MARISTELA BRAGA MARTINS TEIXEIRA

Synthesis of novel anthracycline derivatives containing azido glycosides

Síntese de novos derivados de antraciclinas contendo azido glicosídeos

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Concentration Area: Natural and Synthetic Products

Supervisor: Prof Dr Ivone Carvalho
Co-supervisor: Prof Dr M. Carmen Galan

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1. Anthracycline. 2. Azido glycoside. 3. Glycodiversification. 4.Cytotoxicity. 5. Cardiotoxicity.

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This thesis is dedicated to my beloved parents José Martins (*in memoriam*) and Maria da Graça, my dear brother Matheus, and my inspiring husband Luís Gustavo: my gratitude beyond measure.

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To God, before all things, for the gift of Life, for the opportunities to learn, improve and outgrow, and for the guidance along the way.

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"Science makes people reach selflessly for truth and objectivity;
it teaches people to accept reality, with wonder and admiration,
not to mention the deep awe and joy that the natural order of things
brings to the true scientist." [❁]

Lise Meitner

[❁] Lecture, Austrian UNESCO Commision, 30 March 1953. In: Meitner, L.; Hahn, O. Atomenergie und Frieden. Vienna: Wilhelm Frick-Verlag, 1954. *Schriftenreihe der Österreichischen Unesco-Kommission*, v 9, p 23-4.
Translated by: Sime, R. L. *Lise Meitner: a life in physics*. Berkeley: University of California Press, 1997, p 375.

ABSTRACT

MARTINS TEIXEIRA, M. B. Synthesis of novel anthracycline derivatives containing azido glycosides. 2018. 298 p. Thesis (Doctoral). School of Pharmaceutical Sciences of Ribeirão Preto – University of São Paulo, Ribeirão Preto, 2018.

Anthracyclines are ranked among the most effective chemotherapeutics against cancer. They are glycoside drugs comprised by the aminosugar daunosamine linked to a hydroxyanthraquinone aglycone, and act by DNA-intercalation, oxidative stress generation and topoisomerase II poisoning. Regardless of their therapeutic value, multidrug resistance and severe cardiotoxicity are important limitations arising from anthracycline treatment, prompting the discovery of novel analogues, for instance through glycodiversification. This work aimed to exploit azido glycosides, to be combined with anthracycline aglycone and generate novel glycosides. In a semi-synthesis approach, both daunorubicinone and protected doxorubicinone were glycosylated with conveniently functionalised 2-azido glucosyl and galactosyl donors, as well as glycals. A screening of glycosylation protocols involved glycosyl chlorides, imidates and thioglycosides with the most successful promoters being $HgO/HgBr_2$ (4-52% yield) and TMSOTf (38-41%); for glucals and galactals, Au(I) and Cu(I) catalysts gave moderate yields (15-46%), but thiourea-phosphoric acid was the most efficient catalyst system (18-95%). Cleavage of protecting groups proved challenging, hampering and delaying the obtention of free glycosides. Upon deprotection, the glycosides obtained included glucoside **49** (13%), 2-azido glucoside **51** (34%), 2-deoxyglucoside **58** (11%), and 2-deoxygalactoside **61** (85%), all with the daunorubicin scaffold. In cell proliferation assays, glycosides **61α** and **61β** were tested against human cancer cell lines HeLa, MDA-MB-231 and MCF-7 and a model of healthy cells (HDF), with IC_{50} in the range of 27.1 to 74.6 μM for the α anomer, and higher than 250 μM for the β anomer. Preliminary studies with human cardiomyocytes derived from induced pluripotent stem cells were inconclusive to establish a cardiac toxicity experimental model.

Keywords: Anthracycline. Azido glycoside. Glycodiversification. Cytotoxicity. Cardiotoxicity

RESUMO

MARTINS TEIXEIRA, M. B. Síntese de novos derivados de antraciclinas contendo azido glicosídeos. 2018. 298 p. Tese (Doutorado). Faculdade de Ciências Farmacêuticas de Ribeirão Preto – Universidade de São Paulo, Ribeirão Preto, 2018.

Antraciclinas estão entre os mais eficazes quimioterápicos contra o cancer. São fármacos glycosídicos compostos pelo carboidrato daunosamina ligado a uma aglicona hidróxi antraquinona, e atuam por intercalação ao DNA, geração de estresse oxidative e envenenamento de topoisomerase II. Apesar de sua utilidade terapêutica, multirresistência e cardiotoxicidade grave são importantes limitações decorrentes do tratamento com antraciclinas, estimulando a descoberta de novos análogos, por exemplo através de glicodiversificação. Este trabalho objetivou explorar azido glicosídeos, a serem combinados com agliconas de antraciclinas para gerar novos glicosídeos. Em uma estratégia semi-sintética, daunorrubicinona e doxorrubicinona protegida foram glicosiladas com doadores 2-azido glucosídicos e -galactosídicos, além de glicais. Uma varredura de metodologias de glicosilação envolveu cloretos, imidatos e tioglicosídeos, sendo os promotores com melhores rendimentos $HgO/HgBr_2$ (4-52%) e TMSOTf (38-41%); para glucais e galactais, catalisadores de Au(I) and Cu(I) forneceram moderados rendimentos (15-46%), mas o sistema mais eficiente foi o organocatalisador de tiouréia e ácido fosfórico (18-95%). A clivagem dos grupos de proteção foi desafiadora, dificultando e atrasando a obtenção dos glicosídeos livres. Mediante desproteção, os glicosídeos obtidos incluíram glucosídeo **49** (13%), 2-azido glucosídeo **51** (34%), 2-desóxi glucosídeo **58** (11%) e 2-desóxi galactosídeo **61** (85%), todos com o esqueleto de daunorrubicina. Em ensaios de proliferação celular, os glicosídeos **61α** e **61β** foram testados em linhagens de células tumorais humanas HeLa, MDA-MB-231 e MCF-7 e um modelo de células sadias (HDF), com IC_{50} na faixa de 27.1 a 74.6 μM para o anômero α, e superior a 250 μM para o anômero β. Estudos preliminares com cardiomiócitos humanos derivados de células-tronco induzidas foram inconclusivos para estabelecer um modelo experimental de toxicidade cardíaca.

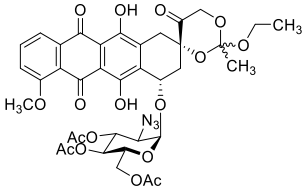
Palavras-chave: Antraciclina. Azido glicosídeo. Glicodiversificação. Citotoxicidade. Cardiotoxicidade

LIST OF ABBREVIATIONS AND ACRONYMS

Ac	acetyl
BINOL	1,1 -bi-2-naphthol
Bn	benzyl
t-Bu	*tert*-butyl
Bz	benzoyl
calcd	calculated
CAN	ceric ammonium nitrate
DAU	daunorubicin
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCE	1,2-dichloroethane
DCM	dichloromethane
DIPEA	diisopropylethylamine
DMAP	4-(*N,N*-dimethylamino)pyridine
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DNS	daunosamine
DOX	doxorubicin
DTBS	di-*tert*-butylsilylene
EDTA	ethylenediaminetetraacetic acid
equiv	equivalent
ESI	electrospray ionisation
Et	ethyl
Et_2O	diethyl ether
Et_3N	triethylamine
EtOAc	ethyl acetate
EtOH	ethanol
FBS	fetal bovine serum
Gal	galactose
Glc	glucose
Hex	hexane
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectrometry
IR	infrared

MALDI	matrix-assisted laser desorption ionization
Me	methyl
MeCN	acetonitrile
MeOH	methanol
MS	mass spectrometry
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
NIS	*N*-iodosuccinimide
NMR	nuclear magnetic resonance
P-gp	P-glycoprotein
Ph	phenyl
*i*Pr	isopropyl
redox	reduction-oxidation
R_f	retention factor
ROS	reactive oxygen species
sat.	saturated
S_N	nucleophilic substitution
TBAF	tetrabutylammonium fluoride
TBAI	tetrabutylammonium iodide
TBDPS	*tert*-butyldiphenylsilyl
TBS	*tert*-butyldimethylsilyl
TCT	trichlorotriazine
Tf	trifluoromethanesulfonyl (triflyl)
THF	tetrahydrofuran
TIPDS	tetraisopropyldisiloxane
TIPS	triisopropylsilyl
TLC	thin-layer chromatography
TMS	trimethylsilyl
TOF	time-of-flight
Tol	toluene
Top2	topoisomerase II enzyme
Tr	triphenylmethyl (trityl)
Troc	2,2,2-trichloroethoxycarbonyl
Ts	*para*-toluenesulfonyl (tosyl)
TTBP	2,4,6-tri-*tert*-butylpyrimidine
UV-vis	ultraviolet- visible
WHO	World Health Organization

LIST OF COMPOUNDS



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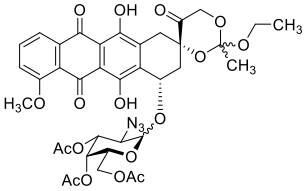

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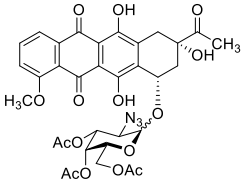

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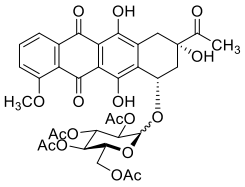

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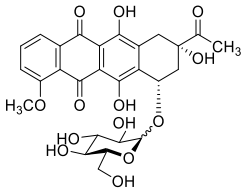

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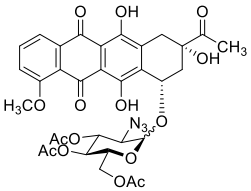

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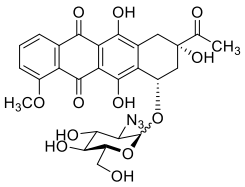

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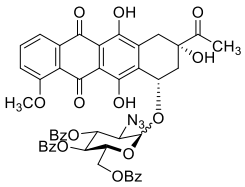

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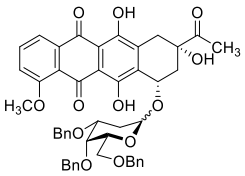

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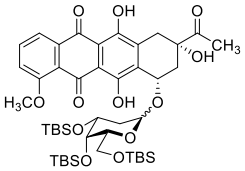

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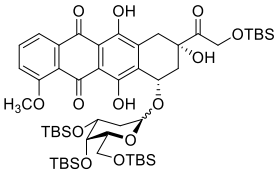

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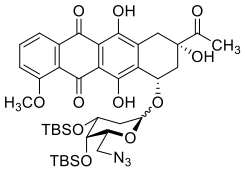

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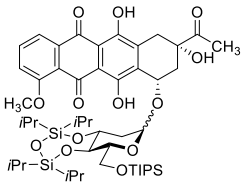

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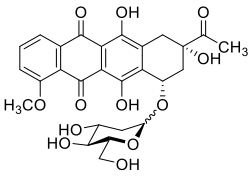

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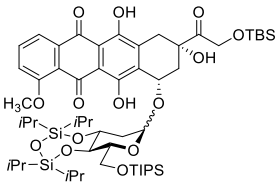

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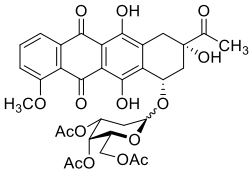

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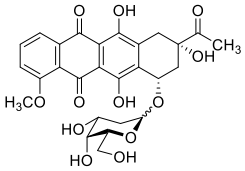

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SUMMARY

Introduction

1 INTRODUCTION

1.1 Cancer and anticancer treatment

The global cancer burden has been continually rising, with predictions to keep growing throughout the coming decades, associated with population ageing and contemporary lifestyle, especially in lower-resource countries that are undergoing major social, demographic, and economic transitions. According to the most current appraisals of cancer incidence and mortality worldwide, the World Health Organization (WHO) estimates more than 18 million new cases diagnosed in 2018, which is projected to increase by over 60% in the next two decades; and at least 9.6 million deaths from cancer during this year, placing the disease as the second leading cause of death globally.[1-3]

Cancer is characterised by abnormal cell transformation with unsuppressed growth and spreading, due to the gradual acquirement of cellular capabilities, such as limitless proliferation and evasion from control mechanisms, known as cancer hallmarks. Ultimately, such multistage process results from cumulative genetic mutations that dysregulate proto-oncogenes and tumour suppressor genes, causing genome instability. In turn, genomic alterations are multifactorial, ensued from the interaction between the individual genetic profile and external agents, including physical, chemical, and biological carcinogens.[4-6]

Among the nearly two hundred existing cancer types, the most common incidence sites are lung, breast, colorectum, prostate, stomach and liver. Although the classification of cancers is traditionally based on the organ of origin combined with histological typing, the increasing knowledge on tumour genomics is providing a deeper refinement of cancer complexity at the molecular level, which is expected to provide better prognostic power and more precisely targeted therapies.[3, 7]

A milestone in anticancer therapy was the discovery of cytotoxic agents in the last century, improving survival rates and the quality of life for cancer patients.[6, 8] Currently, there is a vast therapeutic armoury available for the treatment of a variety of cancer types: alkylating agents, intercalators, antimetabolites, antimitotic, enzyme inhibitors, hormone antagonists, in addition to immuno and gene therapies (Figure 1).

Underlying many of these antineoplastic classes is the disruption of nuclear mechanisms, which kills cancer cells by strategically exploiting their rapid replication as a selectivity feature. However, these drugs also affect healthy tissues that inherently have a

high cell renovation rate, causing recurrent side effects, such as bone marrow suppression, hair loss, mucosal irritation, gastrointestinal disturbances, and many others.[9]

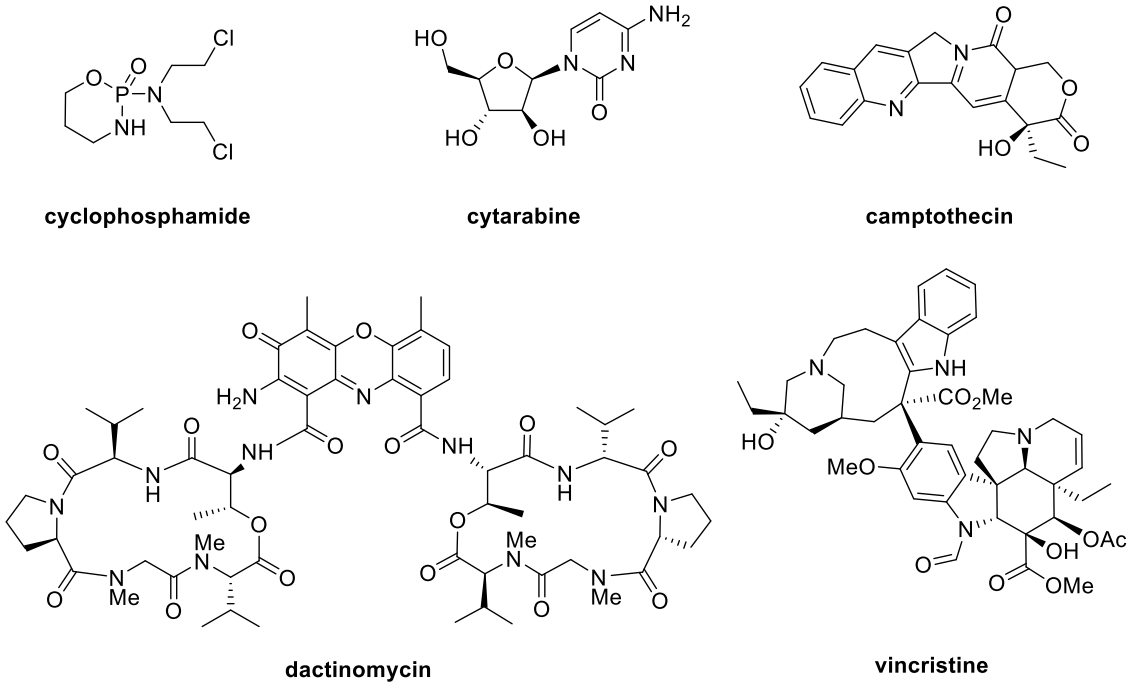


Figure 1. Selected examples of cytotoxic drugs affecting cell replication processes. Cyclofosfamide, a DNA-alkylating agent; cytarabine, a DNA polymerase inhibitor and chain terminator; camptothecin: a topoisomerase I poison; dactinomycin, a DNA intercalator; vincristine, a tubulin-polymerisation inhibitor;

1.2 Anthracyclines

Anthracyclines comprise a class of natural antibiotics among the most effective antineoplastic agents in current clinical use, with few unresponsive cancer types. The first representative compounds of this family were isolated from a mutant strain of the actinobacteria *Streptomyces peucetius* by Arcamone and Di Marco, as part of a systematic search for antitumour agents by an industrial company (Farmitalia Research Laboratories of Milan). Daunorubicin (**DAU**) was described in 1964, followed by doxorubicin (**DOX**) in 1969, when they used to be named "daunomycin" and "adriamycin", respectively (Figure 2). They were soon tested clinically, achieved registration in the early 1970s, and have been marketed since then, becoming the prototypes of the anthracycline class.[10-13]

After half a century of their discovery, the so-called first-generation anthracyclines are still frequently prescribed today and persist as a mainstay in many chemotherapeutic schemes. Ranked among the most potent and widely useful anticancer drugs, **DAU** and **DOX** are

consistently listed by WHO as essential medicines for cancer treatment. While daunorubicin is indicated against acute lymphoblastic and myeloblastic leukaemias, doxorubicin is more active on lymphomas, sarcomas, and a broad spectrum of solid tumours, including breast, lung, bladder, bone, and cervical cancers. [6, 13-15]

Such a distinct range of anticancer activity between daunorubicin and doxorubicin is defined by a minor structural difference, as shown in Figure 2. Overproduced by a number of strains, **DAU** is the immediate biosynthetic precursor of **DOX** in *Streptomyces peucetius caesius* ATCC 27952, the single strain reported to produce doxorubicin, and which complete genome was recently sequenced. However, to date, it proved not to be amenable for scaling up, as strain improvement techniques and genetic engineering have not delivered a cost-effective fermentation process for **DOX** yet. Therefore, on the industrial scale, doxorubicin is prepared by semisynthesis from daunorubicin, through a chemical bromination at C-14, followed by displacement of the bromine by hydroxide with mild base treatment.[13, 16, 17]

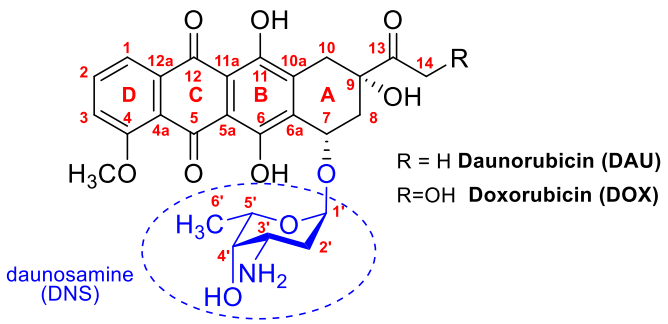


Figure 2. The first generation of anthracyclines: daunorubicin and doxorubicin. The 3-amino-2-deoxyglycosyl unit "daunosamine" is stressed. Conventional structural numbering and naming in accordance with Brockmann (1963), who described the isolation and elucidation of the first anthracycline compounds.[18]

Both daunorubicin and doxorubicin are natural product glycosides derived from microbial polyketide biogenetic intermediates, sharing a polyhydroxy anthraquinone skeleton (rings B, C and D) fused to a fourth saturate substituted ring (A), altogether corresponding to the aglycone moiety. The side chain at C-9 differentiates **DOX** from **DAU**, the former holding an extra hydroxyl group at position 14. The hydroxylated tricyclic quinone system is the chromophore responsible for the red to orange colour characteristic of anthracyclines, which absorb around the 480 nm range of the visible light spectrum. Attached to ring A at the benzylic position 7 is the unique glycone moiety 3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranose, also known simply as daunosamine (**DNS**), an unusual amino deoxy sugar conserved in tens of anthracycline congeners and considered crucial for their anticancer activity.[13, 18]

Many other bioactive natural products contain glycosides, in which the carbohydrate residues are often essential, as illustrated in Figure 3. Some types of carbohydrates are repeatedly present in various classes of glycoconjugates, such as 2-deoxysugars,[19] found in antiparasitic macrocyclic lactones (ivermectin B_{1a}), aureolic acid antibiotics (chromomycin A3), and cardiotonic agents (digoxin), for example. In addition, aminosugars, besides occurring most abundantly in structural polysaccharides, such as glucosamine in chitin and galactosamine in chondroitin, also exist in many rare and complex natural product glycosides, as is the case of 3-amino sugars in antibiotics like vancomycin, amphotericin B and erythromycin (Figure 3). Cytotoxic anthracyclines combine both structural features enclosed in the unique 3-amino-2-deoxyglycosyl residue daunosamine.[20]

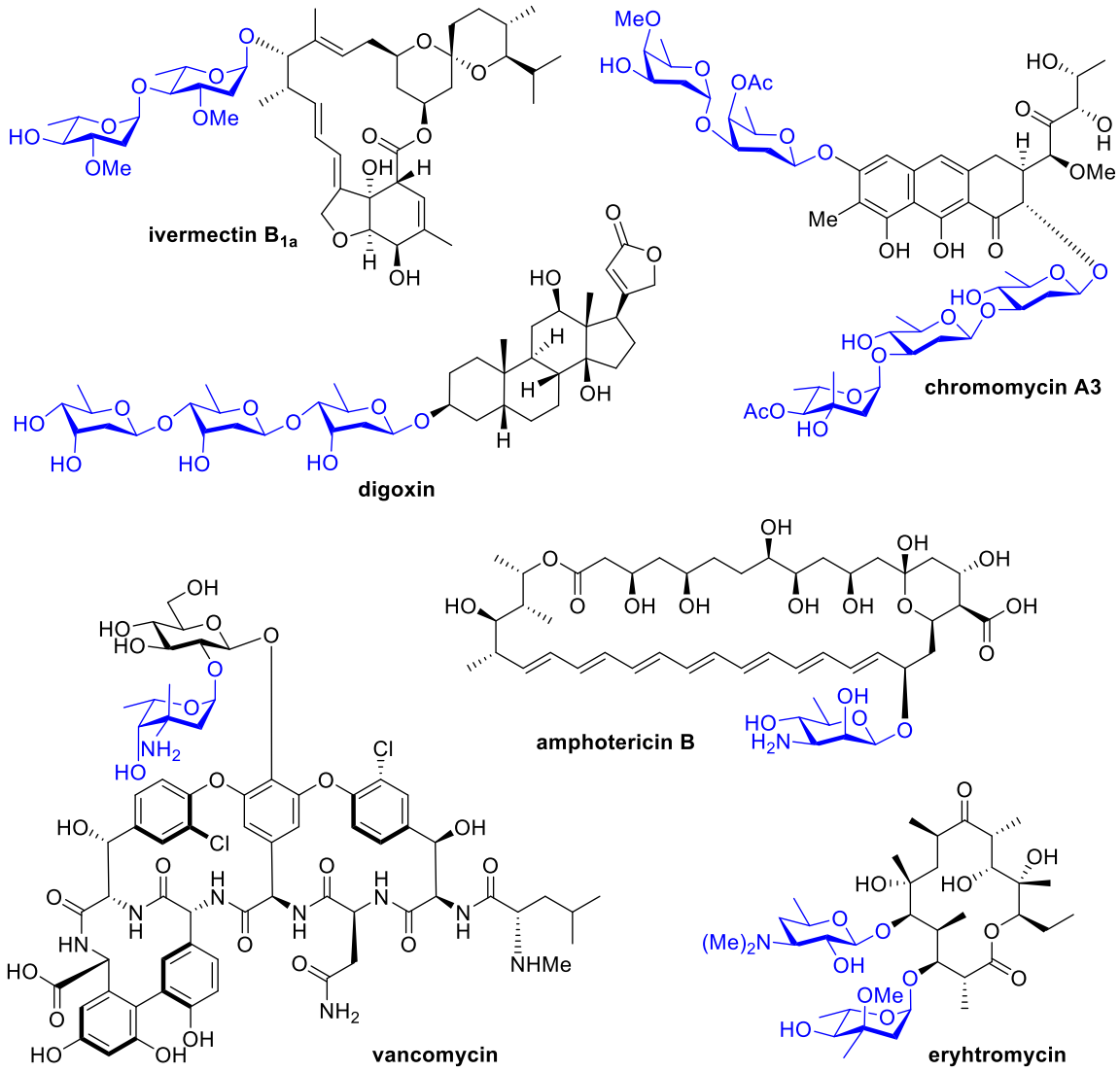


Figure 3. Selected examples of glycoside drugs containing 2-deoxy and 3-amino-3-deoxy carbohydrates, highlighting the glycones.

1.2.1 Mechanisms of action and structure-activity relationship considerations

Anthracyclines cytotoxic activity results from a strong inhibitory effect on nucleic acid synthesis, but there has been substantial controversy and debate over their exact molecular mechanisms of action. It is now generally recognised that anthracyclines act through a combination of multiple mechanisms and that the most consistent are, namely: induction of oxidative stress, intercalation into DNA, and more importantly, poisoning of topoisomerase II enzyme (Top2).[15, 21]

The formation of reactive oxygen species (ROS) induced by anthracyclines can be initiated by one-electron reductions of either the quinone into semi-quinone or chelated ferrous into ferric ions. These electron transfers trigger enzyme-mediated reduction-oxidation (redox) cycles that ultimately produce very destructive hydroxyl radicals, causing protein alkylation, lipid peroxidation, and particularly direct DNA damage and cross-links, followed by all downstream cellular cascades leading to cell death (Figure 4). Interestingly, the ability of anthracyclines to boost ROS production is also largely associated with their toxic effects, predominantly on myocardial tissue.[15, 22, 23]

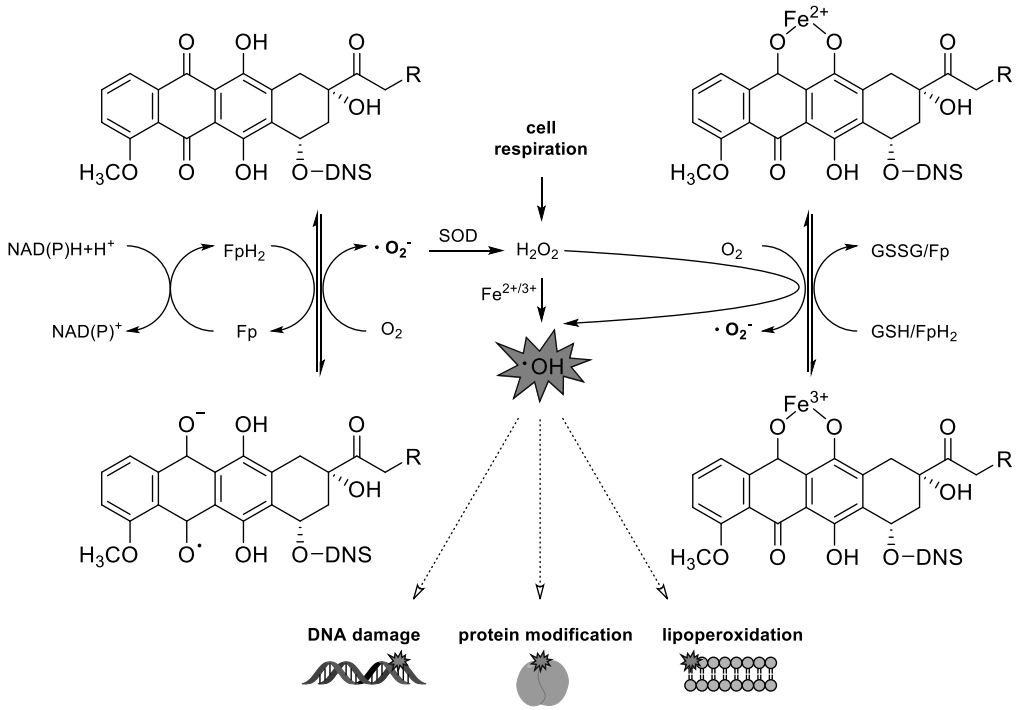


Figure 4. Schematic main pathways of anthracycline-induced and iron catalysed oxidative stress generation. Fp: flavoprotein, GSH/GSSH: reduced/oxidised glutathione, NAD(P): nicotinamide adenine dinucleotide (phosphate), SOD: superoxide dismutase. Adapted from Sterba (2013).[22]

Owing to their aglycone planar structure, comprised by a polyaromatic system, anthracyclines are capable of intercalating DNA. More specifically, rings B and C from the anthraquinone moiety stack between neighbouring base pairs, pushing them apart to result in bidirectional positive torsion of the double helix. Ring D occupies the major groove, while ring A and daunosamine project into the minor groove, as shown by the interaction of doxorubicin with a short DNA sequence in a crystalline structure, illustrated in Figure 5.[24, 25] Although intercalation itself can distort DNA and possibly interfere with the nuclear functions, it is considered necessary but not sufficient for the optimal cytotoxic activity of the anthracyclines, in particular at the *in vivo* concentrations elicited by therapeutic doses.[15, 22]

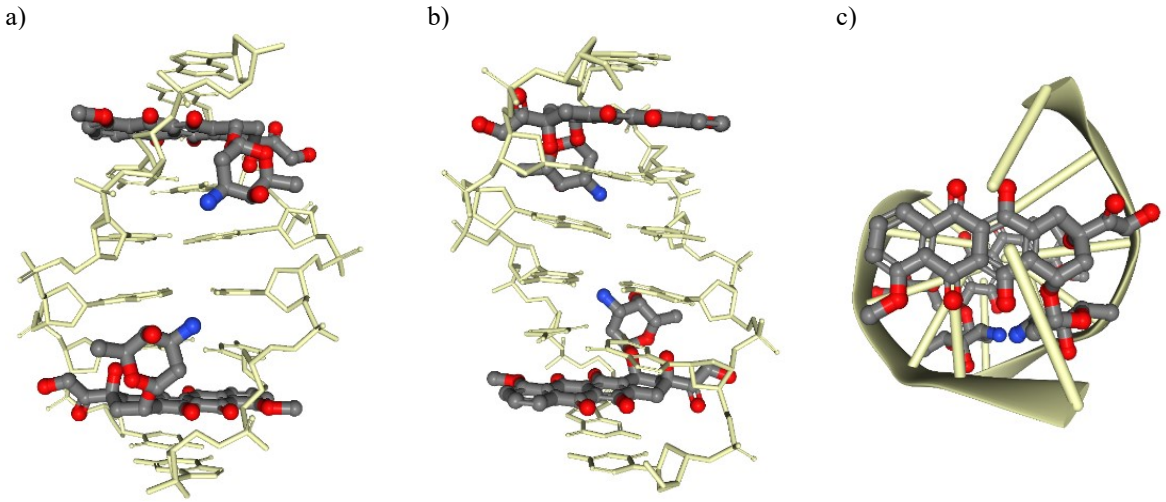


Figure 5. Crystallographic complex of two doxorubicin molecules with DNA sequence CGATCG, PDB 1P20. Adapted from [25] Lateral views from the minor groove (a) and major groove (b) perspectives; c) helix axial view projected through the anthracycline plane. Illustrations generated with web-based NGL Viewer.[26]

Intercalation becomes essential as part of the primary mechanism of action of anthracyclines, the poisoning of topoisomerase II enzyme. Observing other Top2 poisons, an obvious shared structural feature is the polyaromatic intercalating moieties (Figure 6).[27] Topoisomerase II is a nuclear enzyme that adjusts the torsion state of DNA, which tends to form supercoiled structures during replication and transcription over the cell cycle. To relief supercoiling tension, Top2 promotes transient breaks in both strands of a duplex DNA, passing an intact DNA double helix through the open gap, and then reseals the broken strands. In specific recognition sites, Top2 cleaves one phosphodiester bond on each strand of DNA and covalently binds to the 5′ OH of DNA backbone through the active site tyrosyl group, stabilising the broken strands in a cleavable complex. After unknotting, the enzyme promotes relegation of the broken strands and releases the restored duplex DNA (Figure 7).[27-29]

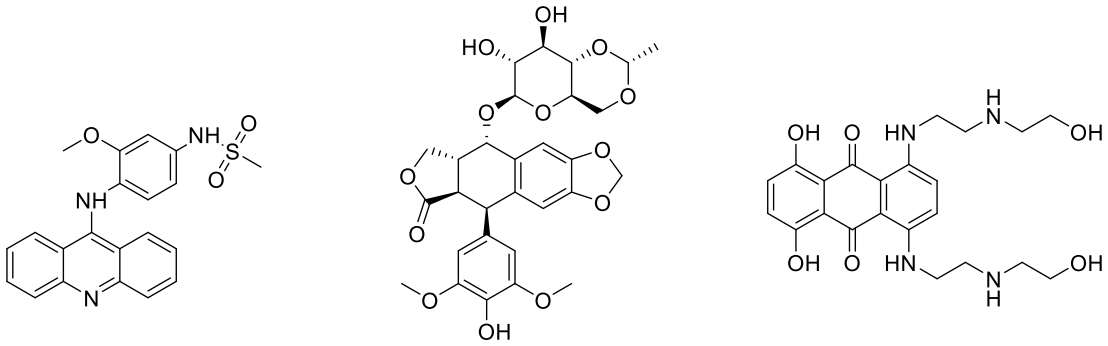


Figure 6. Anticancer drugs that target topoisomerase II. Intercalating moieties are essential for the poisoning of Top2 and are conserved in different poisons.

Anthracyclines stabilise the cleavable complex between Top2 and DNA, trapping the covalent intermediate into a ternary drug-DNA-enzyme complex, which prevents topoisomerase from properly regenerating the broken phosphodiester bonds. These drugs subvert the physiological enzyme functions, converting topoisomerase into a DNA-breaking nuclease, which leads to genomic instability and triggers apoptotic cell death.[15, 23, 30-32]

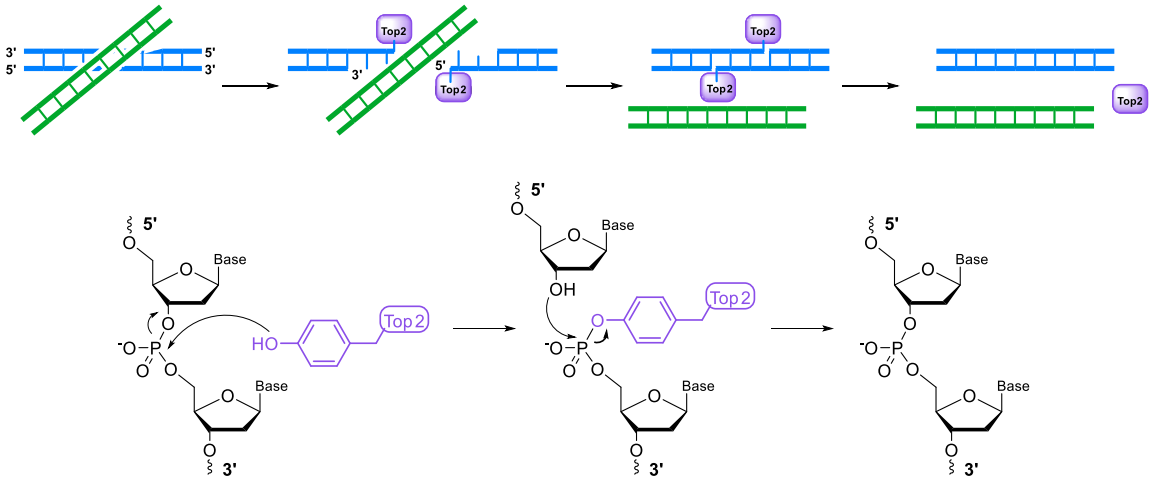


Figure 7. Catalytic mechanism of topoisomerase II. A transesterification occurs between the enzyme tyrosyl residue and DNA phosphodiester, breaking the DNA backbone bond and the forming a covalent enzyme–DNA intermediate. It allows another DNA chain to cross, after which the reversal reaction re-establishes the phosphodiester bond. Anthracyclines stabilise the intermediate in a ternary complex, poisoning the enzyme.

In this mechanism of action, while the planar aromatic aglycone stacks between DNA base pairs, the glycosyl unit plays a crucial role, projecting through the helix groove to bridge the interaction with topoisomerase II and stabilise the ternary complex, the basic amino group being determinant for the stabilisation and binding affinity (Figure 8). Changing the

configuration of daunosamine to the β anomer modifies the mode of binding. Blocking the amino function with amide group reduces cytotoxicity, but the replacement at C-3′ with a small group such as hydroxyl retains comparable activity while averting multidrug resistance.[31, 33] Conformational and crystallographic studies show that daunosamine is the most flexible domain in anthracyclines, possibly adopting numerous stable conformations for an optimal fit of the sugar at the interface between DNA and topoisomerase. Thus, modifications in the amino sugar are not only tolerable regarding bioactivity, but also of potential interest in drug development.[33, 34]

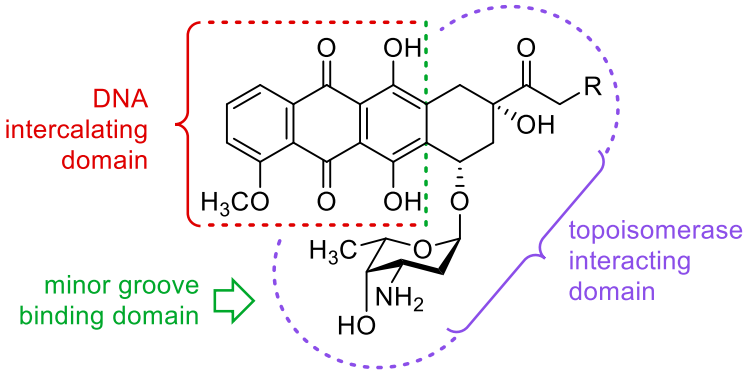


Figure 8. Anthracycline domains relevant for the binding to DNA and topoisomerase enzyme, and consequently to the pharmacological activity.

1.2.2 Limitations of anthracyclines

Despite their widely acknowledged efficacy, anthracyclines clinical use is significantly limited by their toxicity, the most serious side effects manifesting in the myocardial tissue. Chronic cardiomyopathy is dose-dependent and develops in 5-20% of patients receiving cumulative doses higher than 500 mg/m^2 of doxorubicin and 900 mg/m^2 of daunorubicin. Within months or years after treatment, symptomatic congestive heart failure evolves, which is severe, progressive, irreversible, usually refractory to conventional therapy, and associated with high mortality rates.[6, 15, 35]

The mechanisms underlying anthracycline-induced cardiotoxicity are complex, multifactorial, and not fully elucidated yet. The most accepted is the generation of reactive oxygen species in cardiomyocytes, which cells are particularly susceptible to free radical damage due to their deficiency in catalase and dismutase enzymes. ROS are overproduced by one-electron reductions, mediated by chelated iron and subsequent redox cycles of

flavoproteins (NAD(P)H oxidoreductases) (Figure 4). The biotransformation of anthracyclines involves the two-electron reduction of the C-13 carbonyl into secondary alcohol (doxorubicinol or daunorubicinol), via sequential metabolism by aldo-ketoreductase and carbonyl reductase enzymes. These metabolites greatly accumulate in cardiomyocytes, and are particularly harmful to these cells, especially regarding iron-dependent mechanisms. Mitochondrial dysfunction, calcium misbalance and apoptosis induction are other alterations involved in anthracycline cardiotoxicity.[15, 35-39] Moreover, Top2β isoform present in cardiomyocytes was shown to mediate doxorubicin-induced heart damage, whilst Top2α isoform overexpressed in cancers cells is the molecular target of anthracycline therapeutic activity.[40, 41]

A second major limitation related to anthracyclines is the development of resistance by certain cancer cells, which can be rendered by several specific inherent or acquired factors. For example reduced expression or activity of topoisomerase II, decreasing enzyme-mediated DNA damage; overexpression of superoxide dismutase enzyme, reinforcing cell defences against oxidative stress; suppression or mutation of p53, affecting apoptotic signalling pathways to prevent cell death. More importantly, multidrug resistance through altered membrane transport is the primary mechanism behind resistance to anthracyclines, exploited by cancer cells to evade the toxic effects of chemotherapeutics.[42]

In multidrug resistant cells, active drug efflux is mediated by proteins of the ATP binding cassette family, markedly P-glycoprotein (P-gp) and MRP transporters, which are overexpressed on the cell surface. These efflux pumps are capable of recognising and expelling not only the drug which induced the resistance but a great diversity of drugs without any structural or functional similarity. Anthracyclines are known substrates for such nonspecific transporters, decreasing intracellular drug concentrations, diminishing its effectiveness and thus resulting in drug resistance. [43-45]

1.2.3 New generation anthracycline and other analogues

In the pursuit for better anthracyclines that could overcome the cardiotoxicity and resistance limitations of the parent drugs, thousands of analogues have been isolated or synthesised, over the past decades, including modifications in the tetracyclic rings, the side chain and the amino sugar. The initial rationale was to avoid alterations in the general architecture of the molecule and the chemical functionalities, to retain the structural requirements for action, giving rise to second-generation anthracyclines, among which epirubicin and idarubicin proved to be clinically useful and are the most prominent derivatives.[46]

Epirubicin is a doxorubicin analogue in which the configuration of the C-4′ hydroxyl group is inverted, so the substituent lies in the equatorial orientation (Figure 9). It was first synthesised by glycosylation of the aglycone with a protected L-acosamine (3-amino-2,3,6-trideoxy-α-L-*arabino*-hexopyranose), but further development allowed to perform epimerisation directly on the anthracycline glycoside. Epirubicin is about 30% less cardiotoxic than **DOX**, owing to differences in the pharmacokinetic profile, through extensive detoxification as a 4′ glucuronide, without affecting the antitumour properties of the drug. Because it is better tolerated, cumulative doses threshold is roughly twice as much as for doxorubicin.[15, 46]

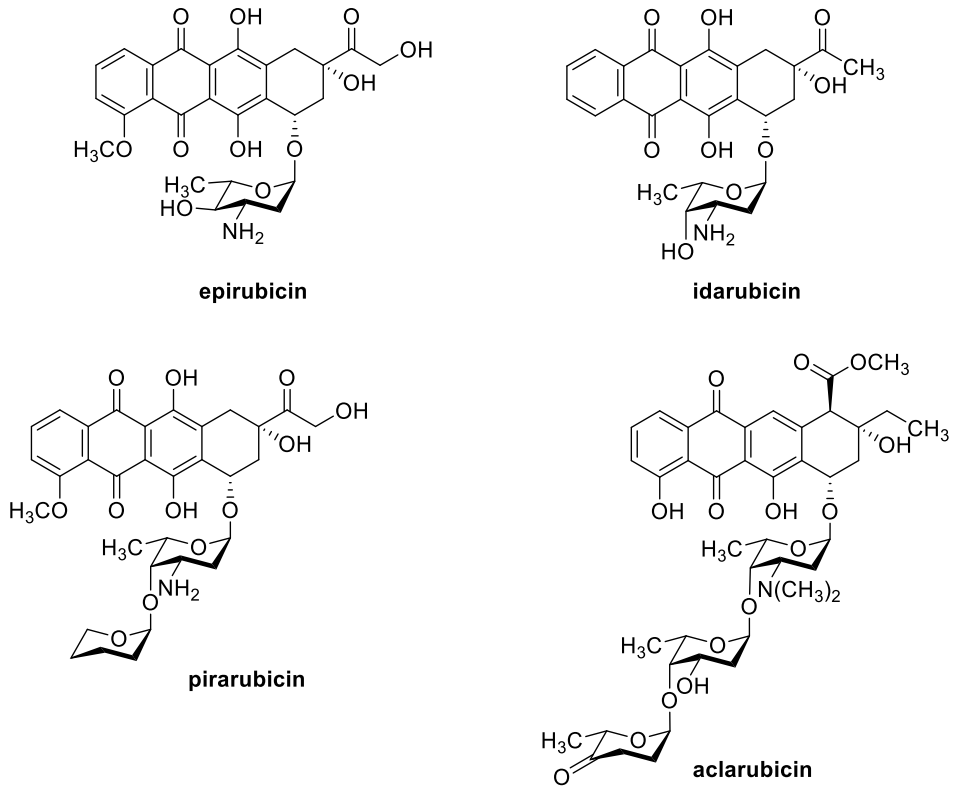


Figure 9. Second-generation anthracyclines.

Idarubicin is the 4-demethoxydaunorubicin (Figure 9), prepared through glycosylation of the non-natural 4-demethoxydaunorubicinone. Originally produced by total synthesis, the aglycone could also be obtained semi-synthetically from daunorubicinone. This drug can be administered orally and shows a broader spectrum of activity compared to **DAU**, including not only leukaemias but also some solid tumours, probably by increased lipophilicity and cellular uptake.[15, 46]

Despite the numerous efforts, only some other analogues reached the stage of clinical development and even fewer achieved approval for marketing.[15, 35] Semi-synthetic pirarubicin (4′-tetrahydropyranyl-doxorubicin, Figure 9) is considered less cardiotoxic than **DOX** and exhibits activity against some doxorubicin-resistant cell lines. Aclarubicin is claimed to have reduced cardiotoxicity than **DOX** and **DAU**, but only moderate improvement in terms of drug resistance. This trisaccharide anthracycline from natural origin (*Streptomyces galilaeus*), comprised by a variant aglycone structure (Figure 9), has different mechanisms of action, including inhibition of Top2 before DNA breakage and histone eviction. These drugs became registered only in a few countries, and do not play a significant role in global terms.[8, 15, 35, 47, 48]

As for the third-generation anthracyclines undergoing clinical development, some are emerging as promising drug candidates. Nemorubicin (PNU 152243) is a semi-synthetic doxorubicin derivative having a more lipophilic 2-(*S*)-methoxy-4-morpholinyl at the 3′ position (Figure 10), discovered by lead optimisation within a series of morpholinyl anthracyclines.[49] In the preclinical phase, it was intensely potent, active against drug-resistant cell lines and xenografts, and not cardiotoxic. It is believed to be bioactivated by P450 CYP3A and to induce DNA strand breaks primarily through topoisomerase I cleavage.[50-52]

Sabarubicin (MEN 10755) derives from the glycosylation of 4-demethoxy-doxorubicinone with a disaccharide, resulting in a glycoside in which a 2,6-dideoxy-α-L-*lyxo*-hexopyranosyl residue is positioned between the aglycone and daunosamine (Figure 10), differently from the natural anthracycline disaccharides, which carry the aminosugar as the first moiety directly attached to the aglycone. The elongated glycone permits the second sugar to interact both at the minor groove and with a DNA base. It is less cardiotoxic than doxorubicin, but it does not show the ability to overcome transport-mediated resistance. [46, 47, 52]

Amrubicin (SM-5887) is a totally synthetic anthracycline analogue, developed through a simplification approach. In comparison with daunorubicin, it lacks the 4-methoxy group of the aglycone and the 3′ amino group of the carbohydrate, which is a minimalist version of daunosamine. On the other hand, an amino group appears at position 9, in replacement to the hydroxyl group. It is a prodrug, with the amrubicinol (13-OH) metabolite being more cytotoxic than the parent drug. Overall, it is less cardiotoxic than doxorubicin and epirubicin.[52, 53]

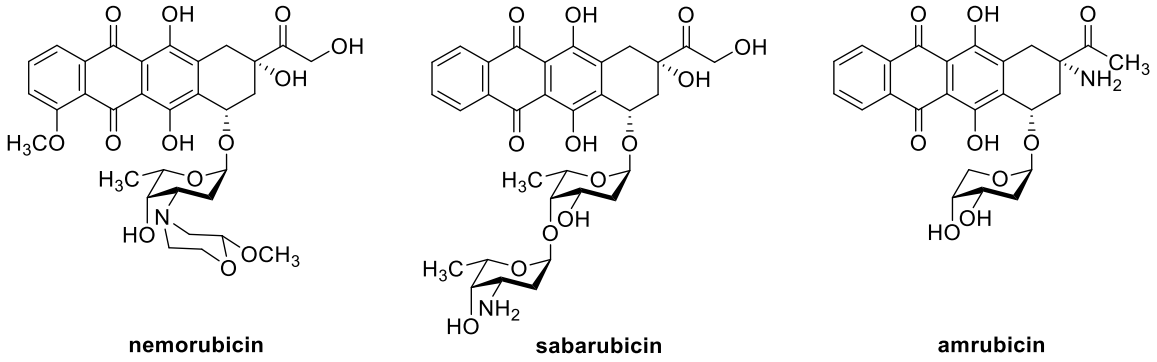


Figure 10. Third-generation anthracyclines.

Specific changes, substituting the amino function in daunosamine by azide, for instance, demonstrated to overcome resistance in specific cancer cells. The 3′-azido derivatives of daunorubicin, doxorubicin and epirubicin (Figure 11) were synthesised from the parent drug and retained antiproliferative activity against drug-sensitive human cancer cell lines of leukaemia (K562) and breast cancer (MCF-7). The first two compounds were further active against drug-resistant cell lines K562/Dox and MCF-7/Dnr, by averting P-glycoprotein binding and active efflux, with consequential intracellular accumulation. Flow cytometry and molecular modelling showed that the chemical modification, from positively ionisable amino group to the more electron-dense and linearly arranged azido group, abolished key interactions with P-glycoprotein. These azides were no longer substrates to P-gp efflux transporter, and were considered new leads for the development of innovative anthracyclines[43, 44, 54, 55] Although the azido group is not found in natural products, it is present in many bioactive compounds, including approved drugs such as azidocillin and azidamfenicol antibacterials, and zidovudine antiviral.

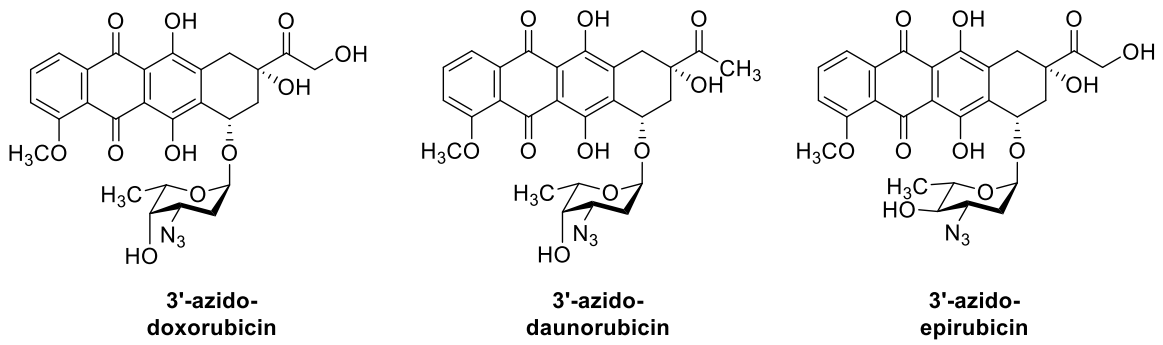


Figure 11. Anthracycline 3′-azido derivatives, some of which can overcome P-gp mediated resistance.

1.3 Glycodiversification and glycosylation reactions

The discovery of novel anthracyclines with wider anticancer spectrum, better heart tolerability and less prone to resistance remains a necessity. Many of the structural variations in new generation anthracyclines took place on the carbohydrate moiety, indicating that the glycone could be successfully replaced without losing the anticancer activity, and at the same time modulating other properties, such as pharmacokinetics, toxicity, and resistance profile. Because anthracyclines are glycoside drugs with demonstrated tolerance to glycone modification, they are promising candidates to the so-called glycodiversification approach. This stands for the replacement of the carbohydrate unit of a bioactive glycoside by sequential glycosidic cleavage, protection, re-glycosylation and deprotection steps, enabling the access to series of glycosides of interest (Figure 12).[56, 57]

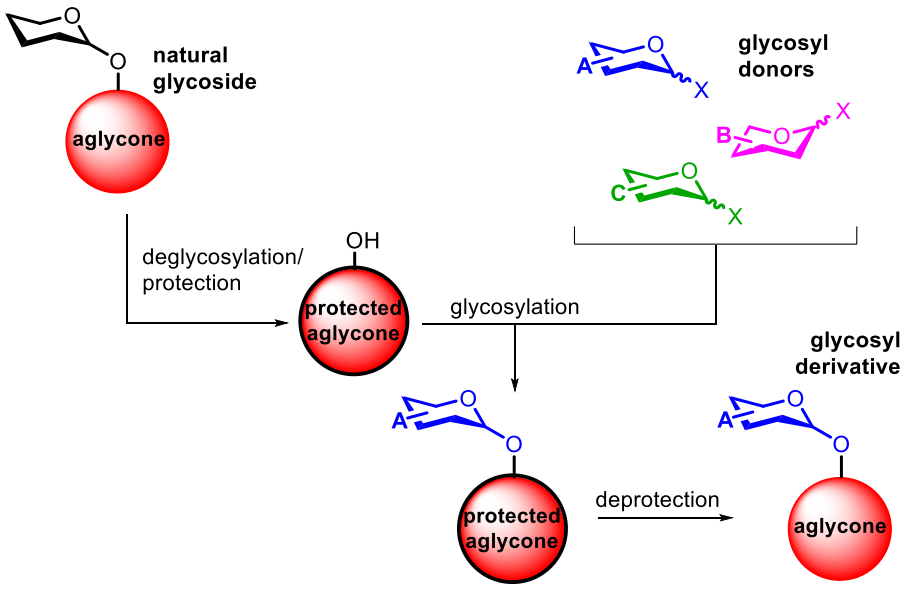


Figure 12. Glycodiversification strategy of natural glycosides, enabling the synthesis of libraries of novel glycoside derivatives. Adapted from Ritter (2003).[57]

This strategy is benefited by the possibility to work out the complexity of the carbohydrate separately, avoiding the valuable polyfunctional aglycone being exposed to unfavourable reaction conditions. Glycodiversification has been successfully applied to several classes of glycoconjugate drugs, such as the macrolide antibiotic erythromycin, the glycopeptide antibiotic vancomycin, the antiparasitic avermectins and the antifungal polyene amphotericin B (Figure 3).[20, 56, 57]

Based on this fruitful approach, it would be interesting to explore a variety of sugars in combination with anthracycline aglycones, with the glycosylation reaction playing a central role in connecting the two moieties. The chemical formation of a glycosidic bond is generally recognised as a challenging reaction, involving the linkage of a glycosyl acceptor, usually through a nucleophilic hydroxyl group, to the electrophilic anomeric carbon of a glycosyl donor equipped with a leaving group. A multitude of factors affects the efficiency and stereoselectivity of the glycosylation reaction, including the configuration of the carbohydrate moiety, the protecting groups on the substituents, the anomeric leaving group, the promoter system, the solvents, etc.[58]

The most important outcome to control in a glycosylation reaction is the stereoselectivity of the glycosyl bond. It is primarily governed by the anomeric effect, which is a stereoelectronic factor describing the tendency of a polar C-1 substituent, adjacent to the oxygen atom in the tetrahydropyran ring, to prefer the axial orientation instead of the equatorial one, despite the latter being favoured by lesser steric hindrance. These improbable observations can be rationalised by the following orbital interaction and electrostatic models. The hyperconjugation of one of the non-bonding electron pairs from the endocyclic oxygen atom with the anti-ligand orbital σ* from the anomeric carbon provides electronic stabilisation. The superimposition is efficient when the orbitals are parallel, which is only enabled when the anomeric bond is in the axial orientation, and the lowest unoccupied molecular orbital has a syn-periplanar relationship with one of the oxygen n orbitals (Figure 13a). Electrostatically, the dipole-dipole interaction between the endocyclic oxygen lone pairs and the electrons of the anomeric substituent is strongly repulsive when it is positioned in the equatorial orientation, but these destabilising interactions do not exist when it is in the axial orientation, and the dipoles are opposite (Figure 13b). [59, 60]

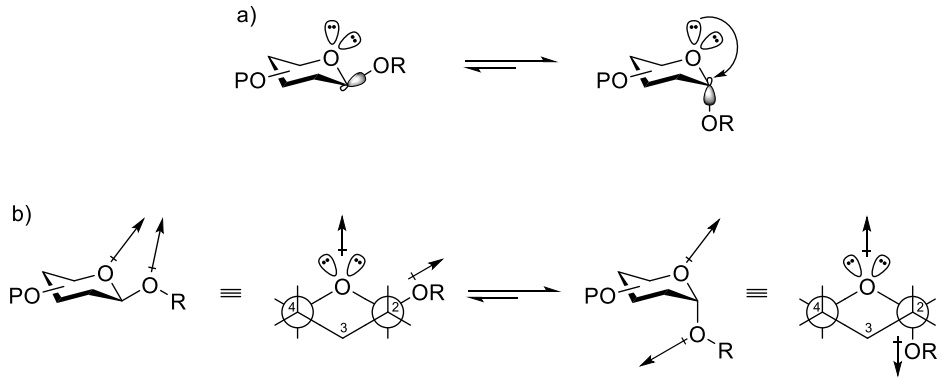


Figure 13. The anomeric effect explained by a) the orbital interaction model, and b) the electrostatic model.

In some cases, the anomeric effect can be outrivaled by other factors, reversing the stereo preference of the forming glycosidic bond from the axial to the equatorial orientation. The most relevant of these factors is known as the neighbouring group participation, in which the substituent at C-2 influences the outcome of the glycosylation reaction. After anomeric activation upon the departure of the leaving group, the anomeric effect prevails if the neighbouring group is non-participant, as is the case of ethers, silyl ethers and azides, and the resulting glycoside is predominantly axial (Figure 14a). When the neighbouring group holds an acyl functionality, such as esters, amides and carbamates, that can stabilise the transient cation through a dioxolenium- or oxazoline-like intermediate, the bottom face is occupied, and the nucleophile can only attack by the top face. This effect is also called anchimeric assistance and results mostly in equatorial-oriented glycoside with high stereoselectivity (Figure 14b). In spite of these directing effects, it is noteworthy that they are not absolute, and commonly a small amount of the opposite anomer is also produced, which can be negligible or not. [60, 61]

Protecting groups in the glycosyl donor, which have the primary function of preventing undesired side reactions, can also interfere by rendering the glycosyl donor more reactive ("armed", with electron donating groups) or less reactive ("disarmed", with electron withdrawing groups), or even influence on the conformation of the intermediate through steric effects, favouring a particular stereo outcome.[60, 62] As every glycosylation reaction is unique, a balance of these factors, combined with solvent, temperature, selection of anomeric leaving group and activation reagents, must be individually optimised.[60, 63, 64]

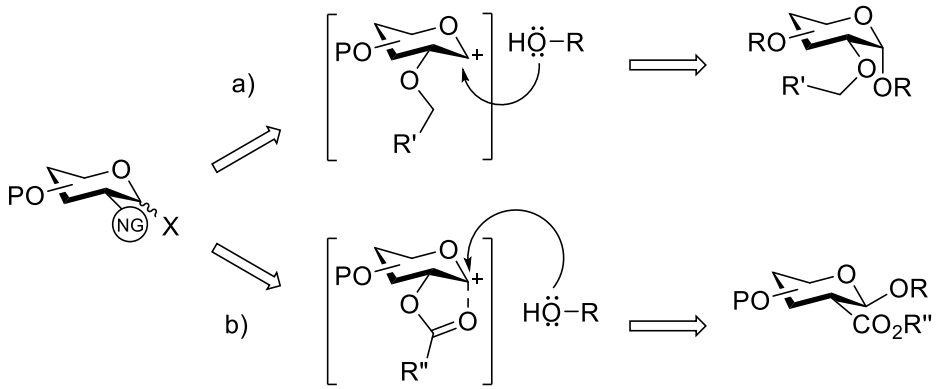


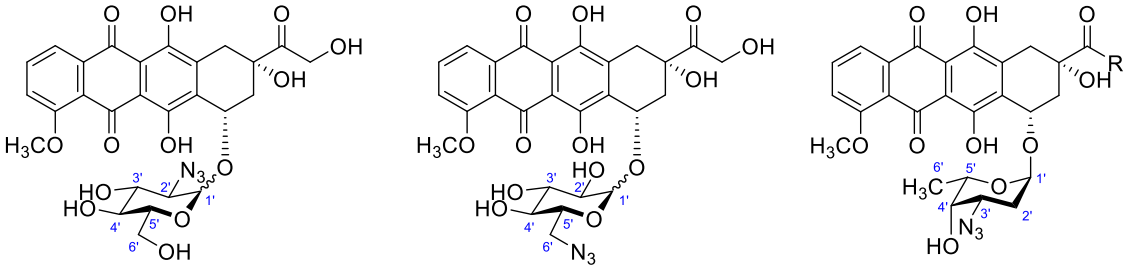
Figure 14. Neighbouring group effect on the glycosylation reaction stereoselectivity. a) Non-participating group at C-2 does not influence, and the anomeric effect governs the formation of the axial glycoside. b) Participating group at C-2 provides anchimeric assistance, reversing the stereoselectivity to the formation of the equatorial glycoside. NG: neighbouring group; P: protecting group; R, R′, R″: alkyl/aryl.

Encouraged by the therapeutic potential of anthracyclines as anticancer agents, and the possibility to develop novel derivatives that could retain antitumor efficacy with reduced toxicity and resistance, given their comprehensive structure-activity relationship, this work envisioned to exploit glycosylation reactions within a glycodiversification strategy to synthesise novel anthracycline derivatives coupled to azido glycosides.

Objectives

2 OBJECTIVES

To obtain by semi-synthesis anthracycline-derived azido glycosides in replacement to daunosamine, as a glycodiversification strategy, namely compounds **1** and **2**, containing 2-azido and 6-azido glucosyl units, respectively:



1 **2** **3** R = CH_2OH **4** R = CH_3

To synthesise known 3′-azido derivatives **3** and **4**, aiming to compare their properties with the novel target compounds.

To test the compounds obtained for *in vitro* cytotoxicity against tumoral cell lines and cardiomyocytes, to reveal their therapeutic and toxic potential.

Experimental

3 EXPERIMENTAL

3.1 Instrumentation and materials

3.1.1 Analytical equipment

Nuclear Magnetic Resonance (NMR) spectroscopy:* 300 MHz (Bruker Fourier 300, Bruker Avance DPX-300), 400 MHz (Bruker Avance DRX-400, Bruker Nano 400, Jeol ECS 400, Varian 400 MR), 500 MHz (Bruker Avance DRX-500, Bruker Avance III HD 500 Cryo, 2 Varian 500 VNMRS), 600 MHz (Varian VNMRS 600).

Mass spectrometry (MS): Electrospray ionisation (Bruker Daltonics micrOTOF QII – hybrid analyser time-of-flight and quadrupole; Bruker Daltonics Focus II micrOTOF – time-of-flight; Bruker Daltonics Esquire 6000 – ion trap, HPLC-MS; Micromass Quattro LC – triple quadrupole), Matrix-assisted laser desorption ionisation (2 Bruker Daltonics UltrafleXtreme 2 – time-of-flight).

Ultraviolet-visible (UV-vis) and fluorescence spectroscopy: Molecular Devices SpectraMax M2 and BMG Labtech CLARIOstar spectrophotometers and plate readers.

Infrared (IR) spectroscopy: Thermo FTIR Nicolet Protege 460.

High-performance liquid chromatography (HPLC): Shimadzu SCL-10AVP chromatographic system, UV-vis detector SPD-M10AVP, software Class-VP 5.0.

3.1.2 Laboratory equipment

Automated flash purification systems: Biotage Horizon, Biotage Isolera Spektra One, Grace Discovery Sciences Reveleris Prep (dual-mode integrated flash chromatography and preparative HPLC).

* NMR spectroscopic data was described as follows: chemical shifts (δ) were indicated in parts per million (ppm) in relation to tetramethylsilane. Coupling constants (*J*) were given in Hertz. Number of hydrogens were deduced from the relative integral. Multiplicities were abbreviated as br (broad), s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or combinations thereof. Assignments for the novel compounds were performed with the aid of bidimensional NMR techniques (COSY, HSQC, HMBC) and others (such as TOCSY(1D), ^{29}Si e ^{15}N).

Rotary evaporators: Büchi R-215, vacuum controller (V-850), diaphragm pump (V-700); Büchi R-215 with dry-ice condenser, vacuum controller (V-850), double diaphragm pump (V-710); Büchi RE-111, diaphragm pump (15 mmHg); Heidolph with dry-ice condenser, oil pump (0.1 mmHg).

Scales: Sartorius BP 121S, Mettler PE 400, Mettler Toledo AX105 DeltaRange, Precisa Gravimetrics AG Series 320XT.

Magnetic stirrers: IKA RCT Basic, Corning PC-320, Heildoph MR Hei-Standard.

Ultraviolet light chamber: Spectroline ENF-260C (254/365 nm).

Centrifuges: Eppendorf 5702, Thermo Scientific Heraeus Biofuge Primo R.

Freeze-driers: Christ Alpha 2-4 LSC Plus, Labconco FreeZone 6L, LTE Lyotrap.

3.1.3 Materials

Thin-layer chromatography (TLC): silica gel on aluminium foils with fluorescence indicator 254 nm

Preparative thin-layer chromatography: silica gel on glass plates with fluorescence indicator 254 nm

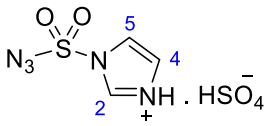
Flash column chromatography: silica gel (Merck, 40−63 / 230−400 mesh) on glass columns or flash cartridges (Biotage® SNAP KP-Sil 50 µm, Grace Reveleris® 40 µm silica and C-18 bonded silica).

HPLC reverse-phase columns: Shimadzu C18 Shim-Pack CLC-ODS(M) (150 mm × 4.6 mm, 5 µm), Shimadzu C18 Shim-Pack CLC-ODS(M) (250 mm × 4.6 mm, 5 µm), Macherey-Nagel Nucleosil C18 (250 × 10 mm, 5 µM), Phenomenex Luna C18(2) AXIA packed (250 × 21.2 mm, 5 µm).

Commercial solvents and reagents were conveniently purified, when necessary, according to standard methods from specialised literature.[65]

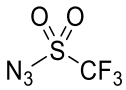
3.2 Synthesis

1-(Azidosulphonyl)-1*H*-imidazol-3-ium hydrogensulfate[66, 67] (5)



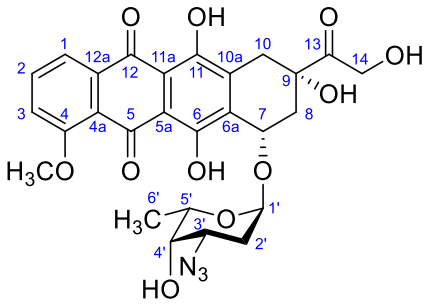
A suspension of sodium azide (NaN_3, 6.50 g, 100 mmol, 1 equiv) in acetonitrile (MeCN, 100 mL) at 0 ºC was treated with sulfuryl chloride (SO_2Cl_2, 8.5 mL, 14.15 g, 105 mmol, 1.05 equiv), slowly added via a dropping funnel. The mixture was stirred for 16 h, when it was again cooled to 0 ºC, and imidazole (12.93 g, 190 mmol, 1.9 equiv, recrystallised) was added in small portions over 40 min. After stirring at room temperature for 5 h, the mixture was diluted with ethyl acetate (EtOAc, 200 mL), washed with water (2 × 200 mL) and $NaHCO_3$ sat. (2 × 200 mL), dried over $MgSO_4$ and filtered. The organic layer was cooled to 0 ºC under stirring, and a solution of H_2SO_4 (5.6 mL, 100 mmol) in EtOAc (53 mL) at 0 ºC was added dropwise. After 18 h, more H_2SO_4 (2.3 mL, 50 mmol) in EtOAc (27 mL) was added, and precipitation of the salt under vigorous stirring occurred upon addition of few crystals of the product previously synthesised. The solid was filtered, washed with cold EtOAc and dried under vacuum, affording a white powder (8.64 g, 31.85 mmol, 32%; contains ~10% of unreacted imidazole). ^{1}H NMR (400 MHz, D_2O) δ: 9.50–9.44 (1H, m, H-2), 8.00 (1H, dd, J=2.2, 1.7 Hz, H-5), 7.61–7.57 (1H, m, H-4). ^{13}C NMR (101 MHz, D_2O) δ: 137.6, 120.2, 118.8. Spectroscopic data in agreement with literature.[66, 67] Appendix 1

Trifluoromethanesulphonyl azide[68] (6)



A solution of NaN_3 (5.76 g, 88.6 mmol, 5 equiv) in water (15 mL) was cooled to 0°C, and dichloromethane (DCM, 23 mL) was added under stirring. Trifluoromethanesulphonic anhydride (5 g, 17.7 mmol, 1 equiv) was added dropwise over a period of 5 min, and the mixture was stirred at 0 °C for 4 h. The organic layer was separated, and the aqueous layer was extracted with DCM (2 × 13 mL). The combined organic layers were washed with Na_2CO_3 sat. (55 mL), resulting in 61 mL of TfN_3 solution in DCM (approximately 0.29 mmol/mL) for further use (not isolated: TfN_3 is recognised as explosive in the absence of solvent and should always be used as a solution[68]).

3′-Azido-doxorubicin (3)

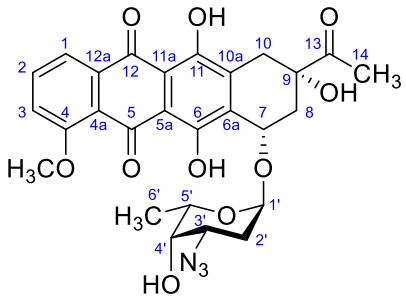


Method I:[69, 70] Doxorubicin hydrochloride (16.3 mg, 0.0281 mmol, 1 equiv) was suspended in methanol (MeOH, 1 mL) under stirring. Potassium carbonate (K_2CO_3, 6.0 mg, 0.0434 mmol, 1.5 equiv), copper sulfate ($CuSO_4$, 0.1 mg, 1 mol%), and the imidazole-1-sulphonyl azide salt **5** (9.3 mg, 0.0342 mmol, 1.2 equiv) were added. The mixture was stirred at room temperature for 48 h, when it was treated with additional K_2CO_3 (12 mg, 0.087 mmol, 3 equiv) and **5** (15.2 mg, 0.056 mmol, 2 equiv), stirred for 72 h (120 h in total), and concentrated under vacuum. The residue was suspended in water (15 mL), extracted with DCM (4 × 10 mL), and the combined organic layers were washed with NaCl sat. (25 mL), dried over $MgSO_4$, and concentrated. It was purified by column chromatography (MeOH:$CHCl_3$ 2-10%), affording **3** as a red solid (3.7 mg, 0.0065 mmol, 23%), R_f 0.56 (MeOH:$CHCl_3$ 1:9). IR: $\nu_{N=N=N}$ 2098 cm^{-1}.

