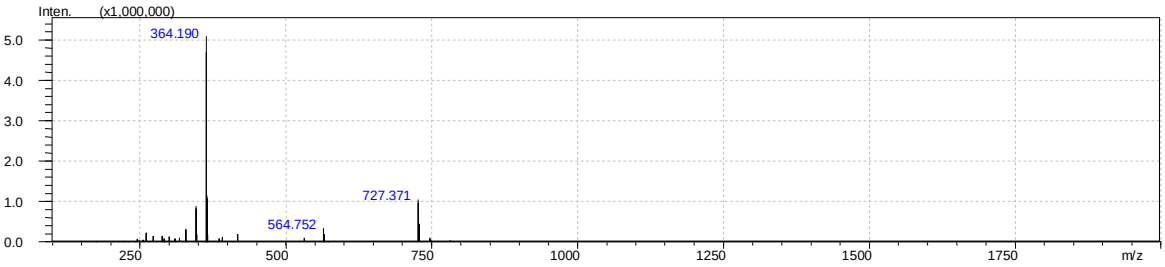


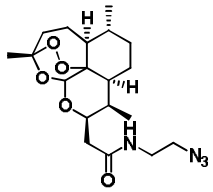


104

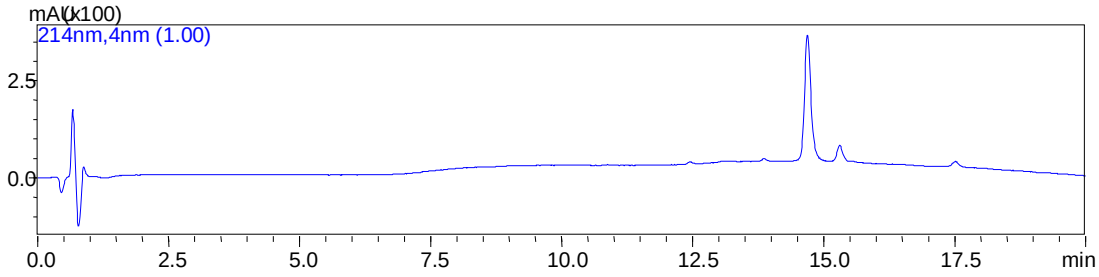


Event#: 1 MS(E+) Ret. Time : 14.933 -> 14.947 - 14.660 <-> 15.287





105



Event#: 1 MS(E+) Ret. Time : 14.767