Method II:[55, 71] Doxorubicin hydrochloride (103.7 mg, 0.179 mmol, 1 equiv) was dissolved in water (3 mL), treated with K_2CO_3 (44.6 mg, 0.323 mmol, 1.8 equiv) and $CuSO_4$ 1.3 mg, 0.008 mmol, 0.04 equiv). MeOH (3 mL) and a freshly prepared solution of TfN_3 **6** (0.55 mmol/mL, 3 mL, 1.64 mmol, 9 equiv) were added, followed by more MeOH (2 mL) to achieve homogeneity, and the mixture was stirred at room temperature for 24 h. It was concentrated under vaccum and purified by column chromatography (MeOH:DCM 0-10%), affording **3** as a red solid (55.4 mg, 0.0973 mmol, 54%), R_f 0.57 (MeOH:DCM 1:9). IR: $\nu_{N=N=N}$ 2115 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 14.01 (1H, s, OH-6), 13.24 (1H, s, OH-11), 8.04 (1H, dd, J=7.6, 1.1 Hz, H-1), 7.79 (1H, dd, J=8.5, 7.7 Hz, H-2), 7.40 (1H, dd, J=8.5, 1.0 Hz, H-3), 5.59 (1H, d, J=3.9 Hz, H-1′), 5.32 (1H, dd, J=4.1, 2.1 Hz, H-7), 4.75 (2H, s, CH_2-14), 4.51 (1H, s, OH-9), 4.09 (3H, s, OCH_3-4), 4.04 (1H, q, J=6.5 Hz, H-5′), 3.73 (1H, s, H-4′), 3.59 (1H, ddd, J=12.8, 4.9, 2.6 Hz, H-3′), 3.28 (1H, dd, J=18.8, 2.1 Hz, H-10a), 3.03 (2H, d, J=18.8 Hz, H-10b, OH-14), 2.35 (1H, dt, J=14.7, 2.2 Hz, H-8a), 2.19 (1H, dd, J=14.8, 4.0 Hz, H-8b), 2.11 (1H, td, J=13.1, 4.1 Hz, H-2′a), 1.92 (1H, ddt, J=13.3, 4.9, 1.2 Hz, H-2′b), 1.34 (3H, d, J=6.6 Hz, CH_3-6′). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 213.6 (C-13), 187.3, 186.9 (C-5,C-12), 161.3 (C-4), 156.3, 155.7 (C-6, C-11), 136.0 (C-2), 135.6, 133.6, 133.5 (C-6a, C-12a, C-10a), 121.0 (C-4a), 120.0

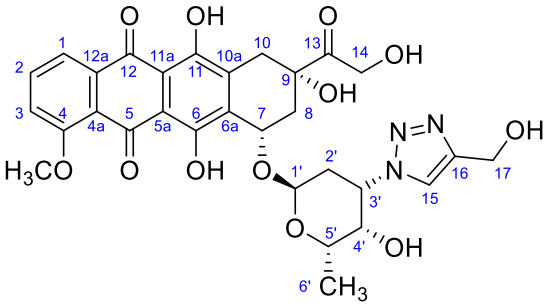
(C-1), 118.7 (C-3), 111.8, 111.7 (C-5a, C-11a), 100.7 (C-1′), 77.4 (C-9), 70.0 (C-7), 69.5 (C-4′), 67.4 (C-5′), 65.6 (C-14), 56.9 (OCH_3-4), 56.8 (C-3′), 35.7 (C-8), 34.1 (C-10), 28.5 (C-2′), 17.0 (C-6′). ESI-HRMS for $C_{27}H_{26}N_3O_{11}^-$ $(M-H)^-$ calcd: 568.1573, found 568.1775. Spectroscopic data in agreement with literature.[44] Appendix 2

3′-Azido-daunorubicin[54, 72] (4)



Daunorubicin hydrochloride (85.0 mg, 0.15 mmol, 1 equiv) was dissolved in water (0.5 mL), treated with K_2CO_3 (37.4 mg, 0.27 mmol, 1.8 equiv) and aqueous 0.1 M $CuSO_4$ (15 µL, 0.0015 mmol, 0,01 equiv). MeOH (0.5 mL) and a freshly prepared solution of TfN_3 **6** (0.29 mmol/mL, 1.1 mL, 0.32 mmol, 2.1 equiv) were added, followed by more MeOH (1 mL) to achieve homogeneity, and the mixture was stirred at room temperature for 17 h. It was concentrated under vacuum and purified by flash column chromatography (MeOH:DCM 0-10%), affording **4** as a red solid (70.6 mg, 0.127 mmol, 84%). R_f 0.59 (MeOH:DCM 1:9). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 13.99 (1H, s, OH-6), 13.25 (1H, s, OH-11), 8.02 (1H, dd, J=7.7, 1.1 Hz, H-1), 7.78 (1H, dd, J=8.5, 7.7 Hz, H-2), 7.39 (1H, dd, J=8.6, 1.1 Hz, H-3), 5.57 (1H, d, J=3.9 Hz, H-1′), 5.29–5.25 (1H, m, H-7), 4.41 (1H, s, OH-9), 4.16–4.09 (1H, m, H-5′), 4.08 (3H, s, OCH_3-4), 3.72 (1H, s, H-4′), 3.63 (1H, ddd, J=12.8, 5.0, 2.6 Hz, H-3′), 3.22 (1H, dd, J=18.8, 2.0 Hz, H-10a), 2.92 (1H, d, J=18.8 Hz, H-10b), 2.40 (3H, s, CH_3-14), 2.32 (1H, dt, J=15.4, 2.1 Hz, H-8a), 2.17–2.05 (2H, m, H-8b, H-2′a), 1.93 (1H, dd, J=13.3, 5.0 Hz, H-2′b), 1.33 (3H, d, J=6.6 Hz, CH_3-6′). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 211.5, 187.0, 186.7, 161.1, 156.3, 155.7, 135.7, 135.5, 134.2, 133.9, 120.9, 119.8, 118.4, 111.5, 111.4, 100.5, 76.7, 70.1, 70.0, 69.4, 67.0, 56.8, 56.7, 34.9, 33.3, 28.5, 24.7, 16.8. Spectroscopic data in agreement with literature.[54, 72] Appendix 3

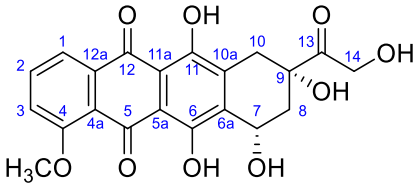
7-(2,3,6-Trideoxy-3-(4-hydroxymethyl-1*H*-1,2,3-triazol-1-yl)-*α*-L-lyxo-hexopyranosyl)doxorubicinone (7)



Method I. 3′-azido doxorubicin **3** (16.4 mg, 0.029 mmol, 1 equiv) and propargyl alcohol (3 μL, 2.9 mg, 0.05 mmol, 1.8 equiv) were dissolved in tetrahydrofuran (THF, 0.5 mL), treated with $(EtO)_3P.CuI$ (2.3 mg, 0.0064 mmol, 0.2 equiv) and diisopropylethylamine (DIPEA, 2 μL, 1.5 mg, 0.01 mmol, 0.4 equiv), and stirred at room temperature for 6 days. Water (1 mL) and 20% $(NH_4)_2S$ aqueous solution (1 mL) were added, the mixture was stirred for 3 h, when it was extracted with $CHCl_3$ (3 × 5 mL), and the combined organic layers were washed with $NaHCO_3$ sat. (10 mL), dried over Na_2SO_4, and concentrated. The residue was purified by preparative thin-layer chromatography (MeOH:DCM 1:19), but the compounds isolated (bands in R_f 0.24, 0.08 and 0.0) did not correspond to the expected product.

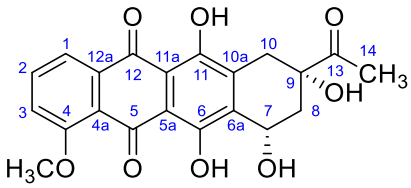
Method II. 3′-azido doxorubicin **3** (26.2 mg, 0.046 mmol, 1 equiv) and propargyl alcohol (5.2 mg, 0.093, 2 equiv), were dissolved in a mixture of THF: H_2O 1:1 (5 mL). 10 mg/mL aqueous solutions of sodium ascorbate (911 μL, 9.1 mg, 0.046 mmol, 1 equiv) and $CuSO_4$ (367 μL, 3.7 mg, 0.023 mmol, 0.5 equiv) were mixed and added to the solution of reactants, followed by more THF (3 mL). The mixture was protected from light and stirred at room temperature for 6 h, when it was concentrated and the residue was purified by column chromatography (MeOH:DCM 0-20%). The eluate was concentrated, dissolved in MeOH (3 mL), treated with Dowex H^+ 50WX8-400 (114 mg) and stirred at room temperature for 1.5 h to remove residual copper. It was filtered and concentrated, to afford product **7** as a red film (5.0 mg, 0.008 mmol, 17%). R_f 0.32 (MeOH:DCM 1:9). ^{1}H NMR (300 MHz, CD_3OD) δ 8.01 (1H, s, CH-15 triazole), 7.65–7.51 (2H, m, H-1, H-2), 7.31 (1H, dd, *J*=7.7, 1.9 Hz, H-3), 5.49 (1H, d, *J*=2.4 Hz, H-1′), 5.09 (1H, dt, *J*=13.3, 3.7 Hz, H-3′), 4.92–4.80 (1H, m, H-7), 4.74 (2H, d, *J*=3.0 Hz, CH_2-14), 4.67 (2H, s, CH_2OH-17), 4.45 (1H, q, *J*=6.6 Hz, H-5′), 3.90–3.83 (4H, m, OCH_3-4, H-4′), 2.93 (1H, d, *J*=18.8 Hz, H-10a), 2.73 (1H, d, *J*=18.6 Hz, H-10b), 2.60 (1H, td, *J*=12.9, 3.8 Hz, H-2′a), 2.35 (1H, d, *J*=14.5 Hz, H-8a), 2.20–2.04 (2H, m, H-2′b, H-8b), 1.32 (3H, d, *J*=6.5 Hz, CH_3-6′). ESI-HRMS for $C_{30}H_{32}N_3O_{12}^+$ (MH^+) calcd: 626.1980; found: 626.1923. Appendix 4.

Doxorubicinone[44, 73] (8)



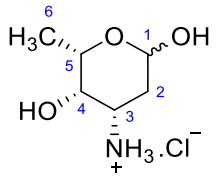
Doxorubicin hydrochloride (506 mg, 0.87 mmol) was dissolved in aqueous 0.2 M HCl solution (25 mL), heated to 90 °C, and stirred under reflux for 1 h. After cooling down to room temperature, the red solid in suspension was separated by filtration and washed throughoutly with cold water. It was resuspended in a small volume of water, centrifuged and freeze-dried, affording **8** as a red solid (346 mg, 0.84 mmol, 95%). R_f 0,63 (MeOH:DCM 1:9). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 13.96 (1H, s, OH-6), 13.20 (1H, s, OH-11), 8.04 (1H, d, J=7.6 Hz, H-1), 7.80 (1H, t, J=8.1 Hz, H-2), 7.41 (1H, d, J=8.5 Hz, H-3), 5.37 (1H, s, H-7), 4.83 (1H, dd, J=20.9, 5.1 Hz, H-14a), 4.75 (1H, dd, J=20.9, 4.8 Hz, H-14b), 4.61 (1H, s, OH-9), 4.10 (3H, s, OCH_3-4), 3.39 (1H, s, OH-7), 3.26 (1H, dd, J=18.6, 1.9 Hz, H-10a), 3.02–2.94 (2H, m, H-10b, OH-14), 2.40 (1H, d, J=14.6 Hz, H-8a), 2.17 (1H, dd, J=14.8, 4.9 Hz, H-8b). Spectroscopic data in agreement with literature.[74] Appendix 5

Daunorubicinone[44, 75] (9)



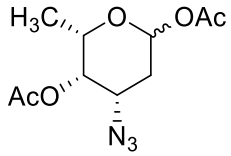
Daunorubicin hydrochloride (521 mg, 0.92 mmol) was dissolved in aqueous 0.2 M HCl solution (20 mL), heated to 90 °C, and stirred under reflux for 1.5 h. After cooling down to room temperature, the red solid in suspension was separated by filtration and washed thoroughly with cold water. It was resuspended in a small volume of water and freeze-dried, affording **9** as a red solid (353 mg, 0.89 mmol, 96%), R_f 0.60 (MeOH:DCM 5:95). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 13.88 (1H, s, OH-6), 13.19 (1H, s, OH-11), 7.98 (1H, dd, J=7.7, 1.0 Hz, H-1), 7.77 (1H, t, J=8.1 Hz, H-2), 7.38 (1H, d, J=8.5 Hz, H-3), 5.30 (1H, t, J=4.3 Hz, H-7), 4.61 (1H, s, OH-9), 4.08 (3H, s, OCH_3-4), 3.78 (1H, d, J=4.9 Hz, OH-7), 3.15 (1H, dd, J=18.6, 2.2 Hz, H-10a), 2.89 (1H, d, J=18.5 Hz, H-10b), 2.43 (3H, s, CH_3-14), 2.34 (1H, dt, J=14.6, 2.1 Hz, H-8a), 2.14 (1H, dd, J=14.5, 4.9 Hz, H-8b). MALDI-MS for $C_{21}H_{18}KO_8^+$ (MK^+) calcd: 437.1; found: 437.1. Spectroscopic data in agreement with literature.[76] Appendix 6

3-Amino-2,3,6-trideoxy-L-*lyxo*-hexose hydrochloride (DNS·HCl)[44]



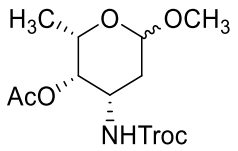
The aqueous layer from the hydrolysis of doxorubicin was extracted with EtOAc (3 × 20 mL) and concentrated to dryness, affording the product as a white solid (116 mg, 0.63 mmol, 85%). ^{1}H NMR (500 MHz, DMSO-*d*6) δ: 8.00 (3H, s, NH_3), 6.32 (1H, d, *J*=2.9 Hz, OH), 5.34 (1H, d, *J*=6.2 Hz, OH), 5.13 (1H, s, H-1), 3.97 (1H, q, *J*=6.5 Hz, H-5), 3.54 (1H, d, *J*=3.3 Hz, H-4), 3.47–3.42 (1H, m, H-3), 1.78 (1H, td, *J*=12.5, 2.2 Hz, H-2a), 1.61 (1H, dd, *J*=12.2, 4.2 Hz, H-2b), 1.07 (3H, d, *J*=6.6 Hz, CH_3-6). Spectroscopic data in agreement with literature.[77] Appendix 7

1,4-Di-*O*-acetyl-3-azido-2,3,6-trideoxy-L-*lyxo*-hexopyranose[66, 78] (10)



Daunosamine hydrochloride **DNS·HCl** (66 mg, 0.36 mmol, 1 equiv) was dissolved in MeOH (0.5 mL) and water (0.5 mL) under stirring. K_2CO_3, (74 mg, 0.054 mmol, 1.5 equiv), $CuSO_4 \cdot 5\ H_2O$ (1 mg, 1 mol%), and the imidazole-sulphonyl azide salt **5** (118 mg, 0.43 mmol, 1.2 equiv) were added. After 19 h, the pH was adjusted to 8 with aqueous Na_2CO_3 sat., the mixture was stirred at room temperature for 5 h (23 h in total), then concentrated under vacuum. Pyridine (3 mL) and acetic anhydride (Ac_2O, 2 mL) were added, and the mixture was stirred at room temperature for 16 h, when it was concentrated under vacuum. The residue was dissolved in EtOAc (15 mL) and washed with HCl 5% (2 × 10 mL) and water (2 × 10 mL) dried over $MgSO_4$, and concentrated. It was purified by column chromatography (EtOAc:Hex 0-30%), affording **10** as colourless oil (3.7 mg, 0.0065 mmol, 23%), R_f 0.56 (MeOH:$CHCl_3$ 1:9). IR: $\nu_{N=N=N}$ 2098 cm^{-1}. ^{1}H NMR (500 MHz, CDCl3): 6.29 (1H, d, *J*=2.6 Hz, H-1), 5.21 (1H, s, H-4), 4.09 (1H, q, *J*=6.5 Hz, H-5), 3.84 (1H, ddd, *J*=12.8, 4.7, 2.9 Hz, H-3), 2.19 (4H, s, H-2a, CH_3CO), 2.10 (3H, s, CH_3CO), 1.95 (1H, dd, *J*=13.4, 4.7 Hz, H-2b), 1.14 (3H, d, *J*=6.4 Hz). ^{13}C (126 MHz, $CDCl_3$) δ: 170.6, 169.3 (CH_3*C*O), 91.5 (C-1), 69.7 (C-4), 67.8 (C-5), 54.4 (C-3), 28.6 (C-2), 21.2, 20.9 (CH_3*C*O), 16.8 (C-6). ESI-HRMS for $C_{10}H_{19}N_4O_5^+$ (MNH_4^+) calcd: 275.1350; found: 275.1349. Appendix 8

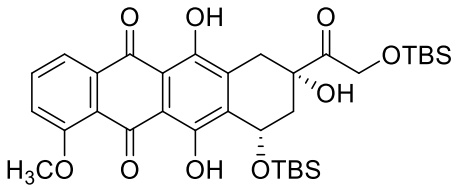
Methyl 2,3,6-trideoxy-3-(((2,2,2-trichloroethoxy)carbonyl)amino)-2-*O*-acetyl-*α/β*-L-*lyxo*-hexopyranoside (12)



The aqueous layer from the hydrolysis of doxorubicin was concentrated to dryness, dissolved in MeOH treated with acetone, filtered, and washed with cold acetone,[79] to afford white crystals of methyl L-daunosaminide hydrochloride **11** (110 mg, 0.56 mmol, 63%), R_f 0.12 (MeOH:DCM 1:9). ^{1}H NMR (400 MHz, D_2O) δ: 4.93 (1H, s, H-1), 4.05 (1H, q, J=6.5 Hz, H-5), 3.81 (1H, s, H-4), 3.74 (1H, ddd, J=11.3, 5.8, 2.6 Hz, H-3), 3.38 (3H, s, OCH_3), 2.06–1.91 (2H, m, H-2a, H-2b), 1.25 (3H, d, J=6.5 Hz, CH_3-6). ESI-MS for $C_7H_{16}NO_3^+$ (MH^+) calcd: 162.1; found: 162.1. Spectroscopic data in agreement with literature. [80, 81] Appendix 9

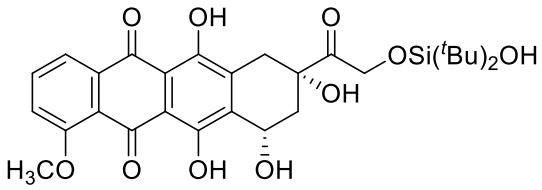
Daunosamine methyl glycoside **11** (85 mg, 0.43 mmol, 1 equiv) was dissolved in anhydrous MeCN (2 mL), $NaHCO_3$ (90.3 mg, 1.07 mmol, 2.5 equiv) was added, followed by 2,2,2-trichloroethoxycarbonyl chloride (TrocCl, 90 µL, 127 mg, 0.60 mmol, 1.4 equiv), and the mixture was stirred at room temperature. After 22 h, more $NaHCO_3$ (47 mg, 0.55 mmol, 1.3 equiv), TrocCl (50 µL, 77 mg, 0.36 mmol, 0.8 equiv) and MeCN (1 mL) were added and stirring was kept for another 6 h (28 h in total). The mixture was concentrated under reduced pressure, the residue was taken up in DCM (5mL), washed with water (15 mL), and the aqueous layer was then extracted with DCM (2 × 10 mL). The combined organic layers were dried over $MgSO_4$, and concentrated. The crude extract was dissolved in pyridine (2 mL), acetic anhydride (0.1 mL) was added and the mixture was stirred at room temperature for 18 h, upon which time it was concentrated under reduced pressure, taken up in DCM (20 mL) and extracted with HCl 1% (20 mL), $NaHCO_3$ (sat) (20 mL), brine (20 mL) and water (20 mL). The organic layer was dried over $MgSO_4$, concentrated, and the residue was purified by column chromatography (EtOAc:Hex 0-40%) to afford **12** as a pale yellow oil (102 mg, 0.27 mmol, 62%), R_f 0.45 (EtOAc:Hex 4:6). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.17 (1H, s, H-4), 4.87 (1H, d, J=8.0 Hz, NH), 4.82 (1H, s, H-1), 4.76 (1H, d, J=12.1 Hz, C*H*HCCl$_3$), 4.66 (1H, d, J=12.0 Hz, CH*H*CCl$_3$), 4.30 (1H, dddd, J=11.3, 8.8, 6.2, 2.9 Hz, H-3), 4.04 (1H, q, J=6.6 Hz, H-5), 3.35 (3H, s, OCH_3), 2.18 (3H, s, CH_3CO), 1.91–1.80 (2H, m, H-2a, H-2b), 1.12 (3H, d, J=6.6 Hz, CH_3-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 170.8 (CH_3*C*O), 153.7 (NH(CO)OCH_2), 98.0 (C-1), 95.6 (*C*Cl$_3$), 74.7 (O*C*H$_2$CCl$_3$), 70.9 (C-4), 65.1 (C-5), 55.0 (OCH_3), 46.1 (C-3), 30.9 (C-2), 21.0 (*C*H$_3$CO), 17.0 (C-6). ESI-HRMS for $C_{12}H_{18}Cl_3NNaO_6^+$ (MNa^+) calcd: 400.0092; found: 400.0109. Appendix 10

7,14-*O*-Di-*tert*-butyldimethylsilyl-doxorubicinone [82, 83] (13)



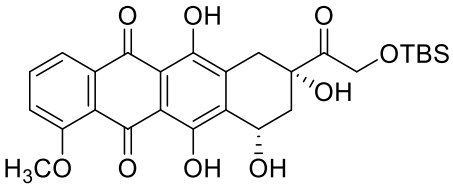
Doxorubicinone **8** (33.2 mg, 0.08 mmol, 1 equiv), previously dissolved and dried from toluene (Tol, 3×), was placed under vacuum in a sealed microwave vial for 1 h, when it was filled with N_2, cooled to -78 °C, and dissolved in anhydrous DCM (0.2 mL). In another vial, 2,6-lutidine (18.6 µL, 0.16 mmol, 2 equiv) and TBSOTf (27.5 µL, 0.12 mmol, 1.5 equiv) were dissolved in anhydrous DCM (0.5 mL) under N_2, and cooled to -78 °C. This solution was stirred and added dropwise to the doxorubicinone solution, in two portions of 0.25 mL, over a period of 10-15 min each. The mixture was stirred at -78 °C for 85 min, allowed to slowly warm up to room temperature, then stirred until 3.5 h. It was diluted with DCM (15 mL), washed with water (15 mL), the aqueous layer was acidified with 5% HCl (15mL) and then extracted twice with DCM. Organic layers were combined, washed with brine (15 mL), dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography (EtOAc:Hex 0-100%; MeOH:EtOAc 10%), to afford major product **13** as a red solid (18.3 mg, 0.03 mmol, 35%), R_f 0.74 (EtOAc:Hex 6:4). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 13.99 (1H, s, OH-6), 13.25 (1H, s, OH-11), 8.01 (1H, dt, *J*=7.8, 1.2 Hz, H-1), 7.80–7.72 (1H, m, H-2), 7.38 (1H, dt, *J*=8.6, 1.0 Hz, H-3), 5.43 (1H, s, OH-9), 5.41 (1H, t, *J*=3.1 Hz, H-7), 5.00 (1H, d, *J*=20.0 Hz, H-14a), 4.86 (1H, d, *J*=20.2 Hz, H-14b), 4.09 (3H, s, OCH_3-4), 3.20 (1H, d, *J*=18.9 Hz, H-10a), 2.99 (1H, d, *J*=18.9 Hz, H-10b), 2.25 (1H, dt, *J*=14.4, 2.6 Hz, H-8a), 2.05 (1H, dd, *J*=14.5, 3.6 Hz, H-8b), 0.96 (9H, s, $SiC(CH_3)_3$), 0.87 (9H, s, $SiC(CH_3)_3$), 0.30 (3H, s, $SiCH_3$), 0.16 (3H, s, $SiCH_3$), 0.14 (3H, s, $SiCH_3$), 0.13 (3H, s, $SiCH_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 212.0 (C-13), 187.2, 186.7 (C-5, C-12), 161.1 (C-4), 156.3, 156.2 (C-6, C-11), 135.8 (C-2), 135.7 (C-12a), 135.5 (C-6a), 134.6 (C-10a), 121.1 (C-4a), 119.9 (C-1), 118.4 (C-3), 111.4, 111.4 (C-5a, C-11a), 77.5 (C-9), 66.7 (C-14), 63.1 (C-7), 56.8 (OCH_3-4), 36.7 (C-8), 34.5 (C-10), 26.0, 25.9 ($SiC(CH_3)_3$), 18.7, 18.3 ($SiC(CH_3)_3$), -4.5, -4.9, -5.1, -5.3 ($SiCH_3$). ESI-HRMS for $C_{33}H_{46}NaO_9Si_2$ (MNa^+) calcd: 665.2573; found 665.2581. Appendix 11

14-*O*-(Di-*tert*-butyl(hydroxy)-silyl)doxorubicinone[84] (14)



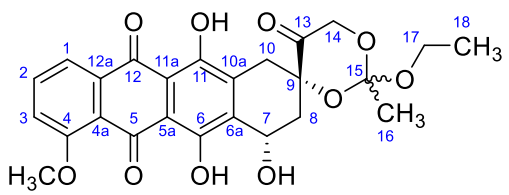
Doxorubicinone **8** (20.4 mg, 0.05 mmol, 1 equiv), previously dissolved and dried from toluene (3×), was placed under vacuum in a sealed microwave vial for 1 h, when it was filled with N_2, cooled to -78 °C, and dissolved in anhydrous DCM (1 mL). In another vial, 2,6-lutidine (20 µL, 0.17 mmol, 3.5 equiv) and DTBSOTf (22 µL, 0.07 mmol, 1.35 equiv) were dissolved in anhydrous DCM (1 mL) under N_2, and cooled to -78 °C. This solution was stirred and added dropwise to the doxorubicinone solution, over a period of 20 min. The mixture was stirred at -78 °C for 1 h, then at 0 °C for 6 h, and finally allowed to warm up to room temperature and stirred for 15 h (22 h in total). It was concentrated and the residue was directly purified by column chromatography (EtOAc:Hex 0-25%), to afford **14** as major product (4.2 mg, 0.007 mmol, 14%) *Rf* 0.34 (EtOAc:Hex 6:4). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 13.90 (1H, s, OH-6), 13.16 (1H, s, OH-11), 8.00 (1H, d, *J*=7.8 Hz, H-1), 7.78 (1H, t, *J*=8.1 Hz, H-2), 7.39 (1H, d, *J*=8.5 Hz, H-3), 5.32 (1H, d, *J*=4.7 Hz, H-7), 5.16 (1H, d, *J*=19.9 Hz, H-14a), 5.07 (1H, d, *J*=19.9 Hz, H-14b), 4.60 (1H, s, OH-9), 4.08 (3H, s, OCH_3-4), 3.52 (1H, s, OH-7), 3.21 (1H, dd, *J*=18.6, 2.2 Hz, H-10a), 2.93 (1H, d, *J*=18.7 Hz, H-10b), 2.39 (1H, dt, *J*=14.6, 2.0 Hz, H-8a), 2.15 (1H, dd, *J*=14.8, 4.8 Hz, H-8b), 1.08 (18H, s, $SiC(CH_3)_3$). ^{13}C (101 MHz, $CDCl_3$): 213.1 (C-13), 187.2, 186.7 (C-5, C-12), 161.2 (C-4), 156.2, 155.6 (C-6, C-11), 136.0 (C-2), 135.6 (C-12a), 135.4 (C-6a), 133.9 (C-10a), 120.8 (C-4a), 120.0 (C-1), 118.6 (C-3), 111.7, 111.3 (C-5a, C-11a), 77.1 (C-9), 67.3 (C-14), 62.4 (C-7), 56.9 (OCH_3-4), 35.8 (C-8), 34.0 (C-10), 27.6 ($C(CH_3)_3$), 20.9 ($C(CH_3)_3$). MALDI-MS for $C_{29}H_{36}NaO_{10}Si$ (MNa+) calcd: 595.2; found 595.3. Appendix 12

14-*O*-*tert*-Butyldimethylsilyl-doxorubicinone[85, 86] (15)



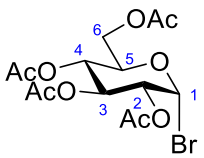
Doxorubicinone **8** (339 mg, 0.82 mmol, 1 equiv), previously dissolved and dried from toluene (3×), and molecular sieves (4 Å, 390 mg) were placed under vacuum for 1 h, when N_2 pressure was applied, anhydrous dimethylformamide (DMF, 4 mL) was added, and the solution was cooled to 0 °C, under stirring. In parallel, imidazole (445 mg, 6.55 mmol, 8 equiv) was placed under vacuum for 1 h, the flask was filled with N_2, TBSCl (494 mg, 3.28 mmol, 4 equiv) was added, and the solids were dissolved in anhydrous DMF (4mL). This solution was stirred vigorously for 5 min, and transferred dropwise into doxorubicinone solution, and the mixture was stirred at 0 °C for 20 min, then at room temperature for 1 h, after which time it was diluted with DCM (150 mL), filtered over Celite and washed with ice + 5% HCl (200 mL). The aqueous layer was extracted with DCM (3×50 mL) and the combined organic layers were washed with water, (200 mL), dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography (EtOAc:Hex 0-100%), affording product **15** as a red solid (210 mg, 0.397 mmol, 48%), R_f 0.30 (EtOAc:Hex 6:4). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 13.95 (1H, s, OH-6), 13.21 (1H, s, OH-11), 8.03 (1H, dd, J=7.7, 1.1 Hz, H-1), 7.79 (1H, dd, J=8.5, 7.7 Hz, H-2), 7.40 (1H, dd, J=8.5, 1.1 Hz, H-3), 5.32 (1H, t, J=3.1 Hz, H-7), 4.92 (1H, d, J=19.5 Hz, H-14a), 4.84 (1H, d, J=19.5 Hz, H-14b), 4.56 (1H, s, OH-9), 4.09 (3H, s, OCH_3-4), 3.53 (1H, d, J=2.3 Hz, OH-7), 3.21 (1H, dd, J=18.6, 2.2 Hz, H-10a), 2.98 (1H, d, J=18.8 Hz, H-10b), 2.38 (1H, dt, J=14.7, 2.1 Hz, H-8a), 2.19 (1H, dd, J=14.7, 4.9 Hz, H-8b), 0.95 (9H, s, $SiC(CH_3)_3$), 0.14 (6H, d, J=0.7 Hz, 2 × $SiCH_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 211.7 (C-13), 187.3, 186.8 (C-5, C-12), 161.2 (C-4), 156.3, 155.7 (C-11, C-6), 136.0 (C-2), 135.7, 135.6 (C-6a, C-12a), 133.8 (C-10a) 120.9 (C-4a), 120.0 (C-1), 118.6 (C-3), 111.7, 111.4 (C-5a, C-11a), 77.4 (C-9), 67.1 (C-14), 62.3 (C-7), 56.9 (OCH_3-4), 35.9 (C-8), 33.9 (C-10), 26.0 ($SiC(CH_3)_3$), 18.7 ($SiC(CH_3)_3$), -5.2 ($SiCH_3$), -5.3 ($SiCH_3$). ESI-HRMS for $C_{27}H_{32}NaO_9Si$ (MNa^+) calcd: 551.1708; found 551.1695. Spectroscopic data in agreement with literature.[85] Appendix 13

9,14-*O*-(1-Ethoxyethane-1,1-diyl)doxorubicinone[87] (16)



Doxorubicinone **8** (31,2 mg, 0,075 mmol, 1 equiv) was dissolved in anhydrous DMF (400 µL) under Ar, and 1,1,1-triethoxyethane (250 µL, 222.3 mg, 1.37 mmol, 18 equiv) and trifluoroacetic acid (25 µL, 37.2 mg, 0.33 mmol, 4.4 equiv) were added. The mixture was stirred at room temperature for 4 h, when it was diluted with DCM and concentrated under vacuum. The residue was dissolved in DCM (20 mL) and washed with water, $NaHCO_3$ sat., and water (20 mL each), then the aqueous layers were extracted with DCM. The combined organic layers were dried over $MgSO_4$, concentrated, and purified by column chromatography (EtOAct:Hex 0-10%; EtOAc/DCM 0-30%), to afford the product **16** as a red film (19.5 mg, 0.04 mmol, 53%). R_f 0.48 (EtOAc/DCM 1:9). ^{1}H NMR (400 MHz, $CDCl_3$) δ:13.57 (1H, s, OH-6), 13.09 (1H, s, OH-11), 7.99 (1H, dd, *J*=7.7, 1.0 Hz, H-1), 7.75 (1H, dd, *J*=8.3, 7.8 Hz, H-2), 7.37 (1H, d, *J*=8.5 Hz, H-3), 5.60 (1H, d, *J*=5.7 Hz, H-7), 4.33 (1H, d, *J*=12.7 Hz, H-14a), 4.07 (3H, s, OCH_3-4), 3.86 (1H, d, *J*=12.7 Hz, H-14b), 3.80 (1H, dq, *J*=9.2 Hz, 7.1 Hz, H-17a), 3.59 (1H, dq, *J*=9.3 Hz, 7.0 Hz, H-17b), 3.35 (1H, s, OH-7), 3.30 (1H, dd, *J*=19.3, 1.4 Hz, H-10a), 3.11 (1H, d, *J*=19.3 Hz, H-10b), 2.64 (1H, ddd, *J*=10.9, 5.7, 1.4 Hz, H-8a), 1.97 (1H, d, *J*=11.0 Hz, H-8b), 1.88 (3H, s, CH_3-16), 1.23 (3H, t, *J*=7.0 Hz, CH_3-18). ^{13}C (101 MHz, CDCl3) δ: 187.1, 186.9 (C-5, C-12), 170.3 (C-13), 161.2 (C-4), 155.4, 153.8 (C-11, C-6), 139.4 (C-6a), 136.0 (C-10a), 135.8 (C-2), 135.6 (C-12a), 121.1 (C-4a), 119.9 (C-1), 118.5 (C-3), 112.2, 111.9 (C-5a, C-11a), 102.2 (C-15), 79.0 (C-9), 68.8 (C-7), 60.0 (C-14), 57.5 (C-17), 56.8 (OCH_3-4), 40.1 (C-8), 33.6 (C-10), 20.6 (C-16), 15.8 (C-18). ESI-HRMS for $C_{25}H_{25}O_{10}^+$ (MH^+) calcd: 485.1442; found: 485.1448. Appendix 14

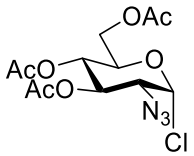
2,3,4,6-Tetra-*O*-acetyl-*α*-D-glucopyranosyl bromide[88, 89] (18)



D-glucose (3.61 g, 20 mmol, 1 equiv) was suspended in acetic anhydride (18 mL), treated with iodine (I_2, 153 mg, 0.6 mmol, 0.03 equiv), and stirred at room temperature for 2 h. The mixture was diluted with DCM (30 mL), washed with ice-$Na_2S_2O_3$ 5% (2 × 50 mL), and Na_2CO_3 sat. (3 × 500 mL), dried over $MgSO_4$, and concentrated. Crystallisation from ethanol (EtOH) afforded 1,2,3,4,6-penta-*O*-acetyl-α-D-glucopyranose **17** (5.87 g, 15 mmol, 75%), R_f 0.46 (EtOAc/Hex 1:1), as a white solid. Per-acetylated intermediate (1.05 g, 2.69 mmol) was dissolved in glacial acetic acid (6 mL), cooled to 0 °C, treated slowly with HBr 33% solution

in acetic acid (2 mL), and stirred at room temperature for 15 h. The mixture was diluted with DCM (25 mL) and poured onto crushed ice-water (100 mL), the aqueous layer was extracted with DCM (2 × 25 mL). The combined organic layers were washed with cold $NaHCO_3$ 5%, dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography (EtOAc:Hex 0-30%, containing 0.1% triethylamine (Et_3N)), to afford 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**18**) as a colourless oil which gave white crystals upon drying under vacuum (807 mg, 1.96 mmol, 73%). R_f 0.59 (EtOAc/Hex 1:1). Overall yield (2 steps) 55%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.61 (1H, d, J=4.0 Hz, H-1), 5.55 (1H, t, J=9.7 Hz, H-3), 5.16 (1H, t, J=9.8 Hz, H-4), 4.83 (1H, dd, J=10.0, 4.1 Hz, H-2), 4.38–4.23 (2H, m, H-6a, H-5), 4.18–4.06 (1H, m, H-6b), 2.10, 2.09, 2.05, 2.03 (12H, 4 s, 4 CH_3CO). Spectroscopic data in agreement with literature.[89] Appendix 15

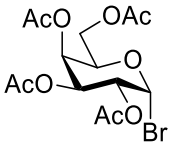
3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-*α*-D-glucopyranosyl chloride[68, 90-92] (20)



Glucosamine (2-amino-2-deoxy-D-glucose) hydrochloride (1.00 g, 4.66 mmol, 1 equiv) was dissolved in water (15 mL) and treated with K_2CO_3 (1.15 g, 8.3 mmol, 1.8 equiv) and $CuSO_4$ (8 mg, 0.05 mmol, 0.01 equiv). MeOH (30 mL) and a previously prepared TfN_3 (**6**) solution in DMC (21.5 mL, 10.20 mmol, 2.2 equiv) were added, followed by more MeOH (10 mL) to achieve homogeneity. The mixture was stirred at room temperature for 20 h, when it was concentrated under vacuum. The residue was dissolved in pyridine (25 mL), treated with acetic anhydride (15 mL), and stirred at room temperature for 17 h. Solvents were removed under vacuum, the residue was dissolved in EtOAc, washed with water, dried over $MgSO_4$, concentrated, and purified by column chromatography (EtOAc:Hex 3:7; 4:6), to afford 1,3,4,6-tetra-*O*-acetyl-2-azido-2-deoxy-*α/β*-D-glucopyranose **19** (1.38 g, 3.7 mmol, 80%) as a mixture of anomers. R_f 0.58 (EtOAc/Hex 1:1). IR: $\nu_{N=N=N}$ 2115 cm^{-1}. Per-acetylated intermediate **19** (872 mg, 2.34 mmol) was dissolved in glacial acetic acid (20 mL) previously saturated by bubbling with HCl (generated by dripping concentrated H_2SO_4 onto crystalline NaCl), and the solution was stirred at room temperature for 5 h. The mixture was diluted with DCM, poured onto crushed ice-water, the organic layer was separated and washed with ice-$NaHCO_3$ 5%, then with ice-$NaHCO_3$ sat., with vigorous shaking. The organic layer was dried over $MgSO_4$,

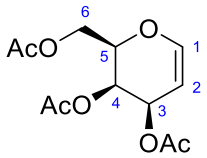
concentrated, and purified by column chromatography (EtOAc:Hex 0-40%), to afford **20** (110 mg, 0.32 mmol, 13%). R_f 0.46 (EtOAc/Hex 4:6). IR: $\nu_{N=N=N}$ 2115 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: 6.12 (1H, d, *J*=3.8 Hz, H-1), 5.52 (1H, t, *J*=9.5 Hz, H-3), 5.12 (1H, t, *J*=9.6 Hz, H-4), 4.41–4.27 (2H, m, H-5, H-6a), 4.12 (1H, dd, *J*=13.9, 3.2 Hz), 3.87 (1H, dd, *J*=10.3, 3.8 Hz), 2.11, 2.09, 2.05 (9H, 3 s, 3 CH_3CO). Spectroscopic data in agreement with literature.[93, 94] Appendix 16

2,3,4,6-Tetra-*O*-acetyl-*α*-D-galactopyranosyl bromide[88, 95] (22)



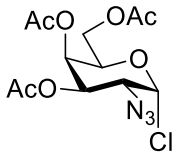
D-galactose (12.00 g, 66.6 mmol) was suspended in acetic anhydride (60 mL), treated with iodine (I_2, 613 mg, 2.4 mmol), and stirred at room temperature for 2.5 h. The mixture was diluted with DCM (60 mL), washed with ice-$Na_2S_2O_3$ 10% (2 × 60 mL), $NaHCO_3$ sat. (3 × 60 mL) and Na_2CO_3 sat. (2 × 60 mL), dried over $MgSO_4$, and concentrated, to give a light yellow oil containing a mixture of 1,2,3,4,6-penta-*O*-acetyl-*α/β*-D-galactopyranose **21** (23.5 g, 60.2 mmol; 90%), R_f 0.43 (EtOAc/Hex 1:1), which was crystallised from EtOH to give the α anomer (10.20 g, 26.1 mmol, 39%) as a white solid. Per-acetylated intermediate (13.31 g, 34.1 mmol) was dissolved in glacial acetic acid (11 mL), cooled to 0 °C, treated slowly with HBr 33% solution in acetic acid (12 mL), and stirred at room temperature for 5 h. The mixture was diluted with DCM (60 mL) and poured onto crushed ice-water (100 mL), the aqueous layer was extracted with DCM (60 mL). The combined organic layers were washed with cold $NaHCO_3$ 5%, dried over $MgSO_4$, and concentrated, to afford a yellowish oil containing the 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**22**) (10.96 g, 26.6 mmol, 78%). R_f 0.61 (EtOAc/Hex 1:1), 0.35 (EtOAc/Hex 3:7). Overall yield (2 steps) 70%. ^1H NMR (500 MHz, $CDCl_3$): δ: 6.69 (1H, d, *J*=4.0 Hz, H-1), 5.51 (1H, dd, *J*=3.3, 1.4 Hz, H-4), 5.39 (1H, dd, *J*=10.6, 3.3 Hz, H-3), 5.04 (1H, dd, *J*=10.7, 4.0 Hz, H-2), 4.47 (1H, t, *J*=6.5 Hz, H-5), 4.17 (1H, dd, *J*=11.4, 6.4 Hz, H-6a), 4.10 (1H, dd, *J*=11.4, 6.8 Hz, H-6b), 2.14, 2.10, 2.05, 2.00 (12H, 4 s, 4 CH_3CO). Spectroscopic data in agreement with literature.[96] Appendix 17

3,4,6-Tri-*O*-acetyl-D-galactal[97, 98] (23)



The galactosyl bromide **22** (10.96 g, 26.6 mmol, 1 equiv) was treated with a solution of sodium acetate (17.86 g, 217 mmol) and glacial acetic acid (80 mL, 84 g, 1.4 mol) in water (57 mL). Under vigorous stirring, $CuSO_4$ (598 mg, 3.7 mmol) and powdered metallic zinc (Zn^0, 23.58 g, 360 mmol) were added, and the mixture was stirred at room temperature for 16 h, when it was filtered over Celite, and the solids washed with water. The filtrate was diluted with EtOAc, washed with $NaHCO_3$ sat., NaCl sat., dried over $MgSO_4$, and concentrated. The residue was purified by flash column chromatography (EtOAc:Hex 0-20% (v/v), to afford galactal **23** as a colourless oil (3.82 g, 14.0 mmol; 53 %). R_f 0.38 (EtOAc/Hex 3:7). ^{1}H NMR (500 MHz, $CDCl_3$,) δ: 6.46 (1H, d, J=6.3 Hz, H-1), 5.57–5.53 (1H, m, H-4), 5.44–5.40 (1H, m, H-3), 4.72 (1H, ddd, J=6.2, 2.5, 1.4 Hz, H-2), 4.34–4.30 (1H, m, H-5), 4.26 (1H, dd, J=11.4, 7.4 Hz, H-6a), 4.21 (1H, dd, J=11.5, 5.2 Hz, H-6b), 2.12, 2.08, 2.02 (9H, 3 s, 3 CH_3CO). Spectroscopic data in agreement with literature.[97, 99] Appendix 18

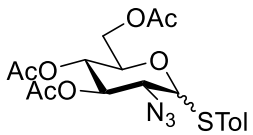
3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-*α*-D-galactopyranosyl chloride[100] (25)



3,4,6-tri-*O*-acetyl-D-galactal **23** (3.82 g; 14.1 mmol, 1 equiv) was dissolved in acetonitrile (MeCN, 60 mL) and cooled to -10 °C. Sodium azide (1.28 g, 19.7 mmol, 1.4 equiv) and ceric ammonium nitrate (CAN, 23.1 g, 42.2 mmol, 3 equiv) were added, and the solution was stirred for 30 min, allowed to reach room temperature and stirred for 8 h. The mixture was diluted with diethyl ether (60 mL), washed with water (60 mL), dried over $MgSO_4$, and concentrated. The residue was purified by flash column chromatography (EtOAc:Hex 0-60%), to afford a mixture of the azidonitrates α-*galacto*-/α-*talo*-/β-*galacto*pyranosides[100] (**24**) with a 8:1.4:1 ratio, as a colourless oil (1.73 g, 4.6 mmol, 33%). R_f 0.60 (EtOAc/Hex 6:4). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 6.34 (d, $J_{1,2}$=4.1 Hz, H-1 α-*galacto*), 6.21 (s, H-1 α-*talo*), 5.57 (d, $J_{1,2}$=8.8 Hz, H-1 β-*galacto*), 4.13 (m, H-2 α-*galacto*), 3.98 (d, $J_{2,3}$=4.2 Hz, H-2 α-*talo*), 3.82 (dd, $J_{1,2}$=9.0

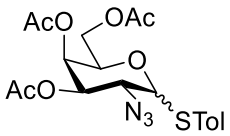
Hz, $J_{2,3}$=10.3 Hz, H-2 β-*galacto*). Azidonitration products **24** were dissolved in MeCN (32.5 mL) containing tetraethylammonium chloride (2.31 g, 13.9 mmol, 3 equiv), and stirred at room temperature for 18 h. The mixture was diluted with toluene, washed with water, dried over $MgSO_4$, and concentrated. The residue was purified by flash column chromatography, (EtOAc:Hex 0-50%), to afford azidochloride **25** as a yellowish oil (1.60 g, 4.6 mmol, 99%). R_f 0.48 (EtOAc/Hex 4:6). Overall yield (2 steps) 32%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 6.21 (1H, d, *J*=3.7 Hz, H-1), 5.56 (1H, dd, *J*=3.1, 1.2 Hz, H-4), 5.43 (1H, dd, *J*=10.5, 3.1 Hz, H-3), 4.60 (1H, t, *J*=6.6 Hz, H-5), 4.30–4.16 (3H, m, H-2, H-6a, H-6b), 2.32, 2.23, 2.22 (9 H, 3 s, 3 CH_3CO). Spectroscopic data in agreement with literature.[100, 101] Appendix 19

_p_-Tolyl 3,4,6-tri-_O_-acetyl-2-azido-2-deoxy-1-thio-_α/β_-D-glucopyranoside[102, 103] (26)



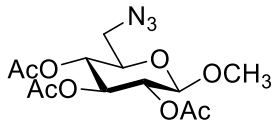
1,3,4,6-tetra-*O*-acetyl-2-azido-2-deoxy-D-glucopyranoside **19** (437 mg, 1.17 mmol, 1 equiv) was dissolved in anhydrous DCM (2.5 mL) under N_2, a solution of 4-methylbenzenethiol (303 mg, 2.44 mmol, 2 equiv) in anhydrous DCM (1 mL) was added, and the system was cooled to 0 °C. The mixture was treated with boron trifluoride diethyl etherate ($BF_3 \cdot Et_2O$, 0.6 mL, 5.41 mmol, 4.6 equiv) dropwise. After 10 min, the cooling bath was removed, the mixture was irradiated by ultrasound in a sonication bath[103] (2 × 40 min, with 20 min interval), then stirred at room temperature for 22 h, when it was neutralised with Et_3N and concentrated under vacuum. The residue was purified by column chromatography (EtOAc:DCM:Hex 1:1:8-1:1:6), to afford **26** as a yellowish oil (243 mg, 0.77 mmol, 66%, α:β =1.4:1). R_f 0.51 (EtOAc:Hex 1:1); 0.22 (EtOAc:DCM:Hex 1:1:6). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.48 (2H, d, *J*=8.2 Hz, CH STol), 7.38 (2H, d, *J*=8.1 Hz, CH STol), 7.18–7.10 (4H, m, CH STol), 5.56 (1H, d, *J*=5.5 Hz, H-1 α), 5.33 (1H, dd, *J*=10.5, 9.3 Hz, H-3 α), 5.11–4.97 (2H, m, H-4 α, H-3 β), 4.90 (1H, t, *J*=9.8 Hz, H-4 β), 4.61 (1H, ddd, *J*=10.2, 5.0, 2.1 Hz, H-5 α), 4.42 (1H, d, *J*=10.1 Hz, H-1 β), 4.33–4.16 (3H, m, H-6a α, H-6a β, H-6b β), 4.10–3.98 (2H, m, H-2a α, H-6b α), 3.67 (1H, ddd, *J*=10.1, 4.7, 2.6 Hz, H-5 β), 3.36 (1H, t, *J*=9.9 Hz, H-2 β), 2.37, 2.33 (6H, 2 s, CH_3 STol), 2.09, 2.09, 2.05, 2.05, 2.04, 2.00 (18H, 6 s, CH_3CO). Spectroscopic data in agreement with literature.[104, 105] Appendix 20

_p_-Tolyl 3,4,6-tri-_O_-acetyl-2-azido-2-deoxy-1-thio-_α/β_-D-galactopyranoside[68, 90, 106] (28)



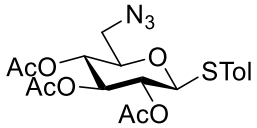
Galactosamine (2-amino-2-deoxy-D-galactose) hydrochloride (905 mg, 4.2 mmol, 1 equiv) was dissolved in water (15 mL) and treated with K_2CO_3 (872 mg, 6.3 mmol, 1.5 equiv) and aqueous 0.1 M $CuSO_4$ (420 µL, 0.042 mmol, 0,01 equiv). MeOH (30 mL) and a previously prepared TfN_3 (**6**) solution in DCM (30 mL, 8.7 mmol, 2.1 equiv) were added, followed by more MeOH (10 mL) to achieve homogeneity. The mixture was stirred at room temperature for 20 h, when it was concentrated under vacuum. The residue was dissolved in pyridine (24 mL), treated with acetic anhydride (15 mL), and stirred at room temperature for 24 h. Solvents were removed under vacuum, the residue was dissolved in EtOAc (120 mL), washed with water (100 mL) and $NaHCO_3$ sat. (100 mL), dried over $MgSO_4$, concentrated, and purified by flash column chromatography (EtOAc:Hex 0-20%), to afford 1,3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-*α/β*-D-galactopyranose **27** (1.10 g, 2.95 mmol, 70%) as a mixture of anomers. R_f 0.62 (EtOAc:Hex 1:1). Per-acetylated intermediate **27** (334 mg, 0.90 mmol; 1 equiv), was dissolved in anhydrous DCM (2.5 mL) under N_2, a solution of 4-methylbenzenethiol (116 mg, 0.93 mmol, 1 equiv) in anhydrous DCM (2.5 mL) was added. The mixture was treated dropwise with $BF_3 \cdot Et_2O$ (345 µL, 3.1 mmol, 3.5 equiv), and stirred at room temperature for 20 h, when it was neutralised with Et_3N and concentrated under vacuum. The residue was purified by column chromatography (EtOAc:Tol 2:8), to afford **28** as a yellowish oil (280 mg, 0.64 mmol, 71%, *α:β* =1.2:1). R_f 0.52 (EtOAc/Hex 1:1). ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 7.52 (2H, d, *J*=8.1 Hz, CH STol), 7.42 (2H, d, *J*=8.1 Hz, CH STol), 7.23–7.11 (4H, m, CH STol), 5.63 (1H, d, *J*=5.4 Hz, H-1α), 5.50 (1H, dd, *J*=3.3, 1.4 Hz, H-4α), 5.36 (1H, dd, *J*=3.3, 1.1 Hz, H-4β), 5.19 (1H, dd, *J*=11.1, 3.2 Hz, H-3α), 4.86 (1H, dd, *J*=10.3, 3.2 Hz, H-3β), 4.79 (1H, t, *J*=6.4 Hz, H-5α), 4.48 (1H, d, *J*=10.1 Hz, H-1β), 4.31 (1H, dd, *J*=11.1, 5.5 Hz, H-2α), 4.21–4.06 (4H, m, CH_2-6α,β), 3.88 (1H, td, *J*=6.6, 1.1 Hz, H-5β), 3.65 (1H, t, *J*=10.2 Hz, H-2β), 2.36 (6H, 2 s, *J*=5.0 Hz, CH_3 STol), 2.17, 2.10, 2.08, 2.06, 2.05, 2.02 (12H, 6 s, CH_3CO). Spectroscopic data in agreement with literature.[106] Appendix 21

Methyl 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-*β*-D-glucopyranoside[107, 108] (30)



Methyl *β*-D-glucopyranoside (194 mg, 1.0 mmol, 1 equiv) was dissolved in anhydrous pyridine (2 mL) under Ar and cooled to 0 °C. A solution of 4-toluenesulphonyl chloride (285 mg, 1.5 mmol, 1.5 equiv) in anhydrous pyridine (2 mL) was added dropwise, and the mixture was stirred at 0 °C for 2 h. Acetic anhydride (1.2 mL) was added, and the stirring was sustained for 12 h, after which time the mixture was concentrated under vacuum by co-evaporation with toluene. The residue was purified by flash column chromatography (EtOAc:Tol 10-30%), to afford the 6-tosylate **29** (320 mg, 0.68 mmol, 68%). Methyl 2,3,4-tri-*O*-acetyl-6-tosyl-6-deoxy-*β*-D-glucopyranoside **29** (90.5 mg, 0.19 mmol, 1 equiv) and sodium azide (188.3 mg, 2.89 mmol, 15 equiv) were suspended in DMF (4 mL) and the mixture was heated at 100-110 °C and stirred for 16 h, when it was diluted with water and extracted with DCM. The organic layer was washed with $NaHCO_3$ sat. and water, dried over $MgSO_4$, and concentrated, to afford product **30** as a colourless oil (50.2 mg, 0.145 mmol, 77%). ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 5.21 (1H, t, *J*=9.6 Hz, H-3), 5.02–4.94 (2H, m, H-2, H-4), 4.47 (1H, d, *J*=8.0 Hz, H-1), 3.70 (1H, ddd, *J*=10.0, 7.5, 2.5 Hz, H-5), 3.53 (3H, s, OCH_3), 3.43 (1H, dd, *J*=13.4, 7.5 Hz, H-6a) 3.19 (1H, dd, *J*=13.4, 2.5 Hz, H-6b), 2.06, 2.04, 2.01 (9H, 3 s, 3 CH_3CO). Spectroscopic data in agreement with literature.[109] Appendix 22

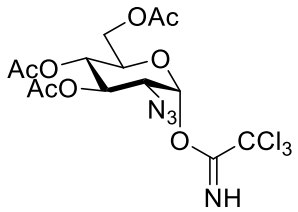
***p*-Tolyl 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-1-thio-*β*-D-glucopyranoside[110] (32)**



Methyl glucoside **30** (786 mg, 2.28 mmol) was dissolved in acetic anhydride (12 mL) and cooled to 0 °C, followed by the slow addition of concentrated sulphuric acid (0.12 mL), and the mixture was stirred at 0 °C for 2 h, when $NaHCO_3$ sat. (20 mL), water (20 mL), and DCM (50 mL) were added. The organic layer was separated, washed with $NaHCO_3$ sat. (2 × 50 mL), brine (50 mL), dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography (EtOAc:DCM 0-3%, containing 0.1% Et_3N), to afford 1,2,3,4-tetra-*O*-acetyl-6-azido-6-deoxy-*α*/*β*-D-glucopyranose[111] **31** as a colourless oil (354 mg, 0.95 mmol, 41%). R_f

0.40 (EtOAc:DCM 2,5%). Glucosyl acetate **31** (128 mg, 0.34 mmol, 1 equiv) previously dried under vacuum, and 4-methylbenzenethiol (120 mg, 0.97 mmol, 2.8 equiv) were dissolved in anhydrous DCM under N_2 and cooled to 0 °C. The mixture was treated dropwise with $BF_3 \cdot Et_2O$ (190 µL, 1.72 mmol, 5 equiv), and stirred at room temperature for 24 h, when it was neutralised with Et_3N and concentrated under vacuum. The residue was purified by column chromatography (EtOAc:DCM/Hex 1:1:8-1:1:6) to afford **32** as a yellowish oil (29.5 mg, 0.067 mmol, 20%). R_f 0.20 (EtOAc:DCM:Hex 1:1:6). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.40 (2H, d, *J*=8.1 Hz, CH STol), 7.14 (2H, d, *J*=7.9 Hz, CH-STol), 5.19 (1H, t, *J*=9.4 Hz, H-3), 4.94 (1H, d, *J*=9.4 Hz, H-4), 4.88 (1H, d, *J*=9.2 Hz, H-2), 4.64 (1H, d, *J*=10.2 Hz, H-1), 3.63 (1H, ddd, *J*=9.8, 5.8, 3.5 Hz, H-5), 3.40–3.23 (2H, m, H-6a, H-6b), 2.35 (3H, s, CH3 STol), 2.10, 2.01, 1.98 (9H, 3 s, 3 CH_3CO). Assignments based on the described phenyl thioglucoside[111] analogue. Appendix 23

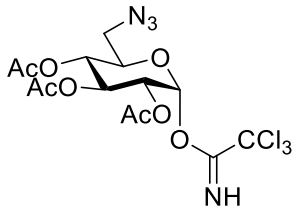
3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-*α*-D-glucopyranosyl trichloroacetimidate[112-114] (34)



1,3,4,6-Tetra-*O*-acetyl-2-azido-2-deoxy-D-glucopyranose **19** (506 mg, 1.36 mmol, 1 equiv) was treated with a 3:7 mixture of 7 M ammonia solution in MeOH (7 mL, 49 mmol, 36 equiv) and THF (14 mL), under N_2. The solution was stirred at room temperature for 75 min, when it was bubbled with N_2 for 5 min to remove the excess NH_3 and concentrated under vacuum. The residue was purified by column chromatography (EtOAc:Hex 0-40%), to afford 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-D-glucopyranose[115] **33** as a colourless oil (264 mg, 0.80 mmol, 58%), R_f 0.40 (EtOAc:Hex 1:1). Hemiacetal **33** (0.80 mmol, 1 equiv), previously dried under vacuum for 30 min, was dissolved in anhydrous DCM (5 mL) under N_2, and the solution was cooled to 0 °C. Trichloroacetonitrile (800 µL, 7.96 mmol, 10 equiv) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 36 µL, 0.24 mmol, 0.3 equiv) were added. The mixture was stirred at 0 °C for 1.5 h, when solvents were removed under vacuum. The residue was purified by column chromatography (EtOAc:Hex, 0-20% containing 0.2% Et_3N), to afford trichloroacetimidate **34** as a yellowish oil which gave a white solid upon drying under vacuum (239 mg, 0.50 mmol, 63%). R_f 0.55 (EtOAc:Hex 1:1). ^{1}H RMN (300 MHz, $CDCl_3$) δ 8.83 (1H,

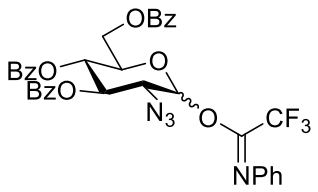
s, NH), 6.49 (1H, d, *J*=3.6 Hz, H-1), 5.52 (1H, dd, *J*=10.6, 9.3 Hz, H-3), 5.15 (1H, dd, *J*=10.2, 9.3 Hz, H-4), 4.27 (1H, dd, *J*=12.2, 4.2 Hz, H-6a), 4.21 (1H, ddd, *J*=10.4, 4.2, 1.9 Hz, H-5), 4.09 (1H, dd, *J*=12.3, 2.0 Hz, H-6b), 3.77 (1H, dd, *J*=10.5, 3.6 Hz, H-2), 2.11 (3H, s, CH_3CO), 2.06 (3H, s, CH_3CO), 2.05 (3H, s, CH_3CO). Spectroscopic data in agreement with literature.[115, 116] Appendix 24

2,3,4-Tri-*O*-acetyl-6-azido-6-deoxy-*α*-D-glucopyranosyl trichloroacetimidate[112, 113, 117] (36)



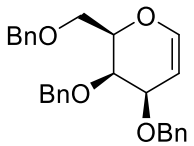
1,2,3,4-Tetra-*O*-acetyl-6-azido-6-deoxy-D-glucopyranose **31** (384 mg, 1.02 mmol, 1 equiv) was treated with a 3:7 mixture of 7 M ammonia solution in MeOH (4.7 mL, 32.9 mmol, 32 equiv) and THF (11 mL), under N_2. The solution was stirred at room temperature for 3 h, when it was bubbled with N_2 for 15 min to remove the excess NH_3 and concentrated under vacuum. The residue was purified by column chromatography (EtOAc:Hex 0-40%), to afford 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-D-glucopyranose[117] **35** as a colourless oil (214 mg, 0.65 mmol, 63%), R_f 0.45 (EtOAc:Hex 1:1). Hemiacetal **35** (256 mg, 0.77 mmol, 1 equiv), previously dried under vacuum for 30 min, was dissolved in anhydrous DCM (5 mL) under N_2, and the solution was cooled to 0 °C. Trichloroacetonitrile (770 µL, 7.67 mmol, 10 equiv) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 34 µL, 0.23 mmol, 0.3 equiv) were added. The mixture was stirred at 0 °C for 2 h, when solvents were removed under vacuum. The residue was purified by flash column chromatography (EtOAc:Hex, 5-60%), to afford trichloroacetimidate **36** as a yellowish oil which gave a white solid upon drying under vacuum (316 mg, 0.66 mmol, 86%). R_f 0.47 (EtOAc:Hex 1:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.72 (1H, s, NH), 6.58 (1H, d, *J*=3.7 Hz, H-1), 5.55 (1H, t, *J*=9.8 Hz, H-3), 5.20–5.06 (2H, m, H-4, H-2), 4.19 (1H, ddd, *J*=10.3, 5.5, 2.8 Hz, H-5), 3.41 (1H, dd, *J*=13.6, 2.8 Hz, H-6a), 3.32 (1H, dd, *J*=13.5, 5.4 Hz, H-6b), 2.06, 2.04, 2.02 (9H, 3 s, 3 CH_3CO). Spectroscopic data in agreement with literature.[117] Appendix 25

2-Azido-3,4,6-tri-*O*-benzoyl-2-deoxy-*α*-D-glucopyranosyl *N*-phenyl trifluoroacetimidate[118, 119] (37)



Title compound had been synthesised by the Carvalho Group following reported methodology[112, 118, 119] and was promptly available in the laboratory. Before use, it was analysed to check for identity and integrity. R_f 0.56 (EtOAc:Hex 3:7), *α:β* =1:0.9. ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 8.07–7.85 (12H, m, *o*-CH Bz), 7.59–7.47 (6H, m, *p*-CH Bz), 7.44–7.32 (12H, m, *m*-CH Bz), 7.29–7.24 (4H, m, *m*-CH Ph), 7.16–7.09 (2H, m, *p*-CH Ph), 6.85–6.73 (4H, m, *o*-CH Ph), 6.64 (1H, br, H-1 α), 6.03 (1H, t, *J*=10.0 Hz, H-3 α), 5.91 (1H, br, H-1 β), 5.68 (1H, t, *J*=9.8 Hz, H-4 α), 5.64–5.56 (2H, m, H-3 β, H-4 β), 4.64–4.42 (5H, m, H-5 α, CH_2-6 α,β), 4.19–4.04 (2H, m, H-5 β; H-2 β), 3.92 (1H, d, *J*=8.8 Hz, H-2 α). ^{13}C NMR (126 MHz, $CDCl_3$) *δ*: 166.1, 166.1, 165.6, 165.6, 165.4, 165.4 (COPh), 143.0, 143.0 (C=N), 142.6, 142.3 (*ipso*-C Ph), 133.8, 133.7, 133.7, 133.7, 133.3, 133.3, 130.1, 130.0, 129.9, 129.9, 129.7, 129.6 (CH Bz, *ipso*-C Bz), 129.0, 129.0 (CH Ph), 128.9, 128.8, 128.6, 128.6, 128.6, 128.5 (CH Bz, *ipso*-C Bz), 124.8, 124.8 (CH Ph), 119.4, 119.3 (CH Ph), 117.3, 117.1, 115.0, 114.8 (CF_3), 95.7 (C-1 β), 93.6 (C-1 α), 73.4 (C-5 β), 72.8 (C-3 β), 70.8, 70.7 (C-3 α, C-5 α), 69.2, 69.1 (C-4 α,β), 63.5 (C-2 β), 62.9 (C-6 β), 62.7 (C-6 α), 61.2 (C-2 α). Full characterisation is ongoing. Appendix 26

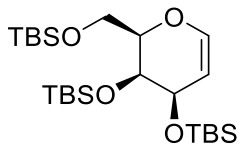
3,4,6-Tri-*O*-benzyl-D-galactal[120] (38)



D-galactal (1.01 g, 6.94 mmol, 1 equiv) was dissolved in anhydrous THF (14 mL) under N_2, and the mixture was cooled in an ice bath. NaH (2.64, 60 wt% dispersion in mineral oil, 66.1 mmol, 9.5 equiv) was added portionwise, followed by stirring at room temperature for 30 min, then cooling to 0 °C. BnBr (3 mL, 4.29 g, 25.1 mmol, 3.6 equiv) was added dropwise over 5 min, after which the ice bath was kept for 40 min. The mixture was stirred at room temperature, and tetrabutylammonium iodide (TBAI) was added after 18 h (40 mg, 0.11 mmol, 1.5 mol%) and 22 h (80 mg, 0.22 mmol, 3 mol%), when it was cooled to 0 °C for the addition

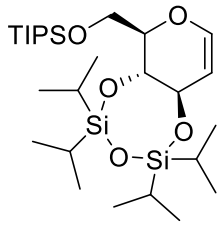
of more BnBr (3 mL, 4.29 g, 25.1 mmol, 3.6 equiv, in 5 mL THF). It was allowed to warm up to room temperature again and kept under stirring for another 19 h (41 h in total). Methanol (10 mL) was added slowly to the mixture at 0 °C to quench the excess NaH, and solvents were evaporated to dryness. The residue was dissolved in DCM (150 mL), washed with water (150 mL) and brine (150 mL), and the aqueous layers were then extracted with DCM. The combined organic layers were dried over $MgSO_4$, concentrated, and purified by flash column chromatography (EtOAc:Hex 0-20%), to afford the product **38** a pale yellow solid (1.98 g, 4.75 mmol, 68%), R_f 0.55 (EtOAc:Hex 2:8). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.39–7.25 (15H, m, Ph), 6.38 (1H, dd, *J*=6.2, 1.4 Hz, H-1), 4.92–4.85 (2H, m, OC*H*HPh, H-2), 4.70–4.60 (3H, m, 3 × OCHHPh), 4.52 (1H, d, *J*=11.9 Hz, OC*H*HPh), 4.44 (1H, d, *J*=11.9 Hz, OCH*H*Ph), 4.23–4.18 (2H, m, H-3, H-5), 3.97 (1H, s, H-4), 3.80 (1H, dd, *J*=10.2, 7.2 Hz, H-6a), 3.67 (1H, dd, *J*=10.1, 5.1 Hz, H-6b). Spectroscopic data in agreement with literature.[121] Appendix 27

3,4,6-Tri-*O*-(*tert*-butyldimethylsilyl)-D-galactal[122] (39)



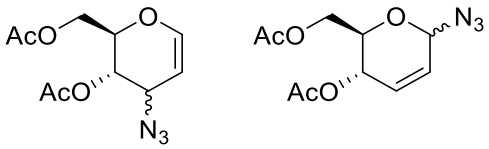
D-galactal (511.3 mg, 3.5 mmol, 1 equiv), imidazole (1.40 g, 21 mmol, 6 equiv) and 4-(*N,N*-dimethylamino)pyridine (DMAP, 45.6 mg, 0.37 mmol, 0.1 equiv) were dried under vacuum for 1 h, when the flask was filled with N_2. TBSCl (3.20 g, 21 mmol, 6 equiv) was added, followed by anhydrous DMF (20 mL), and the solution was heated to 60 °C and stirred for 42 h. Pentane (50 mL) and crushed ice were added, the layers were separated, and the aqueous layer was washed with pentane (2 × 30 mL). The combined organic layers were washed with water (2 × 10 mL), brine (2 × 10 mL), dried over $MgSO_4$, and concentrated under vacuum. The residue was purified by column chromatography (EtOAc:Hex 0-1%) to afford the product **(39)** as a colourless oil (97 mg, 0.20 mmol, 6%), R_f 0.24 (EtOAc: Hex 2:98); 1.0 (EtOAc:Hex 2:8).. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.21 (1H, d, *J*=6.1 Hz, H-1), 4.65 (1H, t, *J*=5.1 Hz, H-2), 4.27–3.77 (5H, m, H-3, H-4, H-5, CH_2-6), 1.03–0.76 (27H, m, 3 × $SiC(CH_3)_3$), 0.18–-0.04 (18H, m, 6 × $SiCH_3$). Spectroscopic data in agreement with literature.[122, 123] Appendix 28

1,2-Dideoxy-3,4-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-6-*O*-triisopropylsilyl-D-*arabino*-1-hexenopyranose [124] (40)



Title compound had been synthesised by the Galan Group following reported methodology[124] and was promptly available in the laboratory. Before use, it was analysed to check for identity and integrity. R_f 0.21 (EtOAc: Hex 2:98); 1.0 (EtOAc:Hex 2:8). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.34 (1H, dd, J=5.9, 1.7 Hz, H-1), 4.63 (1H, dd, J=6.1, 1.9 Hz, H-2), 4.41 (1H, d, J=6.5 Hz, H-3), 4.12 (1H, dd, J=11.2, 1.9 Hz, H-6a), 3.92 (1H, dd, J=11.1, 5.5 Hz, H-6b), 3.87 (1H, dd, J=10.1, 6.8 Hz, H-4), 3.77 (1H, dd, J=10.1, 5.0 Hz, H-5), 1.26–0.85 (49H, m, 7x $Si(CH(CH_3)_2)$). Spectroscopic data in agreement with literature.[124] Appendix 29

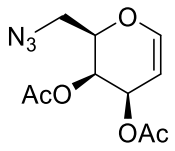
4,6-Di-O-acetyl-3-azido-3-deoxy- D-*arabino*-1-hexenopyranose; 4,6-Di-*O*-acetyl-3-azido-3-deoxy-D-*ribo*-1-hexenopyranose; and 4,6-Di-*O*-acetyl-2,3-dideoxy-*α/β*-D-*erythro*-2-hexenopyranosyl azide[125] (41)



Tri-*O*-acetyl-D-glucal (272 mg, 1 mmol, 1 equiv) and $RuCl_3$ (14 mg, 0.07 mmol, 7 mol%) were placed under vacuum for 1 h, then N_2 was applied. The solids were dissolved in anhydrous MeCN (2 mL) and trimethylsilyl azide ($TMSN_3$, 0.2 mL, 173 mg, 1.5 mmol, 1.5 equiv) was added. The reaction mixture was stirred at room temperature for 30 min, when the solvent was removed under vacuum. The residue was dissolved in DCM, filtered over Celite, concentrated and purified by column chromatography (EtOAc:Hex 0-20%) to afford a colourless oil containing a mixture of C-3 azido glucal epimers and C-1 glucosyl azide anomers (40.5 mg, 0.159 mmol, 16%), R_f 0.26 (EtOAc:Hex 2:8). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.53 (0.25H, d, J=5.9 Hz, H-1 C3 ax), 6.50 (0.35H, dd, J=6.1, 1.8 Hz, H-1 C3 eq), 6.04 (0.1H, ddd, J=10.2, 2.9, 1.9 Hz, H-2 C1 β), 5.95 (1H, dt, J=10.2, 1.6 Hz, H-3 C1 α), 5.86 (0.1H, dt, J=10.3, 1.7 Hz, H-3 C1 β), 5.78 (1H, ddd, J=10.2, 3.0, 2.0 Hz, H-2 C1 α), 5.57–5.55 (1H, m, H-1 C1α),

5.32 (1H, dq, *J*=9.5, 1.9 Hz, H-4 C1 α), 5.27 (0.1H, dt, *J*=6.5, 2.1 Hz, H-1 C1 β), 5.16 (0.35H, dd, *J*=9.0, 7.3 Hz, H-2 C3 eq), 5.09 (0.25H, dd, *J*=10.5, 4.3 Hz, H-2 C3 ax), 4.89 (1H, t, *J*=5.9 Hz, H-4 C3 ax), 4.80 (1H, dd, *J*=6.1, 2.6 Hz, H-4 C3 eq), 4.41–3.98 (6H, m, CH_2-6 C1/C3; H-5 C1/C3; H-3 C3), 2.17–2.06 (10H, m, CH_3CO). TLC-CMS for $C_{10}H_{13}N_3NaO_5^+$ (MNa^+) calcd: 287.1; found: 278.4. Appendix 30

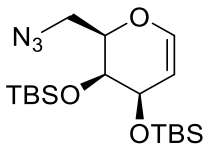
3,4-Di-*O*-acetyl-6-azido-6-deoxy-D-galactal[126, 127] (43)



D-galactal (1.02 g, 7.0 mmol, 1 equiv) and TsCl (2.00 g, 10.5 mmol, 1.5 equiv) were weighted into separate two-necked flasks and placed under vacuum for 1 h, when the flasks were filled with N_2. Galactal was dissolved in anhydrous pyridine (18 mL), while TsCl was dissolved in anhydrous DCM (18 mL), and both flasks were stirred in an ice bath for 10 min. TsCl solution was added dropwise to the galactal solution over a period of 20 min, and the mixture was stirred at 0 °C for 5.5 h. From the crude solution, half of the total volume (18 mL, 3.5 mmol of galactal) was transferred to another flask, treated with Ac_2O (0.5 mL, 19.7 mmol, 5.6 equiv) and the mixture was stirred at room temperature for 16 h. It was poured onto ice-water (50 mL), the organic phase was separated, and the aqueous phase was extracted with DCM (2 × 20 mL). The combined organic layers were washed with brine (60 mL), dried over $MgSO_4$, concentrated, and co-evaporated twice with toluene. The residue was purified by column chromatography (EtOAc:Hex 0-30%), affording the tosylate **42**[99] (421 mg, 1.10 mmol, 31%), R_f 0.62 (EtOAc:DCM 1:9). Tosyl galactal **42** (375 mg, 0.98 mmol, 1 equiv) was dissolved in anhydrous DMSO under an N_2 atmosphere, and NaN_3 was added (317 mg, 4.88 mmol, 5 equiv). The solution was heated to 85 °C and stirred for 5.5 h. After cooling down, the mixture was poured onto ice-water (40 mL) and extracted with diethyl ether (Et_2O, 5 × 15 mL). The combined organic layers were washed with water (40 mL) and brine (40 mL), dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography (EtOAc:Hex 0-30%), to afford the azide **43** as a colourless oil (178 mg, 0.70 mmol, 71%), R_f 0.67 (EtOAc:Hex 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) *δ:* 6.48 (1H, dd, *J*=6.3, 1.8 Hz, H-1), 5.54 (1H, ddt, *J*=4.5, 2.9, 1.5 Hz, H-3), 5.39 (1H, dt, *J*=4.5, 1.7 Hz, H-4), 4.74 (1H, ddd, *J*=6.4, 2.7, 1.4 Hz, H-2), 4.25–4.18 (1H, m, H-5), 3.62 (1H, dd, *J*=13.0, 8.4 Hz, H-6a), 3.31 (1H, dd, *J*=13.1,

4.4 Hz, H-6b), 2.13 (3H, s, CH_3CO), 2.02 (3H, s, CH_3CO). ^{13}C NMR (101 MHz, $CDCl_3$) *δ:* 170.3 (CH_3*C*O), 170.2 (CH_3*C*O), 145.5 (C-1), 99.1 (C-2), 74.2 (C-5), 64.5 (C-4), 64.1 (C-3), 50.7 (C-6), 20.9 (*C*H_3CO), 20.8 (*C*H_3CO). ESI-HRMS for $C_{10}H_{13}N_3NaO_5^+$ (MNa^+) calcd: 278.0747; found: 278.0757. Appendix 31

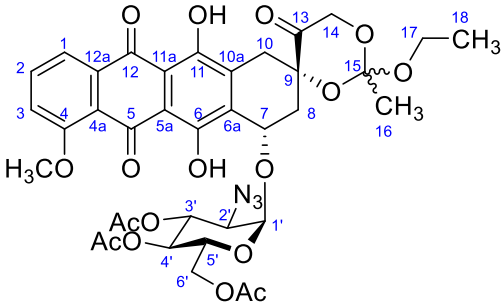
6-azido-6-deoxy-3,4-di-*O*-(*tert*-butyldimethylsilyl)-D-galactal [122, 128] (44)



To a solution of galactal **43** (383 mg, 1.50 mmol, 1 equiv) in EtOH (4 mL), K_2CO_3 (21 mg, 0.15 mmol, 0.1 equiv) was added, and the mixture was stirred at room temperature for 4 h. The solvent was removed under reduced pressure, traces of the alcohol were co-evaporated with $CHCl_3$ (3 × 10 mL) and the residue was dried under high vaccum for 30 min. The flask was filled with N_2, imidazole (613 mg, 9.0 mmol, 6 equiv) and anhydrous DMF (2,5 mL) were added, then a solution of TBSCl (680 mg, 4.5 mmol, 3 equiv) in anhydrous DMF (2,5 mL) was transferred, and the mixture was stirred at room temperature for 16 h. The reaction mixture was poured onto water (30 mL), extracted with Et_2O (4 × 40 mL, and the combined organic layers were dried over $MgSO_4$, and concentrated. The crude mixture was purified by column chromatography (EtOAc:Hex 0-5%), affording the mono-silyl derivative as major product (380 mg, 1.33 mmol, 88%), R_f 0.42 (EtOAc:Hex 1:9). ESI-HRMS for $C_{12}H_{23}N_3NaO_3Si^+$ (MNa^+) calcd: 308.1401; found: 308.1416. After pre-drying under high vacuum for 1 h, mono-silyl galactal (380 mg, 1.33 mmol, 1 equiv) was dissolved in anhydrous DMF (3 mL) under N_2. Separately, imidazole (546 mg, 8.0 mmol, 6 equiv), TBSCl (607 mg, 4.0 mmol, 3 equiv), and DMAP (15 mg, 0.123 mmol, 0.1 equiv) were dissolved in anhydrous DMF (6 mL), added to the galactal solution, and the mixture was heated to 60 °C. After 26 h, more imidazole (364 mg, 5.35 mmol, 4 equiv), TBSCl (405 mg, 2.68 mmol, 2 equiv) and DMAP (10 mg, 0.08 mmol, 0.06 equiv) in anhydrous DMF (2 mL) were added, and the mixture was further stirred at 60 °C until 36 h. It was poured onto crushed ice (100 mL), extracted with Et_2O (50 mL, 2 × 20 mL), the organic layers were washed with water (2 × 15 mL) and brine (2 × 15 mL), dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography (EtOAc:Hex 0-5%) to afford the product **44** as a colourless oil (432 mg, 1.08 mmol, 81%). R_f 0.72 (EtOAc:Hex 1:9). ^1H NMR (500 MHz, $CDCl_3$) *δ:* 6.22 (1H, d, *J*=6.1 Hz, H-1), 4.77 (1H, t, *J*=5.6

Hz, H-2), 4.14–4.09 (1H, m, H-5), 4.08–4.05 (1H, m, H-3), 4.03 (2H, t, *J*=4.0 Hz, H-4), 3.94 (1H, dd, *J*=13.7, 9.6 Hz, H-6a), 3.41 (1H, dd, *J*=13.7, 1.2 Hz, H-6b), 0.97–0.84 (18H, m, 2 × $SiC(CH_3)_3$), 0.16–0.01 (12H, m, 4 × $SiCH_3$). ^{13}C NMR (126 MHz, $CDCl_3$) *δ:* 142.2 (C-1), 103.2 (C-2), 77.3 (C-5), 69.1 (C-4), 64.2 (C-3), 49.7 (C-6), 26.0 ($SiC(CH_3)_3$), 26.0 ($SiC(CH_3)_3$), 18.3 ($SiC(CH_3)_3$), 18.2 ($SiC(CH_3)_3$), -4.1 ($SiCH_3$), -4.2 ($SiCH_3$), -4.7 ($SiCH_3$), -4.9 ($SiCH_3$). ESI-HRMS for $C_{18}H_{37}N_3NaO_3Si_2^+$ (MNa^+) calcd: 422.2266; found: 422.2268. Appendix 32

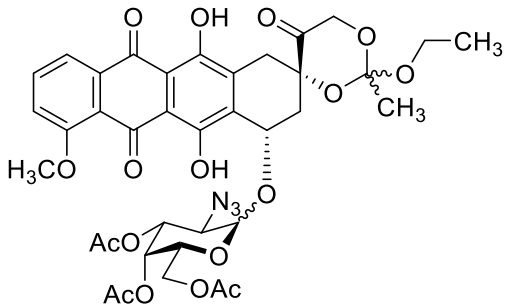
7-*O*-(3,4,6-tri-*O*-Acetyl-2-azido-2-deoxy-α-D-glucopyranosyl)-9,14-*O*-(1-ethoxyethane-1,1-diyl)doxorubicinone (45)



Doxorrubicinone orthoacetate **16** (17.1 mg, 0.035 mmol, 1 equiv) was dissolved in anhydrous DCM (1.5 mL) and transferred to a flask containing molecular sieves (4Å, powder), under N_2. Yellow mercuric oxide (HgO, 38.3 mg, 0.177 mmol, 5 equiv) and mercuric bromide ($HgBr_2$, 13 mg, 0.036 mmol, 1 equiv) were added, and the solution was stirred for 30 min, when a solution of azidochloride **20** (53.4 mg, 0.153 mmol, 4.4 equiv) in anhydrous DCM (0.3 mL) was transferred to the mixture. It was stirred at room temperature for 24 h, then at 40 °C for 18 h (42 h in total), when it was diluted with DCM (10 mL) and filtered over Celite. The filtrate was washed with KI 10% (2 × 10 mL) and water (2 × 20 mL), and the organic layer was dried over $MgSO_4$, and concentrated. The residue was purified by preparative thin-layer chromatography (MeOH:DCM 0,5%, eluted 3 times, R_f 0.24–0.35 band), to afford product **45** as a red solid (14.9 mg, 0.019 mmol, 52%). R_f 0.11 (MeOH:DCM 1:99). IR: $\nu_{N=N=N}$ 2110 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) *δ*: 13.56 (1H, s, OH-6), 13.18 (1H, s, OH-11), 8.03 (1H, dd, *J*=7.7, 1.0 Hz, H-1), 7.79 (1H, dd, *J*=8.4, 7.8 Hz, H-2), 7.40 (1H, dd, *J*=8.5, 0.7 Hz, H-3), 5.92 (1H, d, *J*=3.7 Hz, H-1′), 5.68 (1H, d, *J*=6.0 Hz, H-7), 5.52 (1H, dd, *J*=10.7, 9.2 Hz, H-3′), 5.06 (1H, t, *J*=9.4 Hz, H-4′), 4.38 (1H, d, *J*=12.8 Hz, H-14a), 4.32–4.25 (2H, m, H-5′, H-6′a), 4.13 (1H, d, *J*=12.8 Hz, H-14b), 4.09 (3H, s, OCH_3-4), 4.00 (1H, dd, *J*=14.0,4.2 Hz, H-6′b), 3.78 (1H, dq, *J*=9.3, 7.0 Hz, H-17a), 3.65–3.58 (2H, m, H-10a, H-17b), 3.40 (1H, dd, *J*=10.7, 3.7

Hz, H-2′), 3.13–3.03 (2 H, m, H-8a, H-10b), 2.12–2.01 (10H, 3 s, H-8b, 3 CH_3CO), 1.77 (3H, s, CH_3-16), 1.21 (3H, t, *J*=7.0 Hz, CH_3-18). ^{13}C NMR (101 MHz, $CDCl_3$) *δ*: 187.1, 187.1 (C-5, C-12), 170.7, 170.3, 170.1, 169.9 (C-13, 3 CH_3*C*O), 161.3 (C-4), 155.0, 153.5 (C-11, C-6), 139.3 (C-6a), 135.9 (C-2), 135.6, 135.1 (C-12a, C-10a), 121.2 (C-4a), 120.0 (C-1), 118.7 (C-3), 112.4, 112.1 (C-5a, C-11a), 104.5 (C-15), 94.7 (C-1′, 83.4 (C-9), 70.2 (C-3′), 68.9 (C-4′), 68.3, 68.3 (C-5′, C-7), 62.2 (C-6′), 61.4 (C-2′), 59.6 (C-14), 57.4 (C-17), 56.9 (OCH_3-4), 36.2 (C-8), 34.6 (C-10), 20.9, 20.8, 20.8 (3 *C*H_3CO), 20.5 (C-16), 15.9 (C-18). ESI-HRMS for $C_{37}H_{39}N_3NaO_{17}^+$ (MNa^+) calcd: 820.2172; found: 820.2179. Appendix 33

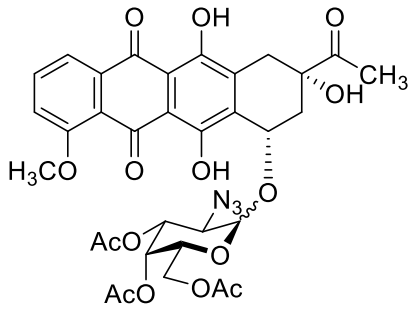
7-*O*-(3,4,6-tri-*O*-Acetyl-2-azido-2-deoxy-*α/β*-D-galactopyranosyl)-9,14-*O*-(1-ethoxyethane-1,1-diyl)doxorubicinone (46)



Doxorrubicinone orthoacetate **16** (23.2 mg, 0.048 mmol, 1 equiv) was dissolved in anhydrous 1,2-dichloroethane (DCE, 2 mL) and transferred to a flask containing molecular sieves (4Å, powder), under Ar. HgO (41.5 mg, 0.19 mmol, 4 equiv) and $HgBr_2$ (19 mg, 0.053 mmol, 1.1 equiv) were added, and the solution was stirred for 30 min, when a solution of azidochloride **25** (67 mg, 0.192 mmol, 4 equiv) in anhydrous DCE (0.6 mL) was transferred to the mixture. It was stirred at room temperature for 6 h, heated to 55 °C for 18 h, then at 90 °C for 24 h, when DCM was added and the mixture was stirred at room temperature for 4 days (6 days in total). It was diluted with DCM (15 mL) and filtered over Celite, the filtrate was washed with KI 10% (2 × 15 mL) and water (2 × 20 mL), and the organic layer was dried over $MgSO_4$, and concentrated. The residue was purified by preparative thin-layer chromatography (MeOH:DCM 0.5%, eluted 3 times, R_f 0.21–0.30 band), to afford product **46** as a red solid (6.3 mg, 0.008 mmol, 16%). R_f 0.33 (EtOAc:Tol 2:8). ^1H NMR (500 MHz, $CDCl_3$) *δ*: 13.57 (1H, s, OH), 13.17 (1H, s, OH), 8.03 (1H, d, *J*=7.7 Hz, H-1), 7.79 (1H, t, *J*=8.1 Hz, H-2), 7.40 (1H, d, *J*=8.4 Hz, H-3), 5.93 (1H, d, *J*=3.6 Hz, H-1′), 5.68 (1H, d, *J*=6.0 Hz, H-7), 5.50–5.33 (2H, m, H-4′, H-3′), 4.47 (2H, dd, *J*=11.5, 5.3 Hz, H-5′, H-6′a), 4.37 (1H, d, *J*=12.8 Hz, H-14a), 4.15–

4.03 (5H, m, H-14b, H-6′b, OCH_3-4), 3.82–3.64 (2H, m, H-17a, H-2′), 3.63–3.55 (2H, m, H-17b, H-10a), 3.11–2.97 (2H, m, H-10b, H-8a), 2.12–1.99 (10H, m, 3 CH3CO, H-8b), 1.77 (3H, s, CH_3-16), 1.22 (3H, t, *J*=7.0 Hz, CH_3-18). ESI-HRMS for $C_{37}H_{39}N_3NaO_{17}^+$ (MNa^+) calcd: 820.2172; found: 820.2171. Appendix 34

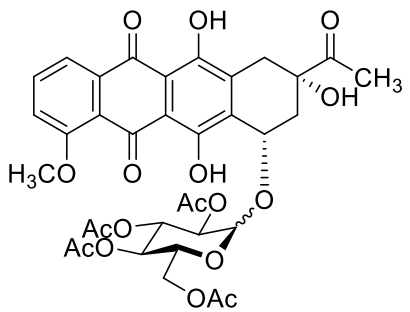
7-*O*-(3,4,6-tri-*O*-Acetyl-2-azido-2-deoxy-*α/β*-D-galactopyranosyl)daunorubicinone (47)



Daunorubicinone **9** (27 mg, 0.067 mmol, 1 equiv) previously dissolved and dried from toluene, HgO (59.5 mg, 0.27 mmol, 4 equiv), $HgBr_2$ (27.6 mg, 0.075 mmol, 1.1 equiv), and molecular sieves (4Å, powder) were placed under vacuum for 1 h, after which time the flask was filled with N_2, and anhydrous DCM (1.5 mL) was added. The mixture was stirred at room temperature for 30 min, when a solution of azidochloride **25** (70 mg, 0.20 mmol, 3 equiv) in anhydrous DCM (3 mL) was transferred. It was stirred at room temperature for 3.5 h, heated to 45 °C for 24 h, then DCE (5 mL) was added and the mixture was heated to 90 °C for 24 h, when heating was stopped stirring was kept for 4 days (6 days in total). It was filtered over Celite and concentrated The residue was dissolved in EtOAc (15 mL), washed with KI 10% (2 × 15 mL) and water (2 × 25 mL), and the organic layer was dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography (MeOH:DCM:Hex 1:50:50, MeOH:DCM 1:50) to afford product **47** as a red solid (2.0 mg, 0.003 mmol, 4%, *α:β* > 10:1). R_f 0.65 (MeOH:DCM 1:99). ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 14.14 (1H, s, OH-6), 13.29 (1H, s, OH-11), 8.05 (1H, d, *J*=7.7 Hz, H-1), 7.79 (1H, t, *J*=8.0 Hz, H-2), 7.40 (1H, d, *J*=8.3 Hz, H-3), 5.48 (1H, dd, *J*=3.0, 1.9 Hz, H-7), 5.40 (1H, d, *J*=2.2 Hz, H-4′), 5.31 (1H, d, *J*=3.9 Hz, H-1′), 5.15 (1H, dd, *J*=10.8, 3.1 Hz, H-3′), 4.75 (1H, t, *J*=6.4 Hz, H-5′), 4.54 (1H, s, OH-9), 4.22 (1H, dd, *J*=11.2, 7.0 Hz, H-6′a), 4.14 (1H, dd, *J*=11.3, 6.4 Hz, H-6′b), 4.12–4.07 (1H, m, H-2′), 4.08 (3H, s, OCH_3-4), 3.30 (1H, d, *J*=19.1 Hz, H-10a), 2.92 (1H, d, *J*=18.9 Hz, H-10b), 2.44 (3H, s, CH_3-14), 2.40 (1H, dt, *J*=15.5, 2.2 Hz, H-8a), 2.16 (6H, 2 s, 2 CH_3CO), 2.07–2.00 (1H, m, H-8b), 1.99 (3H, s, CH_3CO). ^{13}C NMR (126 MHz, $CDCl_3$) *δ*: 212.8 (C-13), 187.3, 187.1 (C-5.C-

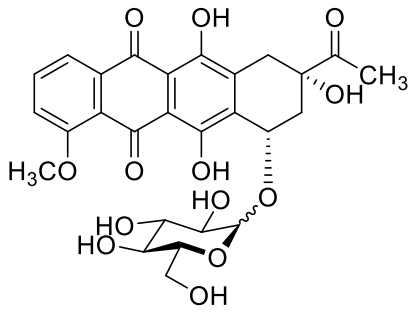
12), 170.8, 170.2, 169.6 (CH_3CO), 161.3 (C-4), 156.3, 155.8 (C-6, C-11), 136.1 (C-10a), 135.9 (C-2), 135.7 (C-12a), 132.2 (C-6a), 121.3 (C-4a), 120.0 (C-1), 118.7 (C-3), 112.0, 111.9 (C-5a, C-11a), 94.3 (C-1), 75.9 (C-9), 70.2 (C-3′), 67.8 (C-5′), 67.7 (C-4′), 65.9 (C-7), 61.7 (C-6′), 58.6 (C-2′), 56.9 (OCH_3-4), 33.8 (C-10), 30.9 (C-8), 25.0 (CH_3-14), 21.0, 20.8, 20.7 (3 CH_3CO). ESI-HRMS for $C_{33}H_{33}N_3NaO_{15}^+$ (MNa^+) calcd: 734.1804; found: 734.1806. Appendix 35

7-*O*-(2,3,4,6-Tetra-*O*-acetyl-*α/β*-D-glucopyranosyl)daunorubicinone (48)



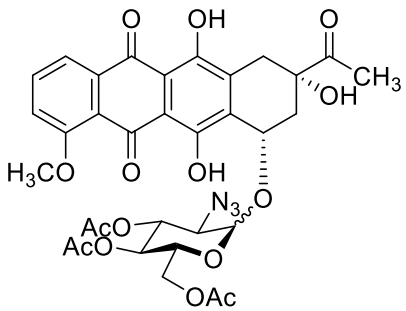
Daunorubicinone **9** (32 mg, 0.08 mmol, 1 equiv) previously dissolved and dried from toluene, HgO (69.3 mg, 0.32 mmol, 4 equiv), $HgBr_2$ (43.4 mg, 0.12 mmol, 1.5 equiv), and molecular sieves (4Å, powder) were placed under vacuum for 1 h, after which time the flask was filled with N_2, and anhydrous DCM (2 mL) was added. The mixture was stirred at room temperature for 40 min, when it was cooled to 0°C, and a solution of bromide **18** (66 mg, 0.16 mmol, 2 equiv) in anhydrous DCM (2 mL) was slowly transferred. It was stirred at 0°C for 1 h, then at room temperature for 5 h, when more bromide **18** (48 mg, 0.12 mmol, 1.5 equiv) was added, and stirring was kept for 1 h (7 h in total). It was filtered over Celite and concentrated The residue was dissolved in EtOAc (25 mL), washed with KI 10% (2 × 20 mL) and EDTA 5% (2 × 20 mL), and the organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography (MeOH:DCM 0-1%) to afford product **48** as a red solid (18 mg, 0.024 mmol, 30%, α:β = 1:4). R_f 0.43 (MeOH:DCM 1:99). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 14.04 (1H, s, OH-6), 13.22 (1H, s, OH-11), 8.02 (1H, dd, *J*=7.8, 1.1 Hz, H-1), 7.78 (1H, dd, *J*=8.4, 7.8 Hz, H-2), 7.39 (1H, dd, *J*=8.5, 0.9 Hz, H-3), 5.37 (1H, dd, *J*=3.9, 2.1 Hz, H-7), 5.24 (1H, t, *J*=9.4 Hz, H-3′), 5.14–5.01 (2H, m, H-4′, H-1′), 4.93 (1H, dd, *J*=9.5, 8.0 Hz, H-2′), 4.35–4.18 (2H, m, H-6′a, H-6′b), 4.08 (3H, s, OCH_3-4), 3.82 (1H, ddd, *J*=10.0, 4.7, 2.8 Hz, H-5′), 3.18 (1H, dd, *J*=19.1, 1.7 Hz, H-10a), 2.90 (1H, d, *J*=19.1 Hz, H-10b), 2.56 (1H, dt, *J*=15.0, 1.8 Hz, H-8a), 2.43 (3H, s, CH_3-14), 2.22–1.93 (10H, m, CH_3CO, H-8b). MALDI-HRMS for $C_{35}H_{36}KO_{17}^+$ (MK^+) calcd: 767.1584; found 767.1586. Appendix 36

7-*O*-α/β-D-glucopyranosyl-daunorubicinone (49)



The mixture of acetyl glycosides **48** (16 mg, 0.022 mmol) was dissolved in THF (3 mL) and cooled down to 0 °C, followed by the addition of cold aqueous 0.1 M NaOH solution (3 mL), and stirring for 2 h, when cold aqueous 1.0 M NaOH solution (0.1 mL) was added, and the ice bath was removed. The mixture was stirred at room temperature for 22 h (24 h in total), when it was neutralised with Dowex H^+ 50WX8-400, filtered and concentrated. The residue was purified by column chromatography (MeOH:DCM 0-15%) and by RP-HPLC (C18, MeCN:H_2O + 0.1% trifluoroacetic acid, 5-60%), affording the glycosides **49** (1.6 mg, 0.003 mmol, 13%, *α:β* < 1:10). ^{1}H NMR (300 MHz, DMSO-*d*6): 13.99 (1H, s, OH-6), 13.22 (1H, s, OH-11), 7.94–7.87 (2H, m, H-2, H-3), 7.70–7.61 (1H, m, H-1), 5.25 (1H, s, H-7), 4.54 (1H, d, *J*=7.9 Hz, H-1′), 3.98 (3H, s, OCH_3-4), 3.74 (1H, d, *J*=11.5 Hz, Glc), 3.21–3.03 (3H, m, Glc), 2.99–2.90 (3H, m, H-10a, H-10b, H-2′), 2.42 (1H, d, *J*=14.6 Hz, H-8a), 2.28 (1H, s, CH_3-14), 2.14 (1H, dd, *J*=14.9, 4.2 Hz, H-8b). Assignments based on the described alpha glucoside[129] analogue. ESI-HRMS for $C_{27}H_{32}NO_{13}^+$ (MNH_4^+) calcd: 578.1868; found 578.1863. Appendix 37

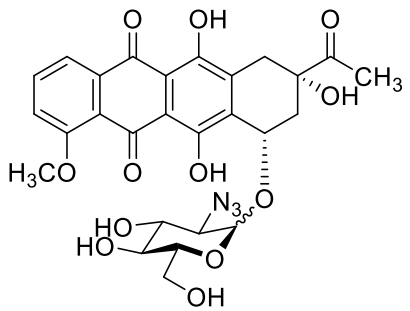
7-*O*-(3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-α/β-D-glucopyranosyl)daunorubicinone (50)



Daunorubicinone **9** (38 mg, 0.095 mmol, 1 equiv) previously dissolved and dried from toluene, imidate **34** (67 mg, 0.14 mmol, 1.5 equiv), and molecular sieves (4Å, powder) were placed under vacuum for 1 h, after which time the flask was filled with N_2, anhydrous DCE (2

mL) was added and it was cooled to 0 °C. TMSOTf (4.3 µL, 0.024 mmol, 0.25 equiv) in DCE (0.1 mL) was added slowly, and the mixture was stirred at room temperature for 48 h, when more imidate **34** (24.2 mg, 0.0.05 mmol, 0.5 equiv) and TMSOTf (9 µL, 0.05 mmol, 0.5 equiv) were added, the solution was heated at 90 °C for 14 h (62 h in total). The mixture was neutralised with Et_3N, filtered over Celite, concentrated, and the residue was purified by column chromatography (MeOH:DCM 0.2-0.8%), affording glycosides **50** as a red film (25.4 mg, 0.035 mmol, 38%, α:β > 5:1). R_f 0.35 (MeOH:DCM 1:99) ^{1}H (300 MHz, $CDCl_3$) δ: 14.22 (1H, s, OH-6), 13.33 (1H, s, OH-11), 8.07 (1H, dd, *J*=7.7, 1.0 Hz, H-1), 7.81 (1H, dd, *J*=8.4, 7.8 Hz, H-2), 7.42 (1H, dd, *J*=8.6, 0.9 Hz, H-3), 5.46 (1H, dd, *J*=3.2, 1.8 Hz, H-7), 5.35–5.23 (2H, m, H-3′, H-1′), 5.07 (1H, dd, *J*=10.2, 9.5 Hz, H-4′), 4.52 (1H, dt, *J*=10.6, 2.2 Hz, H-5′), 4.47 (1H, s, OH-9), 4.44 (1H, dd, *J*=12.4, 3.5 Hz, H-6′a), 4.19 (1H, dd, *J*=12.4, 2.0 Hz, H-6′b), 4.11 (3H, s, OCH_3-4), 3.91 (1H, dd, *J*=10.3, 3.9 Hz, H-2′), 3.31 (1H, dd, *J*=19.1, 1.4 Hz, H-10a), 2.94 (1H, d, *J*=19.1 Hz, H-10b), 2.44 (3H, s, CH_3-14), 2.39 (1H, d, *J*=13.6 Hz, H-8a), 2.14 (3H, s, CH_3CO), 2.18–1.94 (7H, m, H-8b, CH_3CO). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 212.8 (C-13), 187.4, 186.9 (C-5, C-12), 170.8, 170.0, 169.9 (CH_3*C*O), 161.2 (C-4), 156.4, 155.8 (C-6, C-11), 136.5 (C-6a), 136.0 (C-2), 135.6 (C-12a), 132.0 (C-10a), 121.0 (C-4a), 120.0 (C-1), 118.6 (C-3), 111.8, 111.7 (C-5a, C-11a), 94.0 (C-1′), 75.8 (C-9), 72.4 (C-3′), 68.6 (C-5′), 68.1 (C-4′), 66.3 (C-7), 62.1 (C-2′), 61.8 (C-6′), 56.9 (OCH_3-4), 33.8 (C-10), 31.0 (C-8), 24.9 (CH_3-14), 20.9, 20.8, 20.7 (*C*H_3CO). ESI-HRMS for $C_{33}H_{37}N_4O_{15}^+$ (MNH_4^+) calcd: 729.2250; found 729.2242. Appendix 38

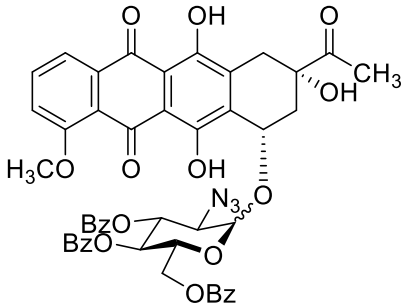
7-*O*-(2-azido-2-deoxy-α/β-D-glucopyranosyl)daunorubicinone (51)



The mixture of acetylated glycosides **50** (22 mg, 0.03 mmol) was dissolved in THF (2 mL) and cooled down to 0 °C, followed by the addition of cold aqueous 0.1 M NaOH solution (2 mL), and stirring for 4 h, when and cold aqueous 1.0 M NaOH solution (0.1 mL) was added, and the ice bath was removed. The mixture was stirred at room temperature for 16 h (20 h in

total), when it was neutralised with Dowex H^+ 50WX8-400, filtered and concentrated. The residue was purified by column chromatography (MeOH:DCM 0-15%), affording the glycosides **51** (6.1 mg, 0.01 mmol, 34%). ^{1}H NMR (400 MHz, DMSO-*d6*) *δ*: 13.86 (s, OH), 13.16 (s, OH), 7.89– 7.84 (2H, m, H-2, H-3), 7.61 (1H, m, H-1), 5.53 (d, *J*=5.8 Hz, OH), 5.26 (1H, d, *J*=3.8 Hz, H-1′), 5.25–5.22 (1H, m, H-7), 5.10 (d, *J*=5.9 Hz, OH), 4.55 (t, *J*=5.7 Hz, OH), 3.96 (3H, s, OCH_3-4), 3.75–3.64 (3H, m, H-5′, H-6′a, H-6′b), 3.49–3.21 (3H, m, H-3′, H-2′, H-4′), 3.01 (1H, d, *J*=17.9 Hz, H-10a), 2.90 (1H, d, *J*=18.1 Hz, H-10b), 2.40 (1H, d, *J*=15.0 Hz, H-8a), 2.28 (3H, s, CH_3-14), 1.98 (1H, dd, *J*=14.9, 4.6 Hz, H-8b). ^{13}C NMR(101 MHz, DMSO-*d6*) *δ*: 212.1 (C-13), 136.5 (C-2), 120.0 (C-1), 119.3 (C-3), 94.5 (C-1′), 75.8 (C-9), 73.4 C-5′), 71.9, 69.8 (C-4′, C-3′), 65.6 (C-7), 63.9 (C-2′), 60.2 (C-6′), 56.8 (OCH_3-4), 32.4, 32.1 (C-10, C-8), 24.4 (CH_3-14). ESI-HRMS for $C_{27}H_{28}N_3O_{12}^+$ (MH^+) calcd: 586.1667; found 586.1639. Appendix 39

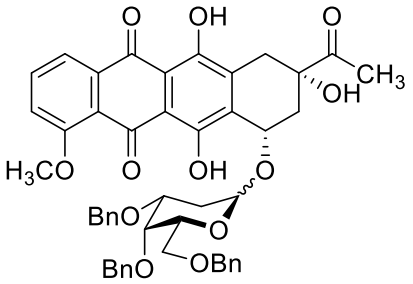
7-*O*-(2-Azido-2-deoxy-3,4,6-tri-*O*-benzoyl-*α/β*-D-glucopyranosyl)daunorubicinone (52)



Daunorubicinone **9** (23.6 mg, 0.06 mmol, 1 equiv) previously dissolved and dried from toluene, imidate **37** (51.6 mg, 0.074 mmol, 1.25 equiv), and molecular sieves (4Å, powder) were placed under vacuum for 1 h, and the flask was filled with N_2. Anhydrous Et_2O (2 mL) and DCM (2 mL) were added, the solution was stirred for 30 min, when it was cooled to -50 °C, and TMSOTf (2.2 µL, 0.012 mmol, 0.2 equiv) in DCM (0.1 mL) was added slowly. The mixture was stirred at -30 to -10 °C for 3 h, when more TMSOTf (0.2 equiv) was added, and stirring continued for 21 h, warming up to room temperature. More imidate **37** (50.5 mg, 0.073 mmol, 1.22 equiv) was added, followed by DCE (5 mL), and the mixture was heated to 60 °C for 44 h (65 h in total). The mixture was neutralised with Et_3N, filtered over Celite, concentrated, and the residue was purified by column chromatography (EtOAc:Hex 30-40%, MeOH:DCM 1-2.5%), affording glycosides **52** as a red film (22 mg, 0.024 mmol, 41%, *α:β* > 10:1). R_f 0.67 (MeOH:DCM 1:99) ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 14.28 (1H, s, OH-6), 13.33

(1H, s, OH-11), 8.16–7.78 (8H, m, CH Bz, H-1, H-2), 7.51–7.29 (10H, m, CH Bz, H-3), 5.83 (1H, t, *J*=9.9 Hz, H-3′), 5.68 (1H, t, *J*=9.9 Hz, H-4′), 5.56 (1H, dd, *J*=3.0, 1.6 Hz, H-7), 5.43 (1H, d, *J*=3.9 Hz, H-1′), 4.83 (1H, dt, *J*=10.3, 2.6 Hz, H-5′), 4.77 (1H, dd, J=12.4, 2.5 Hz, H-6′a), 4.60 (1H, s, OH-9), 4.48 (1H, dd, *J*=12.3, 3.0 Hz, H-6′b), 4.14 (1H, dd, *J*=10.2, 3.9 Hz, H-2′), 4.08 (3H, s, OCH_3-4), 3.33 (1H, d, *J*=18.6 Hz, H-10a), 2.97 (1H, d, *J*=19.1 Hz, H-10b), 2.52–2.41 (4H, m, H-8a, CH_3-14), 2.06 (1H, dd, *J*=15.5, 3.7 Hz, H-8b). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 212.8 (C-13), 187.4, 187.0 (C-5, C-12), 166.3, 165.6, 165.5 (3 COPh), 161.3 (C-4), 156.6, 155.9 (C-6, C-11), 136.5 (C-10a), 135.9 (C-2), 135.7 (C-12a), 133.5, 133.4, 133.1 (CH Bz), 132.3 (C-6a), 130.1, 130.0, 129.9, 129.0, 129.0, 128.5, 128.4 (CH Bz, *ipso*-C Bz), 121.2 (C-4a), 120.0 (C-1), 118.6 (C-2), 112.0, 111.8 (C-11a, C-5a), 94.4 (C-1′), 75.9 (C-9), 72.3 (C-3′), 69.2, 69.1 (C-4′ C-5′), 66.4 (C-7), 62.9 (C-2′), 62.5 (C-6′), 56.9 (OCH_3-4), 33.9 (C-10), 31.3 (C-8), 24.9 (C-14). ESI-HRMS for $C_{48}H_{43}N_4O_{15}^+$ (MNH_4^+) calcd: 915.2719; found 915.2719. Appendix 40

7-*O*-(3,4,6-Tri-*O*-benzyl-2-deoxy-α/β-D-*lyxo*-hexopyranosyl)daunorubicinone (53)



Method I.[130] Tri-*O*-benzyl-galactal donor **38** (82.8 mg, 0.20 mmol, 1 equiv) and aglycone acceptor **9** (52.6 mg, 0.13 mmol, 0.66 equiv) were weighted into a microwave vial and placed under vacuum for 1 h, after which time the vial was filled with N_2. Meanwhile, AgOTf (6.8 mg, 0.009 mmol, 0.05 equiv) and [(*p*CF_3Ph)$_3$P]AuCl (**A**) (5.6 mg, 0.02 mmol, 0.1 equiv) were suspended in anhydrous DCM (2 mL) and stirred for 15 min in a sonicating bath, and then added to the vial containing the donor and acceptor. The reaction mixture was stirred at room temperature for 5 h, followed by TLC and NMR analysis of the crude material. The crude residue was purified by column chromatography (EtOAc:Hex 0-100%, MeOH:EtOAc 20%), to afford a partially separable mixture of glycosides **53** as a red film (50.2 mg, 0.062 mmol, 46%, α:β = 1.6:1), R_f 0.48 (EtOAc:Hex 2:8).

Method II-*S*.[131] Tri-*O*-benzyl-galactal donor **38** (54.0 mg, 0.13 mmol, 1 equiv) and aglycone acceptor **9** (33.0 mg, 0.08 mmol, 0.64 equiv) were weighted into a microwave vial and placed under vacuum for 1 h, after which time the vial was filled with N_2. A mixture of (*S*)-3,3′-Bis(3,5-bis(trifluoromethyl)phenyl)-1,1′-binaphthyl-2,2′-diyl hydrogen phosphate (*S*)-**B** (10.0 mg, 0.013 mmol, 0.1 equiv) and Schreiner's thiourea **C** (13.2 mg, 0.026 mmol, 0.2 equiv) in anhydrous DCM (1 mL) was stirred at room temperature for 30 min, under N_2, and then added to the microwave vial containing the donor and acceptor. The reaction mixture was heated at reflux (45 °C) for 20 h, with one addition of donor (50 mg, 0.12 mmol, 0.9 equiv) at 4 h. It was concentrated under vacuum and purified by column chromatography (EtOAc:Tol 0-20%), to afford a mixture of glycosides **53** as a red film (46 mg, 0.056 mmol, 68%, α:β = 1.5:1), R_f 0.57 (EtOAc:Hex 1:1).

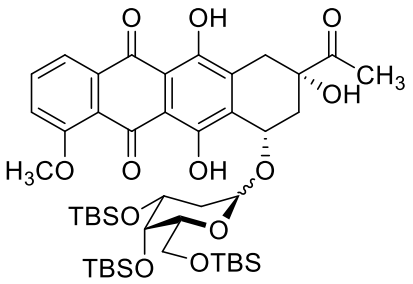
Method II-*R*.[131] Tri-*O*-benzyl-galactal donor **38** (39.0 mg, 0.09 mmol, 1 equiv) and aglycone acceptor **9** (24.5 mg, 0.06 mmol, 0.66 equiv) were weighted into a microwave vial and placed under vacuum for 2 h, after which time the vial was filled with N_2. A mixture of (*R*)-3,3′-Bis(3,5-bis(trifluoromethyl)phenyl)-1,1′-binaphthyl-2,2′-diyl hydrogen phosphate (*R*)-**B** (5.8 mg, 0.008 mmol, 0.1 equiv) and Schreiner's thiourea **C** (9.4 mg, 0.019 mmol, 0.2 equiv) in anhydrous DCM (1 mL) was stirred at room temperature for 30 min, under N_2, and then added to the microwave vial containing the donor and acceptor. The reaction mixture was heated at reflux (45 °C) for 6 h, when more donor (25 mg, 0.06 mmol, 0.66 equiv) was added, and then the mixture was stirred at room temperature until 20 h. It was concentrated under vacuum and purified by column chromatography (EtOAc:Hex 0-100%), to afford a partially separable mixture of glycosides **53** as a red film (42.3 mg, 0.052 mmol, 84%, α:β = 3.7:1).

α: R_f 0.58 (EtOAc:Hex 1:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 14.14 (1H, s, OH-Ph), 13.27 (1H, s, OH-Ph), 8.01 (1H, dd, *J*=7.7, 1.1 Hz, H-1), 7.75 (1H, dd, *J*=8.4, 7.7 Hz, H-2), 7.39–7.19 (16H, m, H-3, Ph), 5.51 (1H, t, *J*=2.6 Hz, H-7), 5.41 (1H, d, *J*=3.6 Hz, H-1′), 4.90 (1H, d, *J*=11.5 Hz, OC*H*HPh), 4.69 (1H, s, OH-9), 4.61–4.46 (5H, m, 5 × OCHHPh), 4.44 (1H, t, *J*=6.6 Hz, H-5′), 4.06 (3H, s, OCH_3-4), 3.97 (1H, s. H-4′), 3.83 (1H, ddd, *J*=12.2, 4.5, 2.4 Hz, H-3′), 3.65 (1H, dd, *J*=9.1, 7.9 Hz, H-6′a), 3.59 (1H, dd, *J*=9.1, 5.5 Hz, H-6′b), 3.24 (1H, dd, *J*=19.0, 1.8 Hz, H-10a), 2.99 (1H, *d*, J=19.0 Hz, H-10b), 2.43 (1H, d, *J*=15.2 Hz, H-8a), 2.38 (3H, s, CH_3-14), 2.34 (1H, dd, *J*=12.7, 3.8 Hz, H-2′a), 1.88–1.84 (2H, m, H-2′b, H-8b). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 212.0 (C-13), 187.1, 186.9 (C-5, C-12), 156.7 (C-6, C-11), 139.0 (4º C), 138.5 (4º C), 138.5 (4º C), 135.8 (C-2), 135.7, 135.6, 133.7 (C-6a, C-12a, C-10a), 128.5 (CH), 128.3 (CH), 128.3 (CH), 127.9 (CH), 127.6 (CH), 127.6 (CH), 127.4 (CH), 121.2 (C-4a), 119.9 (C-1), 118.5 (C-3), 111.6, 111.5 (C-5a, C-11a), 94.8 (C-1′), 74.7 (C-3′), 74.5 (OCH_2Ph), 73.6

(OCH_2Ph), 72.8 (C-4′), 71.0 (C-5′), 70.6 (OCH_2Ph), 69.0 (C-6′), 64.2 (C-7), 56.8 (OCH_3-4), 34.2 (C-10), 31.3 (C-2′), 30.1 (C-8), 24.9 (C-14). ESI-HRMS for $C_{48}H_{50}NO_{12}^+$ (MNH_4^+) calcd: 832.3328; found 832.3327 (α:β mixture). MALDI-MS for $C_{48}H_{46}KO_{12}^+$ (MK^+) calcd: 853.262; found: 853.373 (α anomer). Appendix 41

β (contains ~10% of *α*-anomer): R_f 0.50 (EtOAc:Hex 1:1). ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 14.04 (1H, s, OH-Ph), 13.31 (1H, s), 8.05 (1H, dd, *J*=7.7, 1.0 Hz, H-1), 7.79 (1H, dd, *J*=8.4, 7.8 Hz, H-2), 7.42–7.27 (16H, m, H-3, Ph), 5.28 (1H, dd, *J*=4.0, 1.8 Hz, H-7), 4.98 (1H, dd, *J*=9.8, 2.1 Hz, H-1′), 4.93 (1H, d, *J*=11.7 Hz, OC*H*HPh), 4.66–4.58 (2H, m, 2 × OCHHPh), 4.55 (1H, d, *J*=12.0 Hz, OCH*H*Ph), 4.48 (2H, s, OCH_2Ph), 4.10 (3H, s, OCH_3-4), 3.83 (1H, d, *J*=2.3 Hz, H-4′), 3.67–3.56 (4H, m, CH_2-6′, H-5′, H-3′), 3.23 (1H, dd, *J*=18.8, 2.0 Hz, H-10a), 2.89 (1H, d, *J*=18.7 Hz, H-10b), 2.63 (1H, dt, *J*=14.8, 1.9 Hz, H-8a), 2.41 (3H, s, CH_3-14), 2.13–2.05 (1H, m, H-2′a), 2.04 (1H, dd, *J*=14.9, 4.2 Hz, H-8b), 1.98 (1H, td, *J*=12.1, 9.9 Hz, H-2′b). ^{13}C NMR (126 MHz, $CDCl_3$) *δ*: 213.9 (C-13), 187.4, 186.9 (C-5, C-12), 161.2 (C-4), 156.9, 156.3 (C-11, C-6), 138.9 (4º C), 138.4 (4º C), 138.1 (4º C), 135.8, 135.8 (C-2, C-12a), 135.6, 134.1 (C-6a, C-10a), 128.6 (CH), 128.5 (CH), 128.4 (CH), 128.0 (CH), 128.0 (CH), 127.8 (CH), 127.7 (CH), 127.5 (CH), 121.2 (C-4a), 120.0 (C-1), 118.4 (C-3), 111.5, 111.4 (C-5a, C-11a), 102.9 (C-1′), 77.6 (C-5′), 76.6 (C-9), 74.5 (C-3′), 74.5 (OCH_2Ph), 73.7 (OCH_2Ph), 72.1 (C-4′), 70.5 (OCH_2Ph), 70.3 (C-7), 69.5 (C-6′), 56.9 (OCH_3-4), 35.3 (C-8), 33.4 (C-10), 32.8 (C-2′), 25.4 (C-14). ESI-HRMS for $C_{48}H_{50}NO_{12}^+$ (MNH_4^+) calcd: 832.3328; found 832.3327 (α:β mixture). MALDI-MS for $C_{48}H_{46}NaO_{12}^+$ (MNa^+) calcd: 837.288; found: 837.294 (β anomer). Appendix 42

7-*O*-(3,4,6-Tri-*O*-*tert*-butyldimethylsilyl-2-deoxy-*α/β*-D-*lyxo*-hexopyranosyl)daunorubicinone (54)



Method I.[122] Tri-*O*-*tert*-butyldimethylsilyl-galactal donor **39** (46.1 mg, 0.09 mmol, 1 equiv) and aglycone acceptor **9** (31.3 mg, 0.08 mmol, 0.83 equiv) were weighted into separate microwave vials and placed under vacuum for 1 h, after which time the vials were filled with N_2, the donor was dissolved in anhydrous DCM (0.25 mL) and added to the acceptor vial. A

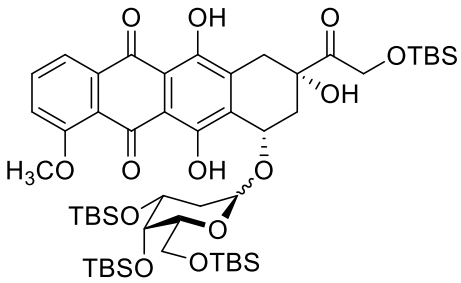
solution of Schreiner's thiourea **C** in anhydrous DCM (~6 mg/mL, 80 µL, 0.48 mg, 0.9 µmol, 1 mol%) was added and the reaction mixture was heated at reflux (50 °C) for 41 h, with one addition of donor (20.6 mg, 0.042 mmol, 0.45 equiv) at 25 h. It was concentrated under vacuum and purified by column chromatography (MeOH:DCM 0-0.4%), to afford a mixture of glycosides **54** as a red film (0.6 mg, 0.68 µmol, 0.8%, α:β = 19:1), R_f 0.37 (EtOAc:Hex 2:8). Intact aglycone was also recovered (29 mg, 0.072 mmol, 92%), R_f 0.0 (EtOAc:Hex 2:8).

Method II.[131] Tri-*O*-*tert*-butyldimethylsilyl-galactal donor **39** (57.4 mg, 0.12 mmol, 1 equiv) and aglycone acceptor **9** (23.0 mg, 0.06 mmol, 0.5 equiv) were weighted into separate microwave vials and placed under vacuum for 1 h, after which time the vials were filled with N_2, the donor was dissolved in anhydrous DCM (0,5 mL) and added to the acceptor vial. A mixture of (*R*)-3,3′-Bis(3,5-bis(trifluoromethyl)phenyl)-1,1′-binaphthyl-2,2′-diyl hydrogen-phosphate (*R*)-**B** (9.6 mg, 0.012 mmol, 0.1 equiv) and Schreiner's thiourea **C** (12.0 mg, 0.024 mmol, 0.2 equiv) in anhydrous DCM (0.5 mL) was stirred at room temperature for 30 min, under N_2, and then added to the microwave vial containing the donor and acceptor. The reaction mixture was heated at reflux (45 °C) for 20 h, when it was concentrated under vacuum and purified by column chromatography (EtOAc:Hex 0-10%), to afford a partially separable mixture of glycosides **54** as a red film (49 mg, 0.055 mmol, 95%, α:β = 4.4:1), R_f 0.39 (EtOAc:Hex 2:8).

α: ^{1}H NMR (500 MHz, $CDCl_3$) δ: 14.10 (1H, s, OH-6), 13.31 (1H, s, OH-11), 8.03 (1H, dd, *J*=7.7, 1.0 Hz, H-1), 7.77 (1H, dd, *J*=8.5, 7.7 Hz, H-2), 7.38 (1H, dd, *J*=8.5, 1.0 Hz, H-3), 5.50 (1H, dd, *J*=3.4, 2.3 Hz, H-7), 5.37 (1H, d, *J*=3.6 Hz, H-1′), 4.85 (1H, s, OH-9), 4.07 (3H, s, OCH_3-4), 4.03 (1H, dd, *J*=7.9, 6.0 Hz, H-5′), 3.94 (1H, ddd, *J*=11.9, 4.3, 2.2 Hz, H-3′), 3.87 (1H, s, H-4′), 3.68 (1H, dd, *J*=9.6, 7.9 Hz, H-6′a), 3.59 (1H, dd, *J*=9.7, 5.9 Hz, H-6′b), 3.24 (1H, dd, *J*=18.9, 1.7 Hz, H-10a), 3.05 (1H, d, *J*=19.0 Hz, H-10b), 2.46 (1H, dt, *J*=15.1, 2.1 Hz, H-8a), 2.40 (3H, s, CH_3-14), 2.25 (1H, td, *J*=12.5, 3.9 Hz, H-2′a), 1.84 (1H, dd, *J*=15.1, 3.5 Hz, H-8b), 1.53 (1H, dd, *J*=13.1, 4.4 Hz, H-2′b), 0.93–0.83 (27H, m, 3 × $SiC(CH_3)_3$), 0.13–-0.02 (18H, m, 6 × $SiCH_3$). ^{13}C NMR (126 MHz, $CDCl_3$): 212.3 (C-13), 187.1, 187.0 (C-5, C-12), 161.2 (C-4), 156.9, 155.9 (C-6, C-11), 135.7 (C-12a), 135.7 (C-2), 135.6 (C-10a), 134.0 (C-6a), 121.4 (C-4a), 119.8 (C-1), 118.6 (C-3), 111.6, 111.4 (C-11a, C-5a), 95.5 (C-1′), 77.0 (C-9), 73.7 (C-5′), 69.8 (C-4′), 68.2 (C-3′), 64.7 (C-7), 61.7 (C-6′), 56.8 (OCH_3-4), 34.3 (C-10), 33.8 (C-2′), 30.7 (C-8), 26.3 ($SiC(CH_3)_3$), 26.3 ($SiC(CH_3)_3$), 26.1 ($SiC(CH_3)_3$), 25.0 (C-14), 18.8 ($SiC(CH_3)_3$), 18.5 ($SiC(CH_3)_3$), 18.3 ($SiC(CH_3)_3$), -3.8 ($SiCH_3$), -4.3 ($SiCH_3$), -4.5 ($SiCH_3$), -4.8 ($SiCH_3$), -5.1 ($SiCH_3$), -5.2 ($SiCH_3$). ESI-MS for $C_{45}H_{70}NaO_{12}Si_3^+$ (MNa^+) calcd: 909.4073; found 909.4084. Appendix 43

a/β (3:1, reported are the distinguishable signals of the β anomer): ^{1}H NMR (500 MHz, $CDCl_3$): 13.92 (1H, s, OH-Ph β), 13.28 (4H, s, OH-Ph α,β), 8.01 (4H, dd, *J*=7.7, 1.0 Hz, H-1 α,β), 7.78–7.73 (4H, m, H-2 α,β), 7.37 (4H, d, *J*=8.5 Hz, H-3 α,β), 5.37 (3H, d, *J*=3.6 Hz, H-1′ α), 5.30 (1H, dd, *J*=3.3, 2.0 Hz, H-7 β), 5.00 (1H, dd, *J*=9.7, 2.5 Hz, H-1′ β), 3.79 (1H, s, H-4′ β), 3.77–3.71 (2H, m, CH_2-6′ β), 3.17 (1H, dd, *J*=19.0, 1.6 Hz, H-10a β), 2.93 (1H, d, *J*=19.1 Hz, H-10b β), 2.76 (1H, dt, *J*=14.6, 2.0 Hz, H-8a β), 1.94 (1H, dd, *J*=14.7, 3.9 Hz, H-8b β), 1.90 (1H, dd, *J*=12.1, 9.8 Hz, H-2′a β), 1.78–1.70 (1H, m, H-2′b β). ESI-MS for $C_{45}H_{70}NaO_{12}Si_3^+$ (MNa^+) calcd: 909.4073; found 909.4070. Appendix 44

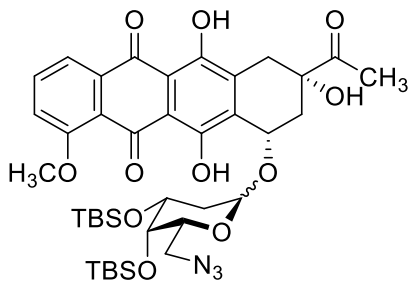
14-*O-tert*-Butyldimethylsilyl-7-*O*-(3,4,6-tri-*O-tert*-butyldimethylsilyl-2-deoxy-*α/β*-D-*lyxo*-hexopyranosyl)doxorubicinone[131] (55)



Tri-*O-tert*-butyldimethylsilyl-galactal donor **39** (25.3 mg, 0.05 mmol, 1 equiv) and aglycone acceptor **15** (11 mg, 0.02 mmol, 0.4 equiv) were weighted into separate microwave vials and placed under vacuum for 1 h, after which time the vials were filled with N_2, the donor was dissolved in anhydrous DCM (1 mL) and added to the acceptor vial. A mixture of (*R*)-3,3′-Bis(3,5-bis(trifluoromethyl)phenyl)-1,1′-binaphthyl-2,2′-diyl hydrogen phosphate (*R*)-**B** (4.3 mg, 0.005 mmol, 0.1 equiv) and Schreiner's thiourea **C** (5.2 mg, 0.01 mmol, 0.2 equiv) in anhydrous DCM (0.5 mL) was stirred at room temperature for 30 min, under N_2, and then added to the microwave vial containing the donor and acceptor. The reaction mixture was heated at reflux (45 °C) for 18 h, when it was concentrated under vacuum and purified by column chromatography (EtOAc:Hex 0-25%), to afford a mixture of glycosides **55** as a red film (3.9 mg, 0.0038 mmol, 18%, *α:β* = 3:1), R_f 0.39 (EtOAc:Hex 2:8). ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 14.10 (3H, s, OH-6 α), 13.99 (1H, s, OH-6 β), 13.29 (3H, s, OH-11 α), 13.24 (1H, s, OH-11 β), 8.03 (4H, dd, *J*=7.8, 1.1 Hz, H-1 α,β), 7.82–7.69 (4H, m, H-2 α,β), 7.38 (4H, dd, *J*=8.6, 1.1 Hz, H-3 α,β), 5.49 (3H, t, *J*=2.4 Hz, H-7 α), 5.35 (4H, d, *J*=3.8 Hz, H-1′ α, H-7 β), 4.94 (4H, d, *J*=20.0 Hz, H-14a α,β), 4.89–4.79 (5H, m, H-14b α,β, OH-9 α), 4.09 (3H, s, OCH_3-4 β), 4.07 (9H, s, OCH_3-4 α), 4.00 (3H, t, *J*=6.9 Hz, H-5′α), 3.92 (3H, ddd, *J*=11.9, 4.4, 2.2 Hz, H-3′ α),

3.88–3.84 (4H, m, H-4′ α,β), 3.70–3.63 (6H, m, H-6′a α,β, H-5′ β, H-3′ β), 3.56 (4H, dd, *J*=9.7, 6.0 Hz, H-6′b α,β), 3.25 (4H, dd, *J*=19.1, 1.7 Hz, H-10a α,β), 3.12 (3H, d, *J*=19.1 Hz, H-10b α), 3.00 (1H, d, *J*=18.6 Hz, H-10b β), 2.45 (3H, dt, *J*=15.1, 2.1 Hz, H-8a α), 2.38 (1H, dt, *J*=14.9, 2.1 Hz, H-8a β), 2.26 (3H, td, *J*=12.5, 3.9 Hz, H-2′a α), 2.20 (1H, dd, *J*=14.8, 5.1 Hz, H-8b β), 2.13–2.06 (1H, m, H-2′a β), 1.90 (4H, dd, *J*=15.1, 3.5 Hz, H-8b α, H-2′b β), 1.51 (3H, dd, *J*=12.9, 4.3 Hz, H-2′b α), 1.00–0.81 (144H, m, 4 × $SiC(CH_3)_3$ α,β), 0.18–-0.03 (96H, m, 8 × $SiCH_3$ α,β). ^{13}C NMR (126 MHz, $CDCl_3$) δ (reported are signals of the α anomer): 211.7 (C-13), 187.4, 186.8 (C-5, C-12), 161.2 (C-4), 156.3, 155.7 (C-6, C-11), 136.0 (C-2), 135.7 (C-12a), 135.6, 133.8 (C-6a, C-10a), 121.0 (C-4a), 120.0 (C-1), 118.6 (C-3), 111.8, 111.4 (C-5a, C-11a), 95.6 (C-1′), 77.4 (C-9), 73.7 (C-5′), 69.7 (C-4′), 68.2 (C-3′), 67.1 (C-14), 64.6 (C-7), 62.6 (C-6′), 56.9 (OCH_3-4), 35.9 (C-10), 33.9 (C-2′), 31.3 (C-8), 26.0 (SiC(*C*H_3)$_3$), 26.0 (SiC(*C*H_3)$_3$), 26.0 (SiC(*C*H_3)$_3$), 26.0 (SiC(*C*H_3)$_3$), 18.8 (Si*C*(CH_3)$_3$), 18.7 (Si*C*(CH_3)$_3$), 18.7 (Si*C*(CH_3)$_3$), 18.7 (Si*C*(CH_3)$_3$), -3.8 ($SiCH_3$), -4.2 ($SiCH_3$), -4.6 ($SiCH_3$), -4.8 ($SiCH_3$), -5.1 ($SiCH_3$), -5.2 ($SiCH_3$), -5.2 ($SiCH_3$), -5.3 ($SiCH_3$). MALDI-HRMS for $C_{51}H_{84}NaO_{13}Si_4^+$ (MNa^+) calcd: 1039.4881; found 1039.4899. Appendix 45

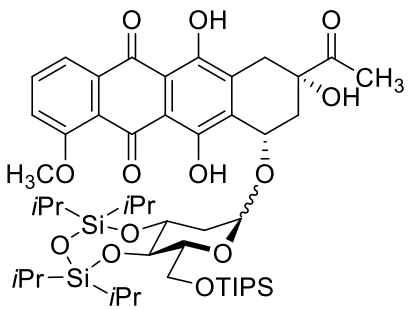
7-*O*-(6-azido-2,6-dideoxy-3,4-di-*O*-*tert*-butyldimethylsilyl-*α/β*-D-*lyxo*-hexopyranosyl)daunorubicinone[131] (56)



Galactal donor **44** (10 mg, 0.25 mmol, 1 equiv) and aglycone acceptor **9** (50 mg, 0.13 mmol, 0.5 equiv) were weighted into separate microwave vials and placed under vacuum for 1 h, after which time the vials were filled with N_2, the donor was dissolved in anhydrous DCM (1 mL) and added to the acceptor vial. A mixture of (*R*)-3,3′-Bis(3,5-bis(trifluoromethyl)phenyl)-1,1′-binaphthyl-2,2′-diyl hydrogenphosphate (*R*)-**B** (19.4 mg, 0.025 mmol, 0.1 equiv) and Schreiner's thiourea **C** (25.6 mg, 0.05 mmol, 0.2 equiv) in anhydrous DCM (1 mL) was stirred at room temperature for 30 min, under N_2, and then added to the microwave vial containing the donor and acceptor. The reaction mixture was heated at reflux (45 °C) for 20 h, when it was concentrated under vacuum and frozen. The residue was

purified by column chromatography (EtOAc:Hex 0-25%), to afford **56** as a red film (49.2 mg, 0.062 mmol, 49%, *α:β* > 30:1), R_f 0.28 (EtOAc:Hex 2:8). ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 14.12 (1H, s, OH-6), 13.26 (1H, s, OH-11), 8.00 (1H, dd, *J*=7.7, 1.0 Hz, H-1), 7.76 (1H, dd, *J*=8.4, 7.8 Hz, H-2), 7.38 (1H, dd, *J*=8.5, 0.8 Hz, H-3), 5.52 (1H, dd, *J*=3.1, 1.9 Hz, H-7), 5.38 (1H, d, *J*=3.3 Hz, H-1′), 4.70 (1H, s, OH-9), 4.32 (1H, t, *J*=6.6 Hz, H-5′), 4.08 (3H, s, OCH_3-4), 3.94 (1H, ddd, *J*=11.9, 4.1, 2.2 Hz, H-3′), 3.73 (1H, s, H-4′), 3.60 (1H, dd, *J*=12.3, 7.3 Hz, H-6′a), 3.26–3.17 (2H, m, H-10a, H-6′b), 2.97 (1H, d, *J*=19.1 Hz, H-10b), 2.45 (1H, dt, *J*=15.2, 1.7 Hz, H-8a), 2.40 (3H, s, CH_3-14), 2.23 (1H, td, *J*=12.7, 3.8 Hz, H-2′a), 1.88 (1H, dd, *J*=15.2, 3.5 Hz, H-8b), 1.53 (1H, dd, *J*=13.0, 4.3 Hz, H-2′b), 0.95–0.80 (18H, m, $SiC(CH_3)_3$), 0.05 (12H, m, $SiCH_3$). ^{13}C NMR (101 MHz, $CDCl_3$) *δ*: 212.0 (C-13), 187.0, 186.8 (C-5, C-12), 161.2 (C-4), 156.7, 155.8 (C-6, C-11), 135.8 (C-10a), 135.7 (C-2), 135.6 (C-12a), 133.6 (C-6a), 121.1 (C-4a), 119.9 (C-1), 118.5 (C-3), 111.6, 111.4 (C-5a, C-11a), 94.9 (C-1′), 77.4 (C-9), 72.7 (C-5′), 71.0 (C-4′), 67.8 (C-3′), 64.6 (C-7), 56.8 (OCH_3-4), 52.1 (C-6′), 34.2 (C-10), 33.4 (C-2′), 30.2 (C-8), 26.3 ($SiC(CH_3)_3$), 24.9 (C-14), 18.7, 18.5 ($SiC(CH_3)_3$), -3.5, -4.2, -4.5, -4.8 ($SiCH_3$). Full characterisation is ongoing. Appendix 46

7-*O*-(2-Deoxy-3,4-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-6-*O*-triisopropylsilyl-*α/β*-D-*arabino*-hexopyranosyl)daunorubicinone[131] (57)



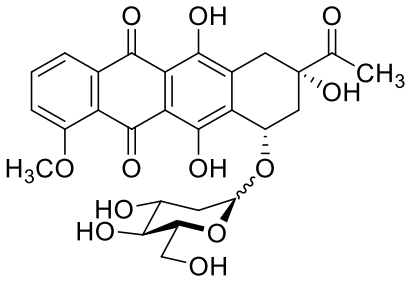
3,4-disiloxane-glucal donor **40** (70.3 mg, 0.13 mmol, 1 equiv) and aglycone acceptor **9** (26.2 mg, 0.07 mmol, 0.5 equiv) were weighted into separate microwave vials and placed under vacuum for 1 h, after which time the vials were filled with N_2, the donor was dissolved in anhydrous DCM (0,5 mL) and added to the acceptor vial. A mixture of (*R*)-3,3′-Bis(3,5-bis(trifluoromethyl)phenyl)-1,1′-binaphthyl-2,2′-diyl hydrogenphosphate (*R*)-**B** (9.6 mg, 0.012 mmol, 0.1 equiv) and Schreiner's thiourea **C** (12.9 mg, 0.025 mmol, 0.2 equiv) in anhydrous DCM (0.5 mL) was stirred at room temperature for 30 min, under N_2, and then added to the microwave vial containing the donor and acceptor. The reaction mixture was heated at reflux

(45 °C) for 20 h, when it was concentrated under vacuum and purified by column chromatography (EtOAc:Hex 0-10%), to afford a partially separable mixture of glycosides **57** as a red film (44.5 mg, 0.047 mmol, 71%, *α:β* = 1.6:1), R_f 0.37 (EtOAc:Hex 2:8).

α (contains ~25% of *β*-anomer): ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 13.96 (1H, s, OH-Ph), 13.35 (1H, s, OH-Ph), 8.05 (1H, dd, *J*=7.7, 1.1 Hz, H-1), 7.78 (1H, dd, *J*=8.5, 7.6 Hz, H-2), 7.40 (1H, dd, *J*=8.6, 1.1 Hz, H-3), 5.52 (1H, t, *J*=2.6 Hz, H-7), 5.34 (1H, d, *J*=3.8 Hz, H-1′), 4.83 (1H, s, OH-9), 4.10 (3H, s, OCH_3-4), 3.97 (1H, dd, *J*=10.9, 3.2 Hz, H-6′a), 3.95–3.87 (2H, m, H-3′, H-6′b), 3.85 (1H, dd, *J*=9.2, 2.4 Hz, H-5′), 3.72 (1H, dd, *J*=9.5, 8.4 Hz, H-4′), 3.26 (1H, dd, *J*=19.2, 1.6 Hz, H-10a), 3.06 (1H, d, *J*=19.1 Hz, H-10b), 2.47 (1H, dt, *J*=15.3, 2.0 Hz, H-8a), 2.41 (3H, s, CH_3-14), 2.01 (1H, d, *J*=5.3 Hz, H-2′a), 1.87 (1H, dd, *J*=15.1, 3.4 Hz, H-8a), 1.80 (1H, ddd, *J*=13.6, 11.5, 4.0 Hz, H-2′b), 1.17–0.91 (49H, m, 7 × $Si(CH(CH_3)_2)$). ^{13}C NMR (126 MHz, $CDCl_3$) *δ*: 212.2 (C-13), 187.1, 187.0 (C-5, C-12), 161.1 (C-4), 156.9, 155.9 (C-6, C-11), 135.8, 135.7, 135.7, 133.9 (C-2, C-12a, C-10a, C-6a), 121.3 (C-4a), 119.9 (C-1), 118.5 (C-3), 111.7, 111.4 (C-5a, C-11a), 94.8 (C-1′), 74.2 (C-5′), 73.8 (C-4′), 71.6 (C-3′), 64.7 (C-7), 62.5 (C-6′), 56.9 (OCH_3-4), 38.4 (C-2′), 34.3 (C-10), 30.6 (C-8), 25.0 (C-14), 18.2, 18.2, 18.1, 17.7, 17.7, 17.6, 17.5, 17.5, 17.5, 17.4 ($Si(CH(CH_3)_2)$), 13.2, 13.0, 12.6, 12.6, 12.4, 12.3, 12.1 ($Si(CH(CH_3)_2)$). MALDI-MS for $C_{48}H_{74}NaO_{13}Si_3^+$ (MNa^+) calcd: 965.4335; found: 965.4357. Appendix 47

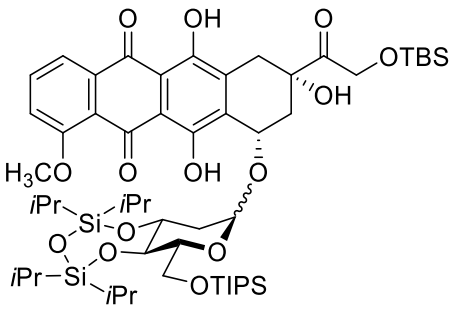
β (contains ~40% of *α*-anomer): ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 13.96 (1H, s, OH-Ph), 13.35 (1H, s, OH-Ph), 8.16–7.94 (1.6H, m, H-1 α,β), 7.78 (1.6H, dd, *J*=8.5, 7.7 Hz, H-2 α,β), 7.47–7.33 (1.6H, m, H-3 α,β), 5.32 (1H, s, H-7), 5.06 (1H, dd, *J*=9.9, 1.9 Hz, H-1′), 4.82 (1H, s, OH-9), 4.15 (1H, dd, *J*=10.7, 1.8 Hz, H-6′a), 4.12–4.07 (4.5H, m, OCH_3-4 α,β), 3.87–3.83 (1H, m, H-6′b), 3.78 (1H, ddd, *J*=11.5, 7.9, 5.3 Hz, H-3′), 3.45 (1H, dd, *J*=9.4, 7.9 Hz, H-4′), 3.41–3.36 (1H, m, H-5′), 3.21 (1H, dd, *J*=18.8, 1.8 Hz, H-10a), 2.95 (1H, d, *J*=18.8 Hz, H-10b), 2.78 (1H, d, *J*=14.9 Hz, H-8a), 2.41–2.40 (3H, m, C-14), 2.19–2.13 (1H, m, H-2′a), 1.98 (1H, dd, *J*=7.2, 4.0 Hz, H-8b), 1.62–1.58 (1H, m, H-2′b), 1.13–0.81 (78H, m, $Si(CH(CH_3)_2)$ α,β). ^{13}C NMR (126 MHz, $CDCl_3$, from HSQC) *δ*: 135.7 (C-2), 120.0 (C-1), 118.4 (C-3), 102.2 (C-1), 70.5 (C-7), 77.9 (C-5′), 74.6 (C-4′), 74.4 (C-3′), 63.7 (C-6′), 56.9 (OCH_3-4), 39.7 (C-2′), 35.3 (C-8), 33.4 (C-10), 25.1 (C-14), 18.2, 18.1, 17.7, 17.5, 17.5, 17.5, 17.4 ($Si(CH(CH_3)_2)$), 12.6, 12.4, 12.3, 12.1 ($Si(CH(CH_3)_2)$). MALDI-MS for $C_{48}H_{74}NaO_{13}Si_3^+$ (MNa^+) calcd: 965.4335; found: 965.4346. Appendix 48

7-*O*-(2-deoxy-*α/β*-D-*arabino*-hexopyranosyl)daunorubicinone[132] (58)



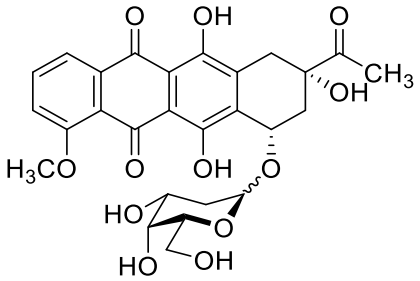
Glycosides **57** (28 mg, 0.029 mmol, 1 equiv) were dissolved in THF (1 mL), treated with KF·2 H_2O (49.5 mg, 0.53 mmol, 18 equiv) and 18-crown-6 (12 mg, 0.045 mmol, 1.5 equiv) and the mixture was stirred at room temperature for 28h. It was concentrated under vacuum, dissolved in DCM and filtered over Celite, wasing with DCM, THF and MeOH. The filtrate was concentrated, the residue was taken up in EtOAc (15 mL), washed with water (20 mL) and brine (20 mL), and the aqueous layers were then extracted with EtOAc. The combined organic layers were concentrated under vacuum and purified by column chromatography (MeOH:DCM 0-6%) to afford **58** as a red solid (1.8 mg, 0.033 mmol, 11%), R_f 0.15 (MeOH:DCM 5:95). ^{1}H NMR (500 MHz, $CDCl_3$): 13.95 (1H, s, OH-6), 13.28 (1H, s, OH-11), 8.02 (1H, d, *J*=7.7 Hz, H-1), 7.78 (1H, t, *J*=8.0 Hz, H-2), 7.39 (1H, d, *J*=8.3 Hz, H-3), 5.42 (1H, dd, *J*=9.5, 1.6 Hz, H-1′), 5.33 (1H, dd, *J*=3.1, 1.9 Hz, H-7), 4.18–4.14 (1H, m, H-3′), 4.08 (3H, s, OCH_3-4), 3.96–3.85 (3H, m, H-6′a, H-6′b, H-5′), 3.70 (1H, dd, *J*=9.2, 2.9 Hz, H-4′), 3.23 (1H, dd, *J*=19.0, 1.6 Hz, H-10a), 2.92 (1H, d, *J*=18.8 Hz, H-10b), 2.58 (1H, d, *J*=14.8 Hz, H-8a), 2.42 (3H, s, CH_3-14), 2.13 (1H, dt, *J*=14.2, 2.6 Hz, H-2′a), 2.08 (1H, dd, *J*=14.8, 3.9 Hz, H-8b), 1.68–1.59 (1H, m, H-2′b). ^{13}C NMR (126 MHz, $CDCl_3$, from HSQC) δ: 136.0 (C-2), 119.9 (C-1), 118.6 (C-3), 100.2 (C-1′), 72.9 (C-5′), 70.3 (C-7), 69.0 (C-4′), 68.1 (C-3′), 64.0 (C-6′), 56.8 (OCH_3-4), 37.7 (C-2′), 35.3 (C-8), 33.6 (C-10), 25.2 (CH_3-14). ESI-HRMS for $C_{27}H_{28}NaO_{12}^+$ (MNa^+) calcd: 567.1473; found: 567.1482. Appendix 49

14-*O*-*tert*-Butyldimethylsilyl-7-*O*-(2-deoxy-3,4-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-6-*O*-triisopropylsilyl-*α/β*-D-*arabino*-hexopyranosyl)doxorubicinone[131] (59)



3,4-disiloxane-glucal donor **40** (164 mg, 0.30 mmol, 1 equiv) and aglycone acceptor **15** (78 mg, 0.15 mmol, 0.5 equiv) were weighted into separate microwave vials and placed under vacuum for 1 h, after which time the vials were filled with N_2, the donor was dissolved in anhydrous DCM (1.5 mL) and added to the acceptor vial. A mixture of (*R*)-3,3′-Bis(3,5-bis(trifluoromethyl)phenyl)-1,1′-binaphthyl-2,2′-diyl hydrogenphosphate (*R*)-**B** (21 mg, 0.03 mmol, 0.1 equiv) and Schreiner's thiourea **C** (31 mg, 0.06 mmol, 0.2 equiv) in anhydrous DCM (1.5 mL) was stirred at room temperature for 30 min, under N_2, and then added to the microwave vial containing the donor and acceptor. The reaction mixture was heated at reflux (45 °C) for 20 h, when it was concentrated under vacuum and frozen. The residue was purified by column chromatography (EtOAc:Hex 0-20%), to afford **59** as a red film (42 mg, 0.04 mmol, 26%, *α:β* > 10:1), R_f 0.55 (EtOAc:Hex 2:8). ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 13.95 (1H, s, OH-6), 13.29 (1H, s, OH-11), 8.02 (1H, d, *J*=7.8 Hz, H-1), 7.76 (1H, t, *J*=8.1 Hz, H-2), 7.38 (1H, d, *J*=8.5 Hz, H-3), 5.50 (1H, t, *J*=2.8 Hz, H-7), 5.32 (1H, d, *J*=3.8 Hz, H-1′), 4.94 (1H, d, *J*=20.0 Hz, H-14a), 4.85 (1H, d, *J*=19.9 Hz, H-14b), 4.82 (1H, s, OH-9), 4.09 (3H, s, OCH_3-4), 4.01–3.80 (5H, m, H-6′a, H-3′, H-6′b, H-5′, H-4′), 3.25 (1H, dd, *J*=19.2, 1.6 Hz, H-10a), 3.11 (1H, d, *J*=19.2 Hz, H-10b), 2.46 (1H, dt, *J*=15.1, 2.2 Hz, H-8a), 2.01 (1H, dd, *J*=13.6, 5.4 Hz, H-2′a), 1.92 (1H, dd, *J*=15.1, 3.4 Hz, H-2′b), 1.81 (1H, ddd, *J*=14.1, 11.5, 4.0 Hz, H-2′b), 1.09–0.98 (49H, m, 7 $SiCH(CH_3)_2$), 0.96–0.94 (9H, m, $SiC(CH_3)_3$), 0.15–0.13 (6H, 2, $SiCH_3$). ^{13}C NMR (126 MHz, $CDCl_3$) *δ*: 211.5 (C-13), 187.0, 186.9 (C-5, C-12), 161.2 (C-4), 156.8, 155.8 (C-6, C-11), 135.7, 135.7 (C-12a, C-2), 135.4, 133.6 (C-10a, C-6a), 121.3 (C-4a), 119.9 (C-1), 118.6 (C-3), 111.7, 111.4 (C-5a, C-11a), 94.9 (C-1′), 77.3 (C-9), 74.3 (C-5′), 73.7 (C-4′), 71.6 (C-3′), 66.7 (C-14), 62.3 (C-6′), 56.9 (OCH_3-4), 38.4 (C-2′), 35.0 (C-10), 31.4 (C-8), 26.0 ($SiC(CH_3)_3$), 18.2, 18.1, 17.7, 17.7, 17.5, 17.5, 17.5, 17.4 ($SiCH(CH_3)_2$), 13.2, 13.0, 12.6, 12.3, 12.2, 12.2, 12.1 ($SiCH(CH_3)_2$), -5.1, -5.2 ($SiCH_3$). Appendix 50

7-*O*-(2-deoxy-α/β-D-*lyxo*-hexopyranosyl)daunorubicinone (61)



Tri-*O*-acetyl-D-galactal donor (28.8 mg, 0.10 mmol, 1 equiv), aglycone acceptor **9**, (28.0 mg, 0.07 mmol, 0.7 equiv) and **Cu(I).L$_n$**† catalyst (1.4 mg, 0.003 mmol, 0.025 eq.) were weighted into a microwave vial and placed under vacuum for 1 h, after which time the vial was filled with N_2. Anhydrous toluene (1 mL) was added and the reaction mixture was stirred at 70 °C for 5 h. It was filtered over a bed of Celite®, concentrated, and purified by column chromatography (EtOAc:Tol 0-100%; MeOH:EtOAc 10%), to afford an anomeric mixture of glycosides **60** as a red solid (7.5 mg, 0.011 mmol, 15%, $\alpha:\beta$ = 1.6:1), R_f 0.67 (MeOH:DCM 5:95). ^{1}H NMR (500 MHz, $CDCl_3$): 8.08–8.00 (2.6H, m, H-1 α,β), 7.82–7.75 (2.6H, m, H-2 α,β), 7.45–7.35 (2.6H, m, H-3 α,β), 5.57 (1.6H, t, *J*=2.8 Hz, H-7 α), 5.46 (1.6 H, d, *J*=3.7 Hz, H-1′ α), 5.10 (1H, dd, *J*=9.8, 2.1 Hz, H-1′ β), 3.32–3.21 (2.6H, m, H-10a α,β), 2.99 (1.6H, d, *J*=19.1 Hz, H-10b α), 2.90 (1H, d, *J*=18.9 Hz, H-10b β), 2.62 (1H, d, *J*=15.0 Hz, H-8a β), 2.21 (1.6H, td, *J*=12.9, 3.9 Hz, H-2′a α), 1.85 (1H, d, *J*=9.8 Hz, H-2′b β), 1.79 (1.6H, dd, *J*=13.0, 5.1 Hz, H-2′b α). ^{13}C NMR (126 MHz, $CDCl_3$): 135.9 (C-2 α,β), 119.9 (C-1 α,β), 118.7 (C-3 α,β), 102.3 (C-1′ β), 94.2 (C-1′ α), 35.1 (C-8 β), 34.2 (C-10 α), 33.3 (C-10 β), 32.1 (C-2′ β), 30.2 (C-2′ α), 30.1 (C-8 α). MALDI-HRMS for $C_{33}H_{34}NaO_{15}$ (MNa^+) calcd: 693.1790; found 693.1799. Appendix 51

The mixture of acetylated glycosides **60** (7.2 mg, 0.0107 mmol) was dissolved in THF (0.2 mL) and cooled down to 0 °C, followed by the addition of aqueous 0.1 M NaOH solution (2.8 mL), and stirring for 6 h, when it was neutralised with 10% w/v citric acid solution (0.2 mL). The mixture was extracted with DCM (3 × 5 mL), the organic phase was washed with $NaHCO_3$ sat. (5 mL) and water (10 mL), and each aqueous layer was then extracted twice with DCM. The combined organic layers were dried over $MgSO_4$, and concentrated. The crude mixture was first purified by column chromatography (MeOH:DCM 0-5%), to give a mixture of deprotected glycosides. The anomers were separated by preparative RP-HPLC (C18,

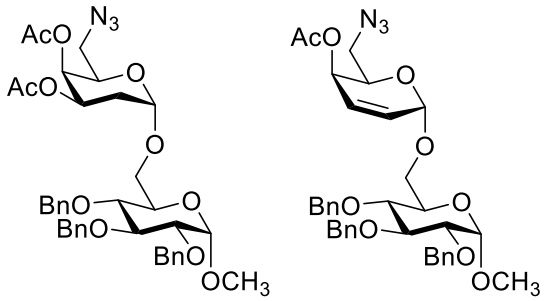
† **Cu(I).L$_n$** refers generically to a copper(I) complex catalyst, which is unpublished work from a collaborator research group, and will not be disclosed herein.

MeCN:H_2O + 0.05% formic acid, 30-60%), affording the glycosides **61*α*** (3.8 mg, 0.0070 mmol, 65%), R_f 0.17 (MeOH:DCM 1:9); and **61*β*** (1.2 mg, 0.0022 mmol, 20%), R_f 0.14 (MeOH:DCM 1:9).

α: ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 14.19 (1H, s, OH-6), 13.30 (1H, s, OH-11), 8.05 (2H, d, *J*=7.6 Hz, H-1), 7.80 (1H, t, *J*=8.1 Hz, H-2), 7.41 (1H, d, *J*=8.4 Hz, H-3), 5.55 (1H, t, *J*=2.8 Hz, H-7), 5.45 (1H, d, *J*=3.7 Hz, H-1′), 4.64 (1H, s, OH-9), 4.27 (1H, t, *J*=3.7 Hz, H-5′), 4.10 (3H, s, OCH_3-4), 4.05 (1H, dd, *J*=11.8, 3.8 Hz, H-6′a), 4.02–3.97 (2H, m, H-6′b, H-4′), 3.90 (1H, ddd, *J*=11.8, 5.0, 3.1 Hz, H-3′), 3.28 (1H, dd, *J*=19.1, 1.8 Hz, H-10a), 3.02 (1H, d, *J*=19.1 Hz, H-10b), 2.46 (1H, d, *J*=15.2 Hz, H-8a), 2.41 (3H, s, CH_3-14), 2.10 (1H, td, *J*=13.0, 3.9 Hz, H-2′a), 1.93 (1H, dd, *J*=15.2, 3.5 Hz, H-8b), 1.82 (1H, dd, *J*=13.3, 5.0 Hz, H-2′b). ^{13}C NMR (126 MHz, $CDCl_3$) *δ*: 212.0 (C-13), 187.4, 186.9 (C-5, C-12), 161.2 (C-4), 156.5, 155.9 (C-6, C-11), 136.0 (C-2), 136.0, 135.7, 133.2 (C-6a, C-12a, C-10a), 121.0 (C-4a), 120.0 (C-1), 118.6 (C-3), 111.7, 111.6 (C-5a, C-11a), 94.8 (C-1′), 76.8 (C-9), 70.7 (C-4′), 70.0 (C-5′), 65.4 (C-3′), 65.0 (C-6′), 64.5 (C-7), 56.9 (OCH_3-4), 34.4 (C-10), 32.9 (C-2′), 30.2 (C-8), 25.0 (C-14). MALDI-HRMS for $C_{27}H_{28}NaO_{12}$ (MNa^+) calcd: 567.1473; found 567.1479. Appendix 52

β: ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 14.07 (1H, s, OH-6), 13.30 (1H, s, OH-11), 8.05 (1H, dd, *J*=7.7, 1.1 Hz, H-1), 7.79 (1H, t, *J*=8.1 Hz, H-2), 7.40 (1H, d, *J*=8.5 Hz, H-3), 5.38 (1H, dd, *J*=4.2, 2.1 Hz, H-7), 5.04 (1H, dd, *J*=9.8, 2.2 Hz, H-1′), 4.50 (1H, s, OH-9), 4.10 (3H, s, OCH_3-4), 4.01 (1H, dd, *J*=11.7, 5.5 Hz, H-6′a), 3.96 (1H, dd, *J*=11.7, 4.4 Hz, H-6′b), 3.89 (1H, d, *J*=3.1 Hz, H-4′), 3.78 (1H, ddd, J=11.8, 4.8, 3.5 Hz, H-3′), 3.56 (1H, t, *J*=4.9 Hz, H-5′), 3.26 (1H, dd, *J*=18.9, 2.0 Hz, H-10a), 2.93 (1H, d, *J*=18.8 Hz, H-10b), 2.60 (1H, d, *J*=14.6 Hz, H-8a), 2.44 (3H, s, CH_3-14), 2.14 (1H, dd, *J*=14.8, 4.1 Hz, H-8b), 2.04 (1H, ddd, *J*=12.4, 4.7, 2.0 Hz, H-2′a), 1.68 (1H, dd, *J*=12.3, 9.8 Hz, H-2′b). ^{13}C NMR (126 MHz, $CDCl_3$) *δ*: 213.0 (C-13), 135.9 (C-2), 118.6 (C-1), 116.5 (C-3), 102.3 (C-1′), 74.6 (C-5′), 70.2 (C-7), 68.7, 68.6 (C-3′, C-4′), 63.5 (C-6′), 56.9 (OCH_3-4), 35.5, 35.3 (C-2′, C-8), 33.6 (C-10), 25.2 (C-14). MALDI-HRMS for $C_{27}H_{28}NaO_{12}$ (MNa^+) calcd: 567.1473; found 567.1485. Appendix 53

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(3,4-di-*O*-acetyl-6-azido-2,6-dideoxy-α-D-*lyxo*-hexopyranosyl)-α-D-glucopyranoside (62) and
Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(4-*O*-acetyl-6-azido-2,3,6-trideoxy-α-D-*threo*-2-hexenopyranosyl)-α-D-glucopyranoside (63)

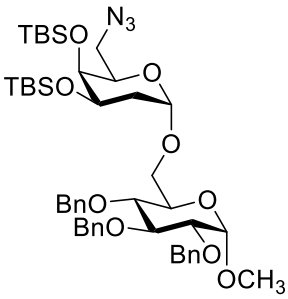


Galactal donor **43** (26.0 mg, 0.10 mmol, 1 equiv), methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside acceptor (35.4 mg, 0.08 mmol, 0.75 equiv) and **Cu(I).L$_n$** catalyst (2.6 mg, 0.005 mmol, 0.05 equiv) were weighted into a microwave vial and placed under vacuum for 1 h, after which time the vial was filled with N_2. Anhydrous toluene (1 mL) was added and the reaction mixture was stirred at 70 °C for 8 h. It was purified by column chromatography (EtOAc:Hex 0-20%), to afford **62** as a colourless oil (19.6 mg, 0.027 mmol, 35%), along with Ferrier product **63** (2.2 mg, 0.003 mmol, 4%).

62: R_f 0.23 (EtOAc:Hex 1:3). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.49–7.14 (15H, m, Ph), 5.25–5.16 (2H, m, H-3′, H-4′), 5.06 (1H, d, *J*=2.9 Hz, H-1′), 4.99 (1H, d, *J*=11.0 Hz, OC*H*HPh), 4.96 (1H, d, *J*=11.1 Hz, OC*H*HPh), 4.82 (1H, d, *J*=10.9 Hz, OCH*H*Ph), 4.79 (1H, d, *J*=11.9 Hz, OC*H*HPh), 4.67 (1H, d, *J*=12.1 Hz, OCH*H*Ph), 4.63–4.58 (2H, m, OCH*H*Ph, H-1), 4.00 (1H, t, *J*=9.2 Hz, H-3), 3.91 (1H, dd, *J*=8.6, 4.1 Hz, H-5′), 3.85–3.74 (2H, m, H-6a, H-5), 3.65 (1H, d, *J*=10.2 Hz, H-6b), 3.53 (1H, dd, *J*=9.6, 3.6 Hz, H-2), 3.50 (1H, t, *J*=9.2 Hz, H-4), 3.38 (3H, s, OCH_3), 3.32 (1H, dd, *J*=12.8, 8.4 Hz, H-6′a), 2.96 (1H, dd, *J*=12.8, 4.1 Hz, H-6′b), 2.13 (3H, s, CH_3CO), 2.06–1.97 (1H, m, H-2′a), 1.98 (3H, s, CH_3CO), 1.88 (1H, dd, J=12.7, 5.0 Hz, H-2′b). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 170.4 (CH_3*C*O), 170.1 (CH_3*C*O), 138.8 (4° C), 138.4 (4° C), 138.3 (4° C), 128.6 (CH), 128.6 (CH), 128.5 (CH), 128.2 (CH), 128.1 (CH), 128.1 (CH), 127.8 (CH), 127.7 (CH), 98.1 (C-1), 97.8 (C-1′), 82.3 (C-3), 80.2 (C-2), 78.1 (C-4), 75.9 (O*C*H_2Ph), 75.2 (O*C*H_2Ph), 73.5 (O*C*H_2Ph), 69.9 (C-5), 68.4 (C-5′), 67.6 (C-4′), 66.4 (C-6), 66.3 (C-3′), 55.3 (OCH_3), 51.4 (C-6′), 30.1 (C-2′), 21.0 (*C*H_3CO), 20.9 (*C*H_3CO). ESI-HRMS for $C_{38}H_{45}N_3NaO_{11}$ (MNa^+) calcd: 742.2946; found 742.2965. Appendix 54

63: R_f 0.26 (EtOAc:Hex 1:3). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.38–7.27 (15H, m, Ph), 6.09 (1H, dd, *J*=10.0, 5.1 Hz, H-2′), 6.03 (1H, dd, *J*=10.1, 2.8 Hz, H-3′), 5.16 (1H, d, *J*=2.8 Hz, H-1′), 4.97 (1H, d, *J*=10.8 Hz, OC*H*HPh), 4.92 (1H, dd, *J*=5.3, 2.5 Hz, H-4′), 4.88 (1H, d, *J*=10.8 Hz, OC*H*HPh), 4.83–4.77 (2H, m, 2 × OCHHPh), 4.69–4.62 (2H, m, 2 × OCHHPh), 4.61 (1H, d, *J*=3.5 Hz, H-1), 4.23–4.17 (1H, m, H-5′), 4.05–3.99 (2H, m, H-6a, H-3), 3.83–3.74 (2H, m, H-6b, H-5), 3.59–3.43 (3H, m, H-4, H-2, H-6′a), 3.37 (3H, s, OCH_3), 3.19 (1H, dd, *J*=12.8, 5.1 Hz, H-6′b), 2.08 (3H, s, CH_3CO). ^{13}C NMR (101 MHz, $CDCl_3$): 170.5 (CH_3*C*O), 138.9 (4º C), 138.4 (4º C), 138.3 (4º C), 130.8 (C-3′), 128.6 (CH), 128.6 (CH), 128.5 (CH), 128.2 (CH), 128.1 (CH), 128.1 (CH), 127.8 (CH), 127.7 (CH), 125.1 (C-2′), 98.2 (C-1), 94.6 (C-1′), 82.1 (C-3), 80.1 (C-2), 78.0 (C-4), 75.9 (O*C*H_2Ph), 75.2 (O*C*H_2Ph), 73.5 (O*C*H_2Ph), 70.2 (C-5), 68.5 (C-5′), 67.2 (C-6), 63.3 (C-4′), 55.3 (OCH_3), 51.0 (C-6′), 21.0 (*C*H_3CO). ESI-HRMS for $C_{36}H_{41}N_3NaO_9$ (MNa^+) calcd: 682.2735; found 682.2734. Appendix 55

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(6-azido-3,4-di-*O*-*tert*-butyldimethylsilyl-2,6-dideoxy-α-D-*lyxo*-hexopyranosyl)-α-D-glucopyranoside (64)



Galactal donor **44** (41 mg, 0.10 mmol, 1 equiv), methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside acceptor (35 mg, 0.08 mmol, 0.75 equiv) and **Cu(I).L$_n$** catalyst (3 mg, 0.006 mmol, 0.05 equiv) were weighted into a microwave vial and placed under vacuum for 1 h, after which time the vial was filled with N_2. Anhydrous toluene (1 mL) was added and the reaction mixture was stirred at 45 °C for 4 h, when it was determined to be complete by TLC. The reaction mixture was purified by column chromatography (EtOAc:Hex 0-10%), affording **64** as a colourless oil (64 mg, 0.074 mmol, 98%). R_f 0.48 (EtOAc:Hex 2:8). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.39–7.27 (15H, m, Ph), 4.99 (1H, d, *J*=2.4 Hz, H-1′), 4.99 (1H, d, *J*=10.9 Hz, OC*H*HPh), 4.90 (1H, d, *J*=10.9 Hz, OC*H*HPh), 4.83 (1H, d, *J*=10.9 Hz, OCH*H*Ph), 4.80 (1H, d, *J*=12.1 Hz, OC*H*HPh), 4.68 (1H, d, *J*=12.1 Hz, OCH*H*Ph), 4.61 (1H, s, H-1), 4.61 (2H, d, *J*=10.7 Hz, OCH*H*Ph), 4.05 (1H, ddd, *J*=11.7, 4.3, 2.4 Hz, H-3′), 4.00 (1H, t, *J*=9.3 Hz, H-3), 3.86–3.75

(3H, m, H-6a, H-5′, H-5), 3.71–3.67 (2H, m, H-6b, H-4′), 3.56–3.50 (2H, m, H-6′a, H-2), 3.45 (1H, t, *J*=9.2 Hz, H-4), 3.37 (3H, s, OCH_3), 3.11 (1H, dd, *J*=12.4, 4.7 Hz, H-6′b), 2.06 (1H, td, J=12.2, 3.5 Hz, H-2′a), 1.68 (1H, dd, *J*=12.5, 4.4 Hz, H-2′b), 0.93–0.88 (18H, 18H, m, 2 × $SiC(CH_3)_3$), 0.14–0.04 (12H, m, 4 × $SiCH_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 138.9 (4º C), 138.4 (4º C), 138.3 (4º C), 128.6 (CH), 128.6 (CH), 128.5 (CH), 128.2 (CH), 128.1 (CH), 128.0 (CH), 127.9 (CH), 127.8 (CH), 127.7 (CH), 98.2 (C-1′), 97.9 (C-1), 82.3 (C-3), 80.2 (C-2), 78.5 (C-4), 75.9 (OCH_2Ph), 75.3 (OCH_2Ph), 73.5 (OCH_2Ph), 71.7 (C-5′), 71.3 (C-4′), 70.4 (C-5), 68.2 (C-3′), 65.9 (C-6), 55.0 (OCH_3), 52.4 (C-6′), 33.4 (C-2′), 26.3 ($SiC(\mathit{C}H_3)_3$), 26.2 ($SiC(\mathit{C}H_3)_3$), 18.7 ($Si\mathit{C}(CH_3)_3$), 18.7 ($Si\mathit{C}(CH_3)_3$), -3.5 ($SiCH_3$), -4.1 ($SiCH_3$), -4.5 ($SiCH_3$), -4.8 ($SiCH_3$). HRMS for $C_{46}H_{69}KN_3O_9Si_2^+$ (MK^+) calcd: 902.4204; found 902.4223. Appendix 56

3.3 Biological assays

3.3.1 *In vitro* cytotoxicity in cancer cell lines

3.3.1.1 HDF, HeLa, MDA-MB-231 and MCF-7

HDF (human primary dermal fibroblast, adult) and MCF-7 (human breast adenocarcinoma) cell lines were grown in Minimal Essential Medium (MEM). HeLa (human cervical adenocarcinoma) and MDA-MB-231 (human breast adenocarcinoma) cell lines were grown in Dulbecco's Modified Essential Medium (DMEM). All growth media were supplemented with antibiotic/antimycotic solution and 10% fetal bovine serum (FBS). Cells from confluent cultures were detached from the surface with trypsin, seeded at a 10^3 cells/well density in flat-bottom 96-well plates, and incubated for 24 h before toxicity assays,

The interference of **DAU, 61α** and **61β** on cell survival after exposure to the testing compounds was quantified by measuring Calcein fluorescence, which is retained within living cells only, because of the esterases acting on the non-fluorescence substrate Calcein AM[133] (Molecular Probes). Alteration in the cell metabolism was evaluated using AlamarBlue[134] (AB, Life Technologies), a cytosolic substrate for the reductive metabolism (conversion of resazurin to resorufin), whose fluorescence spectrum shifts on reduction by cytosolic enzymes.

HDF, HeLa, MCF-7 and MDA cell lines were incubated with 0-250 μM of compounds **DAU, 61α** and **61β** for 72 h. Each experiment was repeated two times, in medium with reduced fetal bovine serum (5%), with eight replicates per concentration point, and each well contained 90 μL of medium and 10 μL of serial dilutions or phosphate buffered saline solution (PBS). After 72 h, the plates were rinsed with PBS, and a solution of AB (5%) and Calcein (2.5 μM) in FBS-free medium was added. After 1 h incubation, the fluorescence of the two dyes was recorded using a plate reader (BMG Labtech CLARIOStar) (AB: λ_{ex}=545 nm, λ_{em}=600 nm; Calcein: λ_{ex}=483 nm, λ_{em}=530 nm). Results were expressed as percentages of the control versus the logarithm of the compound concentration. To determine the absolute IC_{50} absolute, curves were fitted using the non-linear regression function of GraphPad Prism 6, applying the equations [log(inhibitor) vs normalised response – variable slope] or [log(inhibitor) vs response – variable slope], as appropriate.

3.3.1.2 A431

A431 human squamous cell carcinoma cells (ATCC) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% (v/v) fetal bovine serum, 1.5 g/L sodium bicarbonate, and 1% antibiotics,[135, 136] at 37 °C in 5% CO_2 and a humidified atmosphere. The *in vitro* cytotoxicity was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay.[137] Cells were plated at a density of 10.000 cells in 200 μL of medium per well, in a flat-bottomed 96-well microplate. After incubation for 24 h, the medium was removed, and the cells were treated with serial dilutions of compounds **3** and **DOX** in culture medium, in a concentration range of 0.195 μM to 25 μM, in octuplicate. After another 24 h incubation, the medium was replaced with fresh medium, and 20 μL of a solution of the mitochondrial dehydrogenase substrate MTT (2.5 mg/mL) per well were added, followed by 4 h final incubation. 200 μL of DMSO per well were added to solubilise the formazan salts, and absorbance was measured at 570 nm. Cytotoxicity was calculated through the equation: % = 100 × (1 – Abs treated cells) / (Absrvância non-treated cells). Results were expressed as percentages of the control versus the logarithm of the compound concentration. To determine the absolute IC_{50} absolute, curves were fitted using the non-linear regression function of GraphPad Prism 6, applying the equations [log(inhibitor) vs normalised response – variable slope] or [log(inhibitor) vs response – variable slope], as appropriate.

3.3.2 Human induced pluripotent stem cell-derived cardiomyocytes

Cardiomyocytes (PluriCardio™, Pluricell, lot uPC1.2-C007) were plated in 96-well microplates containing matrix GTX, at a cell density of 60.000 cells per well, in PLAQ medium, and incubated at 37 ºC with 5% CO_2 for 48 h, when the medium was replaced for RPMI + SMM, and the plate was incubated for 72 h. On day 6, differentiated contractile cardiomyocytes were observed under a light microscope.

Live-cell imaging was performed using the high content system Operetta (Perkin Elmer) under controlled environment (TCO chamber at 37 °C and 5% CO_2). Before the treatment with compounds, a 16-second video was recorded with the 20× and 10× objective lenses from each well of contracting cells. After renewing the culture medium, 30 µL of the compounds stock solutions were diluted to a final volume of 180 µL per well (1% DMSO), and the plate was incubated for 96 h. Post-treatment live-cell imaging was performed by recording a 16-second with 40×, 20× and 10× objective lenses, from the same well fields. Attempted staining of live cells with far-red dye Draq5 at 1/1000 resulted in poor quality image, and the cardiac movements could not be detected in the fluorescent mode.

Cardiomyocytes contractions were manually counted in both pre-treatment and post-treatment videos, and the ratio was calculated based on the number of contractions divided by the total video time (16 s). The pulse change indicates the percentage reduction or increase in frequency after the treatment.

The plates were fixed for 15 min with 90 µL per well of 12% paraformaldehyde solution (4% final concentration in the well), followed by two washings with PBS, and stained with Draq5 (1/1000 dilution in DPBS, 30µL per well). After staining, the plates were imaged in the High Content Analysis System Operetta (Perkin Elmer) with a 20× objective lens.

The Harmony image analysis software, using the Columbus server (both from Perkin Elmer) was used to identify and count cardiomyocytes nuclei and cytoplasm, thus yielding the number of cells as an indirect measurement of cell viability in the population.

Results and Discussion

4 RESULTS AND DISCUSSION

The strategy of this work involved the semi-synthesis of azido derivatives starting from the drug doxorubicin hydrochloride (**DOX·HCl**), an active pharmaceutical ingredient firstly obtained by the research group by a donation from Prof Dr Alexandre Pinto Corrado (Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo). Daunorubicin hydrochloride (**DAU·HCl**) was also donated to the laboratory by Prof Dr M. Carmen Galan (School of Chemistry, University of Bristol), once the collaboration with her group was established, i.e. at an advanced stage of the development of this project. The available amounts of these anthracyclines were restricted, considering they were costly and not readily accessible from chemical reagents suppliers within the Brazilian domestic market.

Therefore, until the laboratory was able to purchase its own stock by import procedures, which proved rather lengthy, most of the reactions were performed on microscale with minimal mass, in order to use the available drugs sparingly. In some cases, the quantity limitation hampered the complete characterisation analyses of the products. In addition to requiring extra care, the handling of milligram-scale experiments was more laborious and eventually resulted in reduced yields, as any loss in the process became significant.

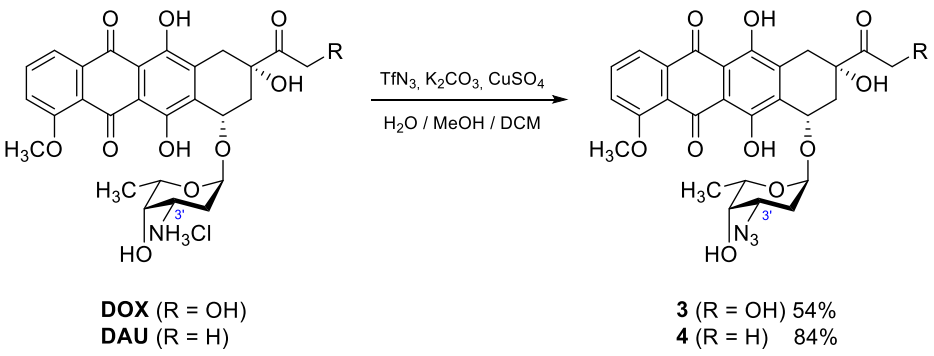
4.1 Anthracycline modification with preservation of the glycosyl unit

Initially, 3′-azido derivatives of anthracyclines **3** and **4** were prepared, in view of the relative synthetic simplicity and for being compounds already described in the literature. For their synthesis, some approaches relied on glycosidic cleavage, parallel preparation of azido daunosamine, followed by glycosylation of the aglycone.[44] In the present work, direct conversion from amino to azido was implemented, through the diazotransfer reaction, as already reported for daunorubicin,[54, 72] and more recently doxorubicin.[55, 71]

Aiming to employ modern and safe donors, with low explosive potential, the first diazotransfer reagent investigated was imidazole-1-sulphonyl azide, which can be crystallised with different acids to form a variety of stable salts, such as hydrochloride or hydrogen sulphate. Not only are they less sensitive to heat, impact, friction, and electrostatic discharge, but also these salts can be isolated and stored for later use, circumventing the main limitations of trifluoromethanesulphonyl azide (TfN_3), also known as triflyl azide, the classical diazotransfer reagent.[138-140]

Imidazole-1-sulphonyl azide hydrogen sulphate (**5**) was prepared from imidazole, sulphuryl chloride, and sodium azide, and precipitated upon addition of sulphuric acid, to form the corresponding salt in 32% yield.[66, 67] Initial attempts to perform the diazotransfer reaction of doxorubicin with **5** (Method I) were unsuccessful, as the product isolated from the reaction mixture, despite bearing the typical red colour of anthracyclines, did not show in ^{1}H NMR spectrum the characteristic signals of daunosamine, indicating that this moiety might have been cleaved from doxorubicin. After several tests comprising pH adjustment, excess of reagents, and reaction time extension, product **3** was obtained. Nonetheless, the best yield achieved was only 23%, considered unsatisfactory compared to the 70% reported in the literature,[69, 70] thus the protocol with imidazole salts was replaced.

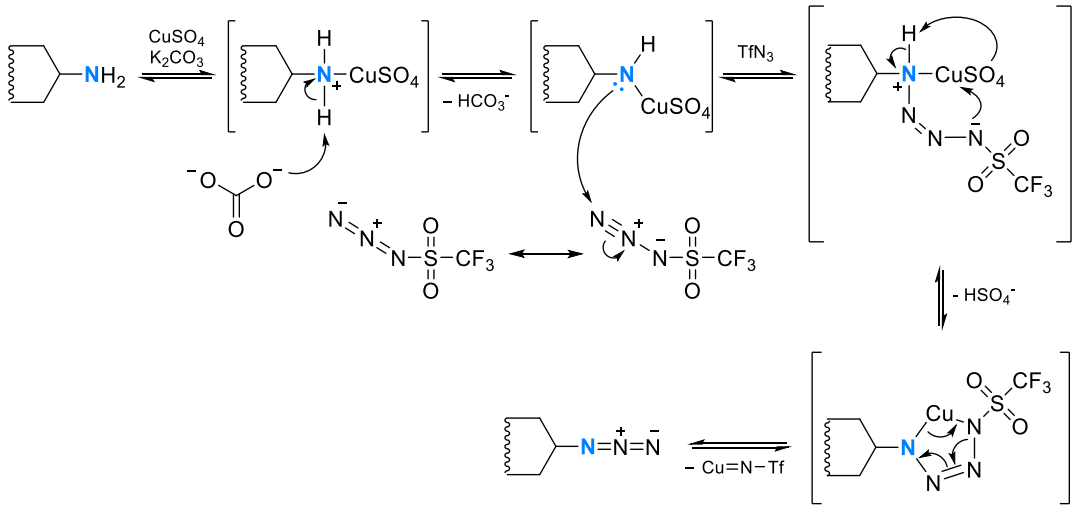
As an alternative, the conventional diazotransfer protocol, by Wong and co-workers (1996)[68], was performed using triflyl azide (Method II), prepared immediately before use from sodium azide and trifluoromethanesulphonic anhydride. The active reagent was not isolated, rather used as a solution because of its explosive potential when not in a solvent.[68, 139] Doxorubicin hydrochloride neutralised with potassium carbonate was treated with TfN_3 solution under catalysis of copper (II), to afford the azido derivative **3** in 54% yield, slightly lower than reported values (65-80%)[55, 71] (Scheme 1). The conversion of the amino functionality into azido reduced the solubility in the aqueous phase, while the remaining polar and ionisable groups disfavoured the dissolution in organic solvents. This is believed to have led to emulsification and precipitation during attempted extraction, which, summed to a tailing effect in column chromatography, caused losses that decreased the yield. In contrast, for daunorubicin hydrochloride, a similar protocol led to the azido derivative **4** in 84% yield. In this case, aqueous extraction was skipped, and chromatographic separation was straightforward, which probably led to yields surpassing literature reports (70%).[54, 72]



Scheme 1. Synthesis of 3′-azido derivatives from daunorubicin and doxorubicin hydrochlorides.

With respect to the spectroscopic data, ^{1}H NMR spectra of compounds **3**[44, 69] and **4**[54, 72] were in accordance with analytical description available. Signals of H-3′, the position to which the azido group is attached, were observed as sharp double double doublets at δ 3.59 ($J_{3',2'a}$ 12.8 Hz, $J_{3',2'b}$ 4.9 Hz, $J_{3',4'}$ 2.6 Hz) for azido doxorubicin **3**, and δ 3.63 ($J_{3',2'a}$ 12.7 Hz, $J_{3',2'b}$ 5.0 Hz, $J_{3',4'}$ 2.6 Hz) for azido daunorubicin **4**. To compare these chemical shifts to the starting material, literature data for doxorubicin free base in $CDCl_3$ can be taken as a reference, which indicates H-3′ at δ 3.02,[141] thus more shielded in relation to the products.

It is noteworthy that the diazotransfer did not cause inversion of the configuration at the C-3 stereocentre where the amino functionality is attached, otherwise the carbohydrate would be converted into its epimer. This is ascribed to the reaction mechanism, as proposed by Wong and co-workers (2002)[142], which proceeds through a nucleophilic attack by the amine at the terminal nitrogen of triflyl azide, followed by deprotonation of the complex to form a copper-stabilised mixed tetrazene. The breakdown of this intermediate via a reverse dipolar cycloaddition releases a copper-triflyl imido complex, producing the new azido compound (Scheme 2).[142] Further studies by Samuelson and co-workers (2014)[143] with ^{15}N labelled amino acids and ^{15}N labelled sulphonyl azide demonstrated the retention of the amine nitrogen atom and the transfer of the two terminal nitrogen atoms from the azidating reagent.[143]



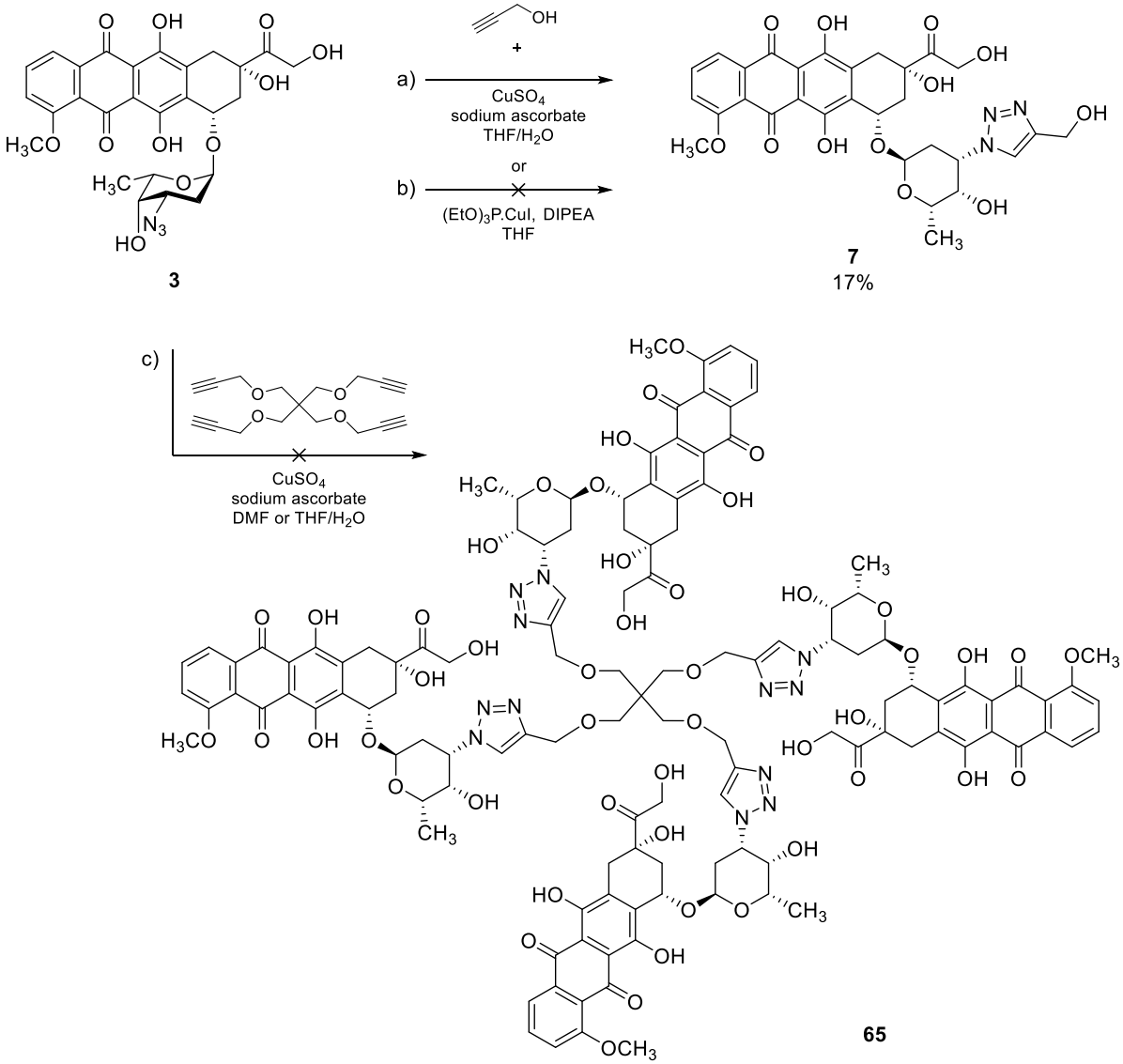
Scheme 2. Possible mechanism for the diazotransfer reaction. Copper(II) catalyst complexes with the amine and stabilises the tetrazene intermediate. The nitrogen atom (in bold) originally comprising the amine is unaffected; only the middle and terminal nitrogen atoms of triflyl azide are transferred to the product as a diazo synthon.

These azido derivatives **3** and **4** are reported as cytotoxic to human tumoral cells, such as leukaemia K562[54], breast MCF-7[44], and ovarian 2008[144], and could be valuable as controls for assessing the cytotoxicity of the novel azido derivatives proposed in this work, providing insights on the structure-activity relationship. Beyond that, they could be exploited as key precursors for bioconjugation or generation of structural diversity, for example, by coupling with terminal alkynes via Huisgen 1,3-dipolar cycloaddition. Well-established since the reports by Meldal[145] and Sharpless[146] groups, copper-catalysed azide-alkyne cycloaddition (CuAAC) was already used for linking 3′-azido doxorubicin to carrier macromolecules, such as somatostatine[69] and human serum albumin,[144] applicable to tumour-targeted drug delivery. Through the same click chemistry approach, bisdaunorubicins were synthesised from 3′-azido daunorubicin and other azido-functionalised congeners, providing dimeric intercalators for an enhanced binding affinity towards DNA.[72]

In connection with other projects ongoing in the research group, such coupling reactions between azide and alkyne, normally versatile, robust, and high-yielding,[140, 147] were studied with compound **3** and propargyl alcohol. Both classical conditions with *in situ* generation of Cu^+ (copper sulphate/sodium ascorbate) or catalysis with the organic complex iodo(triethyl phosphite)copper(I) were tested,[54, 72, 148] but the outcome was not very favourable (Scheme 3a,b), and compound **7** was isolated in 17% yield. The quantity was sufficient only for partial characterisation: the ^{1}H NMR spectrum showed a singlet at δ 8.01 referring to the triazole ring CH, a singlet at δ 4.67, to the hydroxy methylene group, and the H-3′ double triplet at δ 5.09, which was deshielded more than 1 ppm in relation to the precursor, owing to the conversion of azide into triazole.

Seeking multivalent anthracyclines that could intercalate several DNA sites or strands simultaneously, the copper sulphate/sodium ascorbate protocol was then attempted to couple **3** with a tetrameric alkyne derived from pentaerythritol (Scheme 3c). Neither prolonged times nor irradiation by microwaves were successful to get these reactions to completion, possibly because of the steric hindrance the product would present.

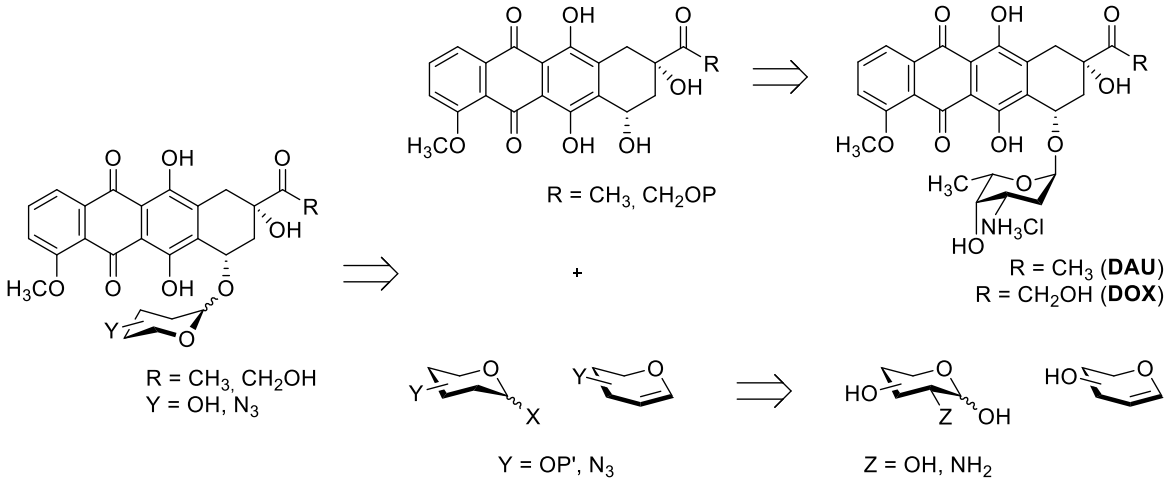
Despite these efforts, the literature demonstrates that triazolic derivatives of daunorubicin had reduced cytotoxicity against human leukaemia K562 cells, in comparison to the intact drug and the corresponding 3′-azido, which was attributed to the former losing ability to poison the topoisomerase II enzyme, due to the introduction of the bulky heterocyclic substituent.[43, 54]



Scheme 3. Azide-alkyne coupling reactions catalysed by inorganic and organic copper (I), leading to triazole derivatives.

4.2 Anthracycline modification with replacement of the glycosyl unit

According to the glycodiversification strategy, the planned semi-synthetic modifications, involving the replacement of the anthracycline glycosidic unit (daunosamine), followed the simplified retrosynthesis shown in Scheme 4. This approach required cleavage of the glycosidic bond in the anthracyclines as the fundamental step to eliminate daunosamine and to isolate the anthracyclinones, to be suitably protected as needed.[44, 73] In parallel, functionalised glycosyl donors were derived from different carbohydrates, including oxy and amino sugars, as well as glycals.



Scheme 4. Simplified retrosynthesis for the semi-synthetic preparation of anthracycline derivatives. Aglycones (glycosyl acceptors) to be obtained from commercial drugs by cleavage of the carbohydrate daunosamine, followed by orthogonal protection, whether necessary. Glycosyl donors to be obtained by the convenient protection and functionalisation of inexpensive carbohydrates, such as glucose, glucosamine and glycals.

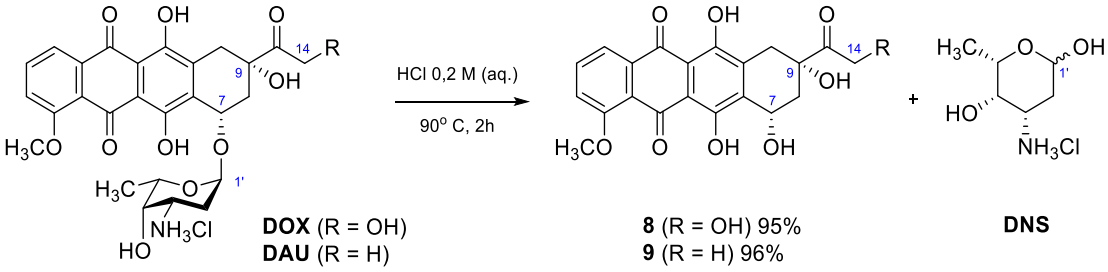
4.2.1 Preparation of anthracyclinones as glycosyl acceptors

4.2.1.1 Hydrolysis reactions

For preparing anthracycline derivatives glycosylated with different sugars, commercial drugs doxorubicin (**DOX**) and daunorubicin (**DAU**), supplied as hydrochloride salts, were initially subjected to acid hydrolysis to remove the original carbohydrate daunosamine, by breaking the glycosidic bond (acetal). This protocol is a mild and general deglycosylation method that has been applied since the first studies on anthracyclines by Arcamone (1964, 1969)[10, 12], in which the glycosidic cleavage was used to separate each moiety of the molecules in the process of their structural elucidation.[10, 12]

Upon heating the original drugs with dilute hydrochloric acid, the aglycones, so-called doxorubicinone **8** and daunorubicinone **9**,[‡] were precipitated in the reaction medium, and could be separated by simple filtration, in yields of 96% and 95% respectively (Scheme 5).[44, 73] The ^{1}H NMR spectra of the aglycones retained the characteristic aromatic and alkylic signals of anthracyclinones, in comparison to the starting materials, but no longer presented the signals of daunosamine, in the regions δ 4.2-3.0 and δ 2.0-1.3, and notably the anomeric hydrogen H-1′ around δ 5.4.[141]

[‡] By conventional naming, the anthracycline aglycones are also referred to by the name of the parent drug followed by the suffix “one”.



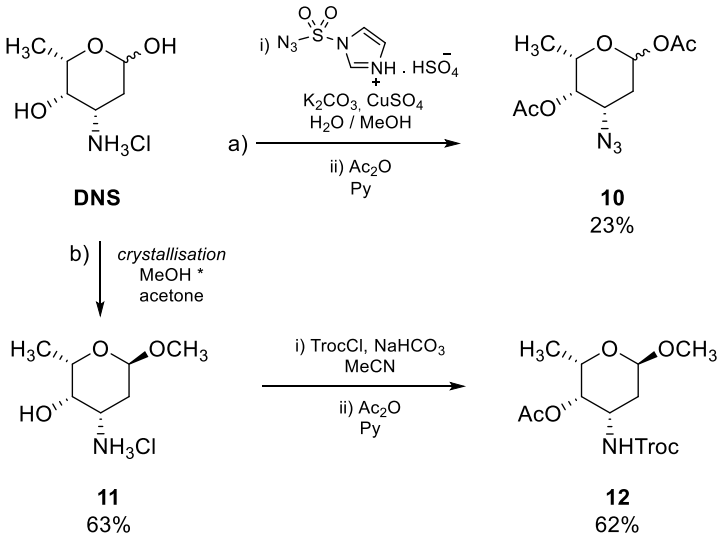
Scheme 5. Acidic hydrolysis of doxorubicin and daunorubicin, releasing the correspondent aglycones **8** and **9** and the carbohydrate daunosamine (**DNS**) as the hydrochloride.

The aqueous filtrate resulting from the hydrolysis was concentrated to dryness to afford the free hemiacetal of daunosamine hydrochloride (**DNS**) in 85%. Because this 3-aminosugar is rare and valuable, resembling the ones present in many antibiotic and antitumour glycosides,[20] it was set aside for other synthetic applications. To that end, the amino functionality was masked by an azide group through a diazotransfer reaction, then the hydroxyl groups were acetylated to give **10** in 23% yield over two steps (Scheme 6a).[78] In ^{1}H NMR, the *O*-Ac signals were singlets at δ 2.19 and 2.10, and H-3 split into a double double doublet at δ 3.84.

When the residue of the aqueous filtrate obtained in the hydrolysis was crystallised from methanol and acetone,[79] the actual product isolated was the respective methyl glycoside **11** (63%), as indicated by a singlet at δ 3.38 in the ^{1}H NMR spectrum. Probably, the glycoside was formed due to residual HCl, which could catalyse the glycosylation in the presence of methanol.[11] It was further protected by the selective installation of a trichloroethoxycarbonyl group on the amino functionality,[149] and acetylation of the remaining hydroxyl group, effected in 62% yield over two steps to give **12** (Scheme 6b). Protecting groups resonance signals were clearly observed as a singlet at δ 2.18 for the *O*-Ac methyl group, and as a pair of doublets at δ 4.76 and δ 4.66 (J 12 Hz) for the diastereotopic protons of *N*-Troc methylene group.

4.2.1.2 Protection reactions

In the subsequent glycosylation reactions, the aglycone would play the role of glycosyl acceptor. Having several sites susceptible to glycosylation, it could lead to a mixture of products glycosylated in different positions. Since the drugs from the anthracycline class present the carbohydrate at position 7, this feature should be maintained in the novel synthesised glycosides, so it was crucial to ensure regioselectivity for that particular position during glycosylation reactions.



Scheme 6. Protections of the daunosamine glycosyl moiety. a) Direct conversion to the azide and per-acetylation gave **10.** b) Crystallisation from MeOH and acetone resulted in the glycosylation of the alcohol, attributable to traces of HCl (*). Methyl glycoside was further protected as the *N*-Troc and *O*-Ac derivative **12**.

It is known that glycosylation of unprotected daunorubicinone (**9**), which does not possess a hydroxyl group at position 14, provides the correspondent 7-*O*-glycoside, even in the presence of phenolic and tertiary hydroxyl groups lacking any protection.[75, 78, 150, 151] That is because the lone electron pairs in phenolic oxygens (positions 6 and 11) are poorly available due to delocalisation through the polyaromatic system, and the steric hindrance on the tertiary hydroxyl at position 9, which restricts its reactivity. Analogously, doxorubicin derivatives were obtained by glycosylating the aglycone protected only in its primary hydroxyl group at position 14.[74, 85, 152, 153]

Following this strategy, some methodologies of selective protection of the OH-14 in doxorubicinone were tested, based on bulky protecting groups, which could react specifically with the primary hydroxyl group directed by steric effects. The triphenylmethyl group, or simply trityl, is routinely used in carbohydrate chemistry for the protection of primary hydroxyl groups and is susceptible to hydrogenolysis with H_2/Pd or dilute acid. The reaction with triphenylchloromethane in pyridine[74, 154, 155] attempting to get **66** was not successful (Scheme 7a), with the isolation of unreacted starting material.

Silyl ethers were then proposed, owing to their susceptibility to mild cleavage in the presence of fluorides, which have a high affinity for silicon. The first attempts utilising *tert*-butylchlorodiphenylsilane (TBDPSCl[152, 156]) and *tert*-butylchlorodimethylsilane (TBSCl[85]), catalysed by imidazole in DMF (Scheme 7b,e), did not give the expected products **67** and **15**. Even extending reaction time, adding excess reagents and heating, the starting aglycone was not

significantly consumed, being recovered from the reaction mixtures. When TBDPSCl and TBSCl were tested for the protection of benzyl alcohol as a model primary hydroxyl group, the corresponding silyl ethers were isolated, discarding any problems with the silylating reagents.

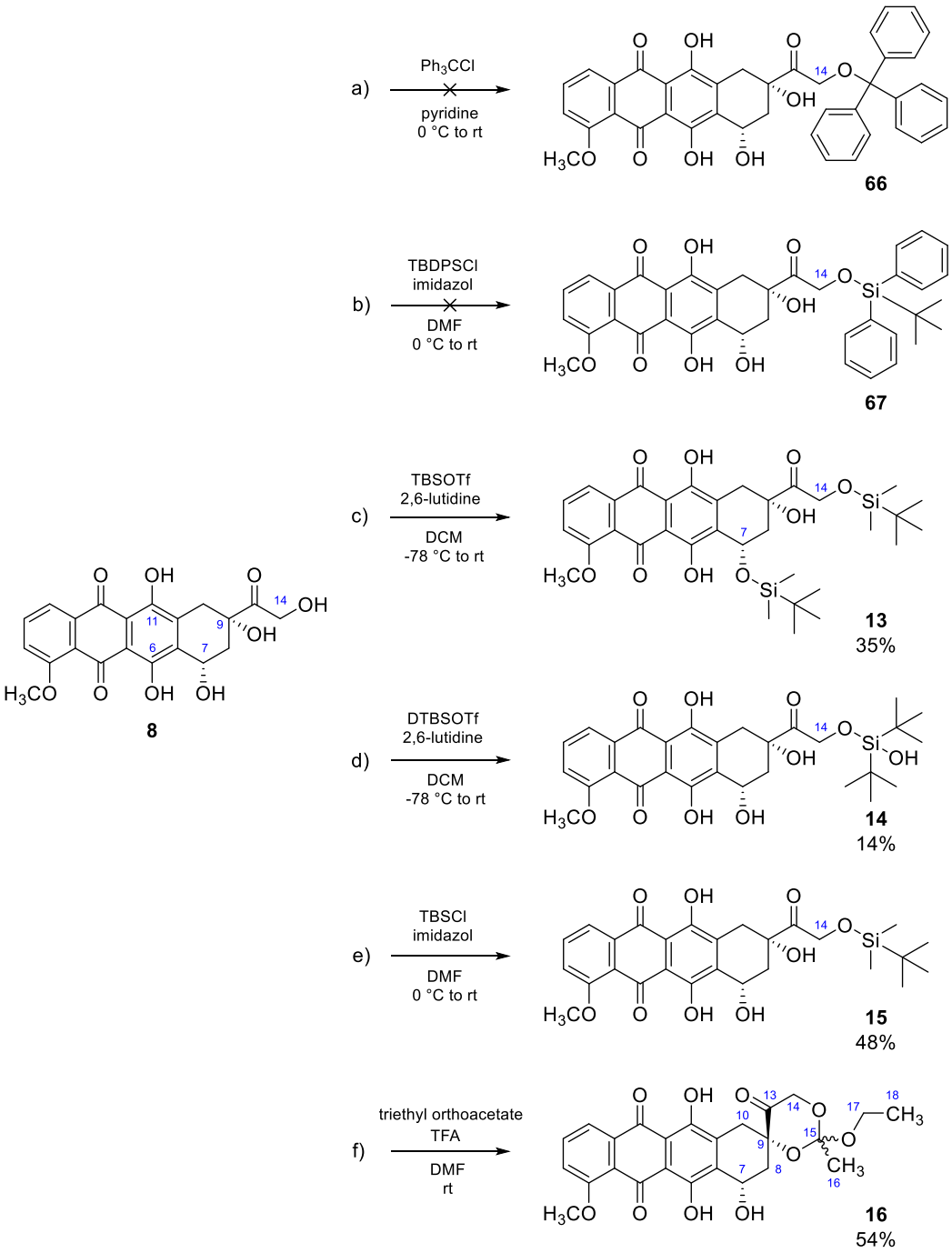
Once the silylation reagent was replaced by *tert*-butyldimethylsilyl trifluoromethane sulphonate (TBSOTf[82, 157]), which has a stronger leaving group, the major product obtained was the 7,14-disilyl derivative **13**, in 35% yield (Scheme 7c). Catalysis by the hindered base 2,6-lutidine at low temperature (-78 °C) for kinetic control was not enough to tune the reactivity of the triflate reagent, and the disilylated product found no direct applicability towards the intended glycosylation reactions. Simultaneous protection of hydroxyl groups at positions 9 and 14 with a bifunctional silyl ether was attempted by treatment with di-*tert*-butylsilylene trifluoromethane sulphonate (DTBSOTf[84]), which can preferably silylate 1,3-diols even when one of the alcohols is tertiary. However, instead of the predicted 9,14-disilylene cyclic derivative, partially hydrolysed di-*tert*-butyl silanols were isolated in low yields, either the monosilyl at position 14 **14** (14%) (Scheme 7d) and the disilyl at positions 7 and 14 (6%).

Turning back to the TBSCl protocol, it was suspected that the very limited amount of aglycone could be a hampering factor for this sensitive protection, as occasional traces of humidity could critically compromise the reaction course when working in microscale. Increasing the quantity of reactant aglycone by 15-fold (340 mg of doxorubicinone), and adjusting some experimental details led to the monosilylated product **15** in 48% yield (Scheme 7e). The particular conditions modified were: previous dissolution/concentration of aglycone in/from anhydrous toluene, inclusion of molecular sieves, dissolution and mixture of imidazole and TBSCl prior to their addition, and use of excess TBSCl (4 equiv). Further increasing the molar ratio of silyl chloride for enhanced yields resulted in a higher proportion of the disilylated byproduct, instead.

The ^{1}H NMR spectrum of **15** showed the TBS *t*-butyl and methyl signals as singlets at δ 0.95 and 0.14, and the absence of the OH-14 signal, previously observed at δ 2.98 for the starting material **8**. Methylenic hydrogens at position 14 had been identified at δ 4.83 and 4.75 in the spectrum of the free aglycone, as overlapping double doublets coupling with the adjacent hydroxyl hydrogen (J 5 Hz). No longer experiencing this coupling in the spectrum of **15**, CH_2-14 split into simple doublets at δ 4.92 and 4.84, coupling with each other with a constant of J 19.5 Hz.

Similarly, the spectra of **13** and **14** presented singlets in the upfield region of δ 1.1-0.1, corresponding to the *t*-butyl and methyl groups attached to silicon atoms, with integrals proportional to the number of silylated sites. In addition, **14** had deshielded signals for CH_2-14

in comparison to the starting material (δ 5.15 and 5.08, previously at δ 4.83 and 4.75), as a consequence of the substitution by the silanol group. Assignments were also confirmed with the aid of ^{1}H-^{29}Si HMBC experiments.



Scheme 7. Selective protection of the primary hydroxyl groups (position 14) of doxorubicinone: a) and b) failed with trityl chloride and TBDPSCl. c) TBSOTf furnished mainly the disilyl product **13**. d) DTBSOTf afforded semi hydrolysed silanol as the principal product **14**. e) TBSCl under optimised conditions resulted in the monosilyl product **15**. f) Triethylortoacetate formed the di-protected cyclic derivative **16**.

In parallel, alternative protecting groups were investigated: an orthoester had been described for the 1,3-diol protection of intact doxorubicin as the orthoformate at positions 9 and 14, in a patented route to convert this drug into epirubicin (C-4′ epimerisation).[87] Taking into account the synthetic sequence of the present work, such protecting group could only be introduced after the hydrolysis of the glycoside, because of the sensitivity of orthoesters to acid conditions. Considering that the C-7 hydroxyl group is also free in doxorubicinone, there would be chances to generate a regioisomeric orthoester connecting positions 9 and 7 (which are *cis*-oriented in relation to the cyclohexene ring). However, the initial reaction should be kinetically favoured at the less hindered primary hydroxyl group, conducting to the expected orthoester involving positions 9 and 14.

Due to the prompt availability of 1,1,1-thriethoxyethane in the laboratory, this reagent was employed to protect doxorubicinone **8**, under catalysis of trifluoroacetic acid in DMF.[87] Indeed, the major product was identified as the orthoacetate at the positions 9 and 14 (**16**), in 54% yield (Scheme 7f). Likely, a single stereoisomer was formed at the quaternary carbon of the orthoester (C-15), since ^{1}H NMR spectrum did not present duplicate signals, but determining the configuration of this centre was irrelevant because it was a transient protecting group.

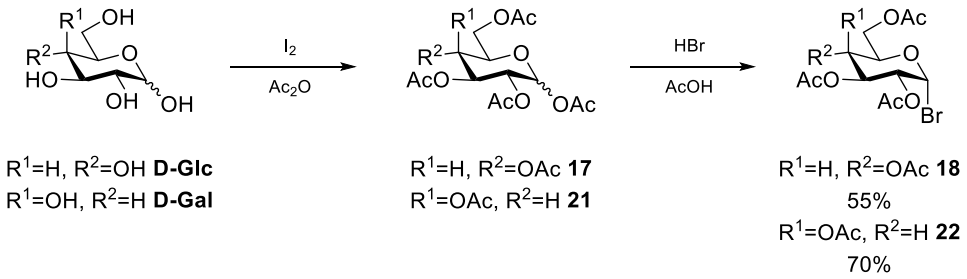
A singlet at δ 1.88 corresponded to the C-16 methyl of the orthoacetate alkyl substituent, and a group of signals indicated the ethoxyl moiety of the orthoacetate: the C-18 methyl triplet at δ 1.23 coupling with the C-17 methylene (J 7.0 Hz), and the latter as a pair of double quartets at δ 3.80 e 3.59, with a geminal J 9.2 Hz. The conformational strain introduced by the new six-member ring caused CH_2-14 methylene signals to change in multiplicity and chemical shift. In the starting material **8**, these signals were close double doublets at δ 4.84 and 4.75 coupled with each other (J 20.9 Hz) and with the adjacent hydroxyl proton (J 5.0 Hz). In the product **16**, these hydrogens were shielded, reflecting the loss of intramolecular hydrogen bonding and the chemical transformation in the vicinity, from a free hydroxyl to an alkoxy group. They differentiated into two well-separated double doublets at δ 4.33 and 3.86, coupling with each other with a typical J 12.7 Hz. The difference between the C-8 methylene hydrogens increased: H-8b shifted upfield to δ 1.97 preserving only the geminal coupling (J 11.0 Hz); H-8a shifted downfield to δ 2.64 and also coupled with H-7 (J 5.7 Hz) and H-10a (^{4}J 1.4 Hz). All these spectroscopic alterations were due to the greater rigidity in the cyclohexene ring induced by the closure of a second ring comprising carbon 9 (*spiro*).

Having got protected doxorubicinones with TBS (**15**) and orthoacetate (**16**), and being unnecessary any protection on the daunorubicinone scaffold (**9**), the glycosyl acceptors became available for glycosylation reactions aiming to the diversification of the glycone unit of these anthracyclines.

4.2.2 Preparation of glycosyl donors

4.2.2.1 Glycosyl halides

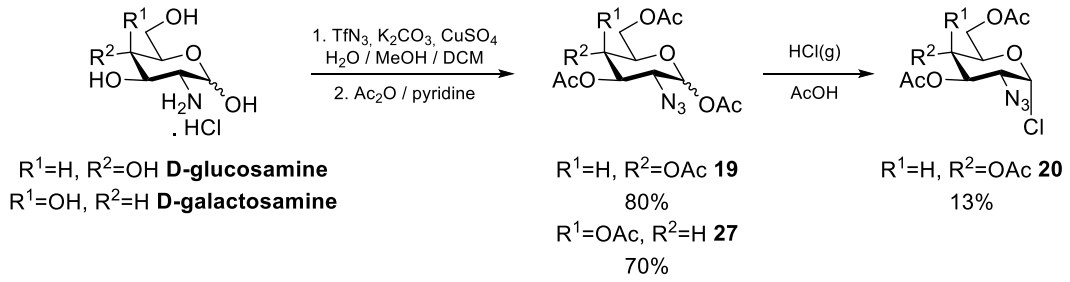
As a starting point, Koenigs-Knorr[158] glycosylation conditions were selected, which utilise anomeric halides as glycosyl donors in reactions promoted originally by silver salts, and later extended to other metals. For initial testing, simple glycosyl bromides were synthesised from D-glucose and D-galactose (Scheme 8), by iodine-catalysed acetylation with acetic anhydride, followed by treatment with hydrogen bromide solution for anomeric substitution, yielding **18** and **22**. Very deshielded anomeric protons (δ 6.61-6.69 ppm) were clearly observed in ^{1}H NMR for both compounds, due to the electronegativity of the bromide. Driven by the anomeric effect, halogenation gave α products exclusively, as indicated by coupling constants $J_{1,2}$ around 4 Hz, compatible with the *cis* equatorial-axial relationship between protons at positions 1 and 2 of the carbohydrate.



Scheme 8. Preparation of glycosyl bromides.

To address compound **1**, the first specific target of this work, glycosyl donor **20**, containing an azido functionality at position 2 of the glucose moiety, was prepared according to Scheme 9. D-Glucosamine (an inexpensive commercial carbohydrate widely available in the laboratory) was neutralised with potassium carbonate and treated with catalytic copper sulphate

and a freshly prepared triflyl azide solution **6**,[68, 115] to perform a diazotransfer reaction. This was followed by protection of the carbohydrate hydroxyl groups with acetic anhydride in pyridine, resulting in the per-acetylated intermediate **19**. Subsequent treatment with glacial acetic acid saturated with hydrogen chloride gas[91, 92] led to the anomeric substitution, affording **20** exclusively with α configuration, evidenced by $J_{1,2}$ 3.8 Hz. The poor 13% yield could have been improved by extending the reaction time from 5 h to at least one or more days to allow full conversion.[91] Attempts to get **20** more efficiently by alternative approaches, such as azidochlorination of glucal[93] and trichlorotriazine-DMF chlorination,[94] were frustrated. Despite the low yield, the former method provided enough glucosyl chloride for glycosylation reactions, and no more efforts were made to improve that step.

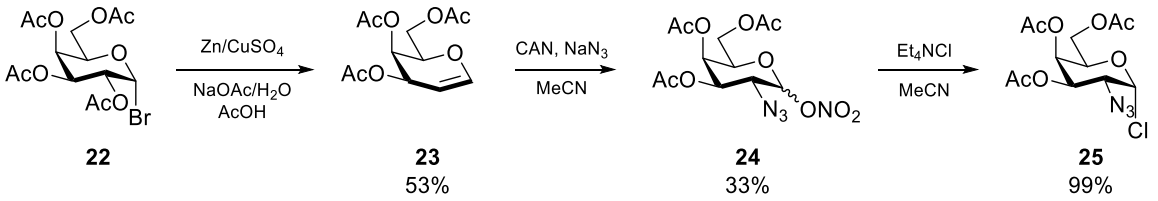


Scheme 9. Synthesis of the glycosyl donors substituted by a 2-azido group. Galactosyl per-acetylated derivative was diverted to a different route.

Aligned with earlier and parallel projects within the research group,[159] galactosamine moiety attracted attention, although it was not originally planned in the present work. It differs from the glucosyl derivatives only in the configuration at position 4, with an axial hydroxyl group, and could be interesting for comparing the influence of the carbohydrate on the structure-activity relationship. Thus, for galactosamine, which was more costly, and available in smaller quantity than glucosamine, the acetylated intermediate **27** was saved for preparing a different galactosyl donor, and the corresponding galactosyl chloride was obtained by a substitute route, as described below.

To exploit the galactosyl residue, the sequence depicted in Scheme 10 was chosen to prepare the chloride containing an azido group at position 2 (**25**). Bromide **22**, obtained in two steps from galactose (Scheme 8), was subjected to a reductive elimination with zinc and acetic acid, generating galactal **23** in 53% yield (Scheme 10), equivalent to literature reports.[97] The signal of the anomeric proton (vinylic) at δ 6.36 was observed as a doublet with a typical coupling constant for *cis* alkenes ($J_{1,2}$ 6.3 Hz).

From intermediate **23**, which is also commercially available, the classical synthetic sequence introduced by Lemieux and Ratcliffe (1979)[100] was followed, involving the azidonitration of galactal, by treatment with excess ceric ammonium nitrate and sodium azide.[100] Azidonitrates **24** were a mixture of the α and β-*galacto*pyranosides, and a minor fraction of the α-*talo*pyranoside (with inversion of the configuration at position 2), as a result of a radical addition mechanism. The mixture was converted to the glycosyl donor **25** by an efficient anomeric substitution promoted by tetraethylammonium chloride, in 99% yield (Scheme 10). The α configuration of the product was confirmed by ^{1}H NMR, with the presence of a doublet at δ 6.12 coupling with characteristic $J_{1,2}$ 3.9 Hz, whereas the shift of H-2 to roughly δ 4, more shielded than the precursors, indicated the influence of the azido group on this proton.

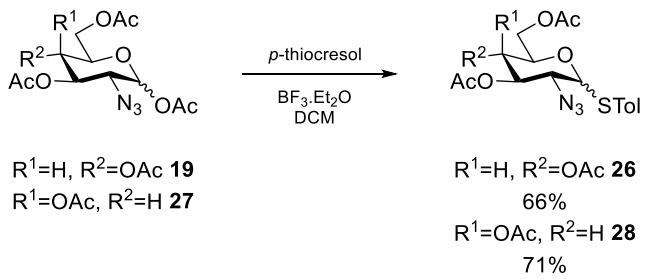


Scheme 10. Synthesis of galactosyl donor substituted by a 2-azido group The azidonitration of galactal route was employed for the introduction of the azido group at position 2.

Owing to the reactivity of the glycosyl halides, and consequently their relative instability, they were prepared on demand for the glycosylation reactions with anthracycline aglycones. To broaden the study of glycosylation conditions, other types of glycosyl donors, namely thioglycosides[160] and imidates,[113] were synthesised and tested as well, due to their practical preparation methods and high-yielding glycosylation reactions.[161]

4.2.2.2 Thioglycosides

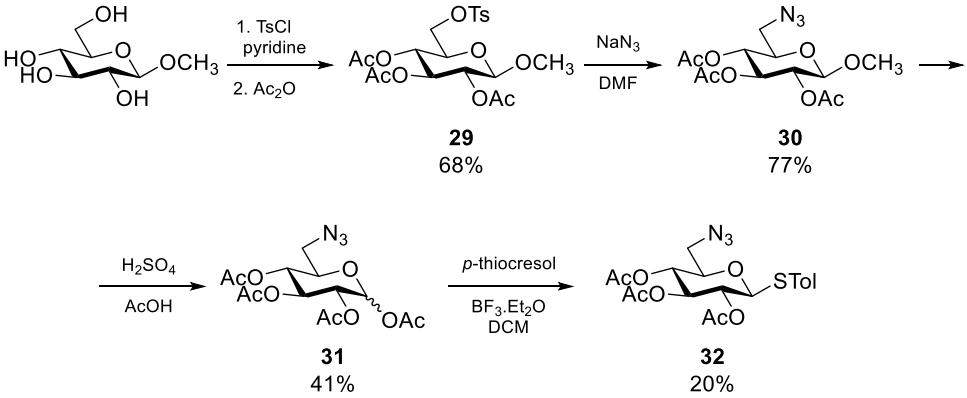
On top of their convenient shelf-stability and orthogonal activation under specific conditions, thioglycosides have been extensively used for the glycosylation of anthracyclines and related scaffolds.[44, 78, 162, 163] They can be prepared directly from anomeric acetates by treatment with a Lewis acid in the presence of a thiol nucleophile. *p*-Thiocresol was preferred over the commonly used thiophenol, inasmuch as the reactivity of the *S*-tolyl and *S*-phenyl thioglucosides do not differ significantly.[104, 105]



Scheme 11. Synthesis of thioglycoside donors from 2-azido per-acetylated glucoside and galactoside, by treatment with *p*-thiocresol and $BF_3 \cdot Et_2O$.

Hence, in a single step, 1-*O*-acetyl intermediates **19** and **27** were activated with $BF_3 \cdot Et_2O$ to react with *p*-thiocresol, furnishing the S-tolyl glucoside (**26**) and galactoside (**28**) in 66% and 71% yields, respectively, in mixtures of anomers (Scheme 11). A protocol including sonication[103] was applied for the synthesis of **26**, intending to reduce the reaction time and increase the yield, but since it did not prove beneficial, the same was not extended to the other thioglycosides. The ^{1}H NMR spectra showed two groups of multiplets in the aromatic region (around δ 7.45 and δ 7.15), consistent with the *para*-substituted ring pattern expected for the S-tolyl moiety. The anomeric protons were identified at δ 5.56 ($J_{1,2}$ 5.5 Hz, α) and δ 4.42 ($J_{1,2}$ 10.1 Hz, β), for glucosides **26**, and at δ 5.63 ($J_{1,2}$ 5.4 Hz, α) and δ 4.48 ($J_{1,2}$ 10.1 Hz, β), for galactosides **28**.

Concerning the second target of this work, azido glycoside **2**, which holds an azido functionality at position 6, the corresponding glycosyl donors were prepared from methyl *β*-glucopyranoside, an affordable commercial starting material. Being the anomeric position already protected as the methyl glycoside, the primary hydroxyl group at position 6 was functionalised with a bulky tosyl group, by treatment with *p*-toluenesulphonyl chloride in pyridine, employing low temperature to favour selectivity.[107, 108] After the one-pot acetylation of the remaining hydroxyl groups by addition of acetic anhydride to the reaction mixture, the tosylate **29** was isolated in 68% yield. Toluenesulphonate is also an excellent leaving group, which was straightforwardly displaced in an S_N2 type reaction, upon heating with NaN_3, to afford 6-azide **30** in 77% yield (Scheme 12).[164, 165] In ^{1}H NMR, two double doublets from the methylene protons at position 6 were observed at δ 3.43 and δ 3.19, evidencing a shielding effect of the azide group, compared to the tosyl group in precursor (CH_2-6 at δ 4.2). H-1 doublet at δ 4.47 ($J_{1,2}$ 8 Hz) confirmed the retention of β configuration.



Scheme 12. Synthesis of the 6-azido thioglucosyl donor. With the anomeric position blocked by a methoxyl group, the primary hydroxyl group was selectively tosylated and then substituted by azide. Acidic cleavage of the methyl glycoside with substitution for an acetate allowed further functionalisation towards the thioglucoside.

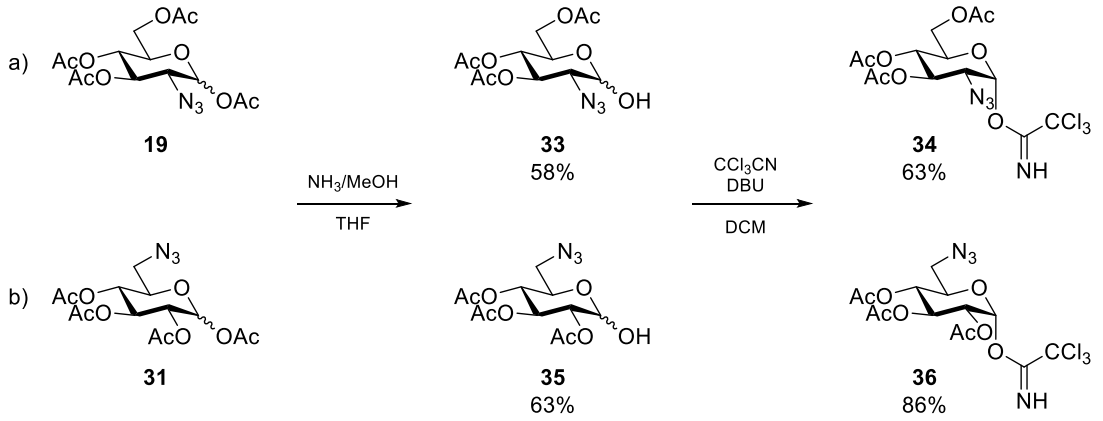
Following Scheme 12, the methyl glucoside, which played the role of a transient anomeric protecting group, was removed by acid treatment with sulphuric acid in acetic acid, and substituted by a reactive acetate group, enabling the conversion of the 6-azido glucoside into more powerful glycosyl donors. In this particular case, the per-acetylated intermediate **31** was subjected to thioglycosidation with *p*-thiocresol and $BF_3 \cdot Et_2O$, under the same conditions described for the synthesis of compounds **26** and **28**. The resulting thioglucoside **32** was obtained in disappointingly low yield (20%), sufficient for a single glycosylation reaction. One of the reasons might have been the formation of dioxolenium intermediate or the thio-orthoester derivative during glycosylation, but this was not investigated. More certain was the anchimeric assistance by the 2-*O*-acetyl group, inducing a complete β stereoselectivity. The ^{1}H NMR spectrum revealed the *S*-tolyl glucoside was formed as a single anomer, since duplicate signals were not present, and the anomeric proton split into a doublet at δ 4.64, with a large coupling constant $J_{1,2}$ 10.1 Hz, consistent with the *trans* diaxial relationship between H-1 and H-2 in β glucosides.

4.2.2.3 Glycosyl imidates

Since the development of trichloroacetimidates as glycosyl donors by Schmidt (1980),[113] they have shown an outstanding versatility and have become increasingly popular for the synthesis of complex natural glycosides, given the mild conditions required for their activation, *i.e.*, catalytic amounts of Lewis acids, such as TMSOTf and $BF_3 \cdot Et_2O$.[166]

Surprisingly, there are fewer reports on the use of imidates for glycosylation of anthracyclines and related molecules, compared to other glycosyl donor classes.[167, 168]

As opposed to the synthesis of previous donors, Schmidt's procedure does not involve a substitution at the anomeric carbon, rather a functionalisation of the oxygen at C-1 by highly electron-deficient nitriles, known to undergo base-catalysed addition of alcohols to the triple bond, transforming the anomeric oxygen atom into a good leaving group. Thus, 2-azido **19** and 6-azido **31** precursors were first selectively deacylated at the anomeric position by treatment with ammonia solution in methanol,[112] although some deprotection of the other acetyl groups was observed before the starting material was consumed entirely, resulting in 58-63% yields. Hemiacetals **33** and **35** were then reacted with trichloroacetonitrile and catalytic DBU,[113, 114] to furnish trichloroacetimidates in 63% and 86% yields, respectively (Scheme 13). Characteristic NH singlets of the imidate were identified in ^{1}H NMR at δ 8.83 and δ 8.72, and the anomeric protons as doublets at δ 6.49 ($J_{1,2}$ 3.6 Hz) and δ 6.58 ($J_{1,2}$ 3.7 Hz), showing the trichloroacetimidates **34** and **36** were isolated in the α configuration only.



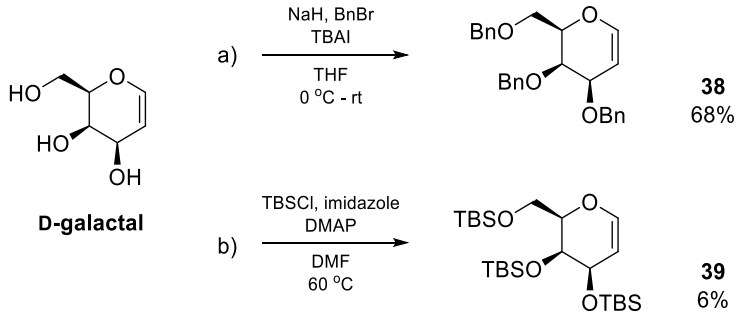
Scheme 13. Synthesis of trichloroacetimidate donors. The anomeric *O*-acetyl group is selectively removed by ammonia solution, then the hydroxyl group is deprotonated by strong base DBU and adds to trichloroacetonitrile.

4.2.2.4 Glycals

As part of a doctoral internship in the laboratory of Prof Dr M. Carmen Galan, the glycodiversification projected in this work was extended towards 2-deoxyglycosides, which resemble more closely the anthracycline's sugar daunosamine. Accordingly, taking advantage of the expertise of the hosting group in glycosylations with glycal donors, a set of glucals and galactals was also prepared.

Tri-*O*-benzyl-galactal **38** was selected as standard glycosyl donor to investigate the 2-deoxy glycosylation with anthracyclines, since part of the vast experience of Prof Galan's research group on the synthesis of 2-deoxyglycosides from glycals has been constructed on optimisation studies with this donor in catalytic glycosylations.[122, 130, 131]

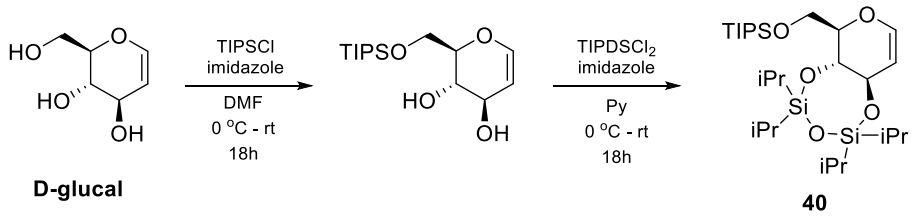
Although it is commercially available, **38** was prepared in-house by alkylation of galactal with benzyl bromide and sodium hydride in THF, with the addition of tetrabutylammonium iodide as phase-transfer catalyst,[120] in 68% yield (Scheme 14-a). Carrying this reaction homogeneously in DMF was avoided to prevent the formation of a tertiary amine impurity, arising from this solvent under classical benzylation conditions, which coelutes with benzylated galactal in column chromatography. Such byproduct was reported to poison the thiourea organocatalysts intended to be used later in the glycosylation reactions.[169] Characteristic benzylic methylene doublets in the region δ 4.9-4.4, with coupling constants in the range of J 11.8-12.1 Hz, accompanied by the aromatic proton signals in δ 7.4-7.2, indicated the formation of **38**.



Scheme 14. Direct protection of D-galactal. a) Benzylation under phase-transfer conditions. b) *tert*-butyldimethylsilylation promoted by imidazole/DMAP.

tert-Butyldimethylsilyl protected galactal **39** was synthesised by heating free galactal with TBSCl, imidazole, and DMAP, the latter as auxiliary base driving to full conversion.[122, 170] In our hands, this particular reaction was very low yielding (6%) (Scheme 14-b), due to partial hydrolysis of galactal. Probably because imidazole was used in equimolar proportion to TBSCl, which might have led to HCl generated during the course of the reaction not being completely neutralised. The formation of the hemiacetal also complicated the separation of these very non-polar compounds from each other. The product was confirmed by the singlets of *t*-butyl (δ 0.93-0.87) and methyl (δ 0.11-0.05), and the signals of the vinylic hydrogens H-1 (δ 6.21) and H-2 (δ 4.65) conserved from the starting material. The reaction was not worked out to achieve the literature yields (73%),[122] since a previously in-house stock of **39** was available.

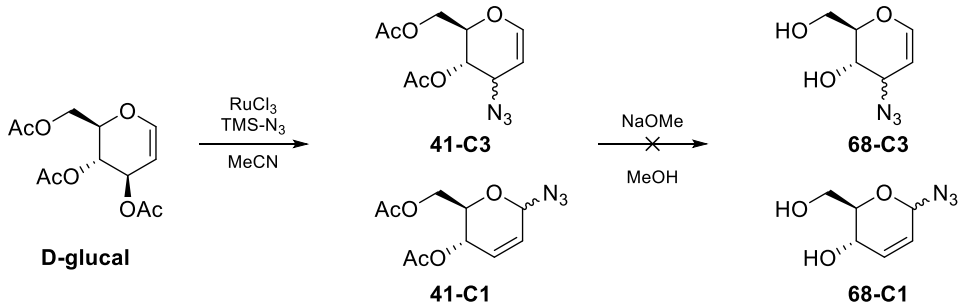
Likewise, the disiloxane glycosyl donor **40** did not need to be synthesised from glucal, for it being ready to use from the Galan laboratory collection of glycosyl donors. Beforehand, it had been obtained following a reported protocol,[124] involving the selective protection of the 6-hydroxyl group with bulky chlorotriisopropylsilane (TIPSCl), then the cyclisation of the 3- and 4-hydroxyl groups into a fused disiloxane by treatment with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane ($TIPDSCl_2$), both silylations promoted by imidazole (Scheme 15).



Scheme 15. Preparation of 40 as described by Balmond et al. (2012)[122], by sequential selective silylation of D-glucal (reported yield 71% over two steps).

To address 2-deoxyglycosides containing azido groups related to the original aims of this work, different substitution positions were exploited. It is known that 3-azido glycals can be obtained via a direct allylic substitution of per-*O*-acetylated or -benzylated glucals and galactals.[125, 171-174] Although the formation of epimers is anticipated, along with a smaller proportion of anomeric azides arisen from Ferrier rearrangement, a mixture of 3-azido glycals should be acceptable to probe the reactivity of these derivatives towards catalytic glycosylation protocols, before investing time and resources in stereoselective multistep synthetic routes.

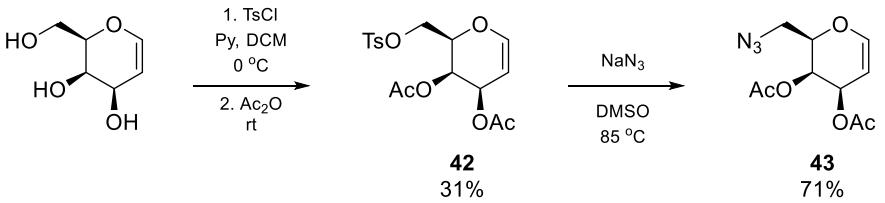
Thereby, direct allylic substitution was attempted as a straightforward strategy to obtain the 3-azido derivative from tri-*O*-acetyl-D-glucal and trimethylsilylazide, in metal catalysis by ruthenium(III) chloride (Scheme 16).[125] The reaction proceeded very fast, completing after 30 min, and decomposition into a probably hydrolysed byproduct was somewhat observed (as judged by TLC). The azide products were obtained as a mixture of at least four components **41**, with *pseudo*-axial and -equatorial epimers of C-3 azido glycal, and α and β anomers of C-1 azido 2-enopyranoside, in the ratio 2.5:3.5:10:1, respectively. Upon standing in $CDCl_3$ solution inside the NMR tube, the proportion changed to 10:5:8.5:2, even having the mixture been purified by column chromatography. The ratios were established by ^{1}H NMR relative integration for the H-1 of each isomer, which signals were observed at δ 6.53 (ax) and δ 6.50 (eq) for the C-3 products, and at δ 5.56 (α) and δ 5.27 (β) for the C-1 products.



Scheme 16. Direct allylic substitution on D-glucal, generating a mixture of four isomers **41**. Attempted deprotection followed by HPLC purification failed to provide isolated deprotected compounds.

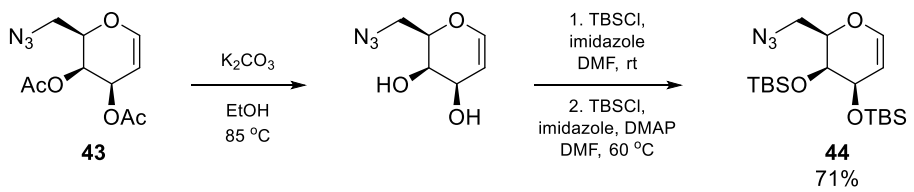
Aiming to isolate the compounds from the former complex mixture, **41** was deacetylated with sodium methoxide solution (Scheme 16). The crude residue was subjected to preparative RP-HPLC separation, but the expected products were not identified in the collected fractions, which was confirmed by mass spectrometry screening (MALDI). This approach revealed to be troublesome, so it was proposed to adopt an alternative route converging cleanly and quickly to a single azido-substituted glycal. For this sake, the placement of the azido group at the primary hydroxyl group (position 6) was preferred because of its differential reactivity.

Exploiting this property, D-galactal was reacted at low temperature with *p*-toluenesulphonyl chloride, which is sterically demanding and forms a sulphonate selectively with the primary OH-6.[127] The reaction mixture was split into two portions, but the attempt to purify the unprotected tosyl galactal from the concentrated crude was unsuccessful. The second half of the reaction mixture (in pyridine:DCM 1:1) was acetylated by simply adding acetic anhydride, and **42** was obtained in 31% overall yield. The tosylate leaving group was displaced by N_3 anion upon heating with sodium azide,[126] to give per-*O*-acetyl 6-azido galactal **43** in 71% yield (Scheme 17). That was evidenced by the shielding effect observed for CH_2-6 in ^{1}H NMR, from δ 4.29 and 4.14 in the tosylate to δ 3.62 and 3.31 in the azido.



Scheme 17. Preparation of 6-azido galactal 43 from D-galactal, by selective tosylation at position 6 and substitution by azide.

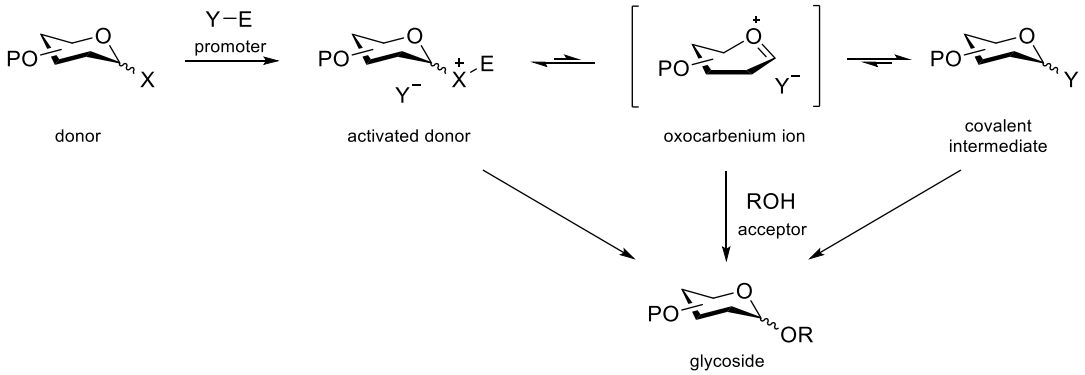
The effect of electron-withdrawing groups, such as esters, in close proximity to the reacting double bond (C-3), was reported by Galan and co-workers (2012, 2017)[122, 130, 131] to be detrimental to the reactivity, yield and stereoselectivity of the intended glycosylation reactions. Then, galactal **43** was subjected to protecting group manipulation, in order to replace *O*-acetyl groups for a more amenable type. TBS was chosen by analogy with the glycosyl donor **39**, so the intermediate **43** was deacetylated with K_2CO_3, and silylated with TBSCl in two steps,[128] the second one under forcing conditions[122] to achieve protection of the more hindered axial OH-4 (Scheme 18).



Scheme 18. Protecting group replacement in acetylated 6-azido galactal **43**. OAc groups were removed by basic cleavage, and TBS was introduced.

4.2.3 Glycosylation reactions

With the building blocks in hand, both the aglycones and the four types of glycosyl donors, the glycosylation reactions could be studied. In a glycosylation process, a glycosyl donor and a glycosyl acceptor react with each other in the presence of a promoter to form a glycosidic linkage. Generically, the anomeric leaving group is polarised by the promoter electrophile, which assists the elimination, leading to a glycosyl cation intermediate, known as oxocarbenium ion (Scheme 19).



Scheme 19. Generic mechanism of glycosylation, through an oxocarbenium ion intermediate. Adapted from Bohe (2016).[175]

Even though the existence of the oxocarbenium ion is still being definitely proved by spectroscopic evidence, the result of a glycosylation reaction is mostly determined by the stability and conformation of this intermediate. Its approximately planar geometry may accept a nucleophilic attack of the glycosyl acceptor by any of the top or bottom faces, being the preference governed by several stereoelectronic factors, such as the anomeric effect, the influence of neighbouring groups and substitution pattern, for instance.[60, 61, 175, 176] Therefore, the primary aim in a glycosylation reaction is to control the anomeric configuration of the product, avoiding mixtures of α and β anomers, which are generally of difficult separation.

Glycosylations are considered very challenging reactions, affected by several different factors, namely: types of donor and acceptor, promoter, temperature, solvent, protecting groups, concentration, and others, which can interfere importantly in the outcome. In this work, various glycosylation reactions were performed to optimise the yields and stereoselectivity, using the collections of glycosyl donors and acceptors prepared, summarised in Figure 15.

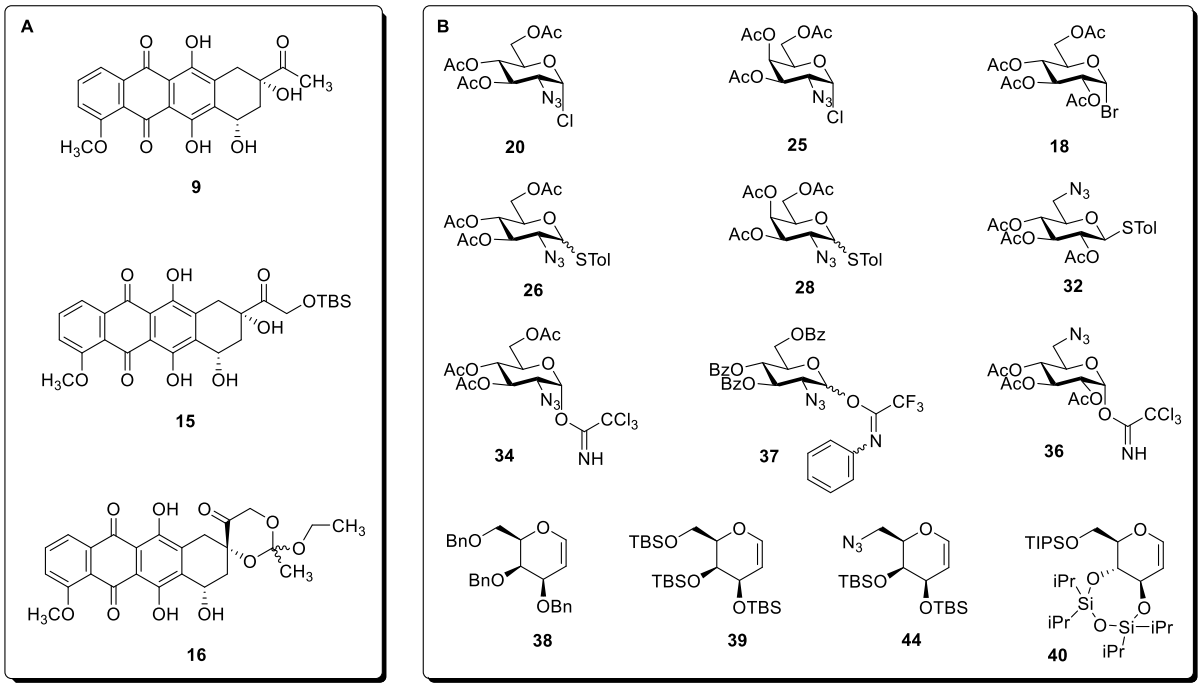
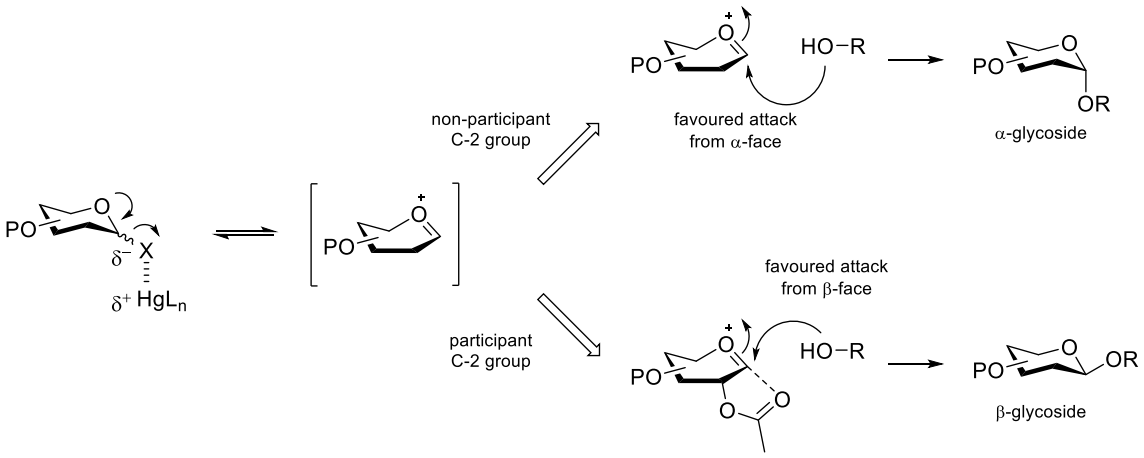


Figure 15. Glycosyl acceptor (A) and donors (B) used in in the study of glycosylation reactions, in order to establish the most favourable conditions.

It is important to emphasise that it was not an exhaustive systematic study comprehending all possible combinations, but the selection of reactants and conditions was based on the best results obtained as the work developed, with the principal intention of getting the desired products.

4.2.3.1 Glycosylation with glycosyl halides

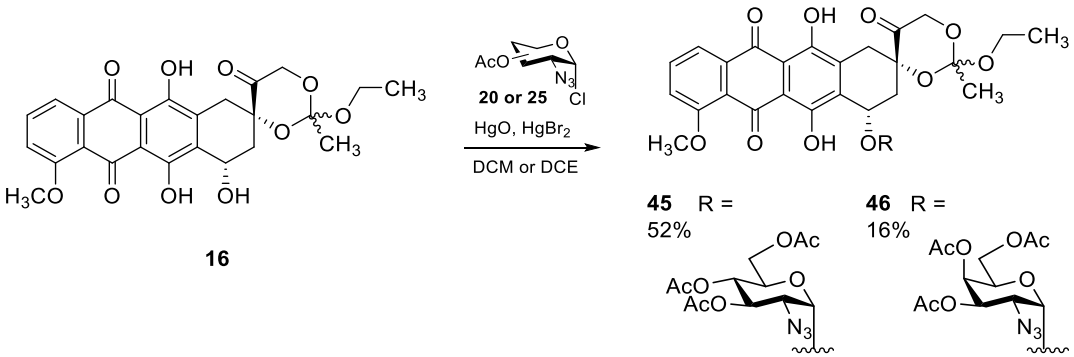
Considering the previous experience of the research group with glycosylation reactions catalysed by mercuric salts,[159, 177] that correspond to variations of the Koenigs-Knorr methodology referred to as Helferich modifications, this transition metal was initially chosen to promote the coupling between the aglycone and glycosyl halides. Because of their electrophilicity, Hg^{2+} ions act as Lewis acid for the elimination of the anomeric halide and consequent glycoside activation as the oxocarbenium ion, with the stereo outcome depending on the balance of stereoelectronic factors, among others (Scheme 20).[176] An association of mercuric bromide and mercuric oxide in DCM was the selected protocol, for it having numerous reports of glycosylation reactions with anthracyclines.[76, 85, 151, 156, 178, 179]



Scheme 20. Proposed activation mechanism of halides. From the formation of the oxocarbenium ion, factors like anomeric effect, neighbouring group participation, solvent, temperature, protecting groups, among others, will determine the α:β selectivity.

As a matter of chronology in obtaining the protected aglycones during the development of this work, the orthoester-protected doxorubicinone **16** was the glycosyl acceptor in such mercury-promoted glycosylation reactions. Because glycosyl chlorides were employed as donors, in contrast with the more reactive bromides used in the literature references, simply stirring at room temperature was not sufficient to promote the reaction, and moderate heating at 40 °C had to be applied to effect glycosylation with glucosyl donor **20**. The glycoside **45** was isolated in 52% (Scheme 21) and unreacted aglycone was recovered, suggesting the yield could still be improved, for example, by increasing the temperature.

The product was characterised by ^{1}H NMR, in which signals for every part of the molecule could be identified. H-7 was a doublet at δ 5.68 ($J_{7,8}$ 6.0 Hz), CH_3-16 and CH_3-18 of the orthoester methyl groups were a singlet at δ 1.77 and a triplet at δ 1.21 ($J_{17,18}$ 7.0 Hz). H-1′ was as a doublet at δ 5.92 ($J_{1',2'}$ 3.6 Hz) coupling with the double doublet of H-2′ at δ 3.40 ($J_{1',2'}$ 3.7 Hz, $J_{2',3'}$ 10.7 Hz), indicating that the glycoside was formed stereoselectively with the α configuration. This observation reflects the expected result for a glycosylation guided by the anomeric effect, given that the azide group is unable to offer anchimeric assistance.



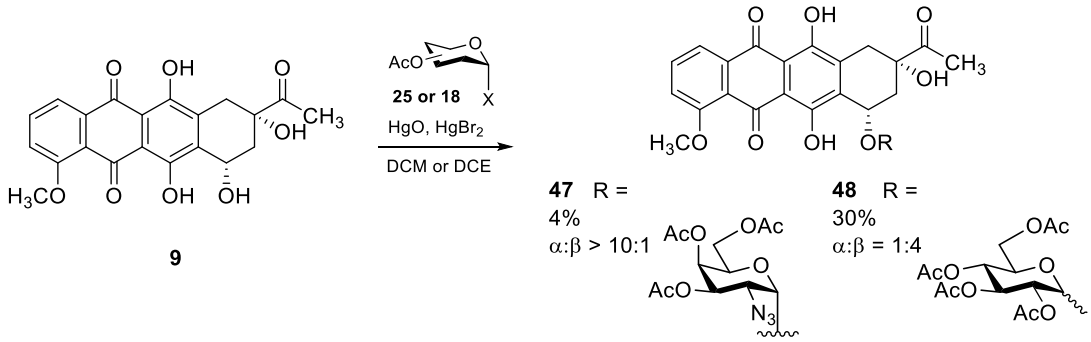
Scheme 21. Glycosylation of the orthoester-protected doxorubicinone **16** with 2-azido glucosyl and galactosyl chloride, under Koenigs-Knorr conditions, promoted by mercuric salts.

As for the galactosyl donor **25**, 1,2-dichloroethane was the choice of solvent, to enable a higher reflux temperature and possibly improve yields. However, even stirring at 90 °C for 2 days did not cause significant conversion of the aglycone starting material **16**. After extending the reaction at room temperature until 6 days, the conversion was still far from complete, but product **46** could be isolated in only 16% yield (Scheme 21.). The small quantity obtained of the galactoside made impracticable the full assignment of the ^{1}H NMR spectrum because of trace impurities (possibly from a minor β galactoside) and overlapping signals, hard to interpret without the aid of bidimensional techniques. Even so, some diagnostic signals of **46** were separated and well resolved, such as the singlet at δ 1.77 from CH_3-16 of the orthoacetate, the doublet at δ 5.68 ($J_{7,8}$ 6.0 Hz) from H-7, and especially the doublet at δ 5.93 ($J_{1',2'}$ 3.6 Hz) from H-1′, which coupling constant confirmed the α anomeric configuration of the galactoside **46**, similarly to glucoside **45**. It is worth mentioning that both glycosides had their formulae confirmed by HRMS.

Before investing beyond in the orthoacetate doxorubicinone, the hydrolysis of the protecting group was tested with **16** as a model for the deprotection of glycosides **45** and **46**. A solution of the protected aglycone **16** in DCM/MeOH was treated with aqueous 1 M HCl

solution,[87] stirred at 0°C, room temperature, then heated to 50 °C until the starting material was consumed, but none of the isolated products corresponded to the original aglycone **8**. Because more forcing conditions in acidic medium, required to hydrolyse the orthoacetate, are likely to cleave the glycosides as well, the orthoester approach was discredited.

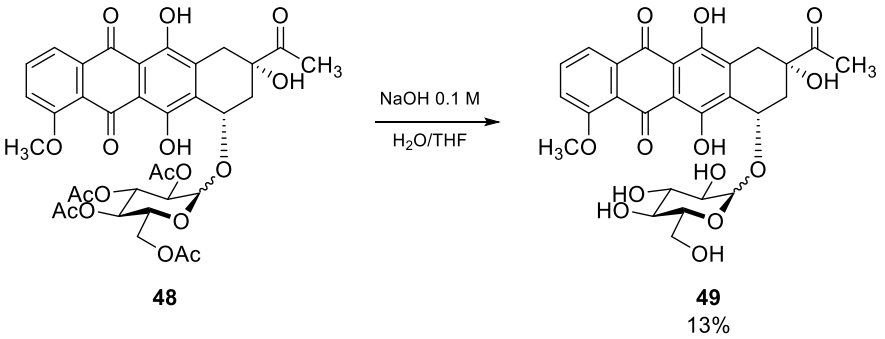
To further investigate Koenigs-Knorr glycosylations, the glycosyl acceptor was replaced for daunorubicinone **9**. With the 2-azido galactosyl donor **25**, even heating to 90 °C and prolonging reaction time to 6 days, a very limited conversion was detected, and the product **47** was isolated in poor 4% yield (Scheme 22), even lower than the result with the doxorubicinone core. ^{1}H NMR showed the α galactoside was the major product, with the H-1′ α at δ 5.31 ($J_{1',2'}$ 3.9 Hz), but probably the β anomer was present in roughly 10% mixture, as suggested by the H-1′ β at δ 4.96 ($J_{1',2'}$ 8.0 Hz).



Scheme 22. Glycosylation of daunorubicinone **9** with glycosyl bromide and chloride, under Koenigs-Knorr conditions, promoted by mercuric salts.

To ultimately prove the effectiveness of the mercuric salts promoters towards anthracyclines glycosylation, the glucosyl bromide **18** was tested using daunorubicinone **9** as acceptor. Although the reaction did not reach completion, it ran much faster than with the chloride donors and could be stopped after 7 h at room temperature. However, after purification, the product was obtained in a modest 30% yield (Scheme 22). The spectrum of product **48** suggested a mixture of anomers, in a 1:4 α:β ratio, because of the duplicate signals in ^{1}H NMR. Since H-1′ signal was overlapped with H-4′ around δ 5.07, the β configuration of the major product was indicated by the coupling constant of the H-2′ double doublet at δ 4.93 ($J_{1',2'}$ 8.0 Hz, $J_{2',3'}$ 9.5 Hz). The stereoselectivity was reversed compared to the previous glycosylation reactions, which is explainable by the presence of an *O*-acetyl substituent at position 2, rendering this glucosyl donor with a neighbouring group participation ability.

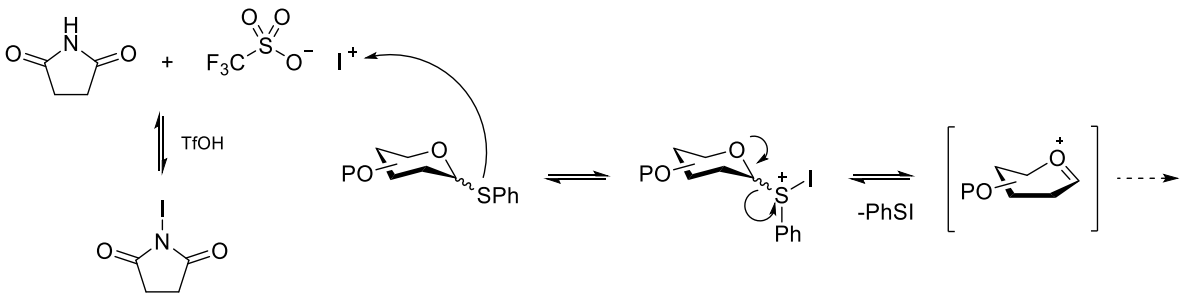
Glycoside **48** was deacetylated with sodium hydroxide in THF/water at 0 °C, instead of using sodium methoxide in methanol to avoid affecting the strong base-sensitive aglycone,[75] Deprotected glucoside **49** was isolated in 13% yield, after purification by HPLC (Scheme 23). In ^{1}H NMR, H-1′ signal split into a doublet at δ 4.54 ($J_{1',2'}$ 7.8 Hz), confirming the β configuration.



Scheme 23. Deacetylation of glycoside **48** with sodium hydroxide.

4.2.3.2 Glycosylation with thioglycosides

Based on the widespread application of thioglycosides for the glycosylation of anthracyclines, attention was turned to this class of glycosyl donor. Thioglycosides are stable to ordinary protecting group manipulation in carbohydrate chemistry and can be activated with a series of thiophilic species. Most frequently, iodonium-generating reagents are applied, such as *N*-iodosuccinimide (NIS) in catalysis by triflic acid or triflates (silver, TMS, etc.), or iodonium di-γ-collidine perchlorate (IDCP). I^+ is released *in situ* and complexes with the sulphur atom, which gains a positive charge that induces the departure of the thio group, with the concomitant formation of the oxocarbenium ion (Scheme 24).[176]

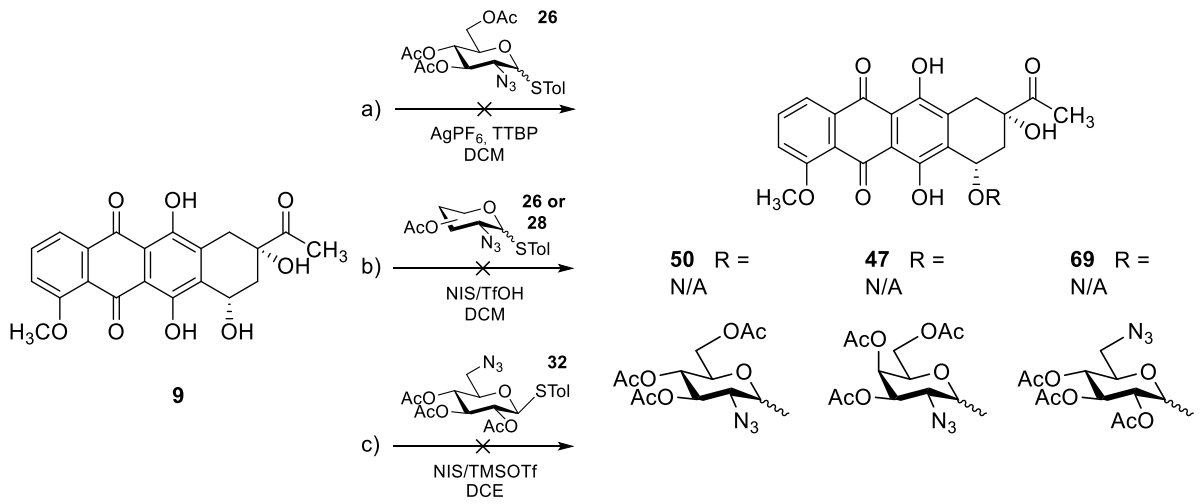


Scheme 24. Activation of thioglycosides with NIS/TfOH.

Since the original daunosamine in anthracyclines is a 2-deoxysugar, without the stereodirecting ability of 2-substituents, a protocol was developed by Sun and Wang[75] to address such stereoselectivity challenge. Pursuing a number of analogues of anthracycline 2-deoxy α glycosides, they have reported silver hexafluorophosphate and 2,4,6-tri-*tert*-butylpyrimidine ($AgPF_6$/TTBP) to efficiently activate 2-deoxythioglycosides, giving anthracycline conjugates with complete α stereoselectivity. The method was successfully implemented in quite a few publications, including azido-containing donors.[72, 75, 78, 180]

Unfortunately, attempting to reproduce the $AgPF_6$/TTBP methodology with the 2-azido thioglucoside **26** did not succeed, since barely any conversion of the aglycone **9** occurred (Scheme 25a). In fact, the original report describing $AgPF_6$ as a remarkable α selective activator of 2-deoxythioglycosides showed that common 2-oxy thioglycosides were unreactive under such glycosylation conditions,[181] so the 2-azido thioglycoside might have suffered from the same lack of reactivity, considering the scope of the method.

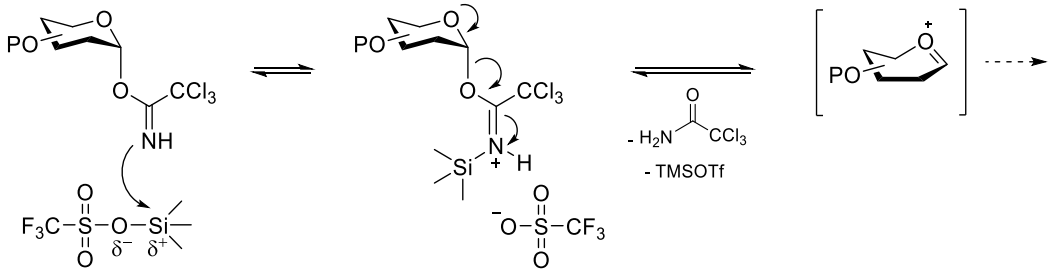
Classical activation with NIS was then investigated, and TfOH was chosen as the acid catalyst, based on a report dealing with the glycosylation of 14-*O*-acetyl doxorubicinone, in which various promoter systems were scrutinised and $AgPF_6$/TTBP failed.[44] Notwithstanding, none of the 2-azido thioglucoside **26** and 2-azido thiogalactoside **28** provided the expected products **50** and **47**, even with the addition of extra TfOH and prolonged reaction times (Scheme 25b). A slightly modified procedure was tried for the reaction with 6-azido thioglucoside donor **32**, by replacing the catalyst for the Lewis acid TMSOTf and the solvent for DCE (Scheme 25c). Changes were unfruitful, as the aglycone **9** did not convert into desired product **69**, even with excess reagents added and heating at 50 °C.



Scheme 25. Glycosylation of daunorubicinone **9** with 2-azido glucosyl, 2-azido galactosyl, and 6-azido glucosyl thioglycosides, promoted by $AgPF_6$ or NIS with TfOH/TMSOTf.

4.2.3.3 Glycosylation with glycosyl imidates

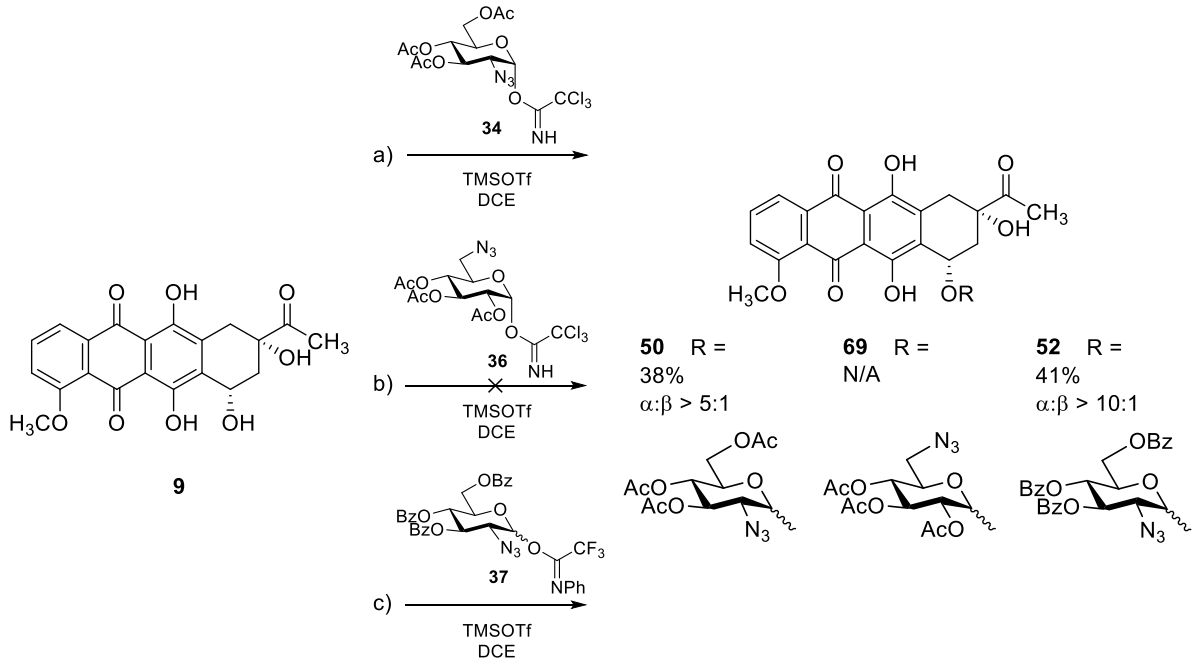
Moving forward in the search for a more efficient glycosylation method, imidates were the obvious choice, although very little is described about anthracycline glycosylation with imidates in the literature so far.[167] Typical glycosylation reactions with trichloroacetimidates are catalysed by Lewis acids, which are supposed to coordinate to the nitrogen of the imidoyl leaving group, assisting the departure and resulting in the formation of trichloroacetamide and the oxocarbenium ion (Scheme 26).[176]



Scheme 26. Proposed mechanism of activation of trichloroacetimidates with TMSOTf.

Schmidt's trichloroacetimidates were employed to glycosylate daunorubicinone **9** under standard conditions, which involved TMSOTf catalysis at -40 to -30 °C in DCM. In the first attempts with 2-azido donor **34**, the reaction was very slow and low yielding (5%), even when DMF was used as a non-conventional α-directing co-solvent to improve solubility of the aglycone.[182]

These downsides prompted the application of more forcing conditions, i.e., doubling the excess of glycosyl donor, and more important, increasing gradually the reaction temperature from 0 °C, to room temperature, to 45 °C, to 90 °C, when finally some conversion could be observed. The glycoside **50** was isolated in 38% yield (Scheme 27a), with the α configuration being indicated by the $J_{1',2'}$ 3.9 Hz coupling constant measured in the H-2′ double doublet at δ 3.91, since the H-1′ signal overlapped with H-3′ in a multiplet around δ 5.3. Other minor products were formed that could be the β anomer, but it was not possible to characterise them as so. Trying to reproduce the above glycosylation procedures with 6-azido trichloroacetimidate donor **36** was fruitless, since adding more donor and TMSOTf, extending reaction time and heating at 90°C did not cause observable conversion of the aglycone **9** into product **69** (Scheme 27b).

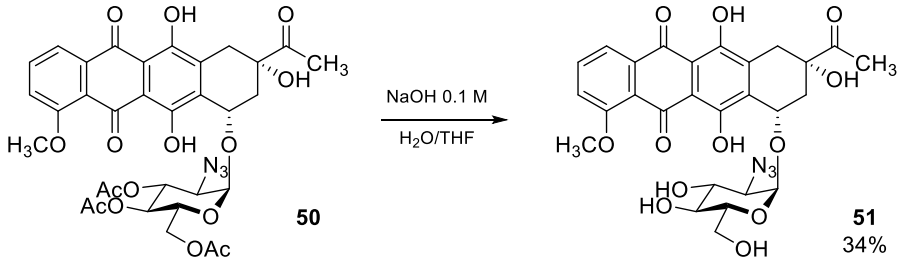


Scheme 27. Glycosylation of daunorubicinone **9** with 2-azido and 6-azido glucosyl trichloroacetimidates, and 2-azido *N*-phenyltrifluoroacetimidate, promoted by TMSOTf.

Deacetylation of glycoside **50** by treatment with NaOH 0.1 M in THF/water provided deprotected glucoside **51** in 34% yield, after purification by HPLC (Scheme 28). In ^{1}H NMR, H-7 signal at δ 5.23 partially overlapped with H-1′ doublet at δ 5.36 ($J_{1',2'}$ 3.8 Hz), so the α configuration was confirmed by H-2′ double doublet at δ 3.36 ($J_{1',2'}$ 3.8 Hz, $J_{2',3'}$ 10.4 Hz).

Novel *N*-phenyltrifluoroacetimidates were introduced by Yu and co-workers (2001),[118, 119] as alternatives to the trichloroacetimidates. Some of these 2-azido glucosyl imidate donors were available from different projects in the research group, and the benzoylated 2-azido donor **37** was chosen to be tested in anthracycline glycosylation. In the same way as with the trichloroacetimidate counterpart, conversion only occurred upon heating at 50 °C, then 80 °C, and excess of donor **37** was added during the reaction. Conversion did not reach completion, and the glycoside **52** was isolated in 41% yield, predominantly as the α anomer (α:β 12:1) (Scheme 27c). H-1′ α was observed as a doublet at δ 5.43 ($J_{1',2'}$ 3.9 Hz) and H-1′ β as another doublet at δ 5.26 ($J_{1',2'}$ 8.0 Hz).

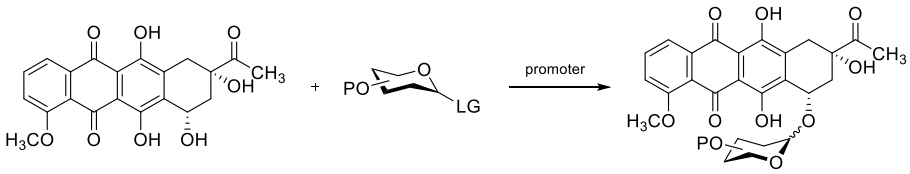
Deprotection of per-*O*-benzoylated 2-azido glucose as a model for glycoside **52** was attempted with sodium hydroxide 0.1 M (Scheme 28) using the same conditions applied to glycosides **48** and **50**, but proved unsuccessful, given the higher stability of the benzoyl esters compared to the acetyl counterparts.

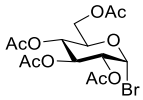
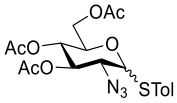
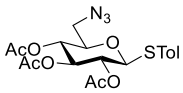
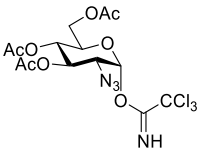
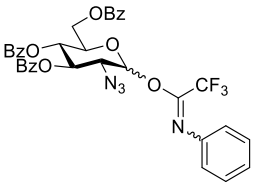


Scheme 28. Deacetylation of glycoside **50** with sodium hydroxide.

To compare the yields and stereoselectivity of the reactions with 2-substituted glycosyl donors and different promoters, Table 1 summarises the best results obtained in glycosylation reactions using daunorubicinone as glycosyl acceptor. The highest yields, although still moderate, were obtained with imidate donors activated by TMSOTf, with the benzoylated trifluoroacetimidate **37** surpassing slightly the acetylated trichloroacetimidate **34**. The greatest stereoselectivity was also achieved for the benzoyl-protected glycoside **52**.

Table 1. Summary of glycosylation reactions with 2-substituted glycosyl donors.



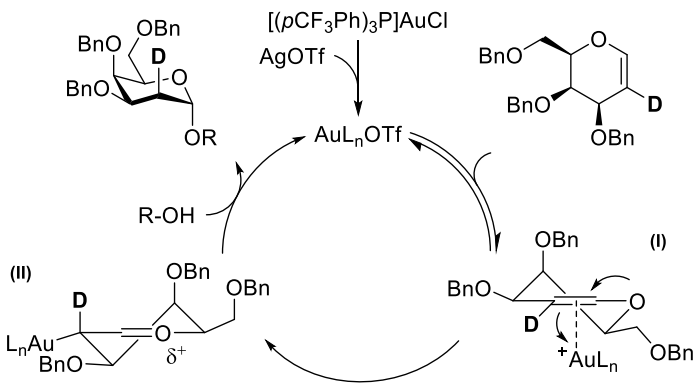
Donor	HgO $HgBr_2$	NIS TfOH or TMSOTf	TMSOTf
	**48** 30% (1:4)	-	-
	-	**50** no product	-
		**69** no product	
	-	-	**50** 38% (>5:1)
	-	-	**52** 41% (>10:1)

4.2.3.4 Glycosylation with glycals

Because 2-deoxyglycosides lack a substituent at C-2 that can direct the stereo outcome of glycosylation reactions, the stereoselective formation of glycosidic linkages is very challenging for these so biologically relevant sugars.[19] Directing groups at C-2 can be transiently installed then removed after glycosylation, but this strategy is inherently inefficient. On the other hand, atom-efficient direct nucleophilic substitution on glycals catalysed by acid is a widely used strategy. However, undesired mixtures of anomers tend to be formed, along with side products,[122, 124] such as Ferrier rearrangement aducts, which result from an allylic shift upon the attack of a nucleophile, producing 2,3-unsaturated glycosides.[183, 184]

To address this issue, Prof Dr M. Carmen Galan research group has been devoted to developing stereoselective synthetic approaches conducting to α-2-deoxyglycosides,[122, 124, 130, 131] and these new methods were considered promising to expand the structural diversity of the anthracycline derivatives within this work. Daunorubicinone **9** was selected as a model anthracycline acceptor to study glycosylation conditions because, lacking the hydroxyl group at position 14, it did not require protection, given that its most nucleophilic and least hindered group was the secondary hydroxyl at position 7, where the glycosylation was desired. To screen for the best glycosylation method, benzyl-galactal **38** was chosen as the standard glycosyl donor, to avoid the deactivating acetyl protecting group.

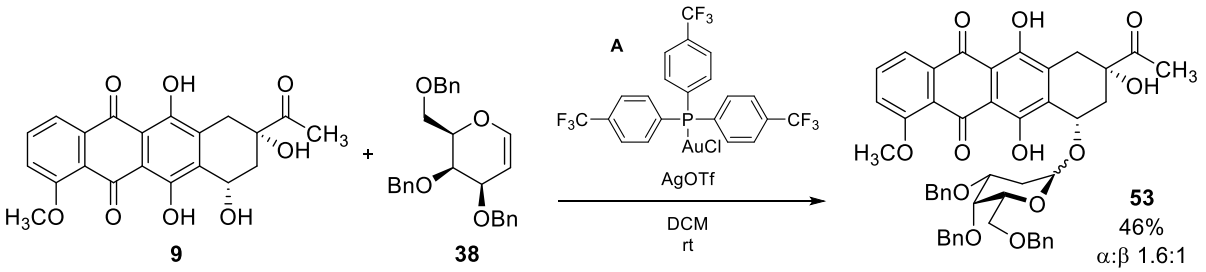
Initially, a protocol using chloro[tris(*para*-trifluoromethylphenyl)phosphine] gold(I) (**A**) activated by silver triflate additive was tested. It reproduced well the literature yields and stereoselectivity (89%, α:β > 30:1) when a model acceptor methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside was utilised.



Scheme 29. Proposed mechanism for the gold-catalysed glycosylation. Coordination of the Au^+ cation to the double bond through a π-complex (I) can lead to the formation of transient oxocarbenium ion (II), that is quickly trapped by the OH nucleophile with concomitant protonolysis of the gold-carbon bond. Adapted from [130]

The system exploited the ability of Au(I) to effect the addition of oxygen nucleophiles across carbon-carbon double bonds, in an α-stereoselective glycosylation. The mechanism was reported to be the hydroalkoxylation of the enol ether, via a *syn*-diastereoselective addition of the OH nucleophile to the double bond. Hence, the new bonds were formed *cis* to each other, as proven by reaction with deuterated donor (Scheme 29).[130]

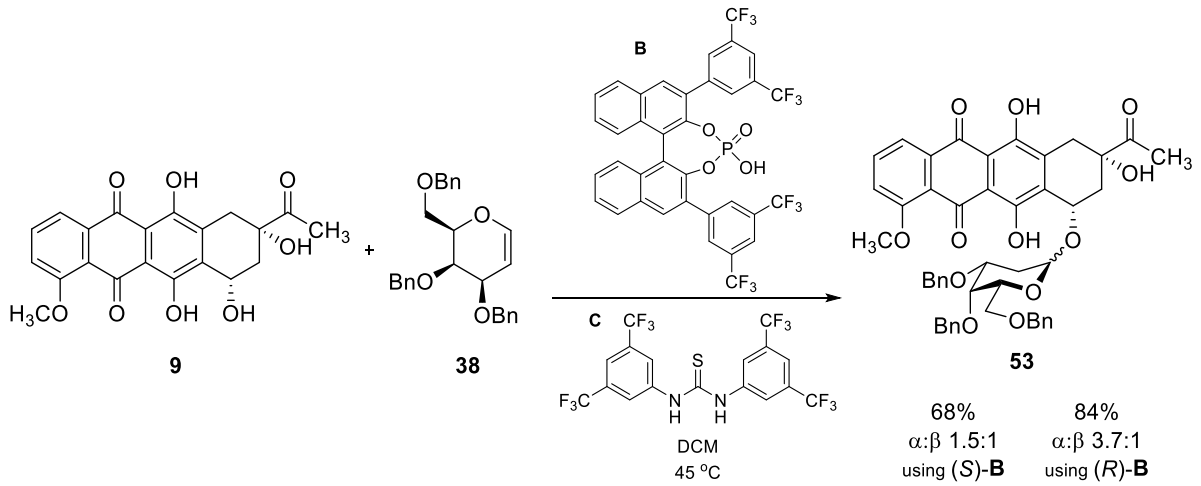
Unfortunately, with the anthracycline core **9**, utilising 5 mol% catalyst **A** and 10 mol% AgOTf, this reaction could not be driven to completion, a mixture of anomers **53** (α:β 1.6:1) was formed in 46% yield (Scheme 30), and unreacted aglycone was recovered. Heating, increasing catalyst load and donor excess, and extending reaction time did not result in better conversion or stereocontrol, suggesting that chelation with the quinone system could be counteracting the catalytic activity of the transition metal.



Scheme 30. Gold(I)-catalysed glycosylation of daunorubicinone **9** with per-*O*-benzyl galactal **38**.

Organocatalysis, an emerging field in carbohydrate chemistry using small organic molecules to effect stereoselective glycosylations under mild conditions, was considered as an alternative.[185] A cooperative organocatalytic protocol with Brønsted acids (*R*)- or (*S*)-3,3′-bis[3,5-bis(trifluoromethyl)phenyl]-1,1′-binaphthyl-2,2′-diyl hydrogen phosphate (**B**) and 1,3-bis[3,5-bis(trifluoromethyl) phenyl]thiourea (**C**, "Schreiner's thiourea") was tried. This catalytic system, with 10 mol% phosphoric acid and 20 mol% thiourea, was well tolerated by the anthracycline core in the glycosylation with **38**, proceeding cleanly to conversions over 70% after 20 h heating at 45 °C (Scheme 31), as appropriate for secondary alcohol glycosyl acceptors.[131] Yields for isolated glycosides **53** were 84% and 68%, according to the stereochemistry of the chiral phosphoric acid, (*R*)-**B** and (*S*)-**B**, respectively. In the same way, better stereocontrol was achieved with (*R*)-**B** catalyst (α:β 3.7:1) over (*S*)-**B** (α:β 1.5:1). Even though these anomeric ratios were still quite far from ideal, the cooperative catalysis with (*R*)-**B** phosphoric acid and Schreiner's thiourea **C** was considered promising for further study.

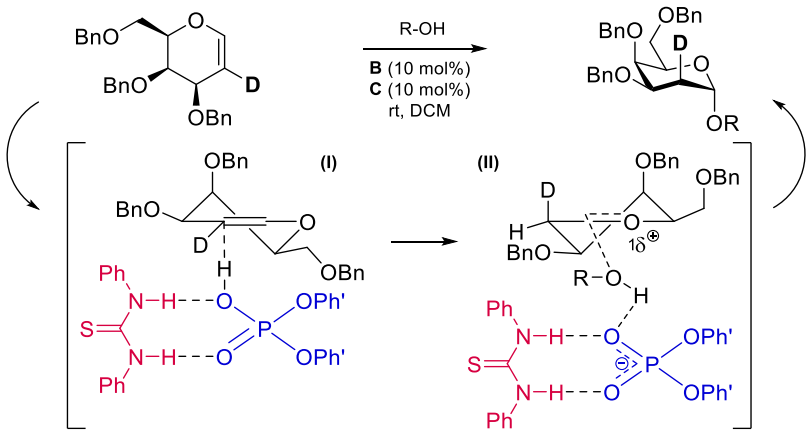
Only partial separation of the anomers was possible by conventional chromatography, but it enabled the characterisation of the glycosides. In the **53** α anomer ^{1}H NMR, H-1′ signal was a doublet at δ 5.41 ($J_{1',2'a}$ 3.6 Hz) coupling with the partly covered H-2′a triple doublet at δ 2.34 ($J_{2'a,2'b}$ 12.7 Hz, $J_{1',2'a,}$ 3.8 Hz). H-2′b was underneath a multiplet at δ 1.86, overlapped with H-8b, which split apart from H-8a (δ 2.43, $J_{8a,8b}$ 15.2 Hz), compared to their chemical shifts of the free aglycone (δ 2.34 and δ 2.14, $J_{8a,8b}$ 14.6 Hz). H-7 was deshielded to δ 5.51, as opposed to δ 5.30 (in daunorubicinone). For the **53** β anomer, H-1′ was a double doublet at δ 4.98 ($J_{1',2'b}$ 9.8 Hz, $J_{1',2'a}$ 2.1 Hz). Multiplet of H-2′a at δ 2.09 and triple doublet of H-2′b at δ 1.98 ($J_{2'b,2'a}$ 12.1 Hz, $J_{2'b,1'\sim3'}$ 9.9 Hz) were much closer together then their α counterparts, flanking the H-8b double doublet at δ 2.04 ($J_{8b,8a}$ 14.9 Hz, $J_{8b,7}$ 4.2 Hz). H-8a was a broad double triplet ($J_{8a,8b}$ 14.8 Hz, $J_{8a,7\sim10a}$ 1.9 Hz) even more deshielded at δ 2.63 than it was in the α anomer, whereas H-7 remained at δ 5.28, changing only in multiplicity to a double doublet ($J_{7,8b}$ 4.0 Hz, $J_{7,8a}$ 1.8 Hz). Regarding ^{13}C NMR, C-1′α peak was found at δ 94.8, whilst C-1′β turned up at δ 102.9. These ^{1}H and ^{13}C NMR signal splitting and chemical shifts patterns were consistently observed in all the forthcoming glycosides spectra, assisting their assignment.



Scheme 31. Orgalocatalysed glycosylations with 1,1′-bi-2-naphthol (BINOL)-derived phosphoric acids (*R* or *S*)-**B** and Schreiner's thiourea **C**. Benzyl galactal **38** was the glycosyl donor employed.

Mechanistic investigations performed by Galan and co-workers (2017), by reacting deuterated galactal, suggested that C−H and C−O bonds were formed in *syn*-diastereoselective steps, to give 1,2-*cis* addition products. Their extensive ^{1}H NMR spectroscopy studies demonstrated an interaction between the thiourea and the phosphoric acid by themselves, and with each of the glycosyl donor and acceptor. Separately, downfield H-shifts of both the glycal

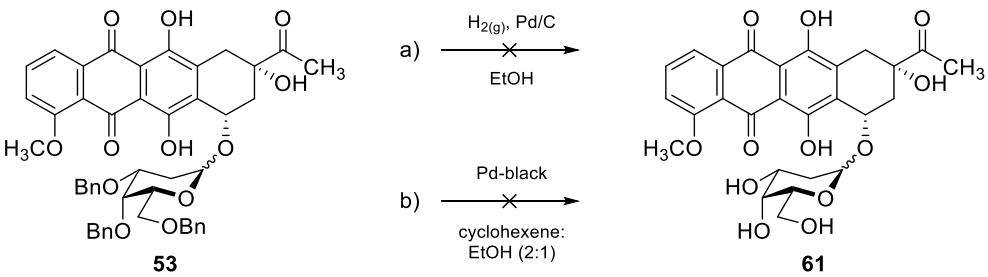
anomeric proton and the hydroxyl from the nucleophile acceptor were observed in the presence of the cocatalysts, rendering a synergistic behaviour that activates both the enol ether and OH nucleophile, enhancing reaction rate and stereocontrol.[131]



Scheme 32. Mechanism of cooperative organocatalysis proposed by Palo-Nieto et al. (2017). It proceeds through a hydrogen bond-mediated complex between Brønsted acid B and Schreiner's thiourea C, whereby the enhanced catalytic activity of the thiourea acts as an acid amplifier. Adapted from [131]

In view of these findings, the mechanism depicted in Scheme 32 was proposed: acid amplification induced by urea, via a hydrogen-bonded complex, results in a proton transfer to the less hindered face of the enol ether (I) to form a short-lived oxocarbenium ion (II); the phosphate intermediate that is generated *in situ* activates the OH nucleophile, which rapidly traps the oxocarbenium intermediate, to give the glycoside product.[131]

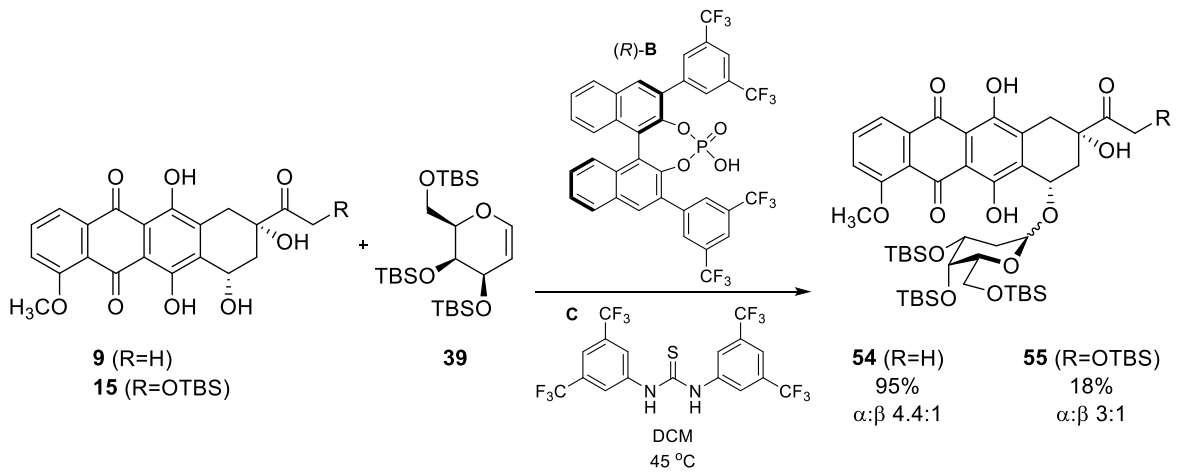
Attempted deprotection of benzyl groups from **53** by conventional hydrogenolysis[186] was unsuccessful (Scheme 33a). Although starting material was consumed, the formation of the deprotected glycosides **61** was not detected. Similarly, a milder method based on heterogeneous catalytic transfer hydrogenation from cyclohexene[187] also failed to cleave the benzyl groups without affecting the glycosides (Scheme 33b). Hydrogenation with palladium might have reductively eliminated the carbohydrate from position 7, which is also benzylic.[188]



Scheme 33. Attempted debenzylation of glycosides **53**, by palladium-catalysed hydrogenolysis.

Considering the inability to get deprotected glycosides from the benzylated precursors, efforts were turned to finding substitute protecting groups that could simultaneously permit efficient glycosylation and later deprotection. Silyl-protected glycals had been reported to be pretty suitable glycosyl donors for the cooperative organocatalysis glycosylation method, with yields from 79 to 82% and α:β ratio higher than 30:1.[131] In addition, the TBS-protected doxorubicinone **15** was available, and the use of silylated donors would supposedly enable a single step for the final deprotection of glycosides.

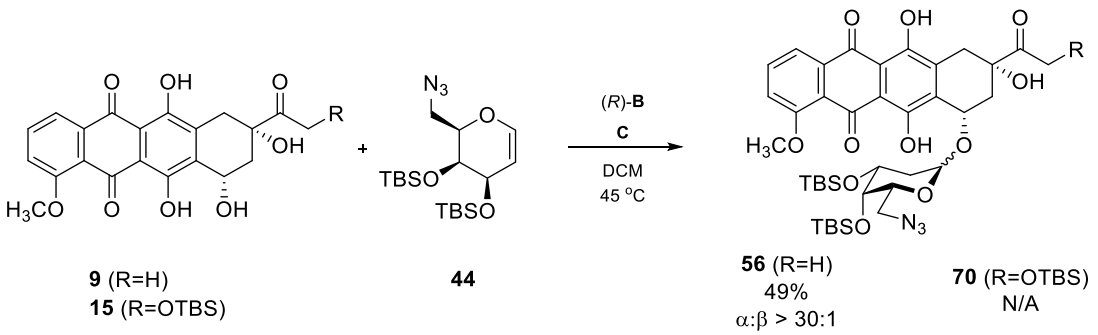
The combination of phosphoric acid (*R*)-**B** and thiourea **C** was then used in glycosylations with the TBS-protected galactal **39**. The best result obtained was in reaction with daunorubicinone **9**, yielding the glycosides in 95%, and α-selectivity of 4.4 to 1. In contrast, under the same conditions, the doxorubicinone acceptor **15** afforded the glycoside products in only 18% yield (α:β 3:1) (Scheme 34), most likely because of the bulkiness of TBS group in the aglycone side chain, in the vicinity of the OH-7 nucleophile, which might have prevented the rate-enhancing cooperative interaction of the catalysts with the acceptor. Moreover, when 1 mol% thiourea **C** alone[122] was employed towards the glycosylation of **9**, less than 1% product was formed, proving that the synergistic action of the two cocatalysts is essential for effective catalysis. In the absence of phosphoric acid, an increased percentage of thiourea, to equal the 10 mol% of the cooperative protocol, would probably be unproductive as well, according to the findings of Galan and co-workers (2017), wherein high catalyst loading resulted in starting material only, which was credited to catalyst inactivation via self-association.[131] Furthermore, the same study showed that phosphoric acid alone was also able to activate the model donor, but at a much slower reaction rate.



Scheme 34. Orgalocatalysed glycosylations with BINOL-derived phosphoric acid (*R*)-**B** and Schreiner's thiourea **C**, using scaffolds **9** and **15** as acceptors, and TBS galactal **39** as donor.

The ^{1}H NMR followed the same pattern of the benzyl-protected glycosides. For the α anomer of **54**, H-1′ was found as a doublet at δ 5.37 ($J_{1',2'a}$ 3.6 Hz). Assigning the signals of the **54** β anomer was harder since it could not be totally separated from the α glycoside, but H-1′ was recognised as a double doublet at δ 5.00 ($J_{1',2'b}$ 9.7 Hz, $J_{1',2'a}$ 2.5 Hz). Concerning **55**, just the signals of the α anomer could be readily distinguishable, and H-1′ α was distinguished as a doublet at δ 5.35 ($J_{1',2'a}$ 3.8 Hz).

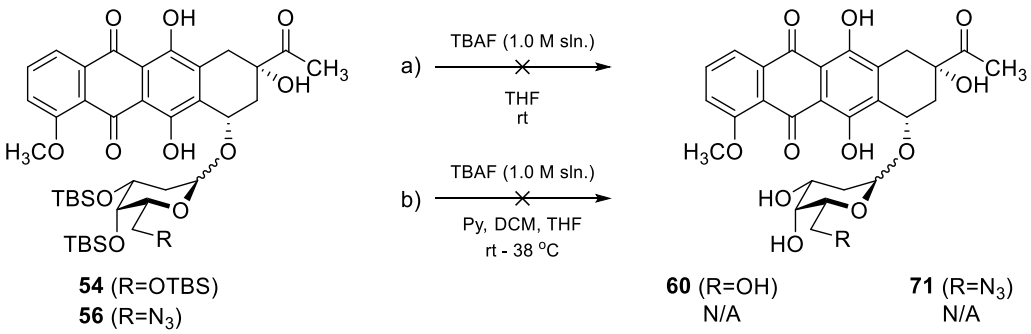
Turning to azido-containing galactal donor **44**, it had never been tested for glycosylation under the cooperative organocatalysis conditions above. So, the standard protocol was applied with daunorubicinone **9** as acceptor and, after refluxing with the cocatalysts mixture for 20 h, the corresponding glycoside **56** was isolated in 49% yield, with a satisfying α:β ratio higher than 30:1 (Scheme 35). On the other hand, with doxorubicinone **15**, barely any consumption of the starting material could be noticed, neither was any strongly arising product, indicating a low-yielding reaction, presumedly. Little could be concluded because the crude mixture was not further worked out. The ^{1}H NMR spectrum of **56** showed H-1′ as a doublet at δ 5.38 ($J_{1',2'}$ 3.3 Hz), coupling with H-2′a triple doublet at δ 2.23 ($J_{1',2'a}$ 3.8 Hz), confirming the α configuration. In comparison to the per-*O*-silylated glycoside **54**, it was noticeable the shift upfield to δ 3.21 of H-6′b (from δ 3.59) and the shift downfield to δ 4.32 of H-5′ (from 4.03).



Scheme 35. Organocatalysed glycosylations with phosphoric acid (*R*)-**B** and thiourea **C**, using scaffolds **9** and **15** as acceptors, and 6-azido-3,4-di-*O*-TBS galactal **44** as donor.

Alike the benzyl glycosides, the attempts to deprotect TBS glycosides were ineffective under conventional conditions to cleave silyl groups. **54** and **56** were treated with tetrabutylammonium fluoride (TBAF) in two different solvent systems: THF or a mixture of pyridine, DCM and THF (Scheme 36-a,b).[85, 156, 189]. Disappointingly, a complex mixture was produced, and efforts to separate the individual components did not succeed, or the isolated products did not correspond to the deprotected products.

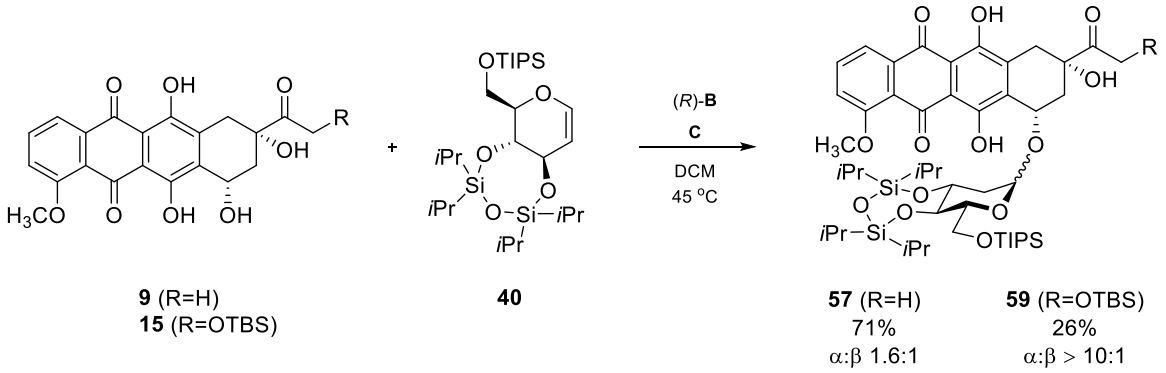
Considering the molecular complexity and the somewhat cumbersome protecting groups, optimised deprotection will require testing a number of alternative reagents, as was true for many examples of silyl groups cleavage in natural products total synthesis.[190] Besides, finding an effective method to remove TBS from the anthracycline glycosides shall be convenient, since it is the same group used to protect the side chain in the doxorubicinone scaffold; thus a full deprotection could be performed in a single step.



Scheme 36. Attempted desilylation of glycosides **54** and **56** with tetrabutylammonium fluoride cleavage.

In regard to catalytic glycosylations with glucal donor, Galan and co-workers (2014)[124] had demonstrated that practicable reaction rates and stereocontrol could only be accomplished by the effect of protecting groups inducing conformational constraints in the oxocarbenium intermediate. The *trans*-vicinal 3,4-*O*-disiloxane glucal was also the only protecting group to prevent Ferrier rearrangement.[124] Accordingly, glucal **40** was chosen as the source for glycosides with the *gluco* configuration.

Despite the predicted conformational lock that should favour the nucleophilic attack on the α-face of the donor, the cooperative organocatalysed reaction of daunorubicinone **9** with donor **40** afforded an inseparable anomeric mixture of glucosides with poor stereoselectivity (α:β 1.6:1). At least, the total yield was satisfactorily 71% (Scheme 37). As a consequence of the difficult separation of the glycosides from one another, no single fraction was got that had a pure anomer, so the NMR spectra contained a certain proportion of the opposite stereoisomer. For **57** α-glycoside, H-1′ was a doublet at δ 5.34 ($J_{1',2'b}$ 3.8 Hz) and H-7 was an apparent triplet at δ 5.52, whereas **57** β glycoside had H-1′ double doublet at δ 5.06 (J 9.9 Hz, J 1.9 Hz), and H-7 was a broad singlet at δ 5.32.



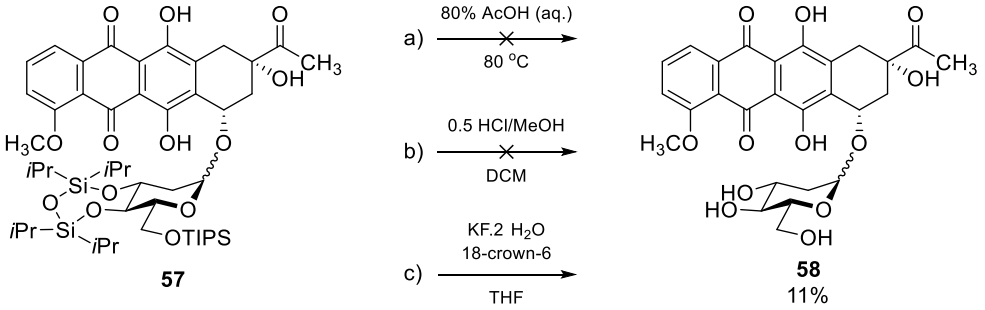
Scheme 37. Orgalocatalysed glycosylations with phosphoric acid (*R*)-**B** and thiourea **C**, using scaffolds **9** and **15** as acceptors, and disiloxane glucal **40** as donor.

The reaction of doxorubicinone **15** conducted under the same conditions ran slower and achieved a lower conversion, but the product glycoside **59** was still isolated in 26% yield, with α:β ratio higher than 10:1 (Scheme 37). Probably the stereoselectivity before purification was inferior, but the β glycoside could not be found and characterised. The ^{1}H NMR spectrum of **59** showed more cleanly the α anomer, with a doublet at δ 5.32 ($J_{1',2'}$ 3.8 Hz) corresponding to H-1′, and a broad triplet at δ 5.50 for H-7.

The cleavage of the silyl groups in glycosides **57** was also troublesome. Hydrolysis though aqueous acetic acid protocol[189, 191] (Scheme 38-a) furnished a product lacking signals of the sugar moiety in the region of δ 4, while the treatment with methanolic HCl[192] (Scheme 38-b) led to the recovery of the intact aglycone, meaning the 2-deoxyglycoside was highly susceptible to acid conditions, under which it was rapidly cleaved from the aglycone.

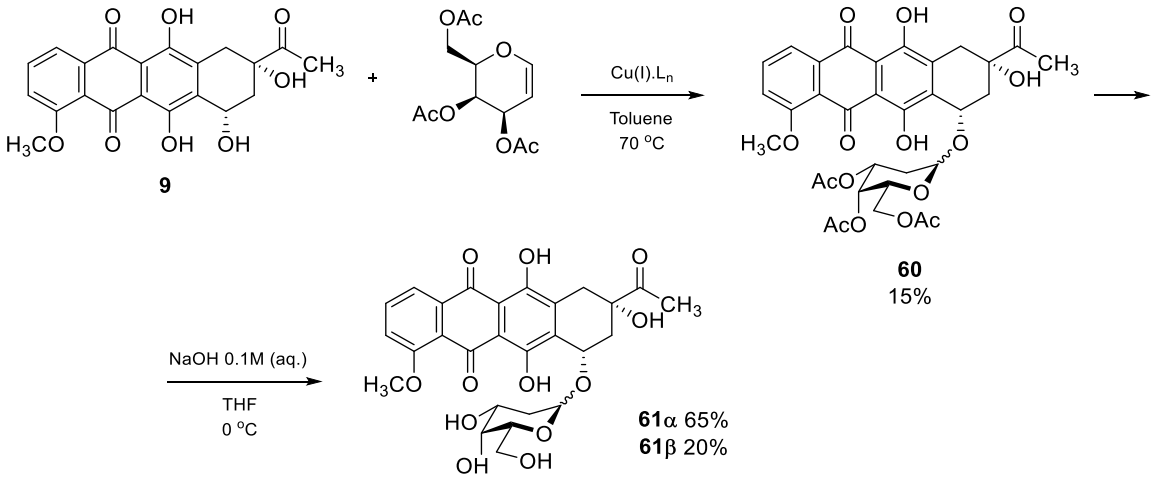
Solubilisation of potassium fluoride in aprotic organic solvents with the phase transfer catalyst 18-crown-6 and its reaction with organic substrates had been reported.[193] The combination of this K^+-chelator ether and KF offering a "naked" fluoride anion was used as a desilylating reagent,[132] and effected the deprotection of **57** into **58** in 11% yield. Even with a low yield, and the products still needing to be resolved by high-performance chromatography, this method accomplished another final deprotected glycoside, a daunorubicin analogue of one target compound of this work, still to be tested on cancer cell lines.

The product was a 1:5 α:β mixture, likely not by anomerisation, but just a matter of the fractions selected during chromatographic purification. For the β glycoside, the ^{1}H NMR revealed H-1′ double doublet at δ 5.42 ($J_{1',2'b}$ 9.6 Hz, $J_{1'2'a}$ 1.6 Hz) and H-7 double doublet at δ 5.33. The distinguishable signals of the α glycoside were the doublet of H-1′ δ 5.38 ($J_{1',2'}$ 2.6 Hz) and the apparent triplet of H-7 at δ 5.52.



Scheme 38. Desilylation of glycosides **57**. Attempted cleavage of the disiloxane and TIPS groups under acidic conditions (a, b) resulted in hydrolysis of the glycosidic bond. Fluoride-based desilylation was achieved by treatment with KF/18-crown-6.

Finally, a novel glycosylation method was examined, which was a hot topic under development by the Galan group, based on a copper(I) complex (**Cu(I)·L$_n$**) catalyst (unpublished work). The fundamental improvement over the preceding protocols was the ability to activate simply peracetylated glycals, with no need of elaborated protecting groups in the glycosyl donors. With daunorubicinone **9** as acceptor and commercial tri-*O*-acetyl-D-galactal as donor, this methodology afforded 2-deoxygalactosides in 15%, as a mixture of anomers (α:β 1.6:1). Such low yield was not improved by increasing catalyst load and donor excess, extending reaction time, and lowering the temperature. As with other metal catalysts, possibly because of chelation with the anthracycline quinone system, the conversion was incomplete, slight degradation was observed, and purification proved complicate. Because ^{1}H NMR of the purified mixture exhibited signals indicative of the products, such as H-1′α doublet at δ 5.46, H-1′β double doublet at δ 5.10, and acetyl singlets around δ 2.1, and MALDI-MS detected the aduct corresponding to the acetylated glycosides, further effort was invested.

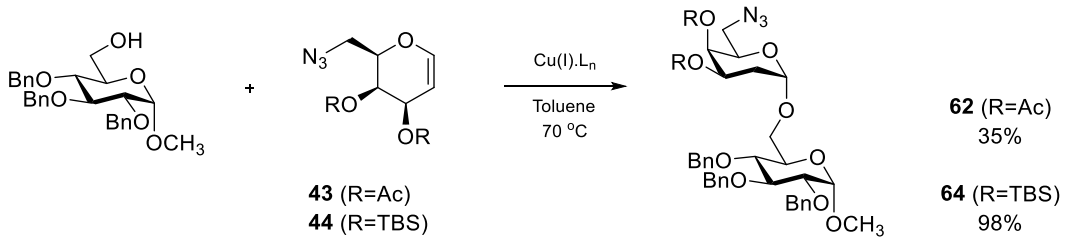


Scheme 39. Catalytic glycosylation with tri-*O*-acetyl-galactal promoted by Cu(I), followed by basic deprotection of the glycosides.

The anomeric mixture above was deacetylated with aqueous sodium hydroxide in THF at 0 °C (Scheme 39), as previously, to avoid affecting the strong base-sensitive aglycone.[75] Pleasantly, the resulting deprotected glycosides could be separated by RP-HPLC[§], to afford each of the α and β anomers in 65% and 20% yields, respectively. Regardless of the poor yield in the glycosylation step, this route provided deprotected glycosides that could be tested against tumour cell lines.

In the ^{1}H NMR of the α glycoside, H-1′ was a doublet at δ 5.45 ($J_{1'2'a}$ 3.7 Hz), H-2′a was a triple doublet at δ 2.10 ($J_{2'a,2'b}$ 13.0 Hz, $J_{2'a,1'}$ 3.9 Hz), and H-2′b was a double doublet at δ 1.82 ($J_{2'b,2'a}$ 13.3 Hz, $J_{2'b,3'}$ 5 Hz). H-7 was a typical apparent triplet at δ 5.55 ($J_{7,8a\sim8b}$ 2.8 Hz), H-8a was a broad doublet at δ 2.46 ($J_{8a,8b}$ 15.2 Hz) coupling with H-8b double doublet at δ 1.93 ($J_{8b,8a}$ 15.2 Hz, $J_{8b,7}$ 3.5 Hz). In relation to the β glycoside spectra, a double doublet at δ 5.04 for H-1′ ($J_{1'2'b}$ 9.8 Hz, $J_{1'2'a}$ 2.2 Hz), and partly obscured signals for H-2′a ($J_{2'a,1'}$ 2.0 Hz) and H-2′b ($J_{2'b,2'a}$ 12.3 Hz, $J_{2'b,1'}$ 9.8 Hz) were observed. H-7 appeared as a double doublet at δ 5.38 ($J_{7,8b}$ 4.2 Hz, $J_{7,8a}$ 2.1 Hz), H-8a as a broad doublet at δ 2.60 ($J_{8a,8b}$ 14.6 Hz) and H-8b as a double doublet at δ 2.14 ($J_{8b,8a}$ 14.8 Hz, $J_{8b,7}$ 4.1 Hz).

Copper(I) catalysis was also tested for glycosylations of the model acceptor methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside with azido containing galactals **43** and **44**, aiming to broaden the glycosyl donor scope of the method, and its synthetic utility.



Scheme 40. Copper(I) catalysed glycosylation of model acceptor methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside with 6-azido glycosyl donors, protected either by *O*-acetyl **43** or *O*-TBS (**44**).

For the acetyl protected 6-azido galactal (**43**), the reaction did not get to full conversion (57% after 8 h, 60% after 24 h), reflecting its much lower rate compared to the peracetylated galactal, which was complete after 3 h under the same conditions, in 65% purification yield (unpublished results from the Galan group). The α glycoside **62** was isolated in 35% yield, but 4% of the Ferrier **63** product was also formed. A suspected interaction of the azido group

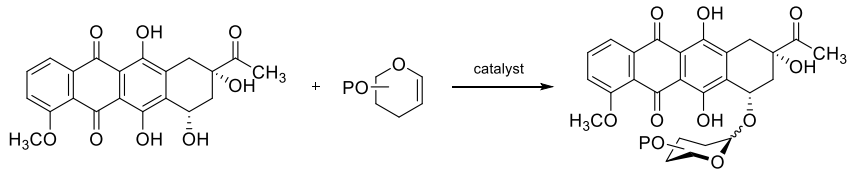
[§] It is noteworthy that RP-HPLC purification of the benzyl (**53**) and TBS (**54**) 2-deoxyglycosides was attempted, but failed to deliver separate anomers. In fact, no product was recovered, revealing that reverse phase was not suitable for purifying such compounds.

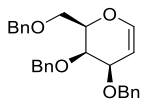
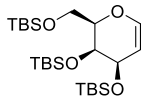
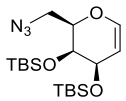
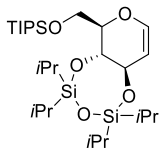
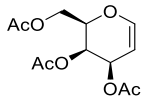
poisoning the copper catalyst might have caused the detrimental effect on the glycosylation efficiency. Nevertheless, the reaction using TBS protected 6-azido galactal (**44**) was complete after 4 h, and the α glycoside **64** was isolated in surprising 98% yield, without detectable Ferrier rearrangement (Scheme 40).

The anomeric configuration of the glycosides was confirmed by ^{1}H NMR. Representative doublets of the H-1′ α were observed at δ 5.06 ($J_{1',2'a}$ 2.9 Hz) for the acetyl derivative **62**, and at δ 4.99 ($J_{1',2'a}$ 2.4 Hz) for the silyl derivative **64**.

To sum up the catalytic glycosylation reactions involving anthracyclines and glycals, yields and anomeric ratios for daunorubicinone 2-deoxyglycosides are compared in Table 2.

Table 2. Summary of glycosylation reactions with daunorubicinone **9** as glycosyl acceptor



Donor	Au(I) (A)	Thiourea (B) Phosphoric acid (C)	Cu(I)
	**53** 46% (1.6:1)	**53** 68%[a] (1.5:1) 84%[b] (3.7:1)	-
	-	**54** <1%[c] (19:1) 95%[b] (4.4:1)	-
	-	**56** 49%[b] (>30:1)	-
	-	**57** 71%[b] (1.6:1)	-
	-	-	**60** 15% (1.6:1)

[a] (*S*)-phosphoric acid. [b] (*R*)-phosphoric acid. [c] no phosphoric acid

With metal complexes catalysts, low to moderate yields resulted from copper(I) (15%) and gold(I) (46%), accompanied by a poor stereoselectivity of 1.5:1. Cooperative organocatalysis was much more fruitful, with high yields in the range of 49-95%, irrespective of the glycosyl donor employed. Phosphoric acid was indispensable as cocatalyst, and the (*R)*-isomer outperformed the (*S*), regarding yield and α:β ratio. The stereoselectivity with the siloxane glucal donor was unawares low (1.6:1), while the largest proportion of the α glycoside (>30:1) was achieved with the 6-azido TBS protected galactal donor. The highest-yielding reaction was provided by the tri-TBS donor. Contrarywise, the same promising results did not reproduce with the protected doxorubicinone scaffold, as reflected by lower conversion rates and yields. Unquestionably, the thiourea-phosphoric acid protocol was the optimal approach to address anthracycline glycosylation, among the examined methodologies.

4.3 Biological assays

Reviewing all the anthracycline modifications, seven final deprotected products were obtained, which are gathered in Figure 16. Because of the timeframe, not all compounds could be tested in biological assays: **3** was tested on A431 cells and cardiomyocytes, whereas **61α** and **61β** were tested on cancer cells lines HeLa, MDA and MCF-7, and on healthy fibroblasts.

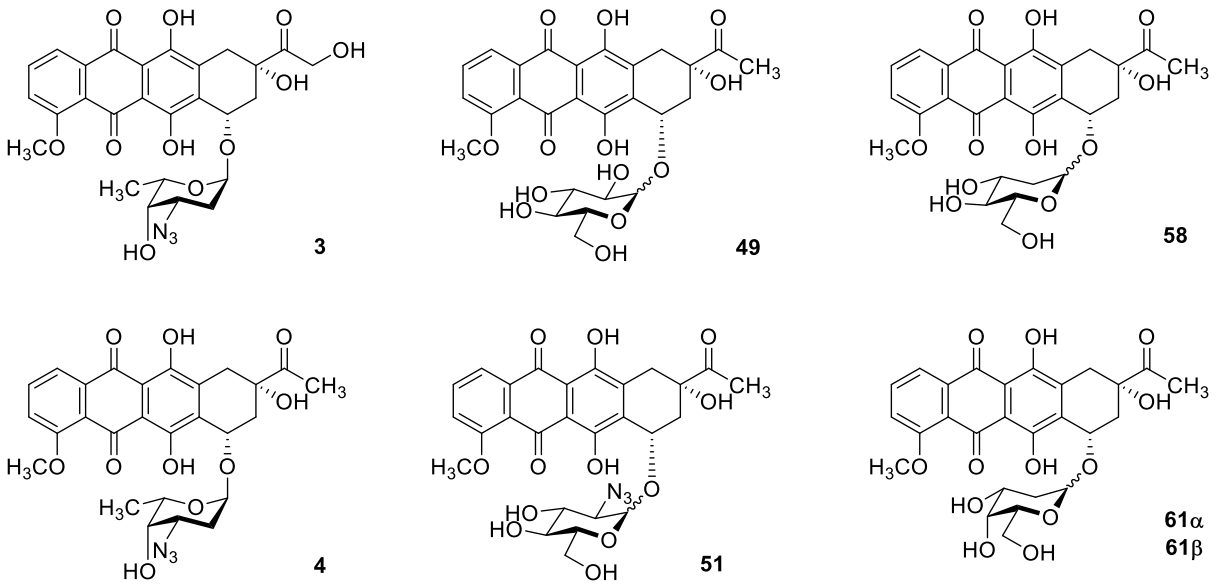


Figure 16. Final products of anthracycline glycodiversification: 3′-azido derivatives of doxorubicin (**3**) and daunorubicin (**4**); daunorubicinone glucosides with hydroxyl (**49**) and azido (**51**) groups in position 2; daunorubicinone 2-deoxyglucosides (**58**) and 2-deoxygalactosides (**61**).

4.3.1 *In vitro* cytotoxicity in cancer cell lines

4.3.1.1 HDF, HeLa, MDA-MB-231 and MCF-7

Glycosides **61α** and **61β** were tested on the following cultured cancer cells lines: HeLa (human cervical adenocarcinoma), MDA-MB-231 (human breast adenocarcinoma), MCF-7 (human breast adenocarcinoma), and a model of healthy cells HDF (human primary dermal fibroblast, adult). As a positive control, clinically used drug **DAU**[**194] was also tested under the same conditions, for it being a precursor of the novel glycosides and keep structural analogy to their anthracycline scaffold.

Cytotoxicity assays determined cell viability (Calcein, for the number of living cells) and metabolic competence (AlamarBlue, for the measure of reductive metabolism), evaluated through non-linear regression of dose-response inhibition curves (Graph 1). Results expressed as the 50% inhibitory concentration (IC_{50}) are summarised in Table 3 and Table 4, respectively.

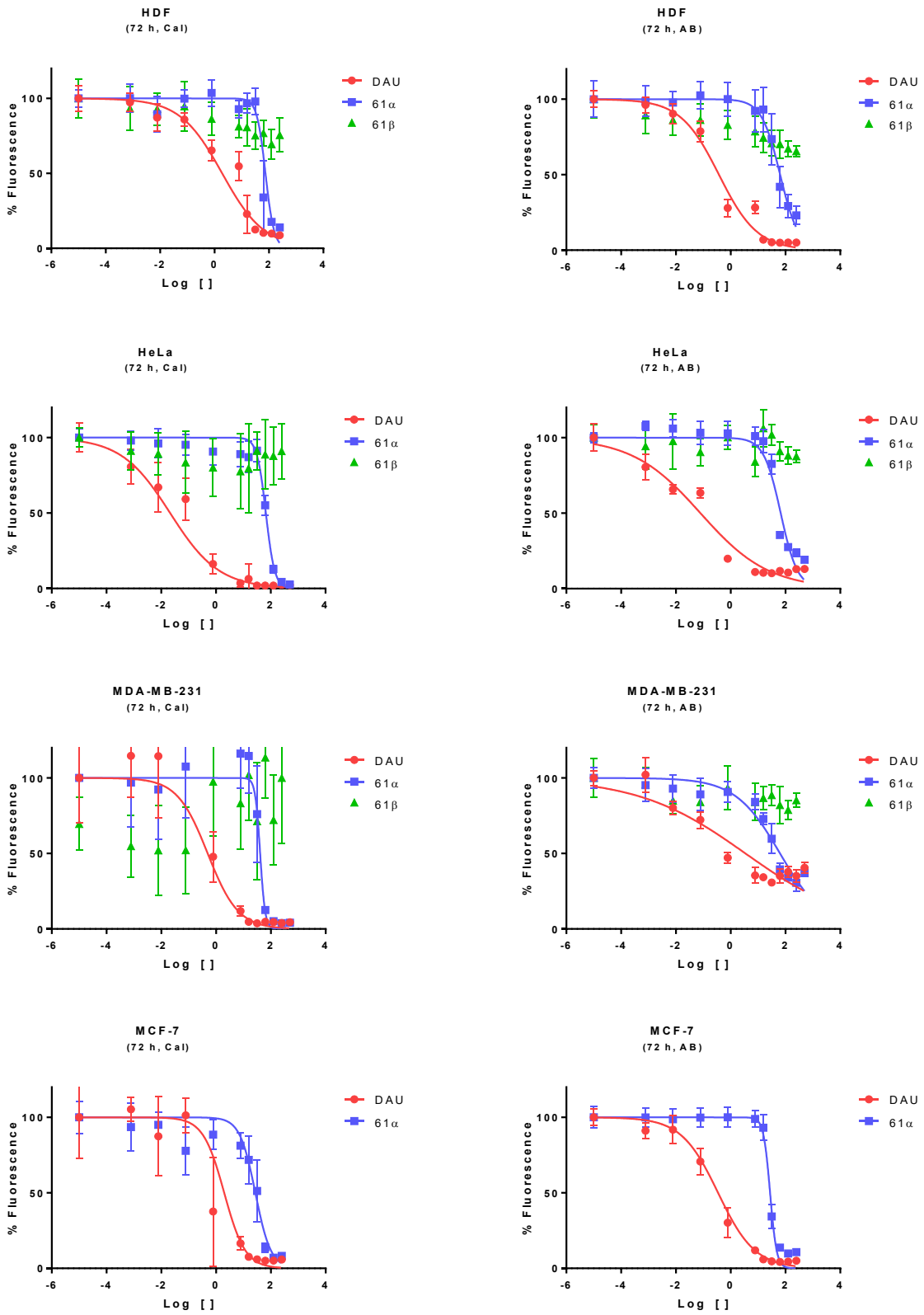
Table 3. Cytotoxicity quantified by Calcein (number of living cells), expressed by IC50 in μM (95% confidence interval in parentheses)

Cell line	HDF	HeLa	MDA-MB-231	MCF-7
DAU	1,91 (1,50-2,44)	0,021 (0,018-0,024)	0,49 (0,38-0,65)	1,98 (1,49-2,63)
61α	74,59 (67,51-82,41)	66,51 (61,70-71,70)	40,64 (33,90-48,72)	27,12 (23,19-31,71)
61β	> 250	> 250	> 250	-

Table 4. Cytotoxicity quantified by AlamarBlue (measure of reductive metabolism), expressed by IC50 in μM (95% confidence interval in parentheses)

Cell line	HDF	HeLa	MDA-MB-231	MCF-7
DAU	0,37 (0,30-0,46)	0,079 (0,056-0,110)	3,46 (2,16-5,54)	0,35 (0,30-0,40)
61α	63,88 (54,81-74,44)	67,09 (59,50-75,64)	63,36 (51,21-78,41)	27,40 (25,82-29,07)
61β	> 250	> 250	> 250	-

[**] IC_{50} (**DAU**/HeLa): 1.09 nM (Takara et al. 2002)



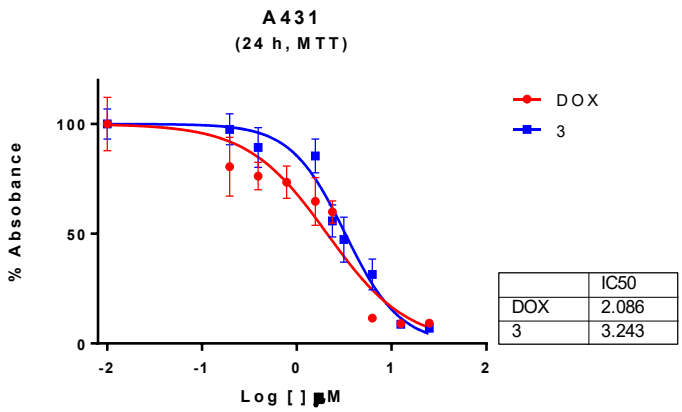
Graph 1. Cell viability inhibition curves (Cal, left) and reductive metabolism inhibition curves (AB, right), for compounds **DAU**, 61α and 61β, against cell lines HDF, HeLa, MDA-MB-231 and MCF-7.

While **DAU** presented an IC_{50} of low or submicromolar (between 0.021 and 1.98 μM for Calcein (Cal), and between 0.079 and 3.46 μM for AlamarBlue (AB), the α 2-deoxygalactoside **61α** had IC_{50} in the range of tens of micromolar (between 27.1 and 74.6 μM for Cal, and between 27.4 e 63.9 μM for AB). These results indicated that this derivative has approximately 10 to 1000 fold less potency than the parental drug, against these particular cancer and healthy cell lines. In turn, compound **61β** showed such low activity that even the highest concentration tested did not inhibit more than 50% cell viability or reductive metabolism, impeding the calculation of its IC_{50} values (Graph 1).

The absence of a nitrogenated functionality in these novel derivatives can explain their lesser cytotoxicity since it is considered essential for anthracycline anticancer activity, even if converted to the azido group. It will be important to obtain the corresponding analogues containing azido glycosides, to confirm definitely the relationship between structure and cytotoxic activity.

4.3.1.2 A431

The doxorubicin derivative **3** was tested in cultures of the cancer cell line A431 (human epidermoid carcinoma) in cell viability assays, with detection by the MTT method,[137] in a collaborator laboratory (Prof Renata Fonseca, FCFRP-USP). In parallel, the drug **DOX** was also tested for control and comparison. The minimum inhibitory concentration for 50% of cell viability (IC_{50}) was determined 2.1 μM for **DOX** and 3.2 μM for the azido derivative **3**, indicating that for this cell line, under the experimental conditions, was slightly less potent than the parent compound, but still in the same order or magnitude (Graph 2).



Graph 2. Cell viability curves for compound DOX and **3**, in A431 cells, after 24 h exposition. Quantification by the MTT method.

Although IC_{50} values between 0.08 μM[195] and 0.086 μM[196] are reported in the literature for doxorubicin cytotoxicity in A431 cells, the difference compared to the result obtained in the present assay (2.1 μM), in which **DOX** was used as control, may be related to the experimental conditions. For instance, in the aforementioned reports, the cell culture was incubated for 70-72 h in contact with the compounds under testing, while in the experiment here described, the incubation time was 24 h, which might not have been sufficient to demonstrate the full cytotoxic potential of the analysed compounds.

Still, it is possible to compare the results between compounds **3** and **DOX** tested under the same conditions, according to Graph 2, their cytotoxicity was comparable. This was already expected since the azido is a known compound, which had been tested in other cancer cell lines MCF-7 (human breast adenocarcinoma) and K562 (human myeloid leukaemia), showing cytotoxicity against them. Although with a higher IC_{50} than **DOX**, compound **3** presented activity against induced DOX-resistant cell lines.

Additionally, **3** is a promising compound, considering that its counterpart **4** was also cytotoxic to anthracycline-resistant cancer cell lines, in addition to a decreased overall toxicity in an animal model.[43, 54]

4.3.2 Preliminary assessment of cardiotoxicity by high content analysis of human iPSCs-derived cardiomyocytes

The studies were conducted by Dr Lúcio Freitas-Júnior (LNBio-CNPEM), with samples of novel human induced pluripotent stem-cell derived cardiomyocytes, which retain the contraction capability in culture plates and can be observed by video imaging. The method quantified cell function based on how much the pulse rate was affected by a treatment, reflecting the cardiac muscular activity.

In spite of a great potential to become a good experimental model for human cardiotoxicity, the cell line was under development by a start-up company, and presented experimental limitations, such as indefinite morphological aspect and cell size, significant presence of debris, non-homogeneous confluent multilayers, with non-coordinate pulsing. These factors hampered the cell culturing and result interpretation. Besides, the number of cells made available by the supplier was not enough to perform replicate experiments, so it was not possible to evaluate repeatability and run statistical processing.

Regarded these limitations, reduction of the pulse rates of contractile cardiomyocytes was determined in the presence of compound **DOX** and **3**, according to Table 5. This is an image-based alternative methodology for the measurement of the cardiac cell function. Pulse reduction indicates a decrease or suppression is of the muscular activity of the cells under observation.

Table 5. Comparison of pulse pre- and post-treatment, and pulse reduction (decrease in contraction after exposure to compound, in relation to pre-treatment condition).

Sample	Pulse (pre-treatment)	Pulse (post-treatament)	Pulse Reduction
H_2O	0,81	0,69	14,8%
DMSO	0,94	0,25	73,4%
DMSO	0,38	0,19	50%
3 (10 µM)	0,88	0	100%
3 (100 µM)	0,44	0,44	0%
DOX (10 µM)	0,50	0	100%
DOX (100 µM)	0,94	0	100%

Results were not consistent with a dose-response behaviour, considering that there was complete pulse reduction for compound **3** at 10 µM, but no pulse reduction was observed at 100 µM. On the other hand, for **DOX**, 100% pulse reduction occurred in both concentrations, while unexpected reductions were observed in the negative controls (water and DMSO). Altogether, these results rendered the study inconclusive.

The images of cells fixed after treatments showed qualitative aspects of the morphological changes induced on the cardiomyocytes by the tested compounds. Cells treated with **3** at 10 µM presented star-shaped elongated cytoplasm. Treatment with **DOX** at 10 µM caused more dramatical changes, with cell damage, resulting in a decreased number of spread-cytoplasm cells (Figure 17).

Image data suggest that compounds **3** and **DOX** had moderate to high toxicity on the cardiomyocytes. However, the unacceptable variation in the measured or evaluated parameters, pulse reduction, cell morphology and population, even in the controls, will demand optimisation of the experimental protocol to extract any conclusion concerning the cardiotoxicity of the compounds, in particular **3**, which toxicity profile has not been described yet.

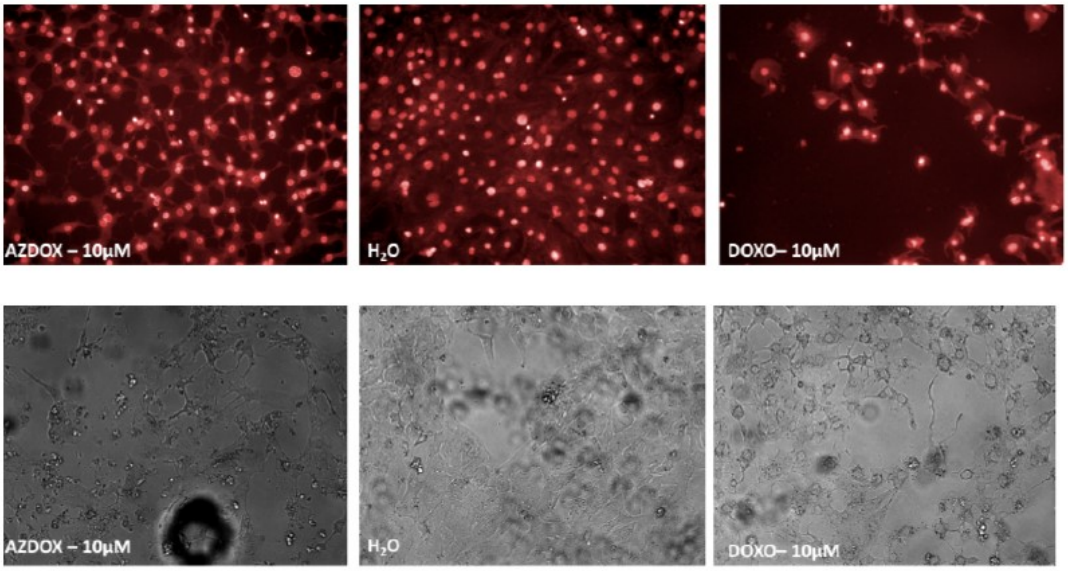


Figure 17. Photomicrography of selected treatment wells. Distinct cellular morphological aspects can be observed with Draq5, a DNA staining probe (top) and in bright field (bottom). Images were acquired with a 20× WD objective in the Operetta system.

Alternatively, other experimental models can be considered, for example, H9C2 cardiomyoblasts, an embryonic murine cell line with the ability to differentiate into cardiac muscle, which is robust and well-established. The experimental model selection depends on the expertise of the collaborator group to perform the assays.

Conclusion

5 CONCLUSION

The proposed glycodiversification route, involving cleavage of the glycosidic bond in daunorubicin and doxorubicin, the regioselective protection of latter, and the reaction with glycosyl moieties allowed to get a series of novel anthracycline protected glycosides, including 2-azido glycosides **45**, **46**, **47**, **50**, **52**, 2-deoxyglycosides **53**, **54**, **55**, **57**, **59**, **60**, and 6-azido-2,6-dideoxy glycoside **56**, achieving and extending the structural diversity originally planned.

The best glycosylation methodologies were the trimethylsilyl trifluoromethane-sulphonate catalytic activation of 2-azido glycosyl imidates, and the cooperative thiourea-phosphoric acid organocatalysis for glycal donors.

Deprotection of compounds **50**, **57** and **60** afforded, respectively, 2-azido glucoside **51**, the daunorubicin counterpart of target compound **1**, 2-deoxyglucoside **58** and 2-deoxygalactoside **61**. Some deprotection reactions are to be optimised, in order to produce a variety of final products from the available protected glycosides, including the deprotected form of 6-azido galactoside **56**, the structurally closest analogue of target compound **2**. The synthesis of 3′-azido-anthracyclines **3** and **4** was successfully reproduced from literature protocols, to serve as a comparison to the novel target compounds.

3′-azido-doxorubicin **3** showed comparable potency to the parent doxorubicin in antiproliferative assays against A431 cells. Compound **61α** was 10 to 1000 fold less potent than the parent daunorubicin in antiproliferative assays against HeLa, MDA-MB-231 e MCF-7 cell lines, reinforcing the interest for testing the azido-containing derivatives against the same panel of cancer cells.

A model of human cardiomyocytes derived from induced pluripotent stem cells could not be standardised for evaluating the synthesised compounds. An adequate experimental model still lacks for the assessment of their cardiotoxicity, which is to be addressed by means of collaboration.

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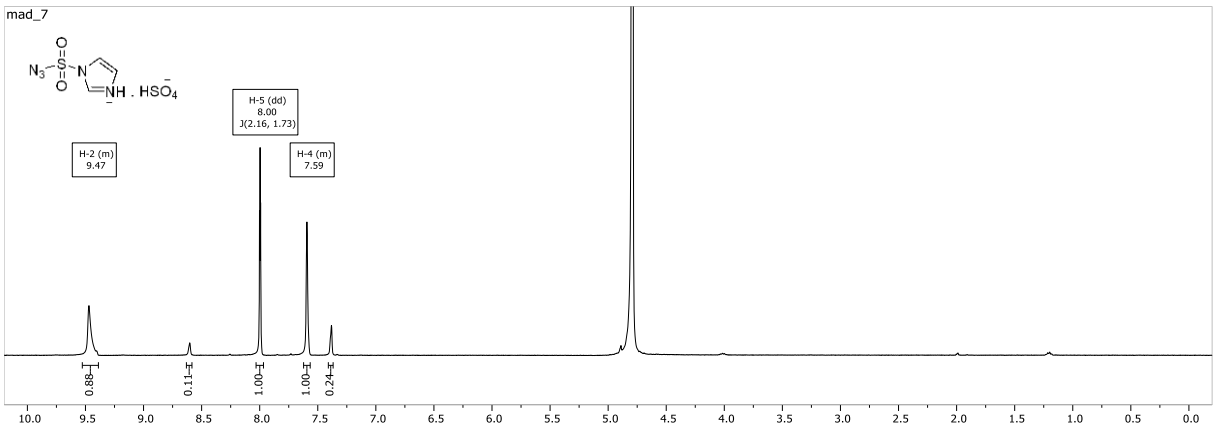
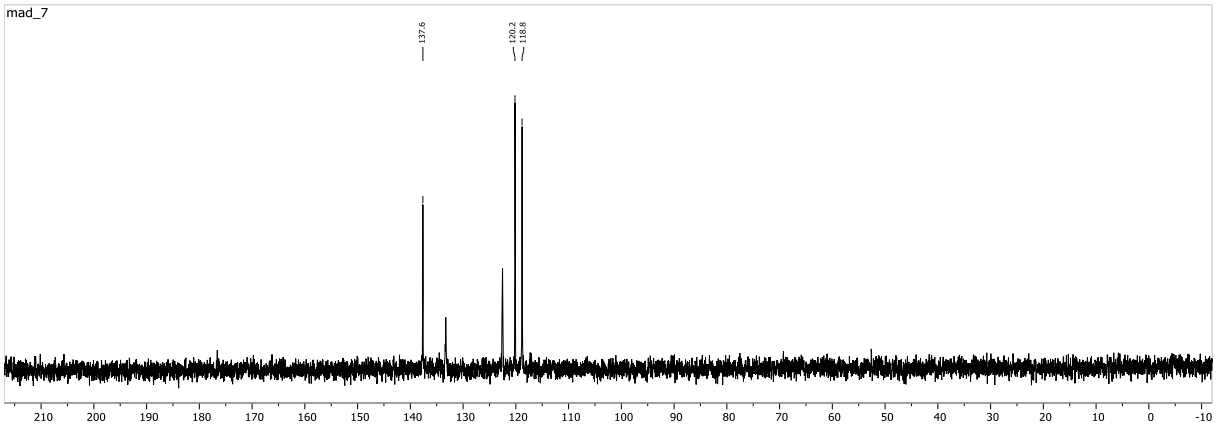
Appendices

APPENDIX 1. Compound **5**





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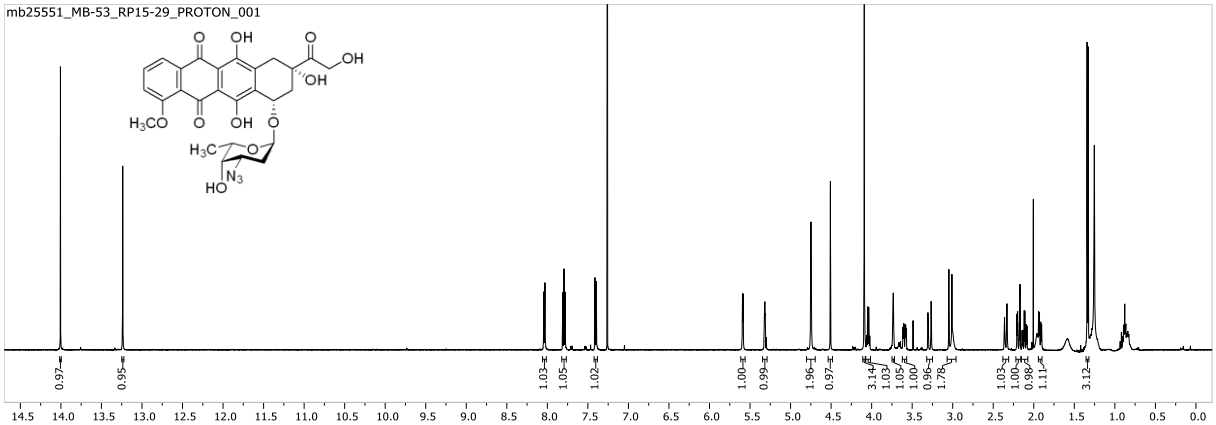

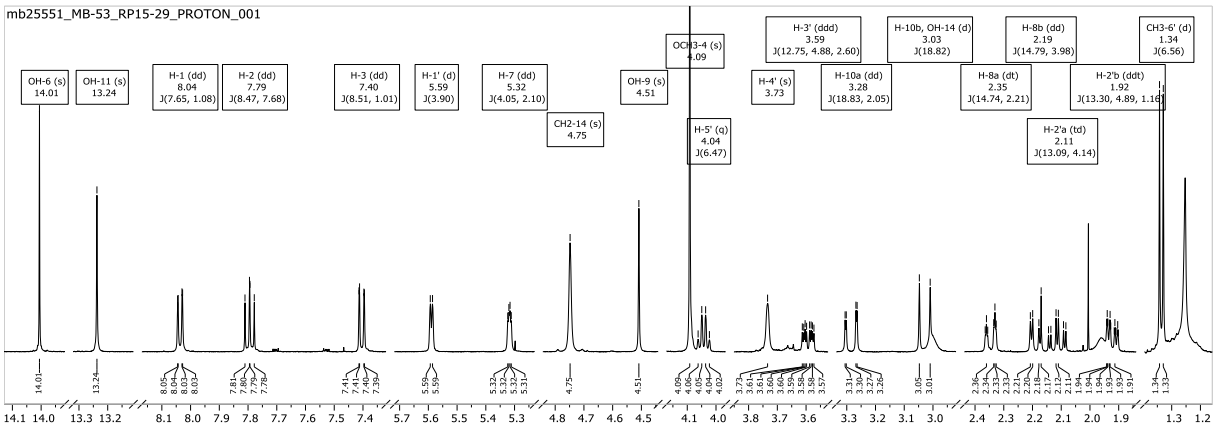

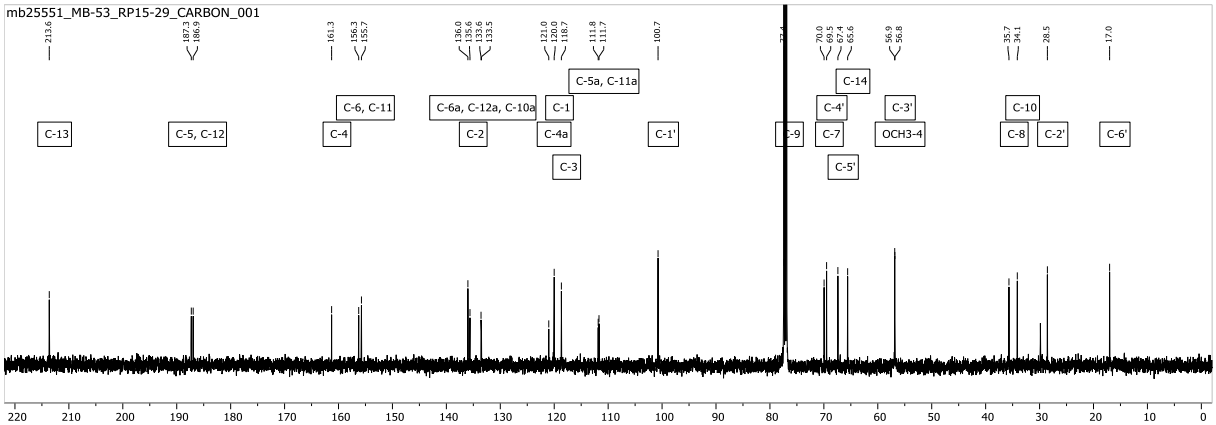

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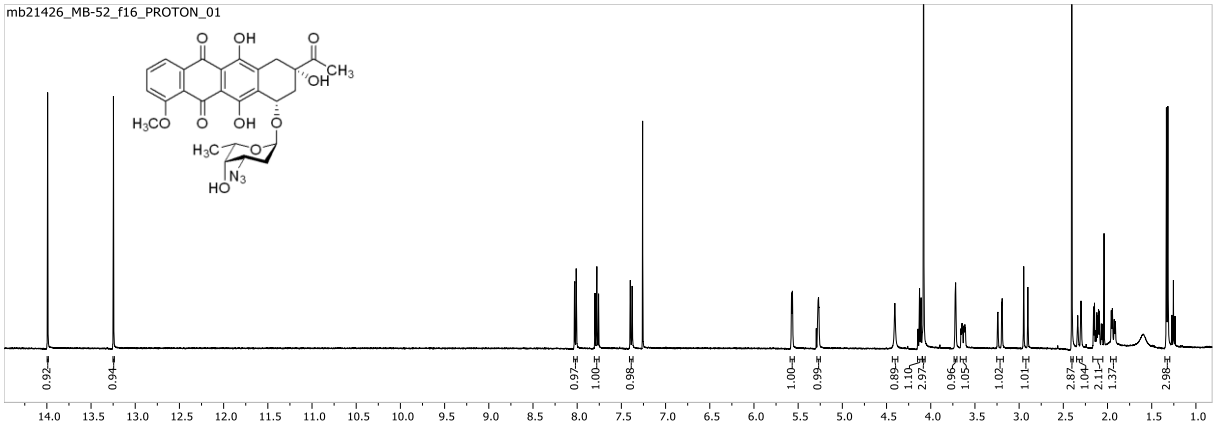

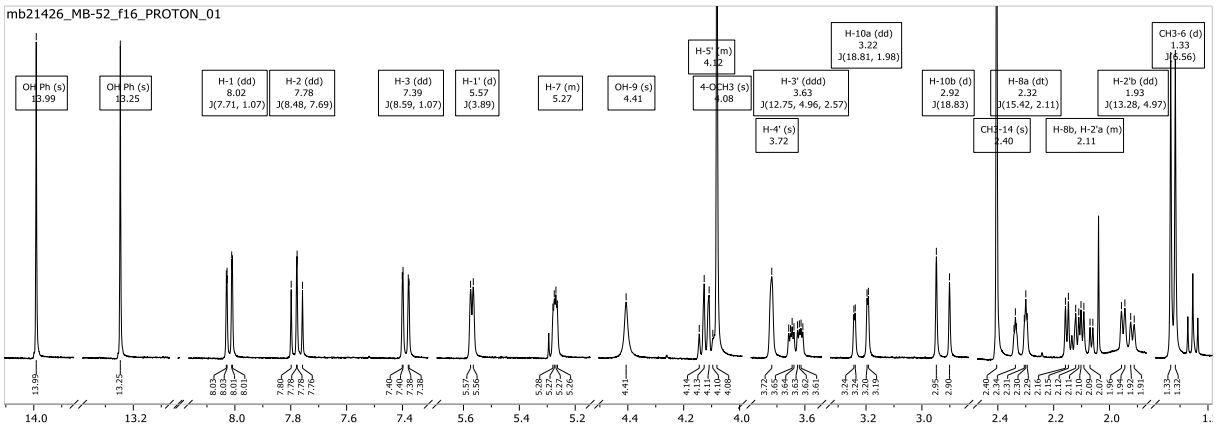

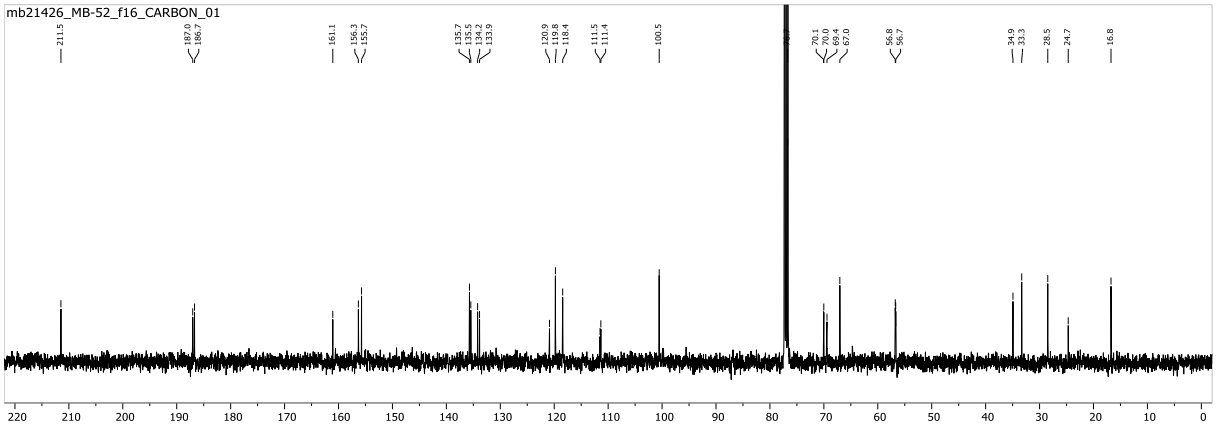

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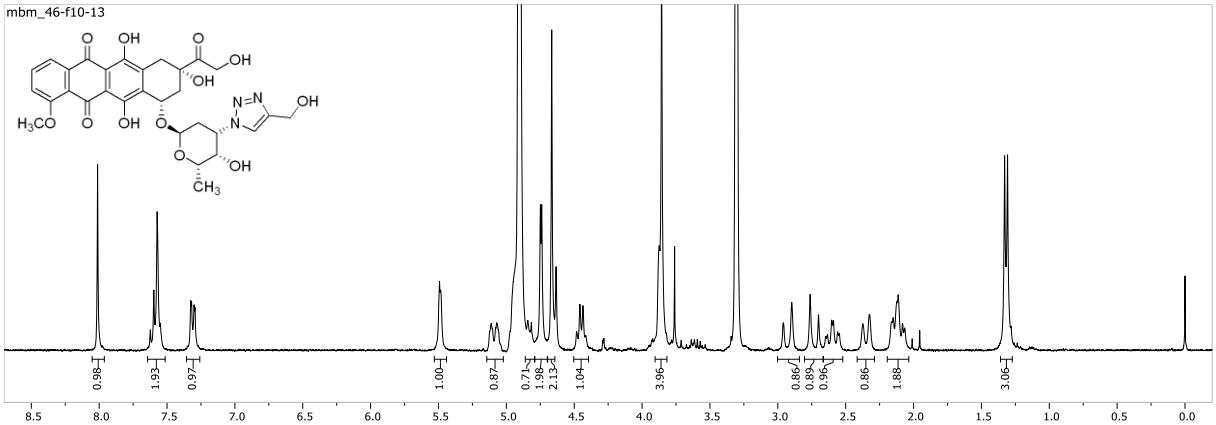

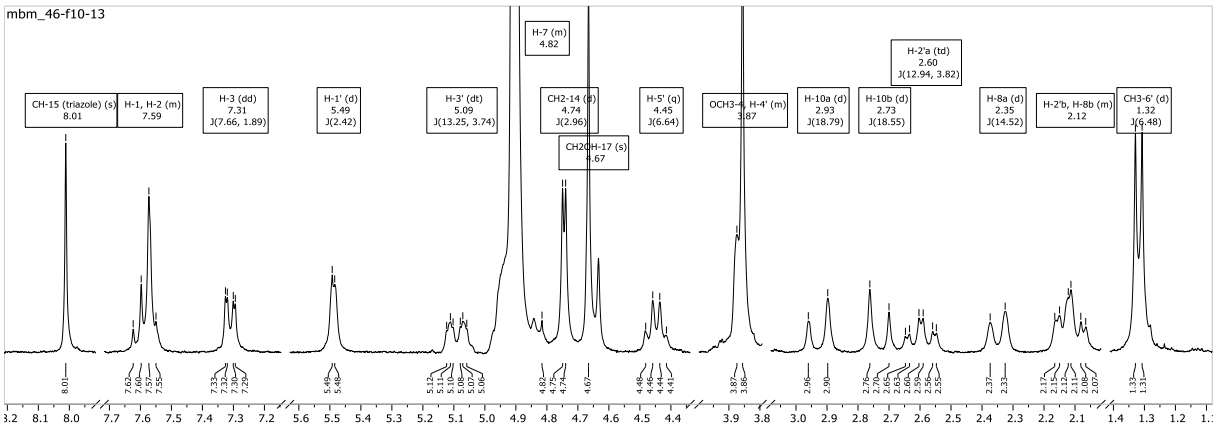

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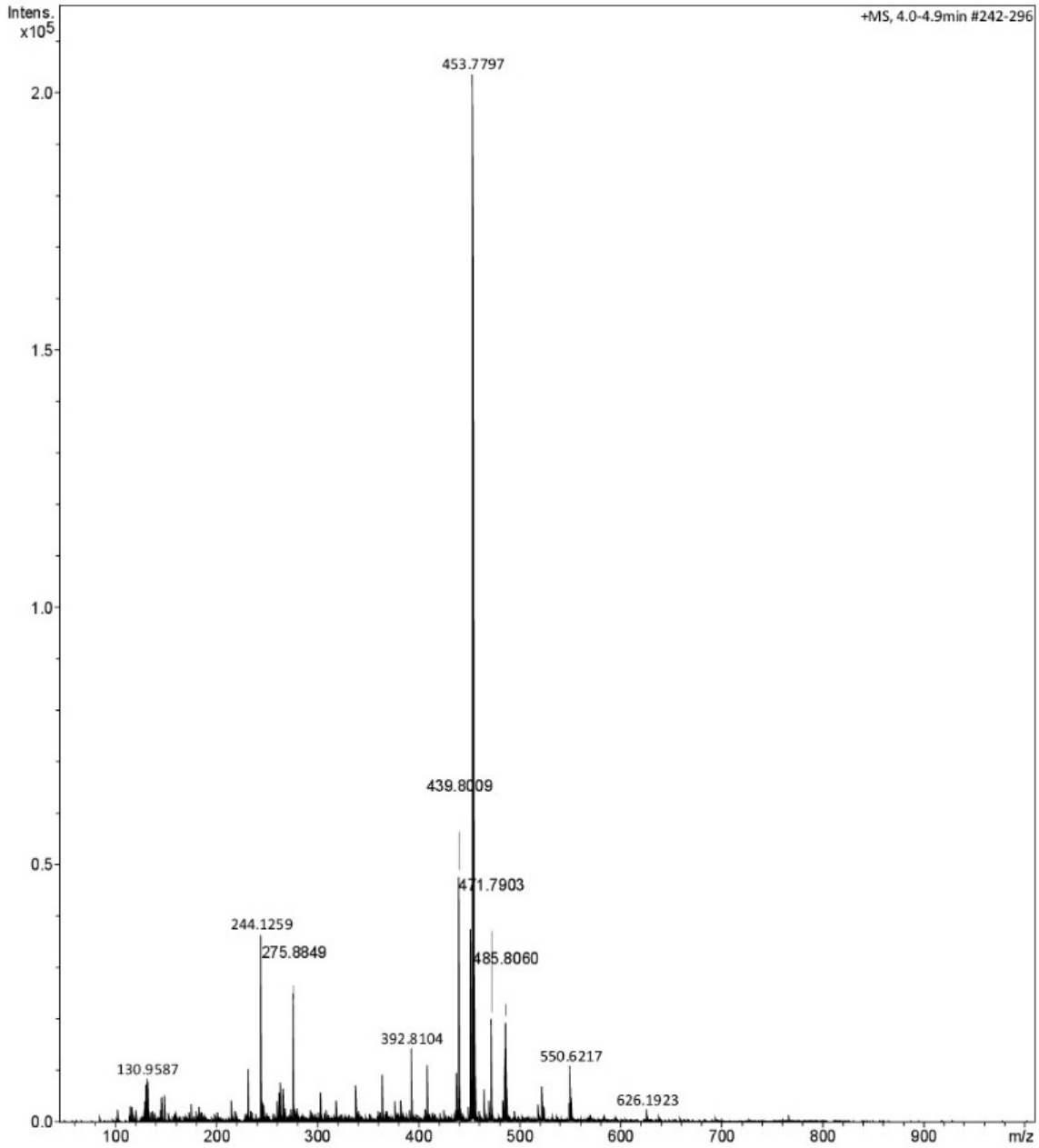
Comment			



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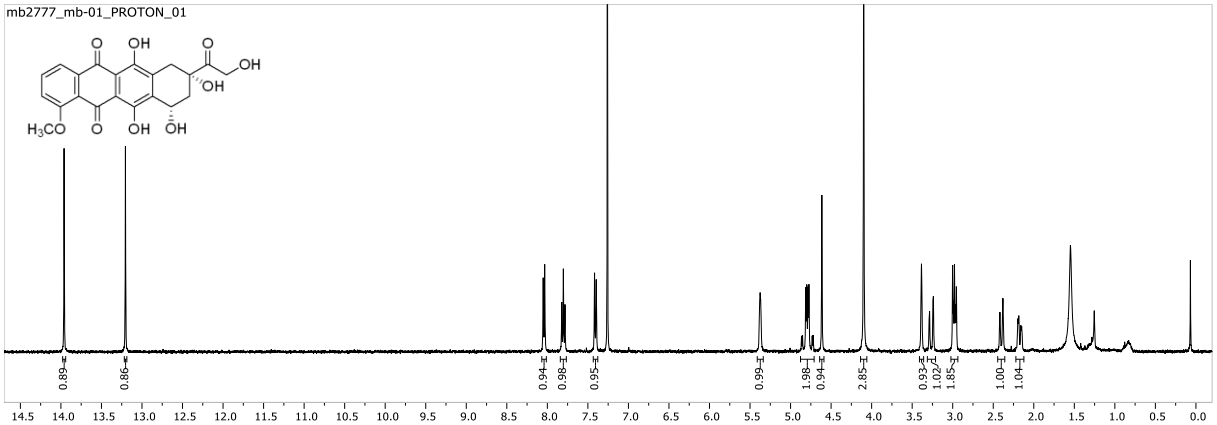

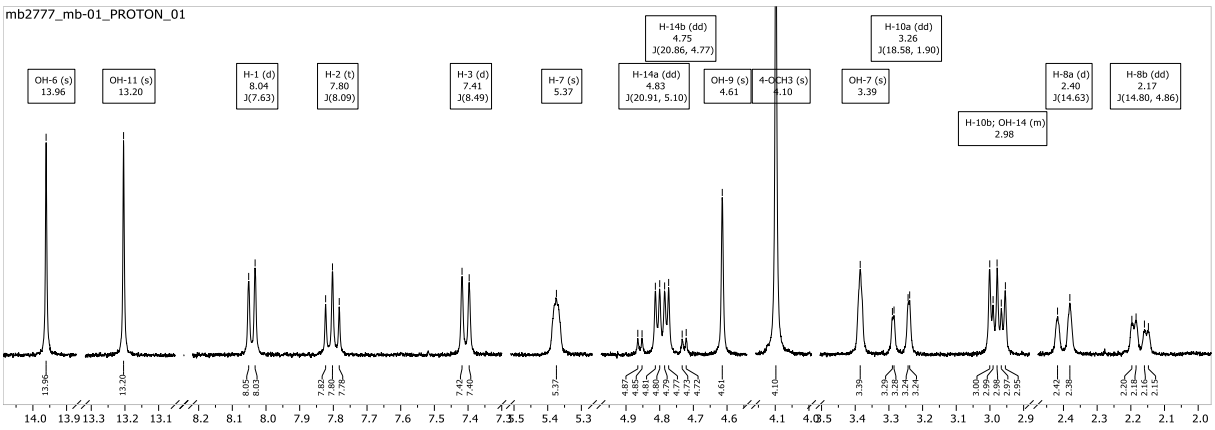

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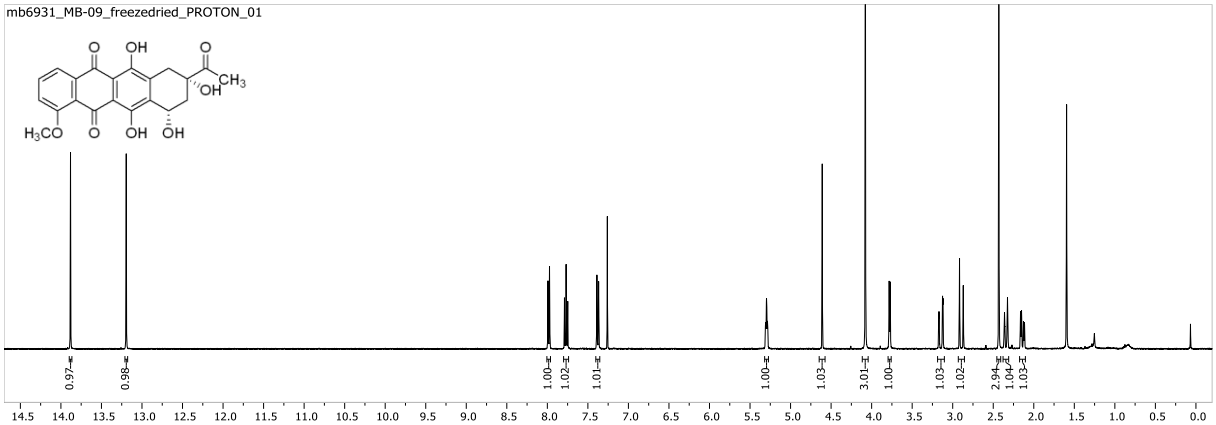

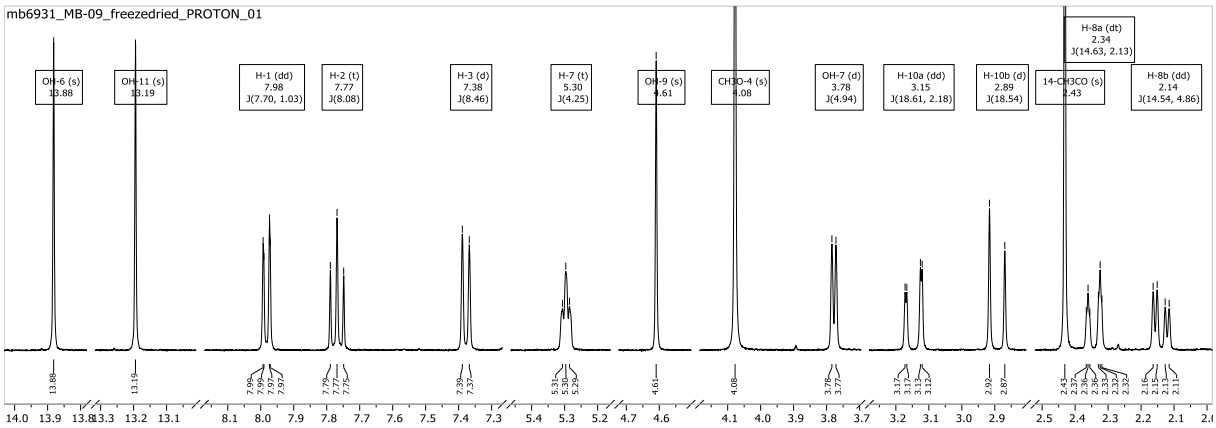

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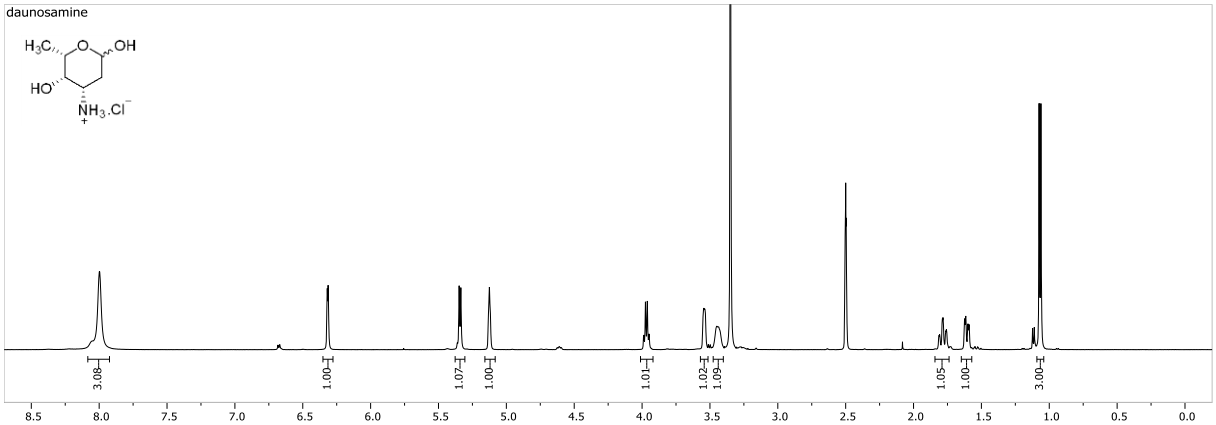

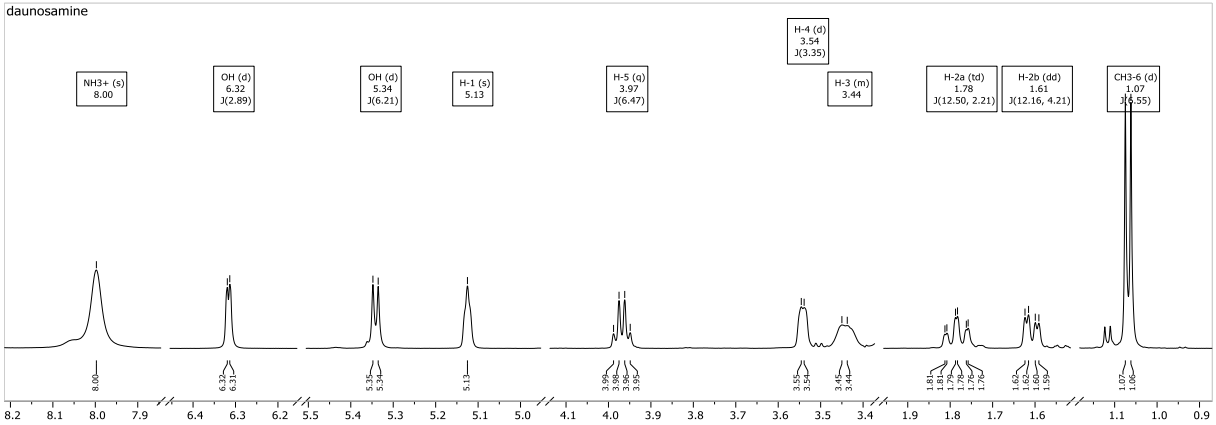

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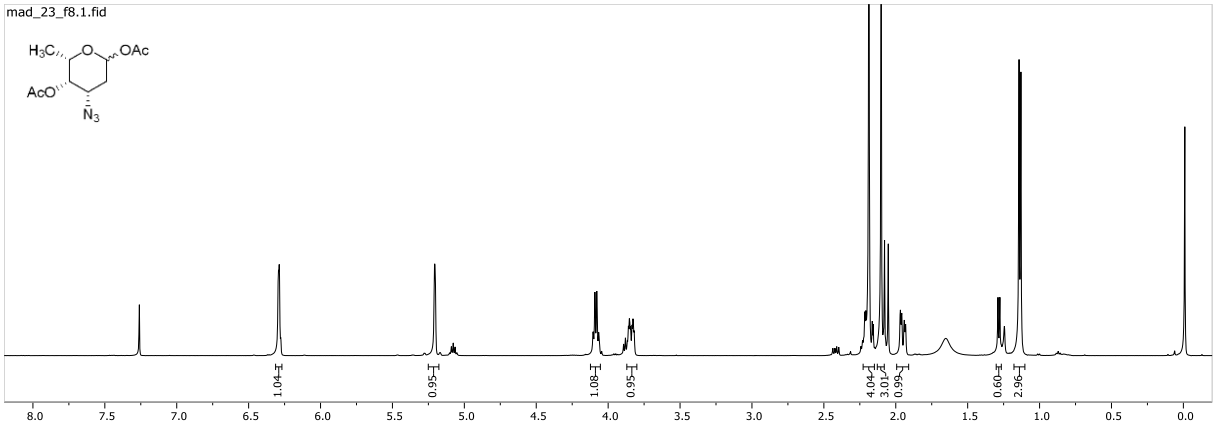

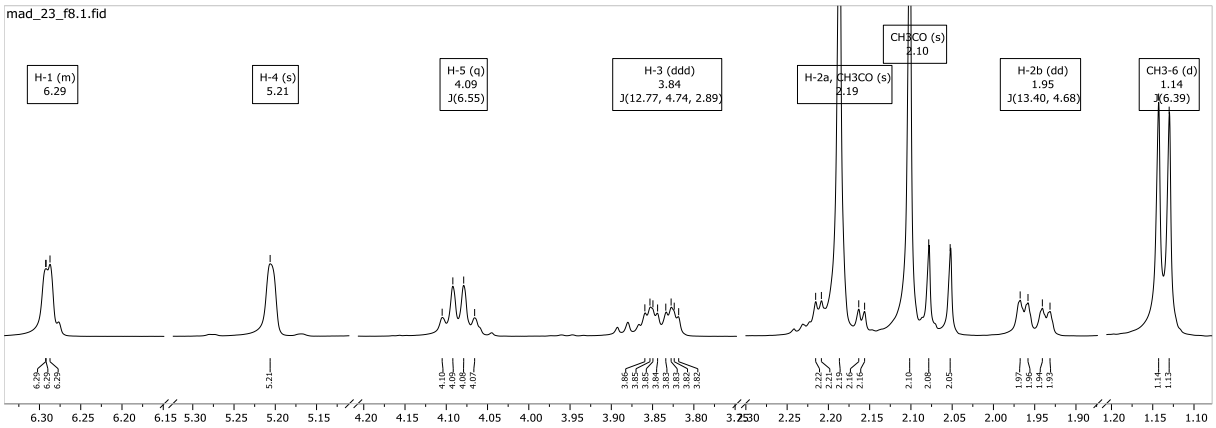

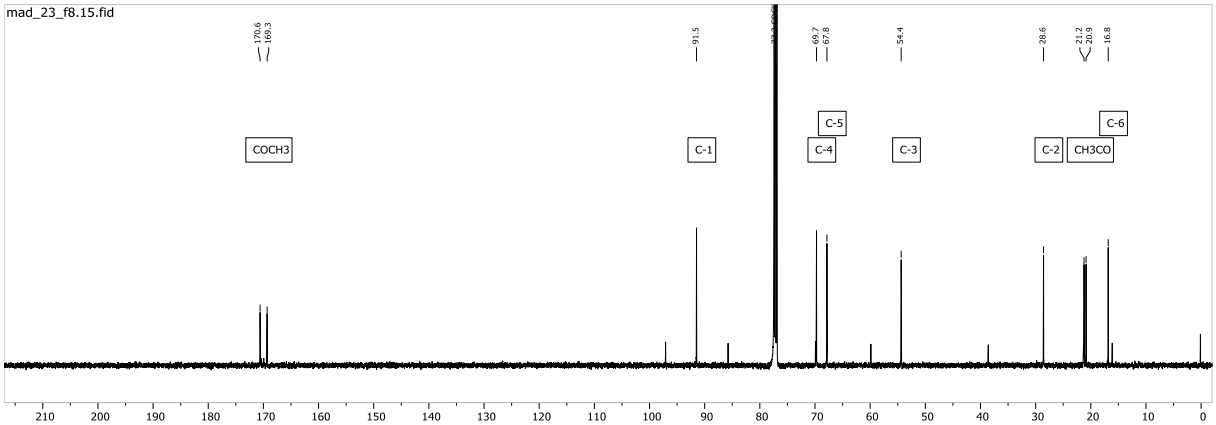

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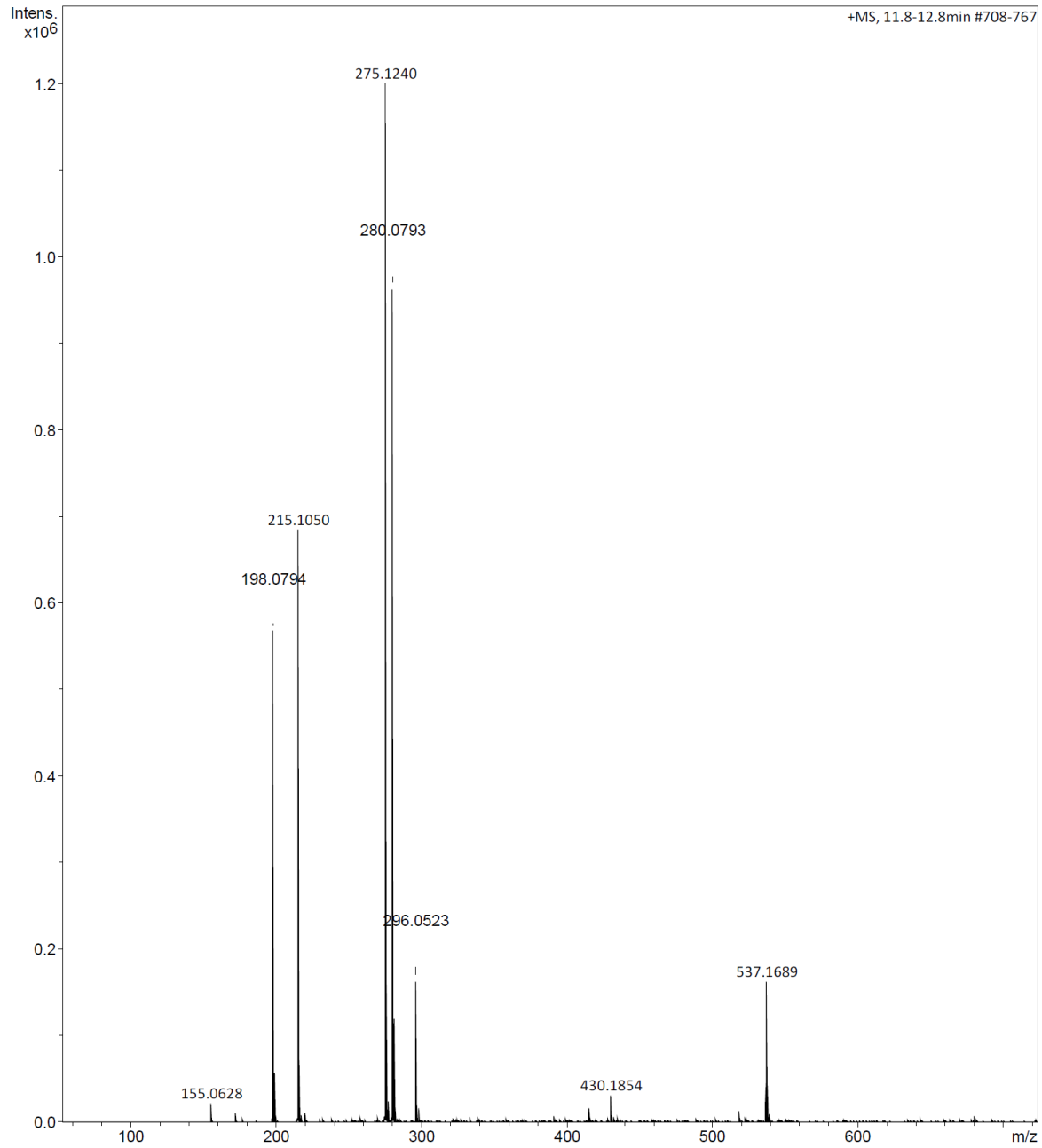
Comment			



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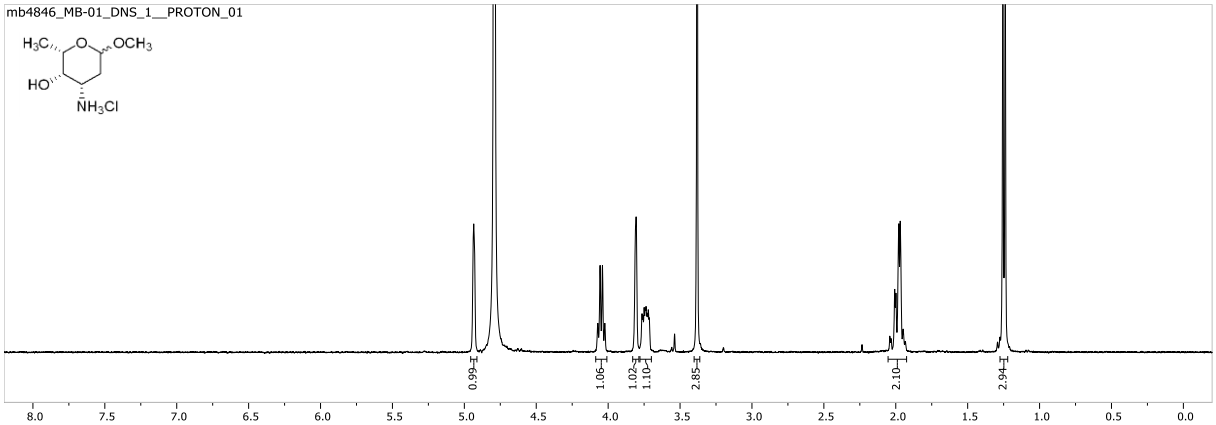

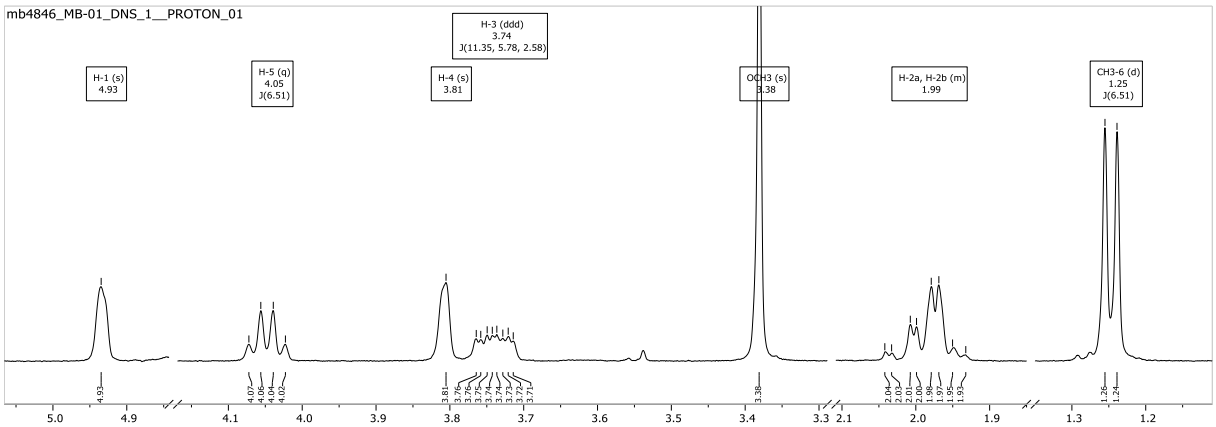

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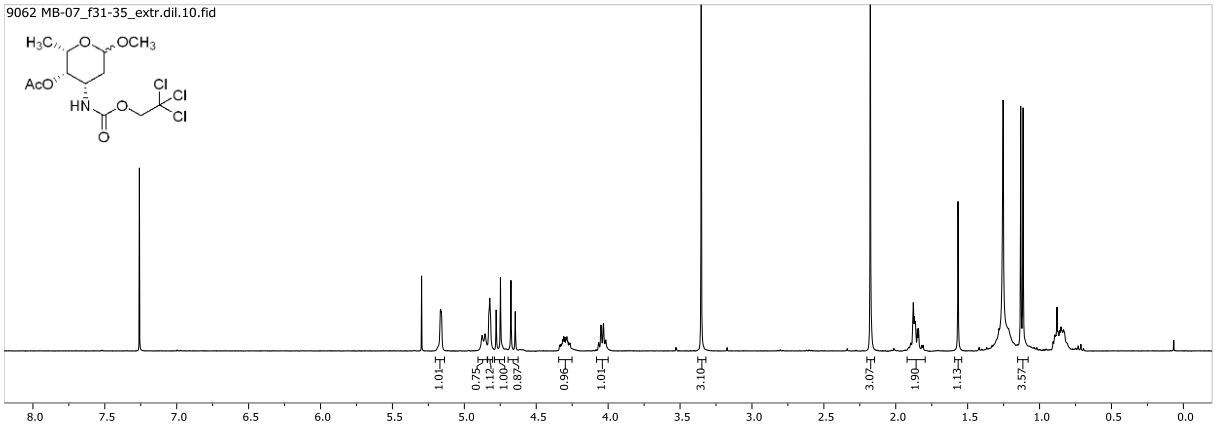

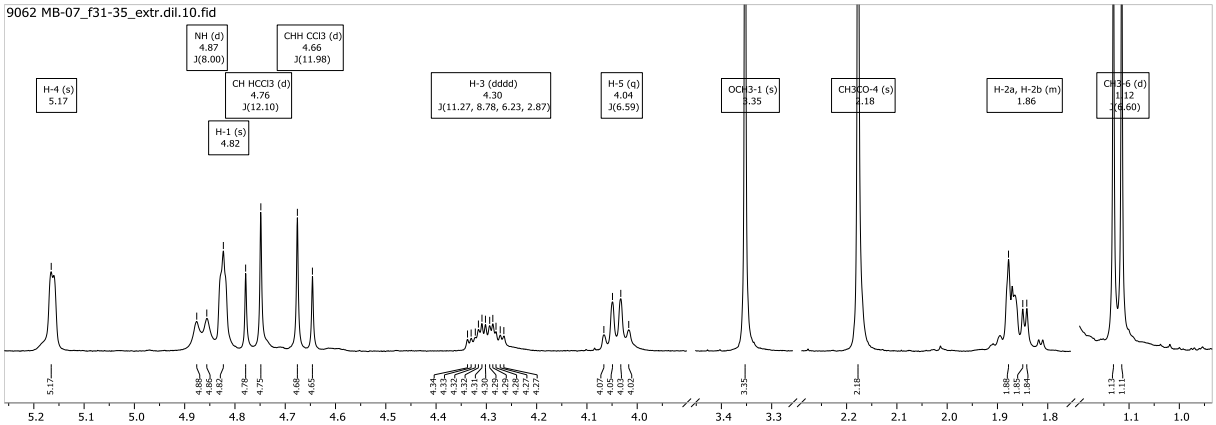

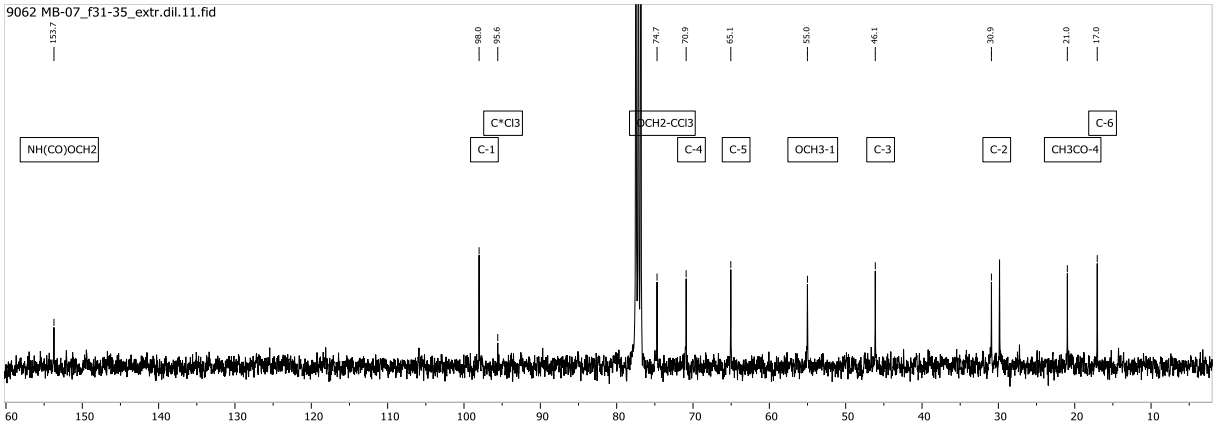

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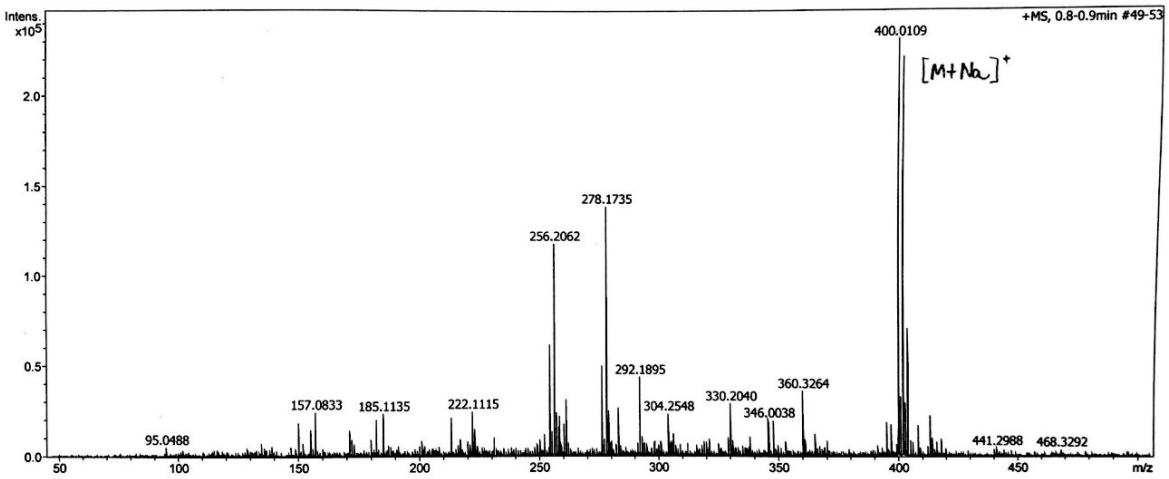

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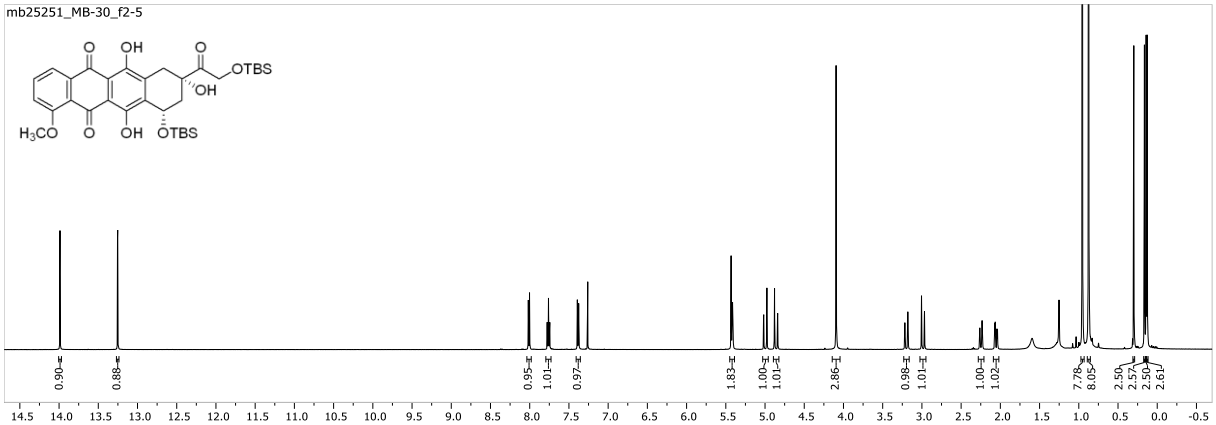

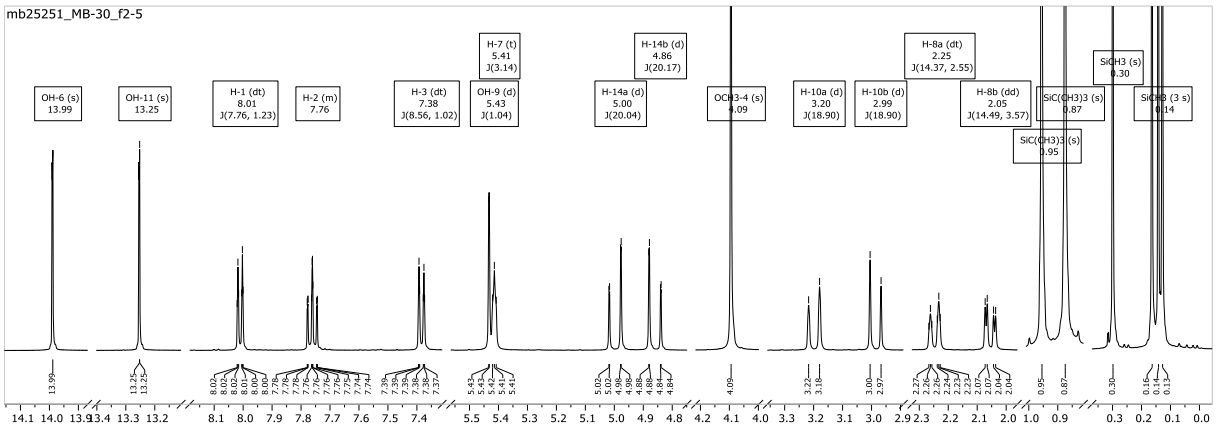

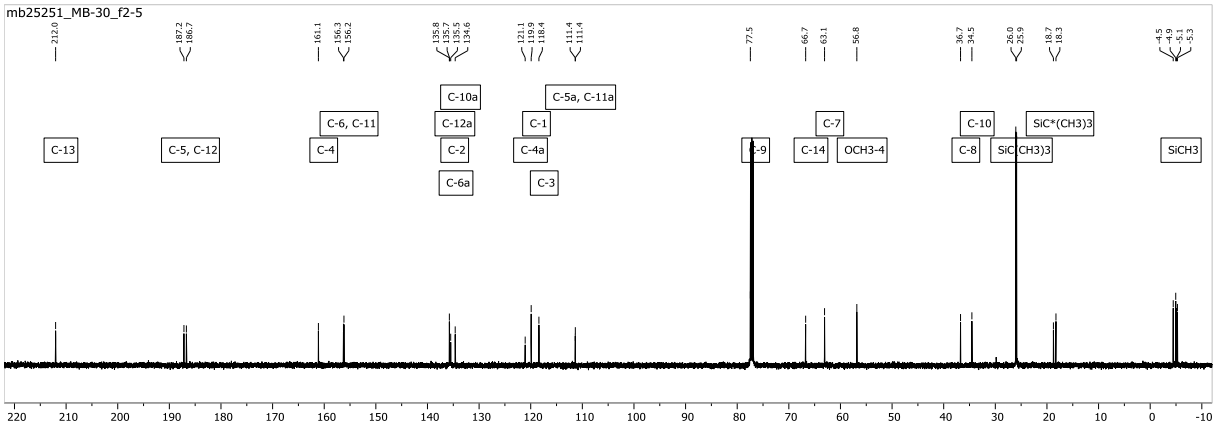

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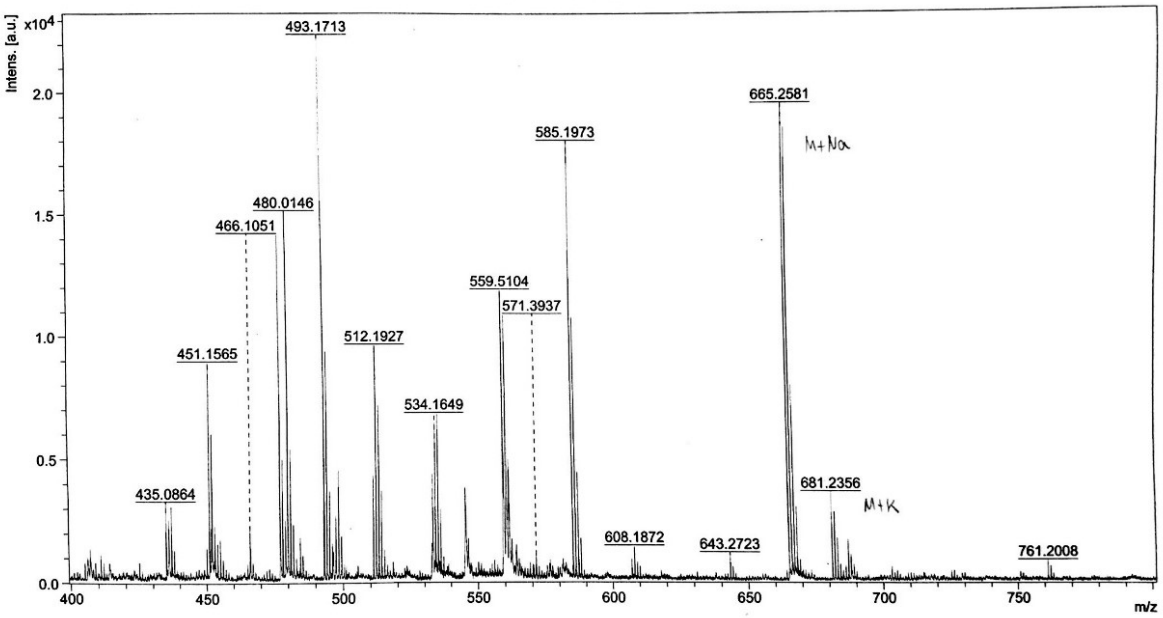

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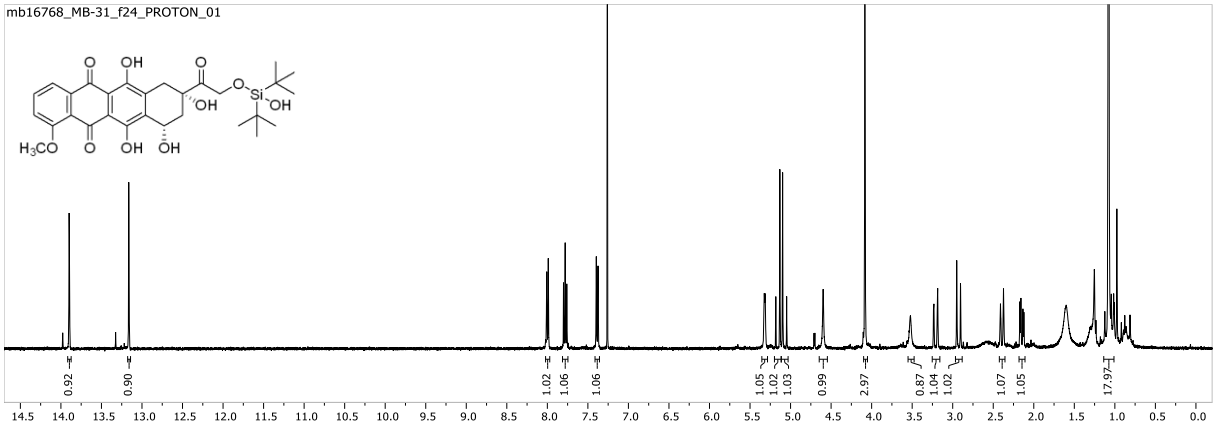

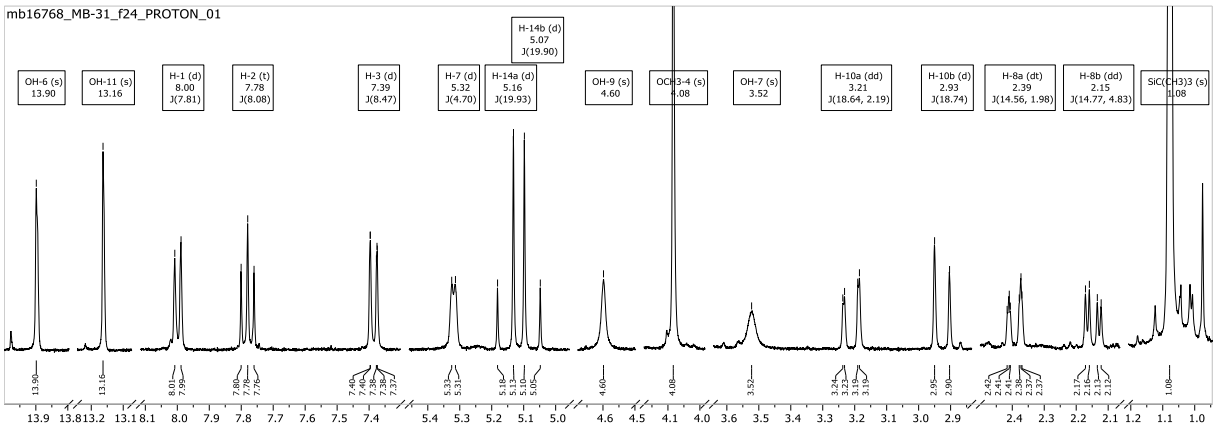

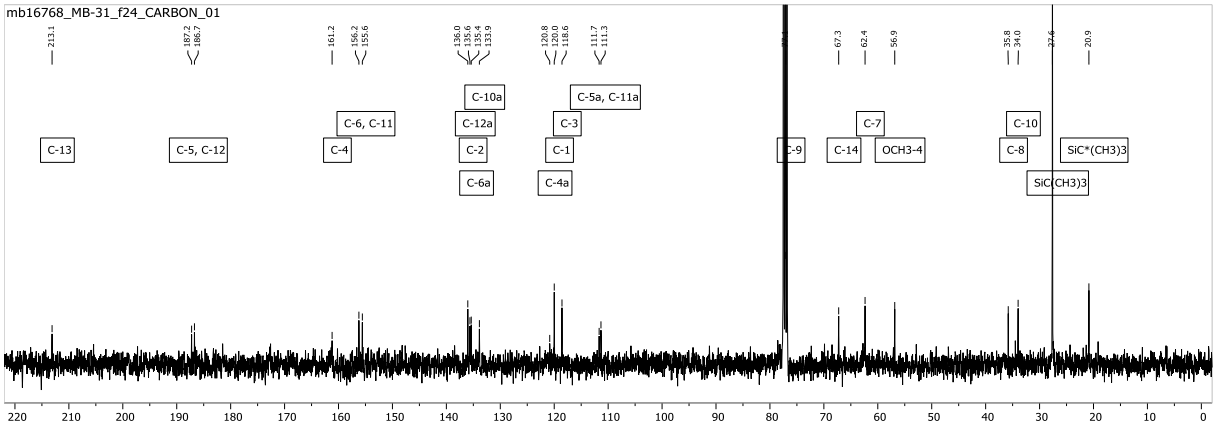

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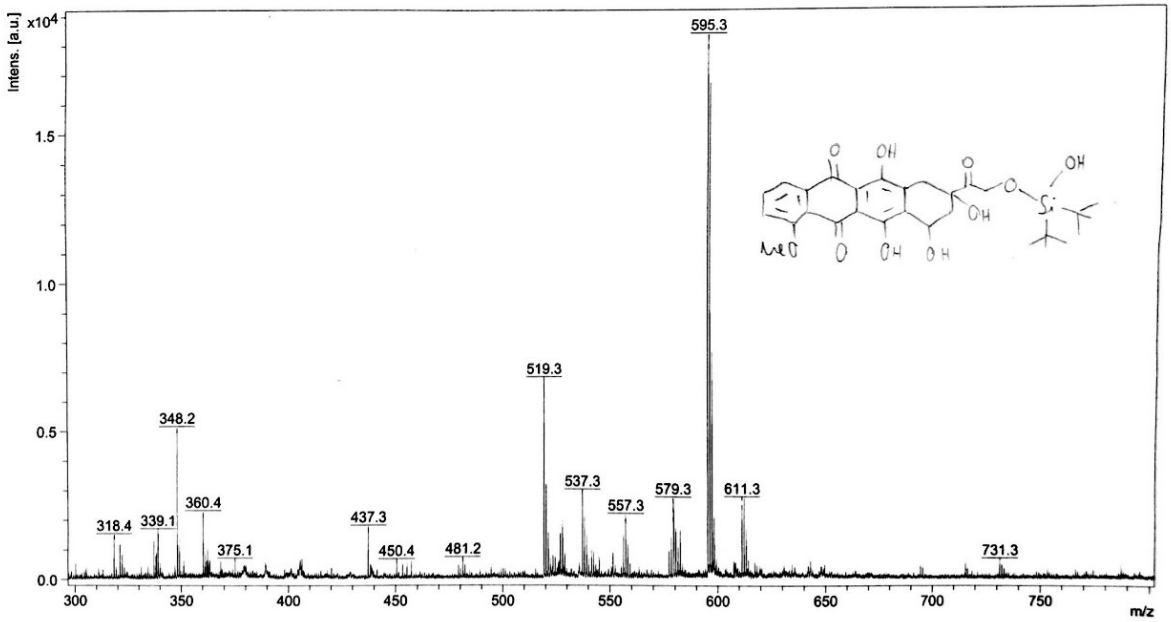

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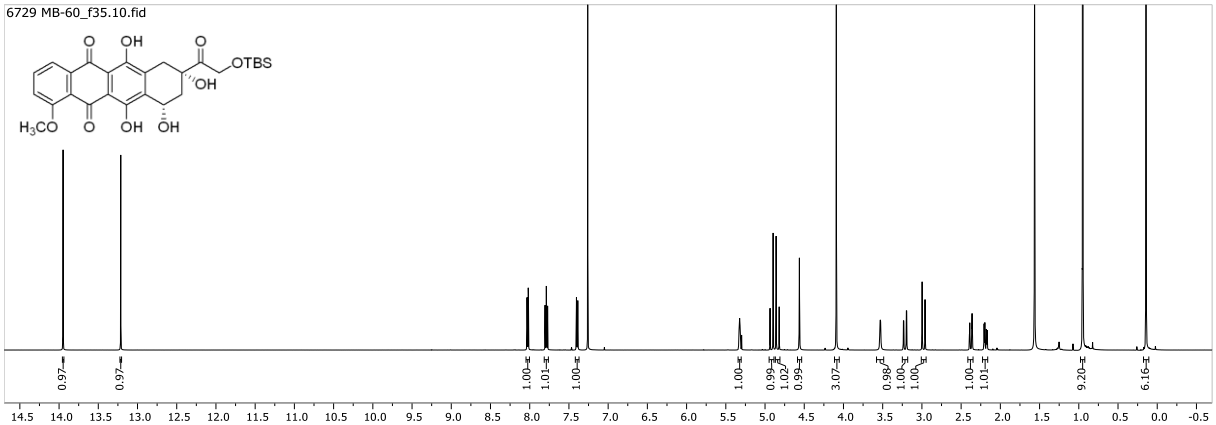

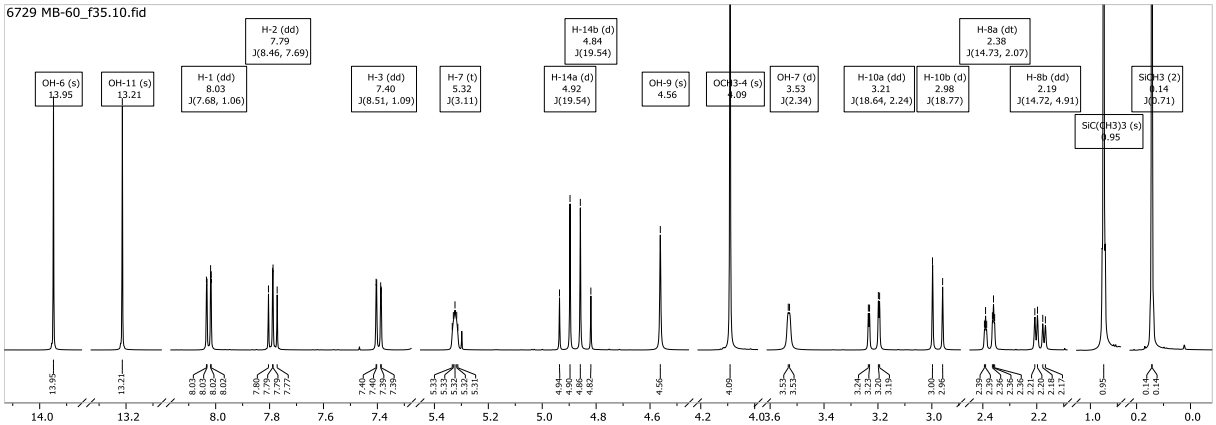

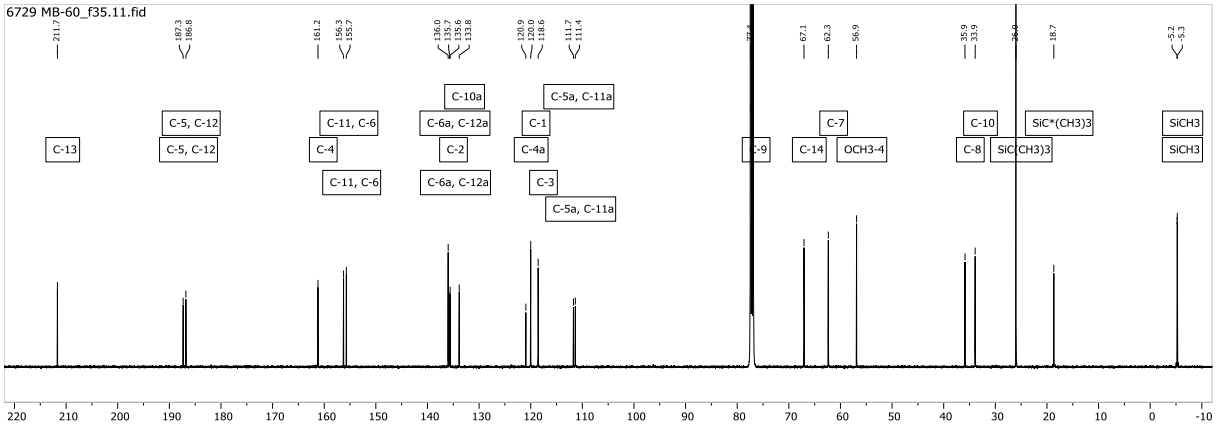

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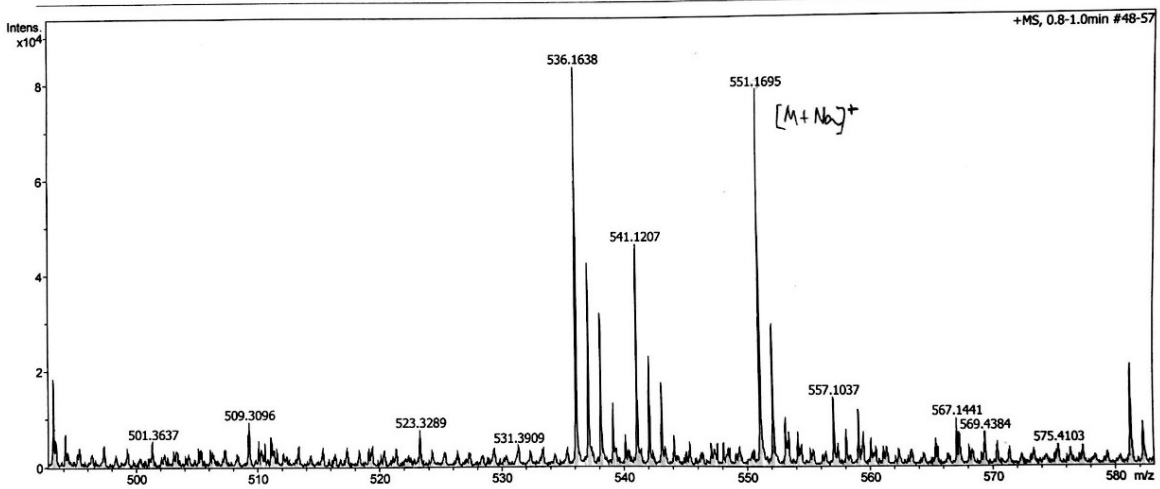

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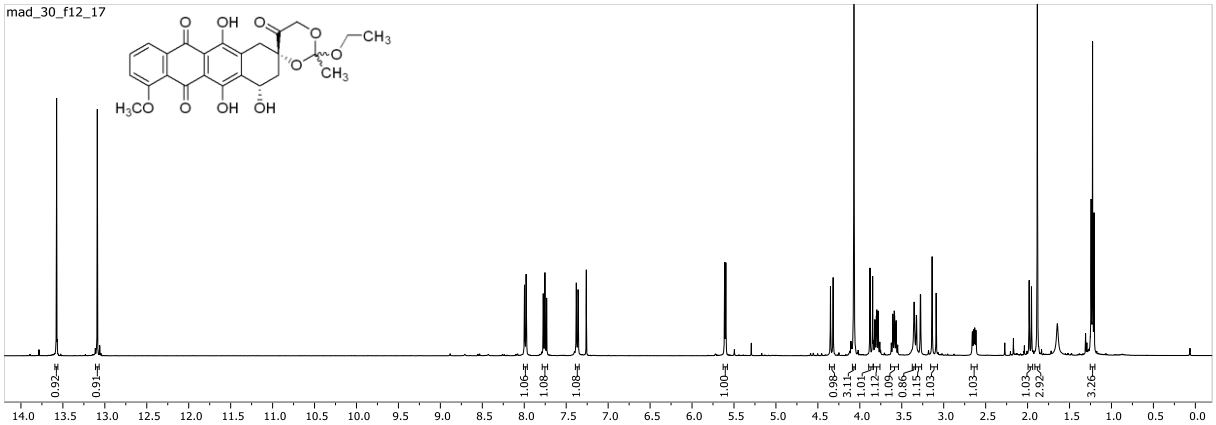

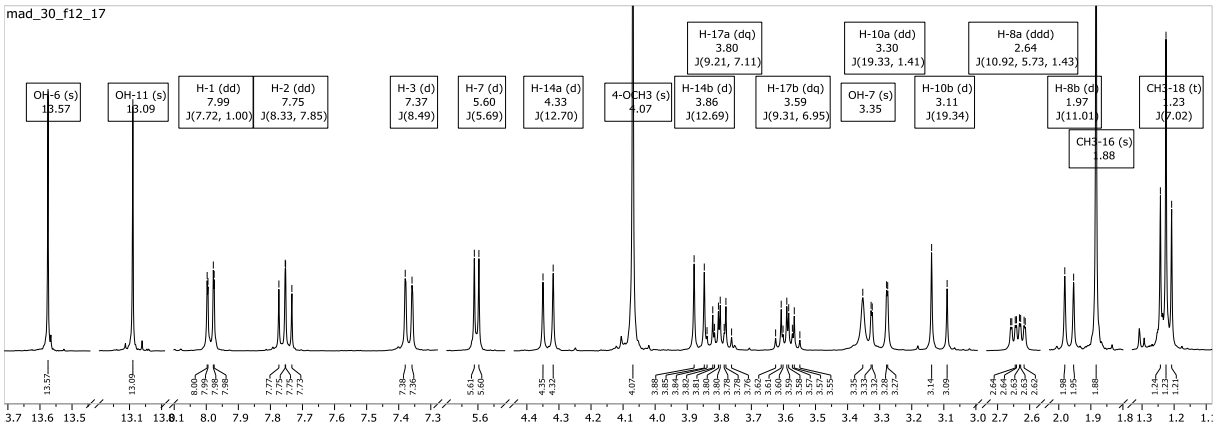

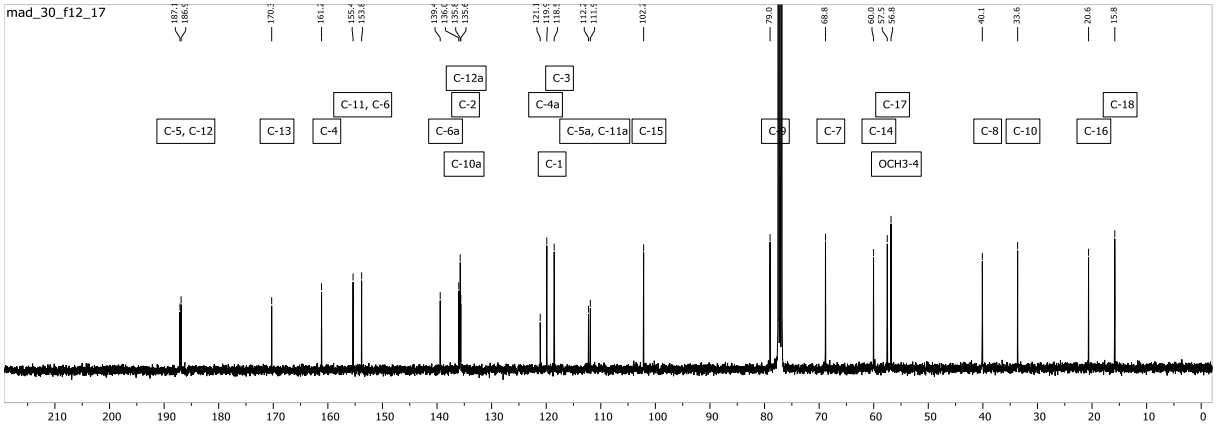

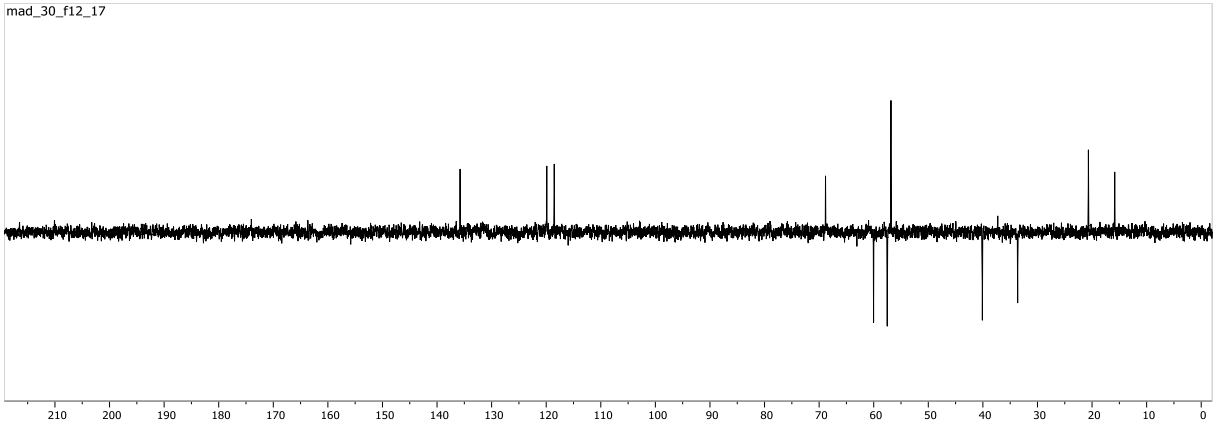

Generic Display Report

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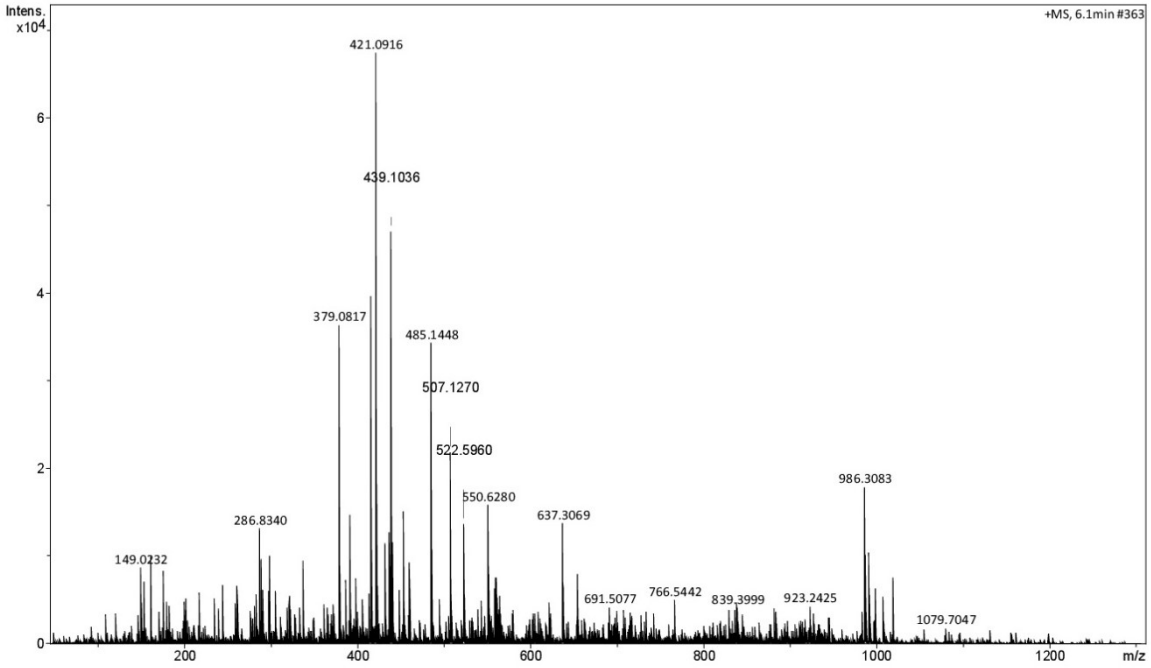
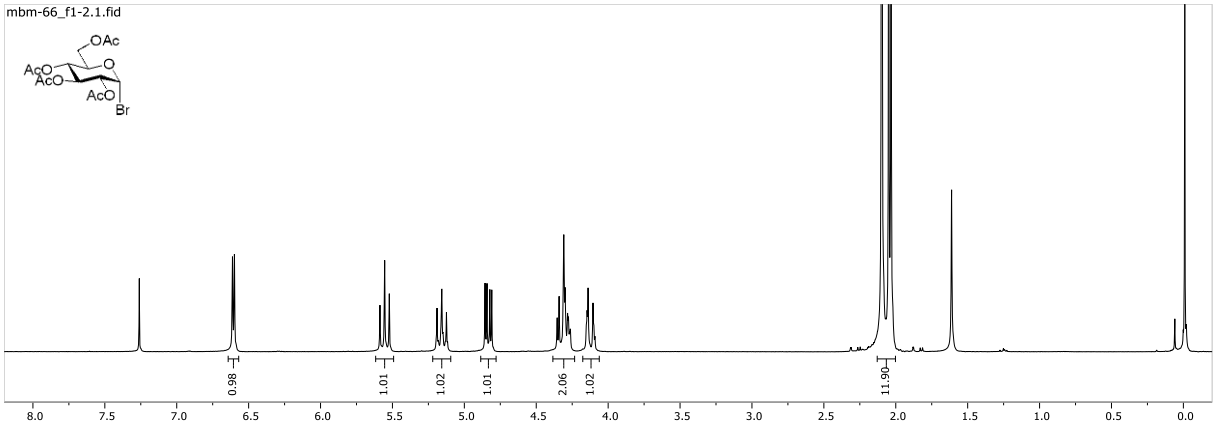
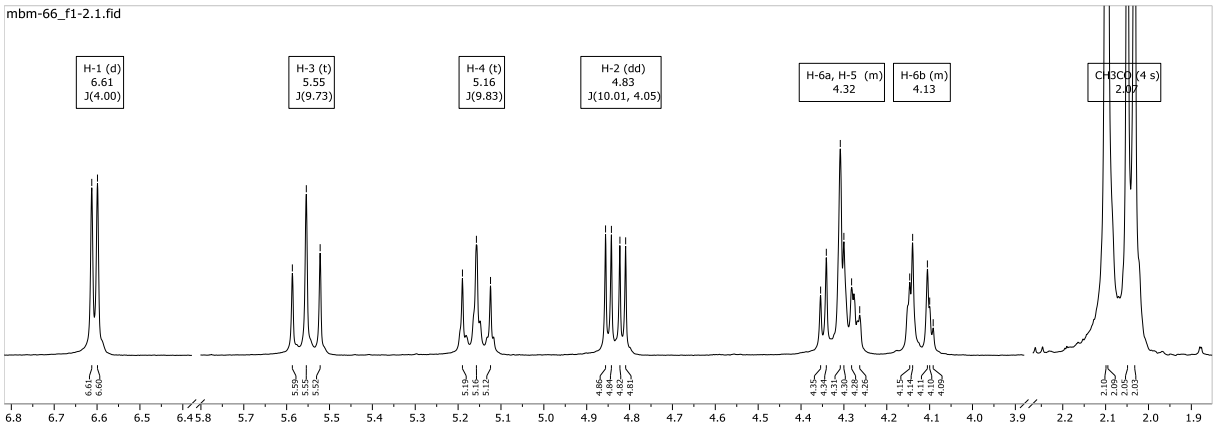
Sample Name	MAD - 30_f 12-17_POS	Instrument	micrOTOF
Comment			

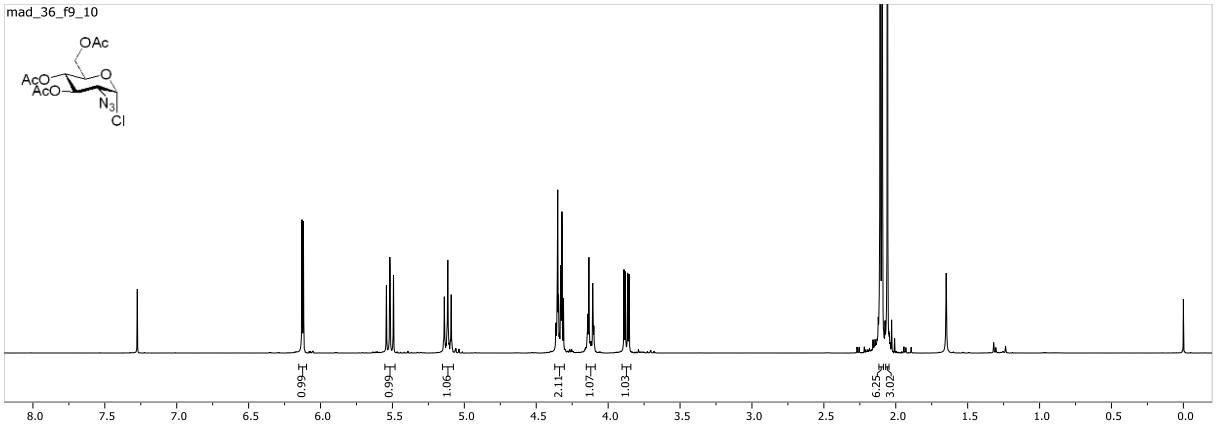


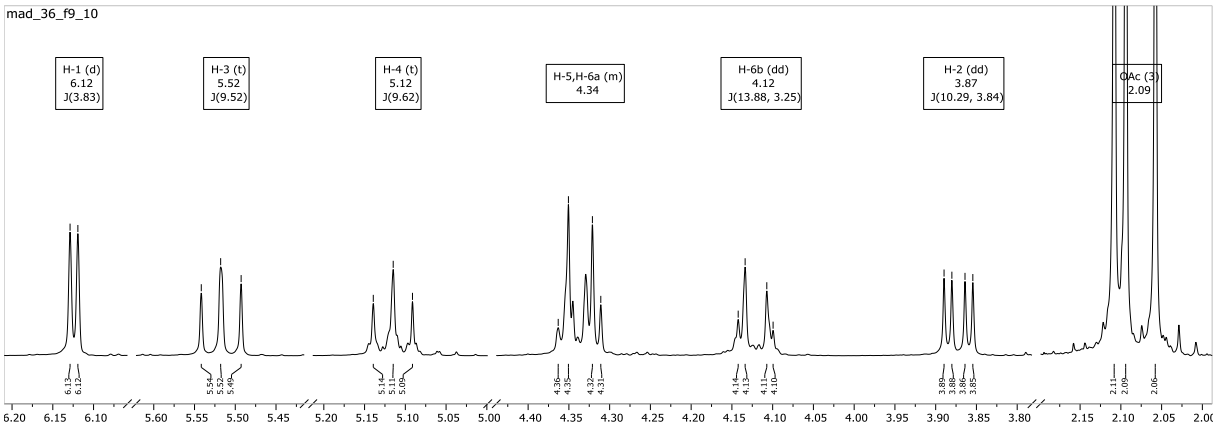
APPENDIX 15. Compound **18**



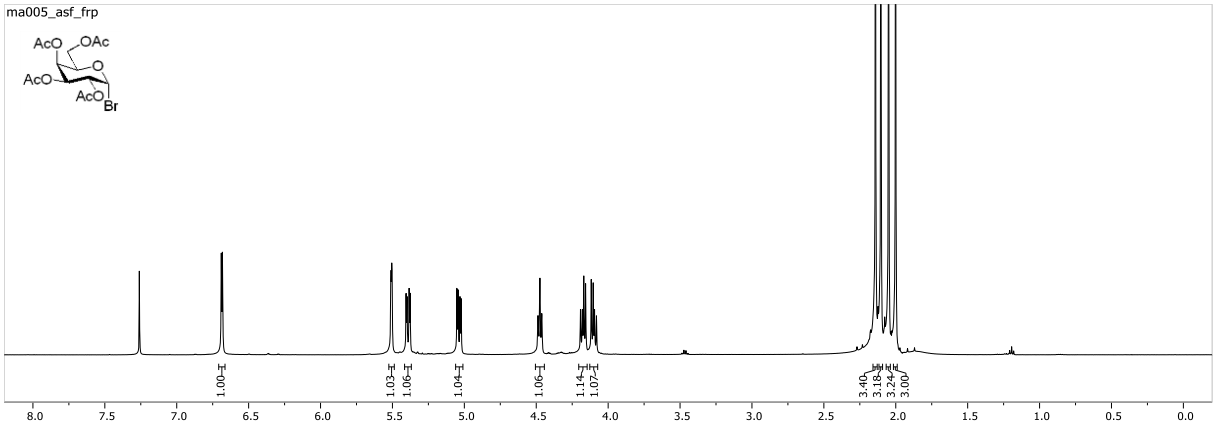


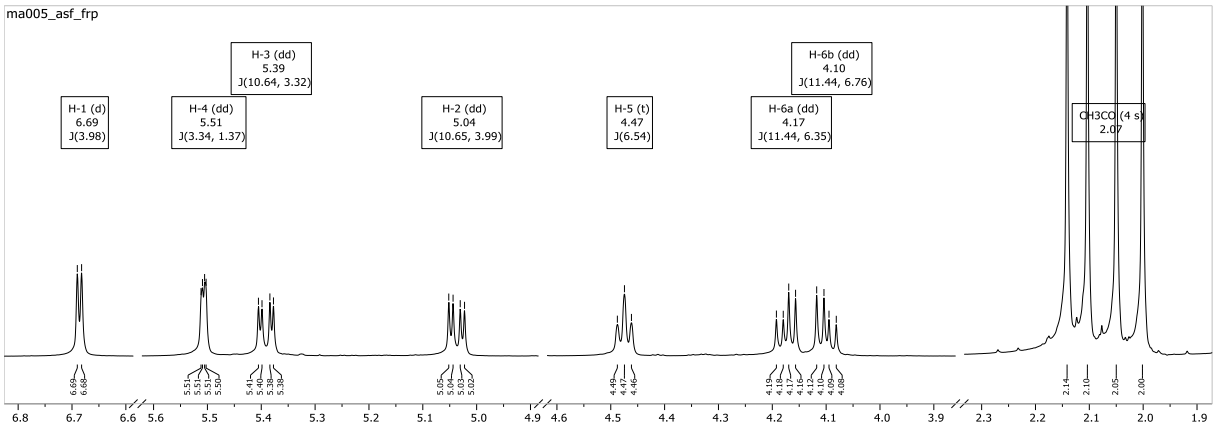
APPENDIX 16. Compound **20**



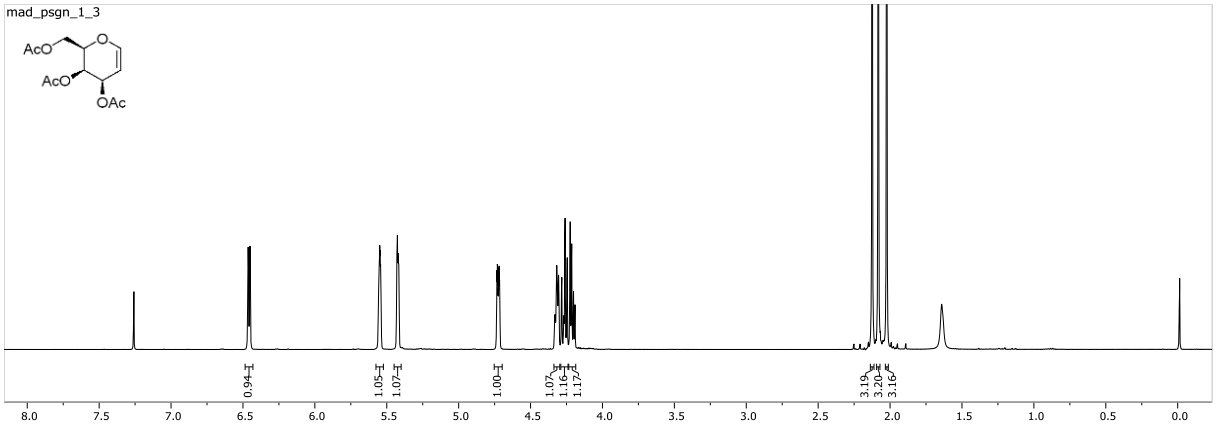


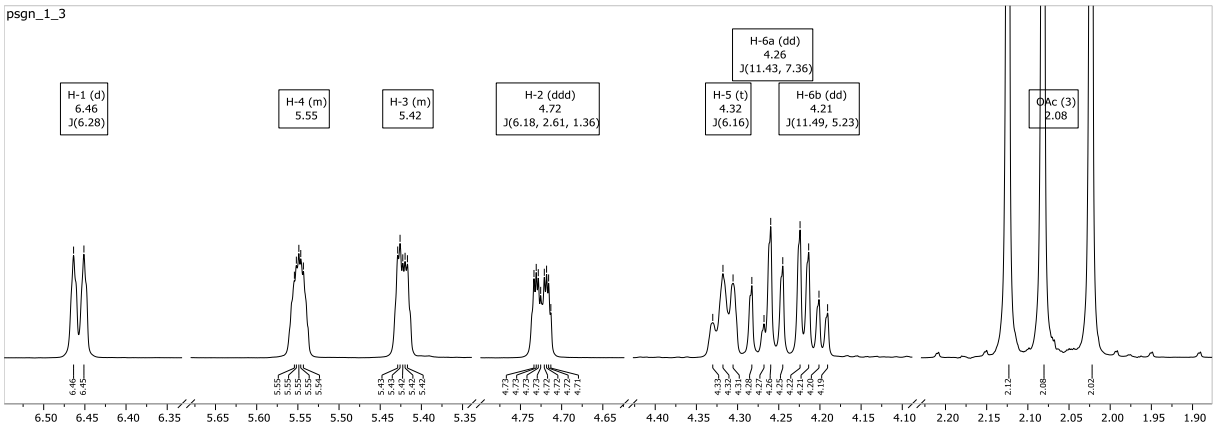
APPENDIX 17. Compound **22**



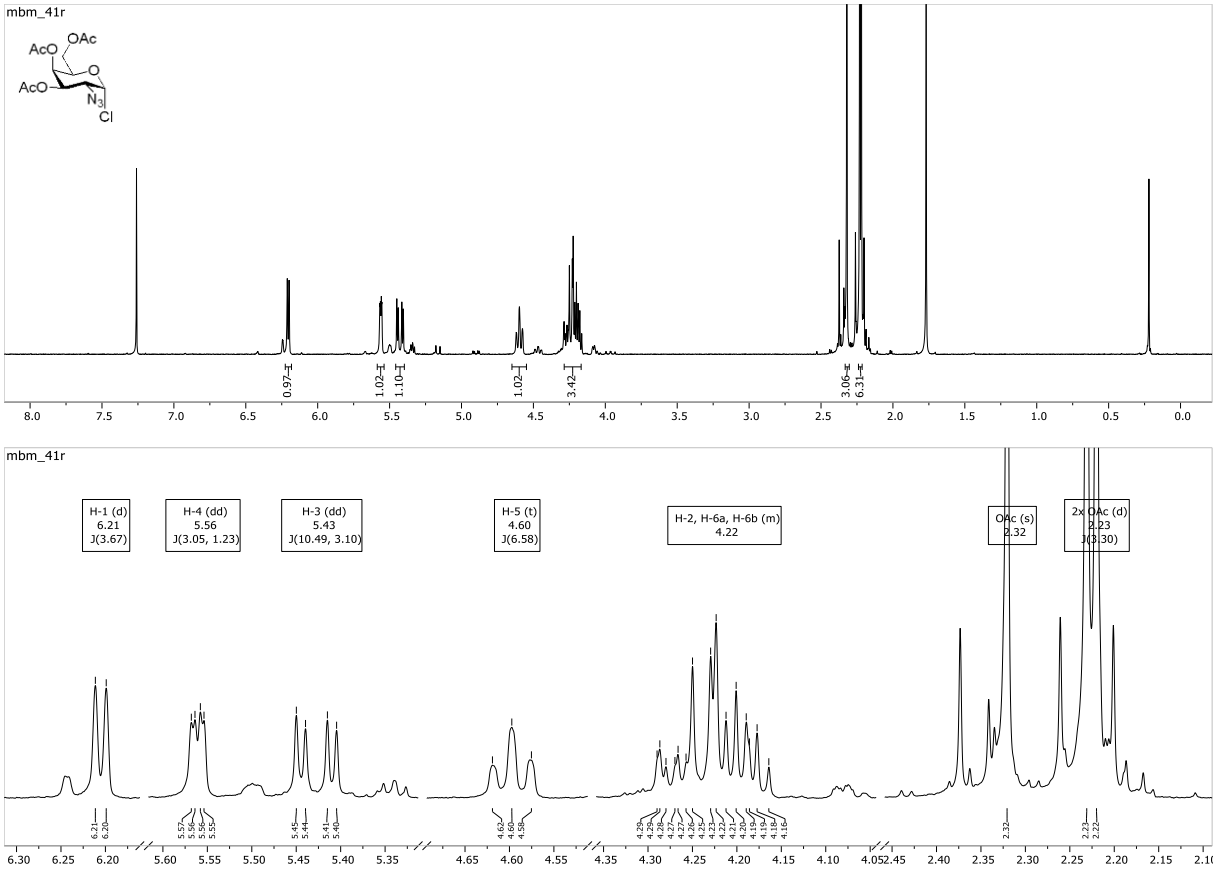


APPENDIX 18. Compound **23**

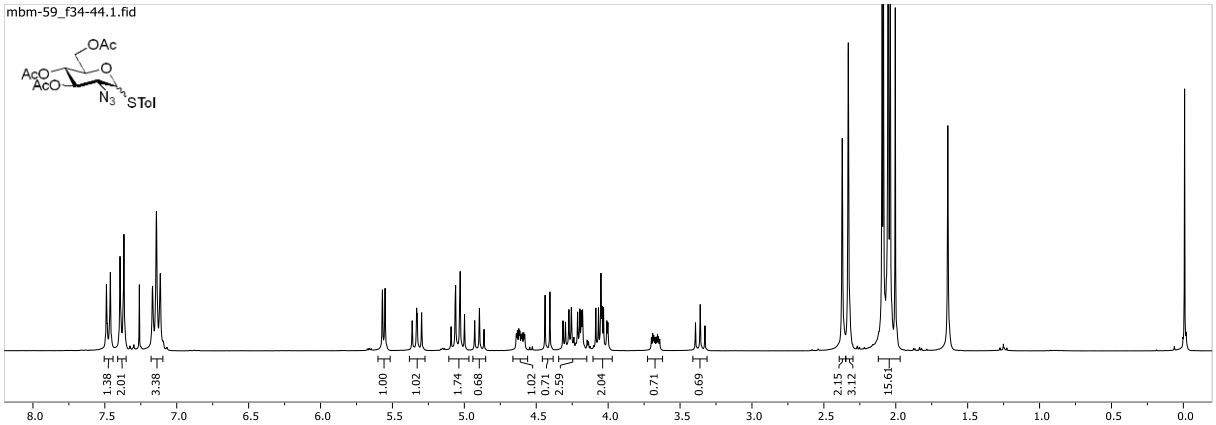


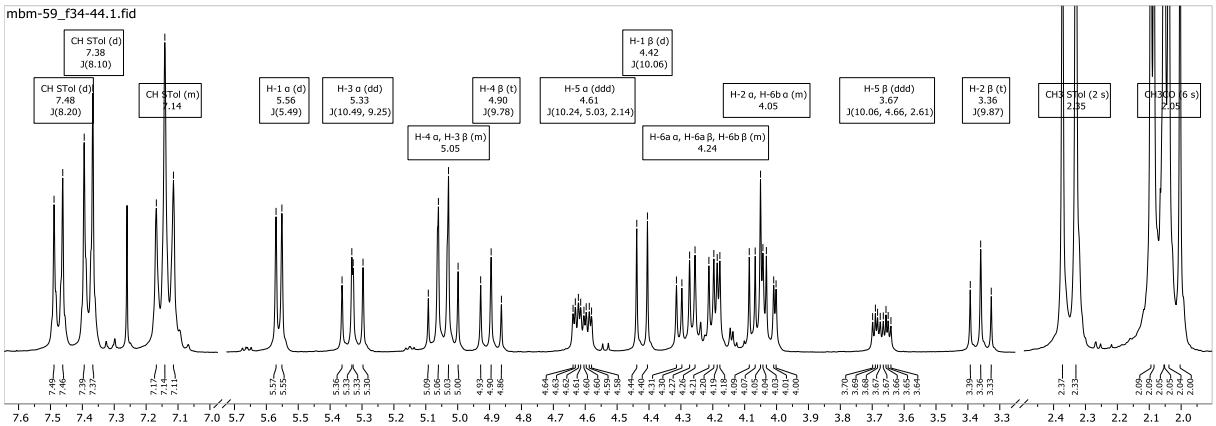


APPENDIX 19. Compound **25**

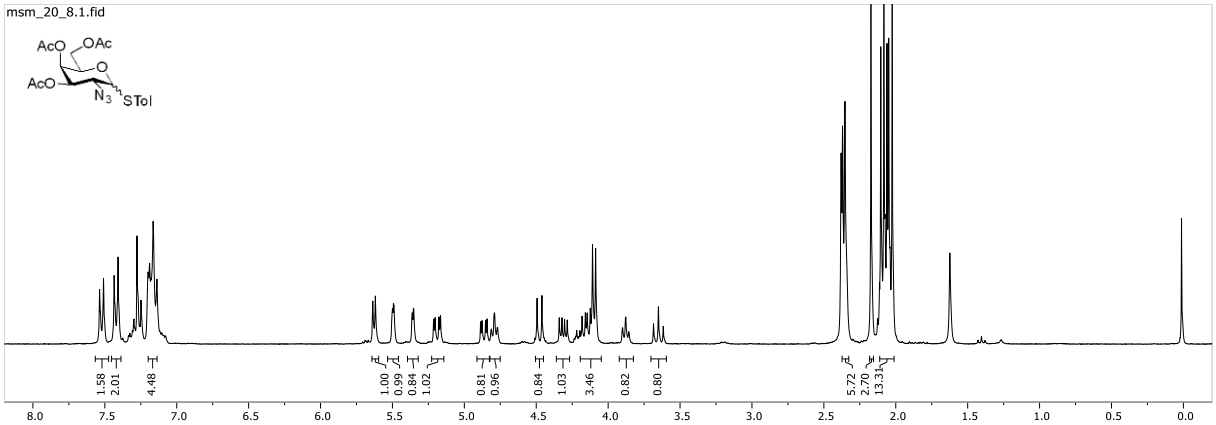


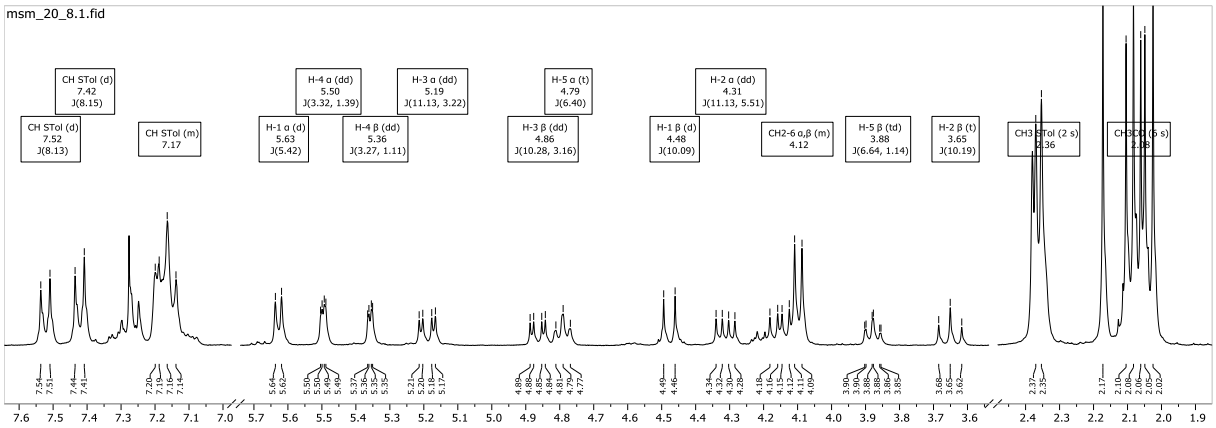
APPENDIX 20. Compound **26**



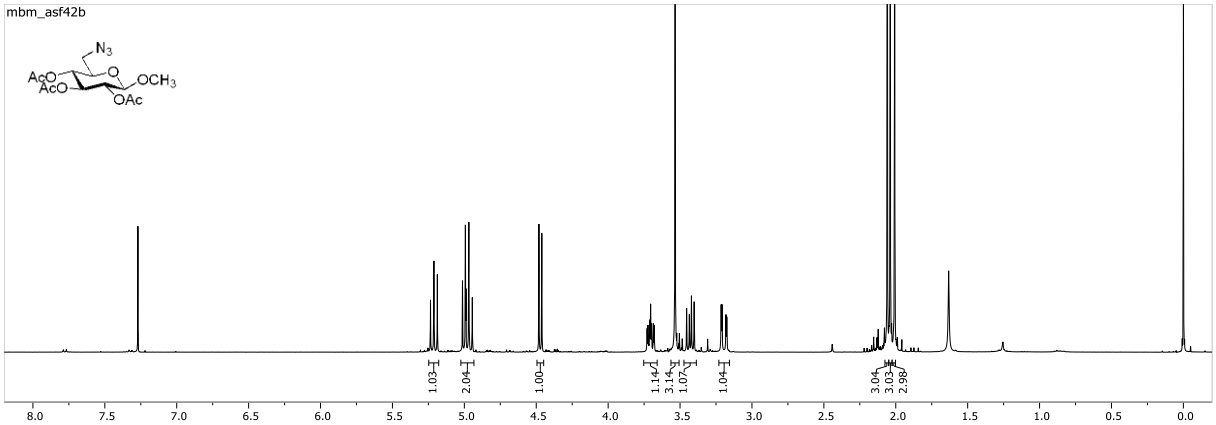


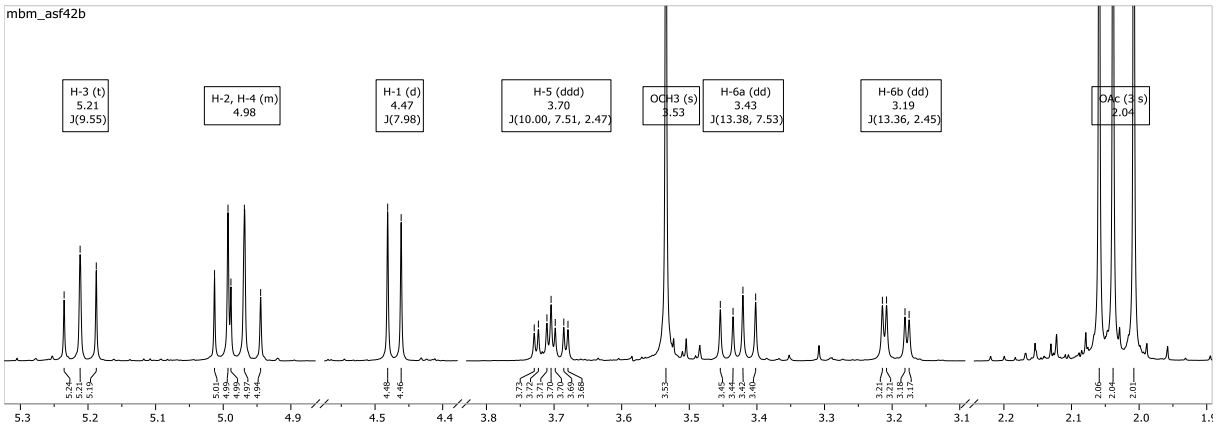
APPENDIX 21. Compound **28**



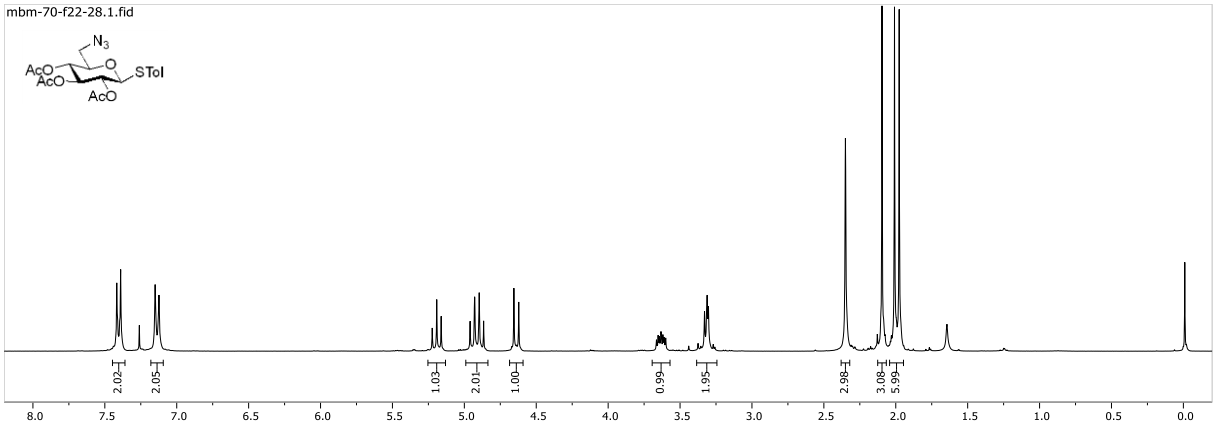


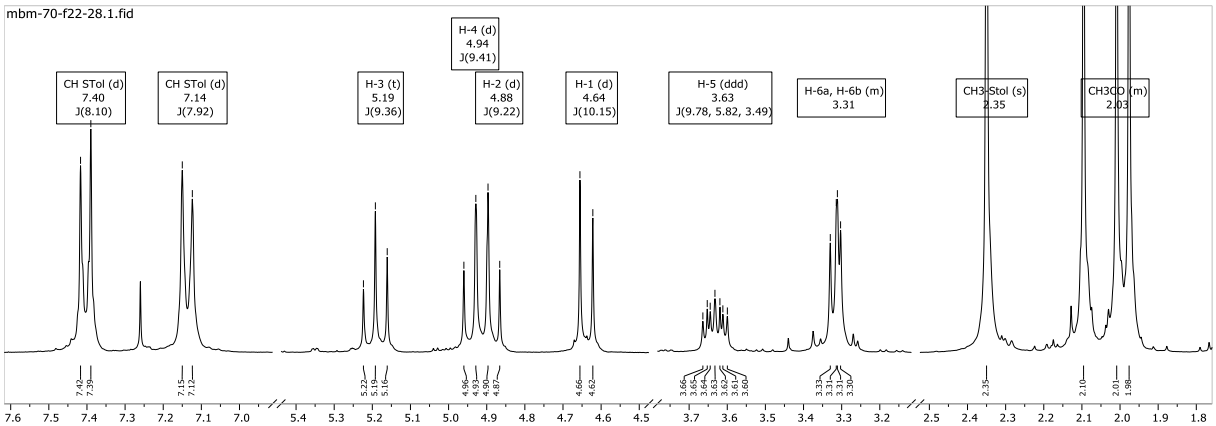
APPENDIX 22. Compound **30**



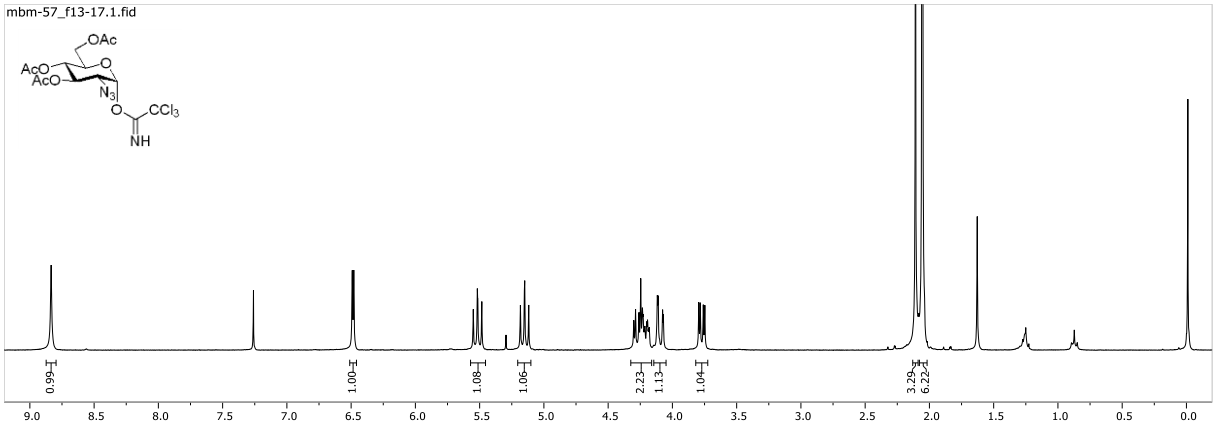


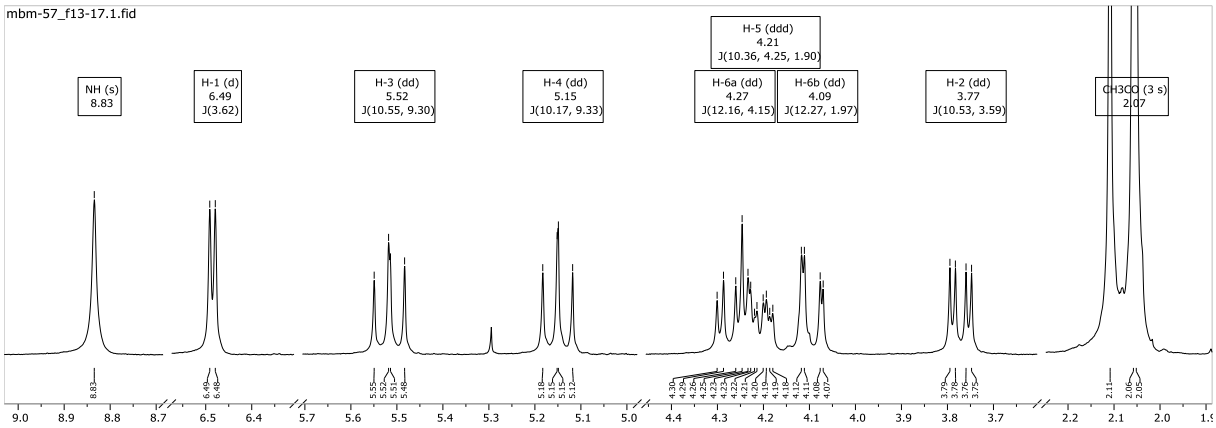
APPENDIX 23. Compound **32**



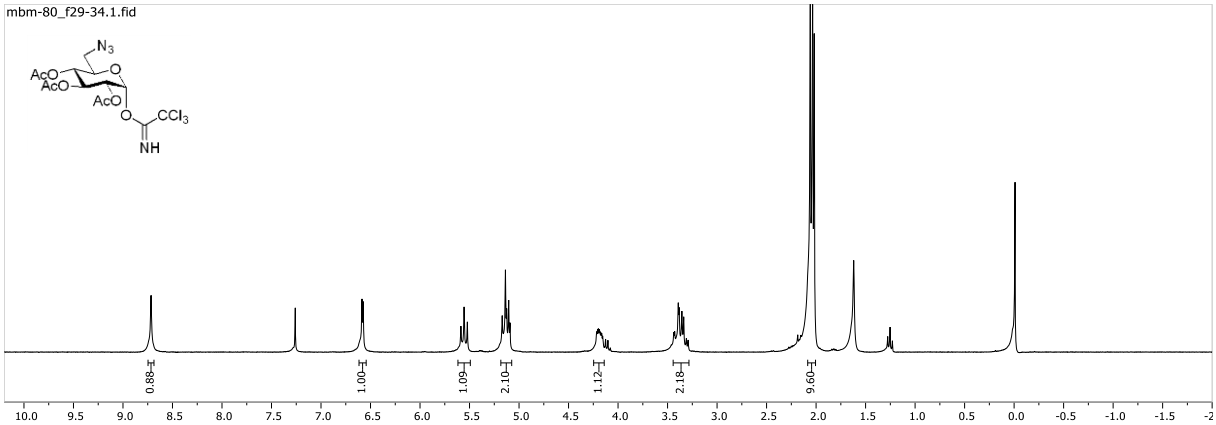


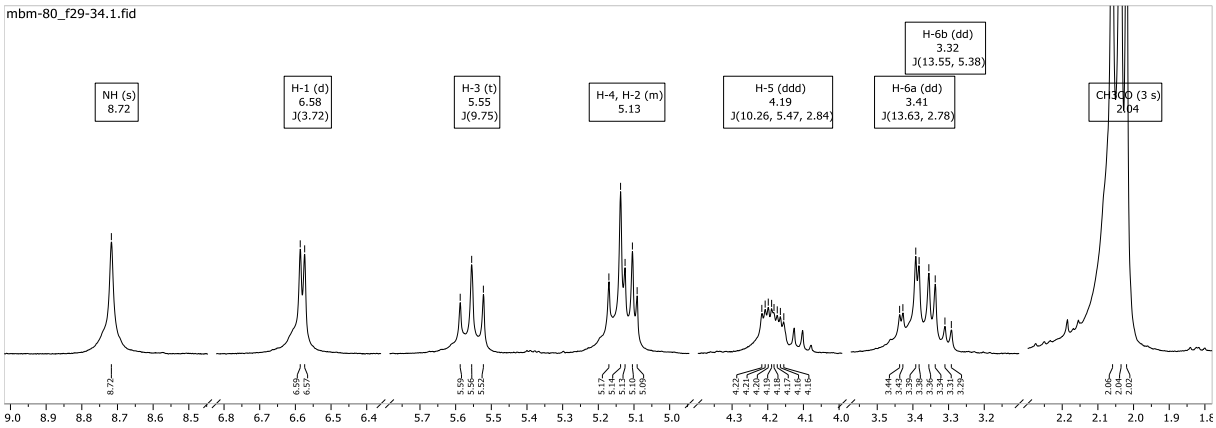
APPENDIX 24. Compound **34**



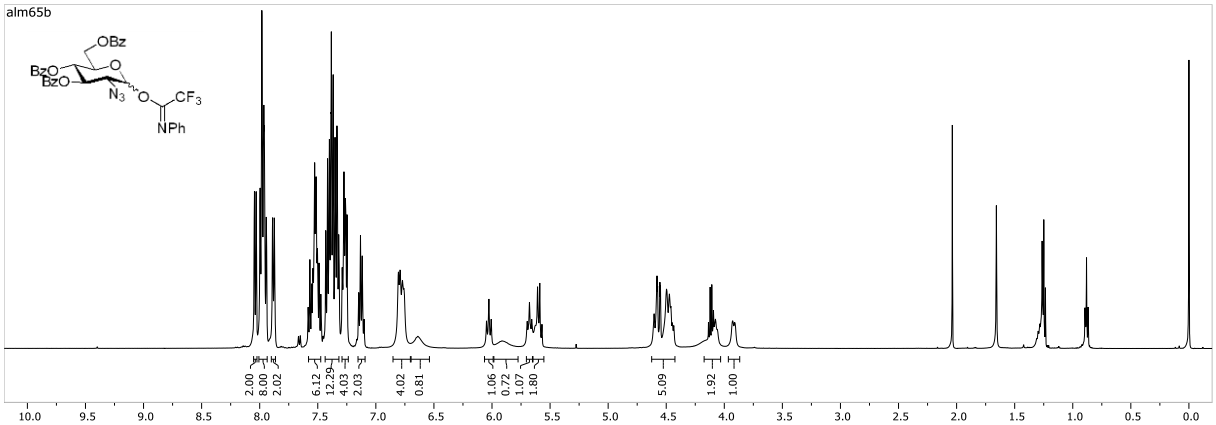


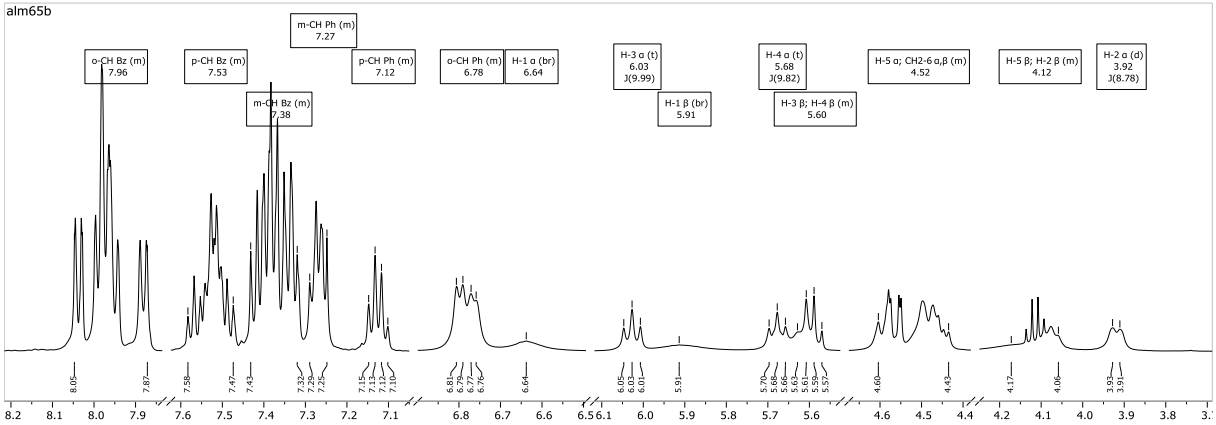
APPENDIX 25. Compound **36**

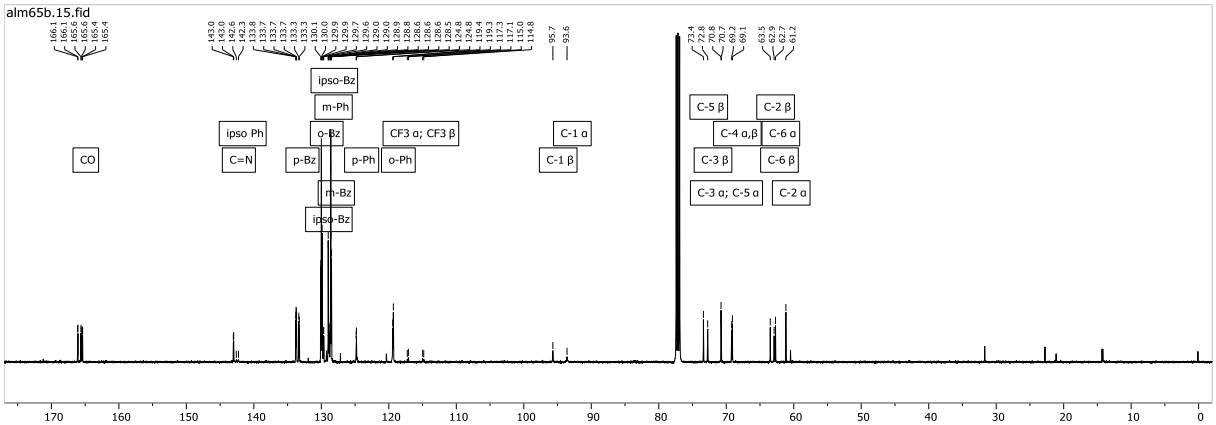


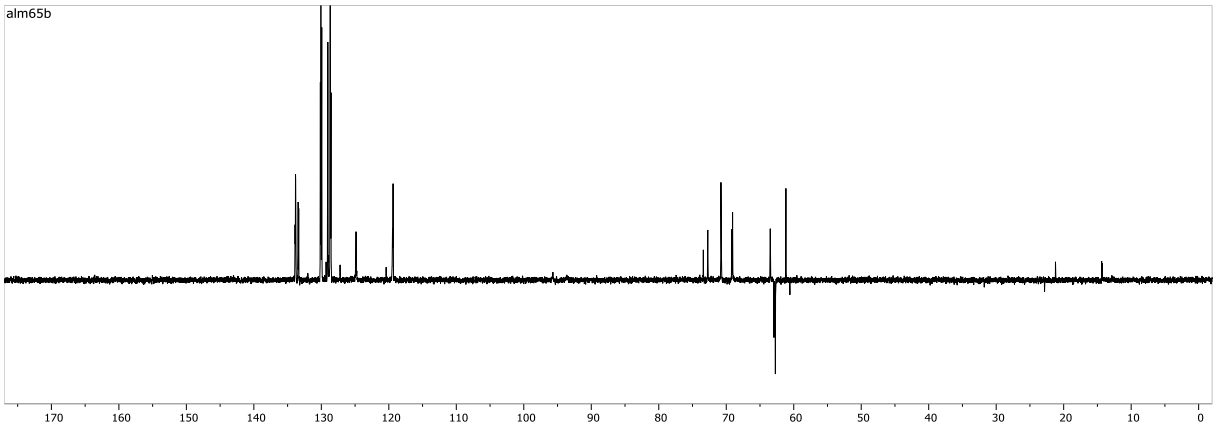


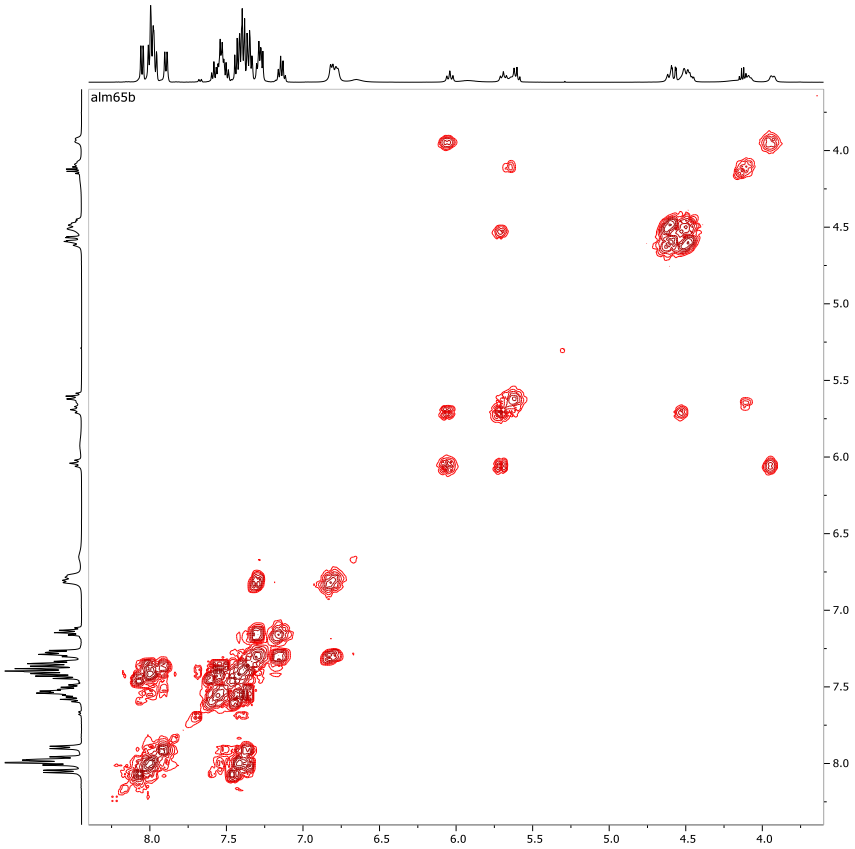
APPENDIX 26. Compound **37**










alm65b
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4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.0
7.5
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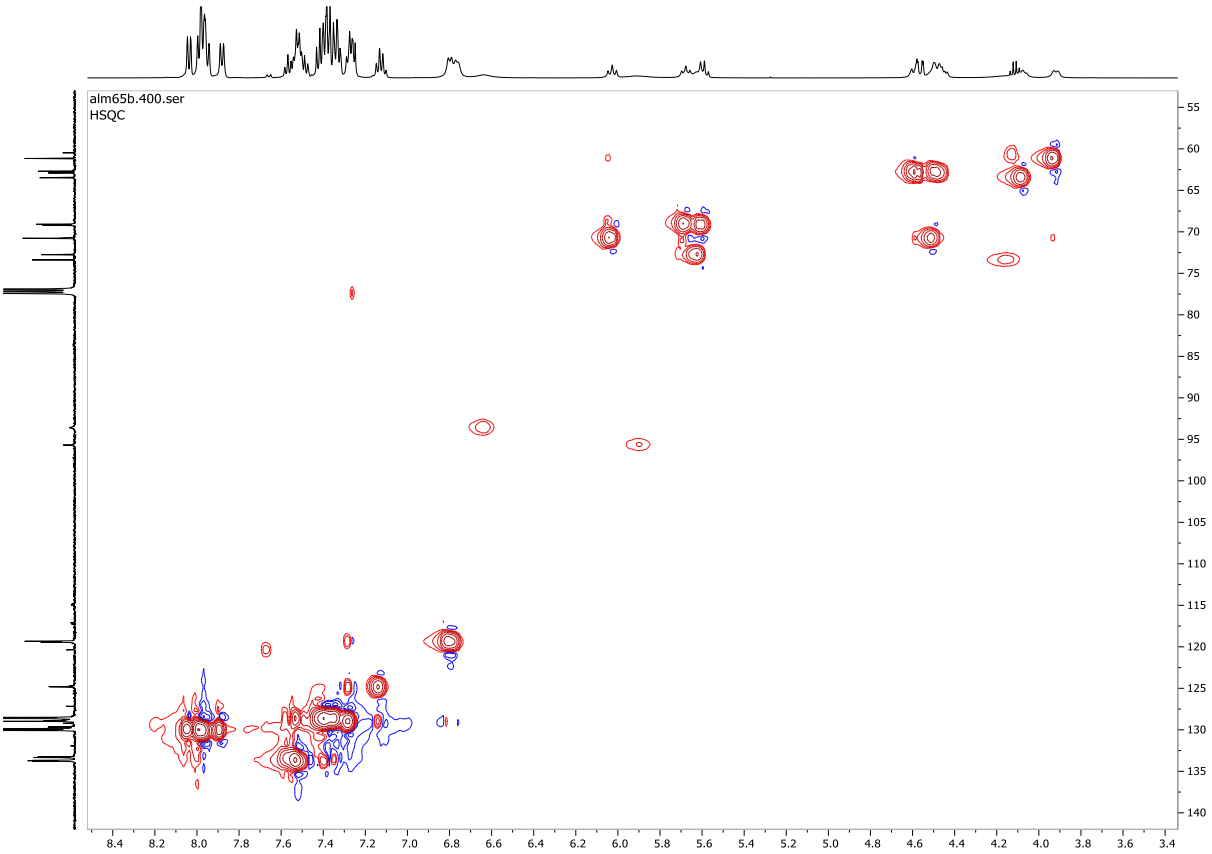
6.5
6.0
5.5
5.0
4.5
4.0


alm65b.400.ser
HSQC
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60
65
70
75
80
85
90
95
100
105
110
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120
125
130
135
140
8.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
5.8
5.6
5.4
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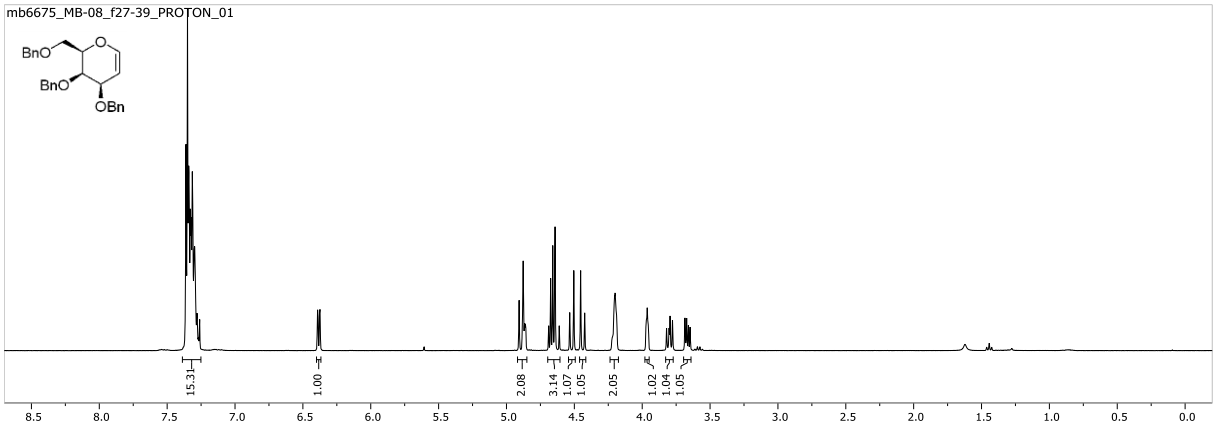
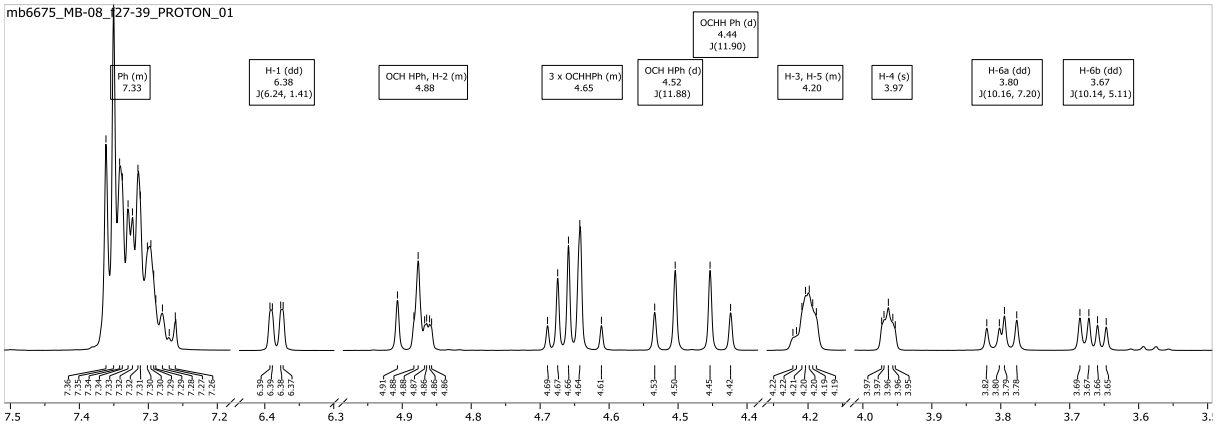
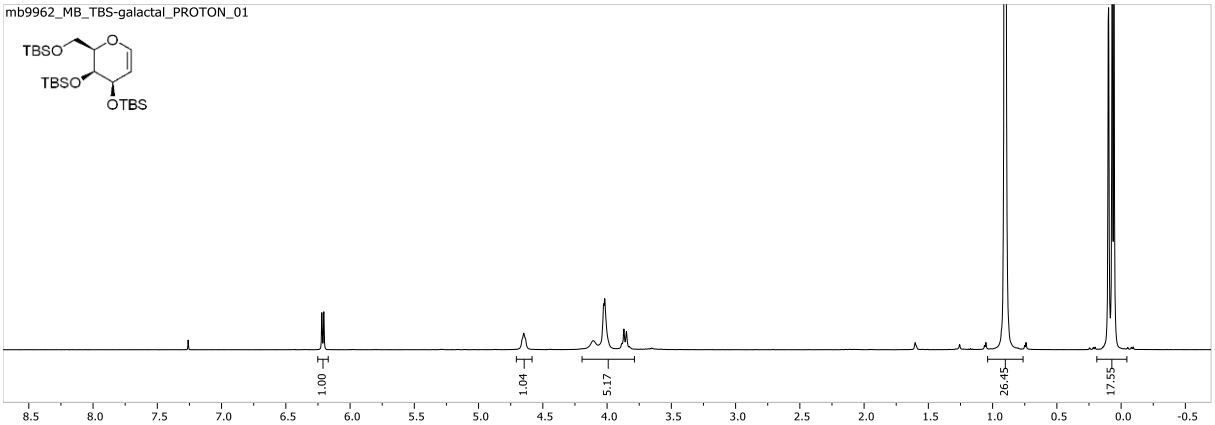
4.0
3.8
3.6
3.4

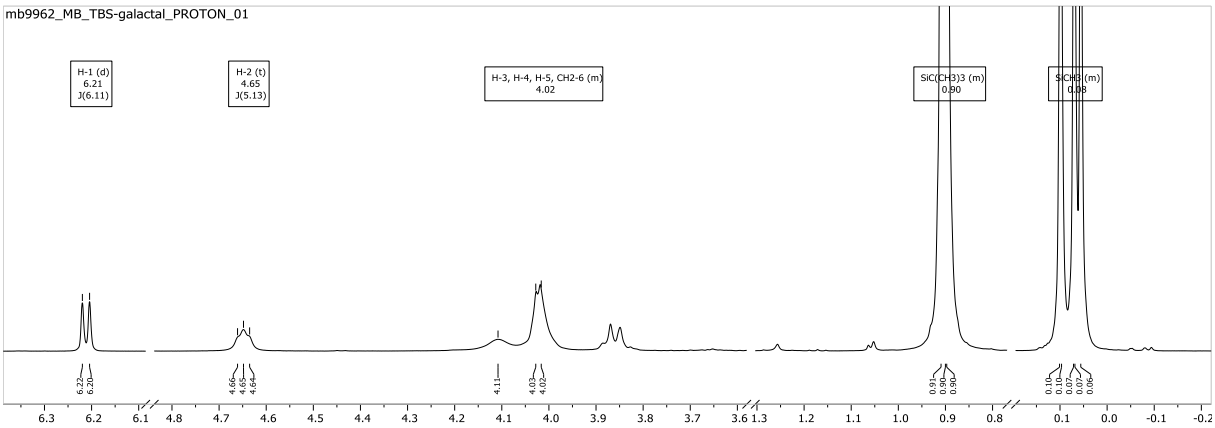
APPENDIX 27. Compound **38**



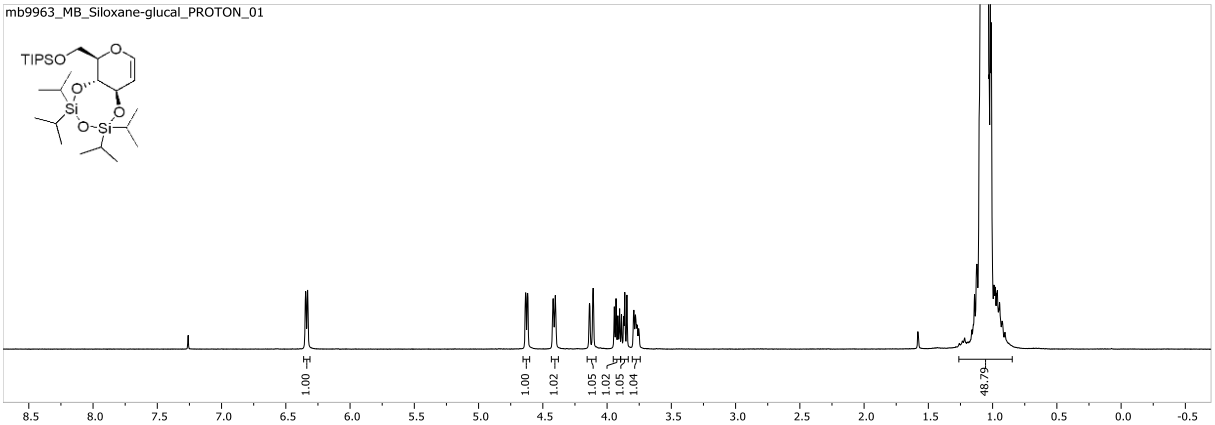


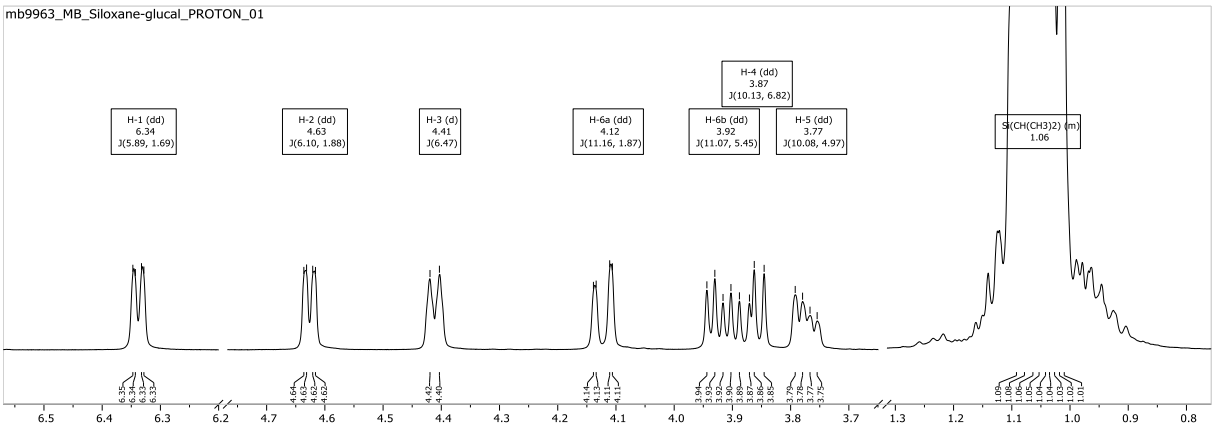
APPENDIX 28. Compound **39**



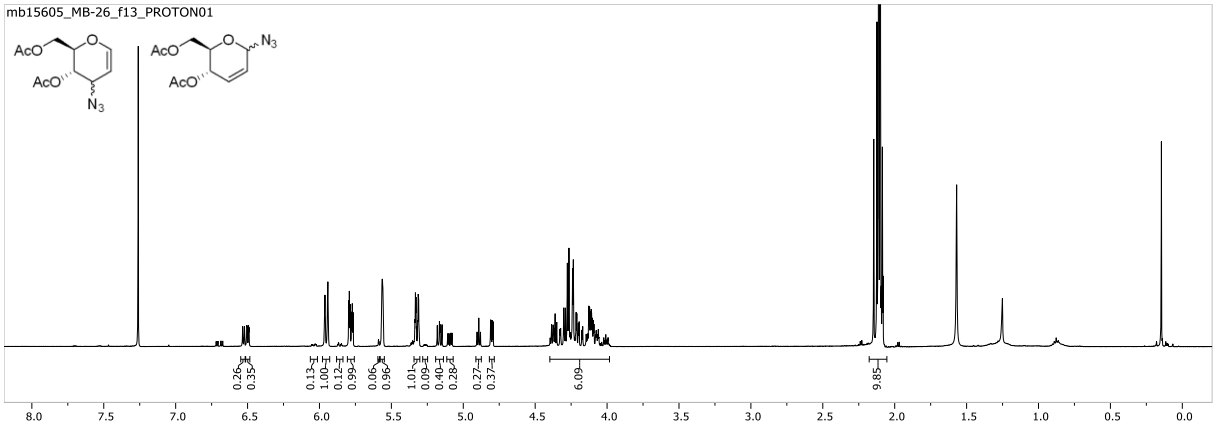


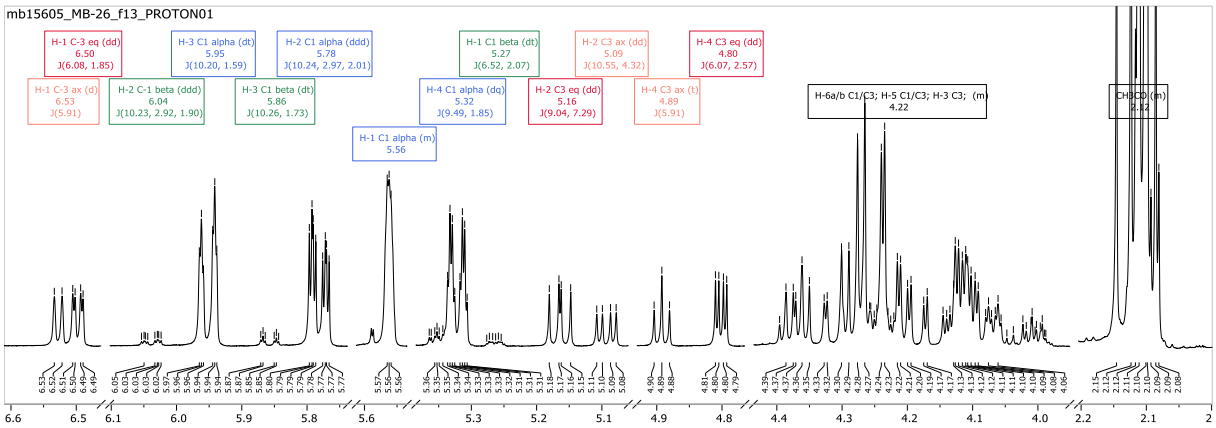
APPENDIX 29. Compound **40**



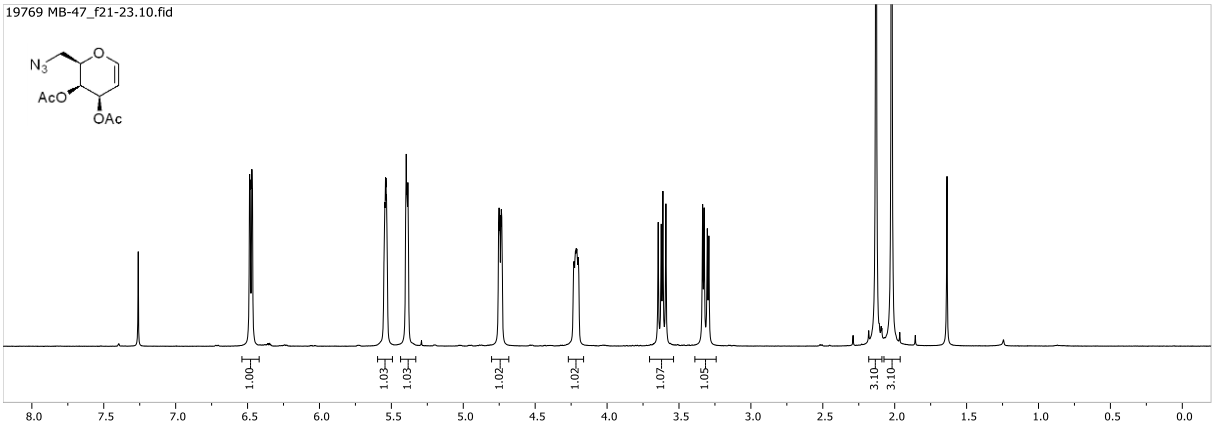


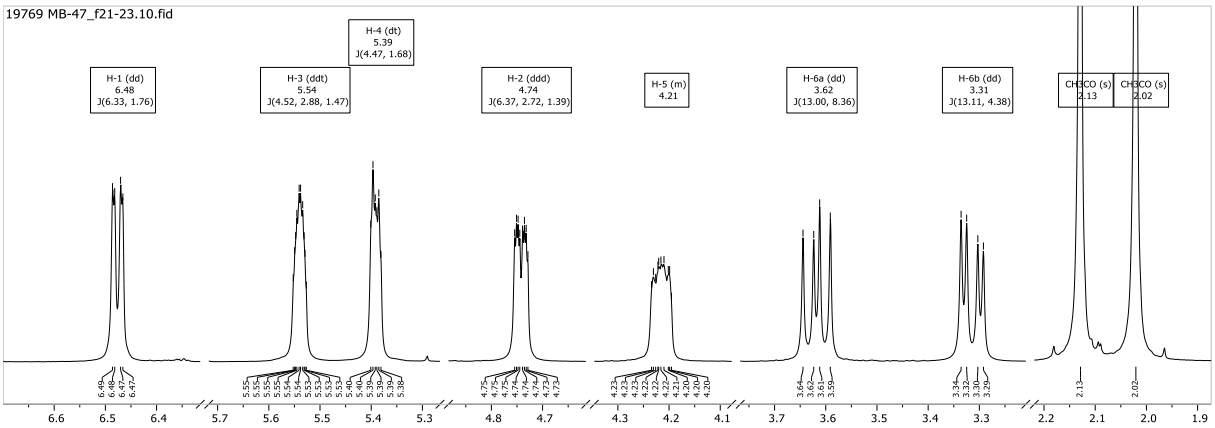
APPENDIX 30. Compounds **41**

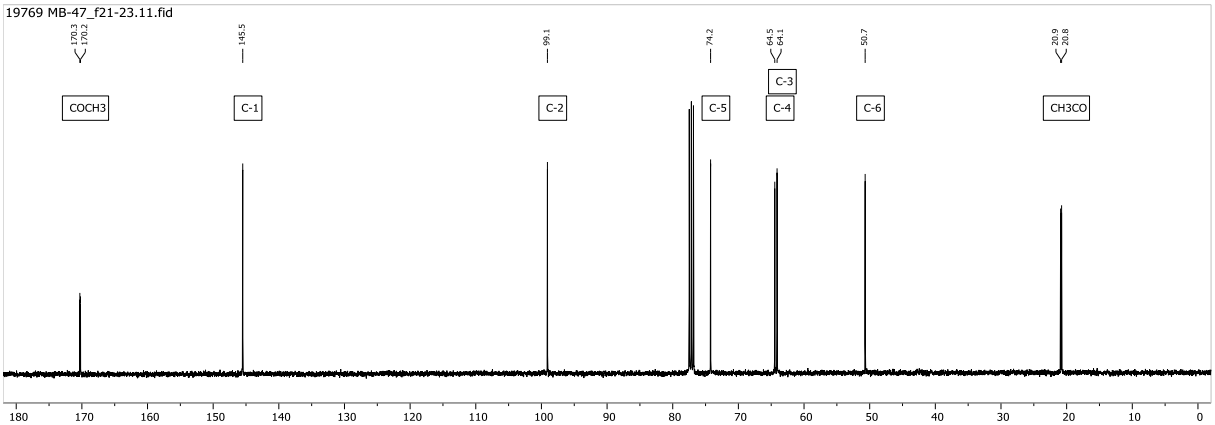




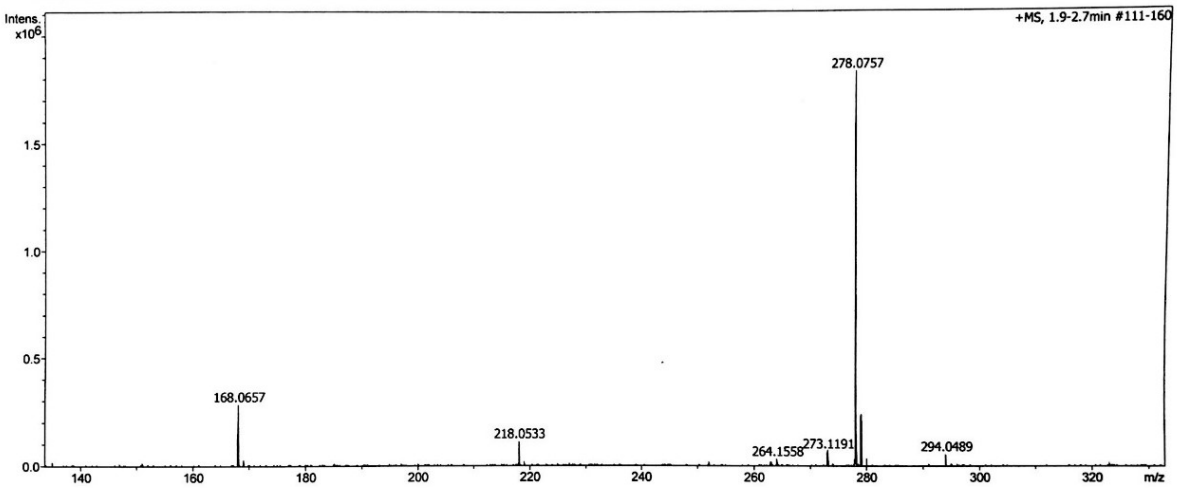
APPENDIX 31. Compound **43**





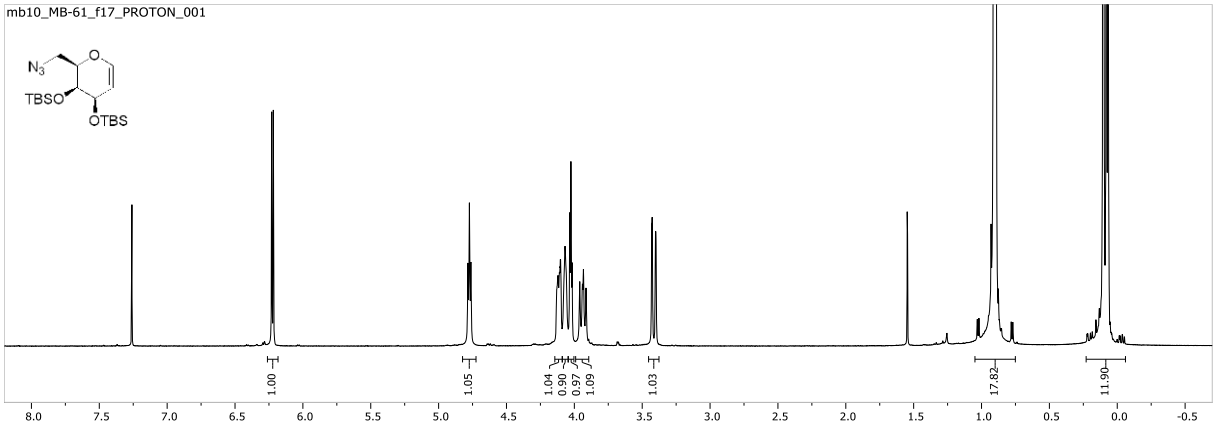


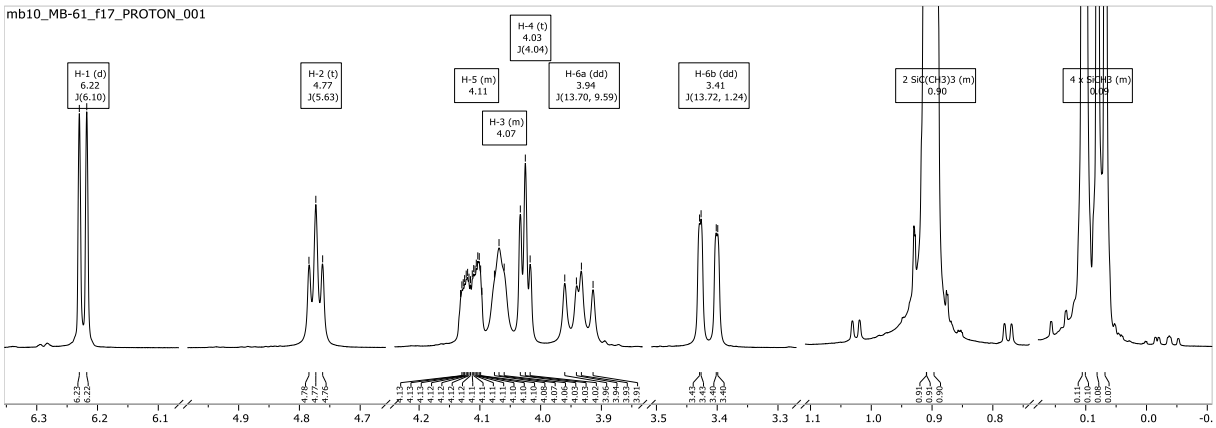
Analysis Name	D:\Data\msservice\cg-mb-11656.d				
Method	Verify Low Mass Compound by Loop MS Positive.m		Operator	MS Service	
Sample Name	cg-mb-11656_13855		Instrument	micrOTOF	213750.10405
Comment	Test sample 1				
Source Type	ESI	Ion Polarity	Positive		

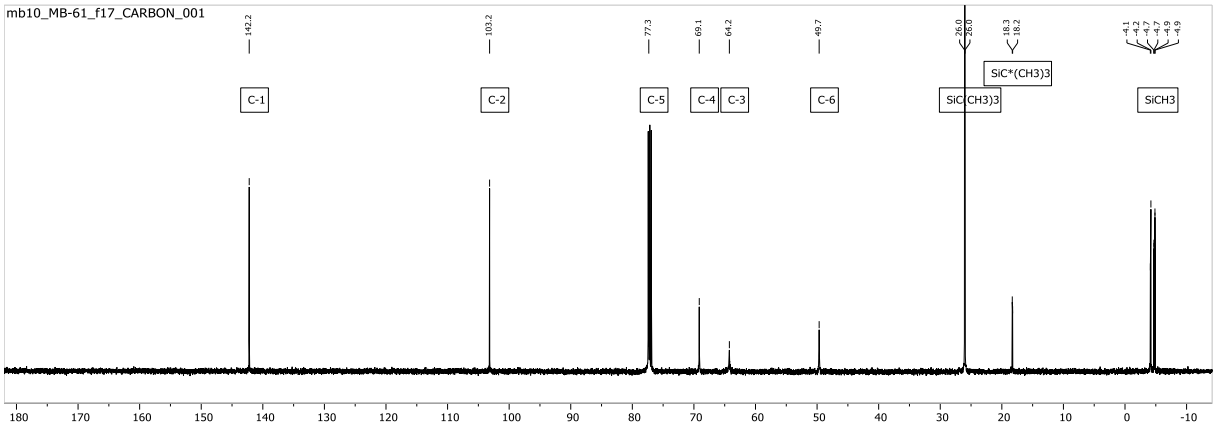


Meas. m/z	#	Ion Formula	m/z	err [ppm]	err [mDa]	mSigma	rdb	N-Rule	e⁻ Conf
278.075704	1	C10H13N3NaO5	278.074741	3.5	1.0	4.6	5.5	ok	even

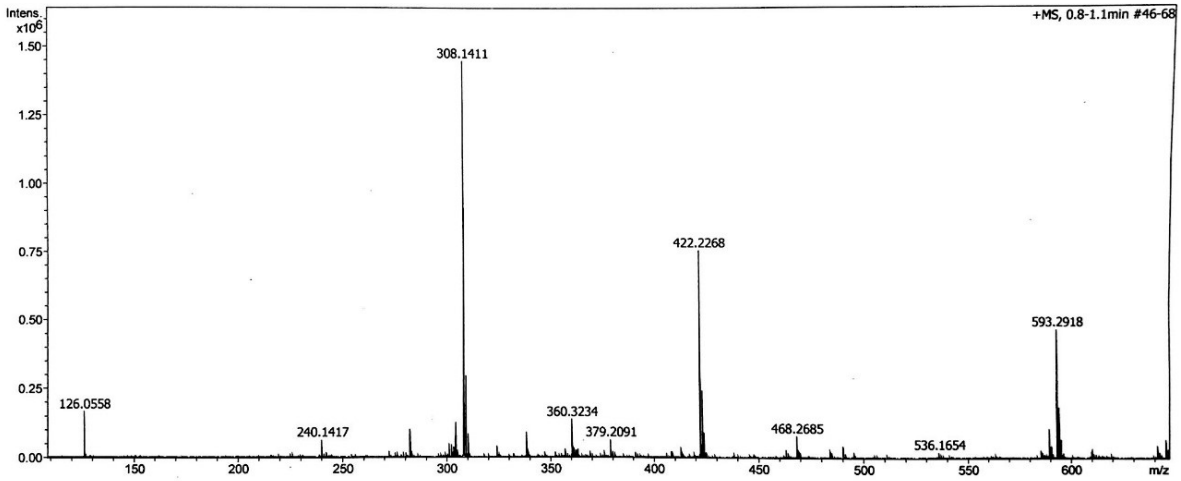
APPENDIX 32. Compound **44**





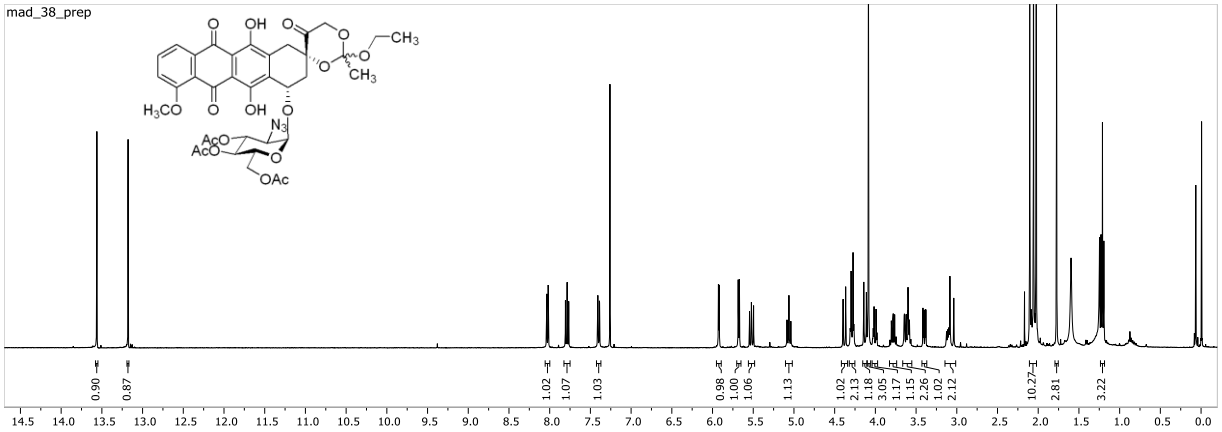


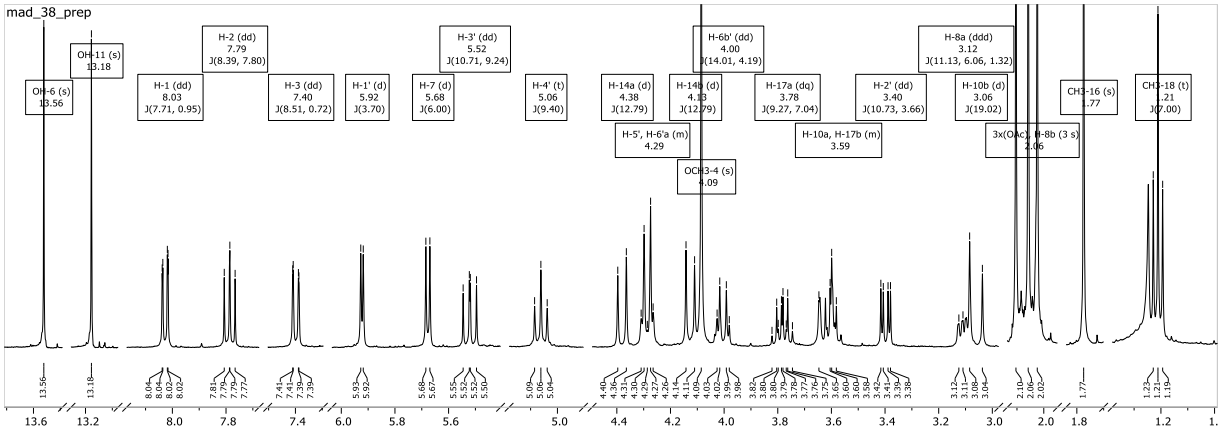
Analysis Name	D:\Data\msservice\cg-mb-12176.d				
Method	Verify Compound by Loop MS Positive.m		Operator	MS Service	
Sample Name	cg-mb-12176_14337		Instrument	micrOTOF	213750.10405
Comment	Test sample 1				
Source Type	ESI	Ion Polarity	Positive		

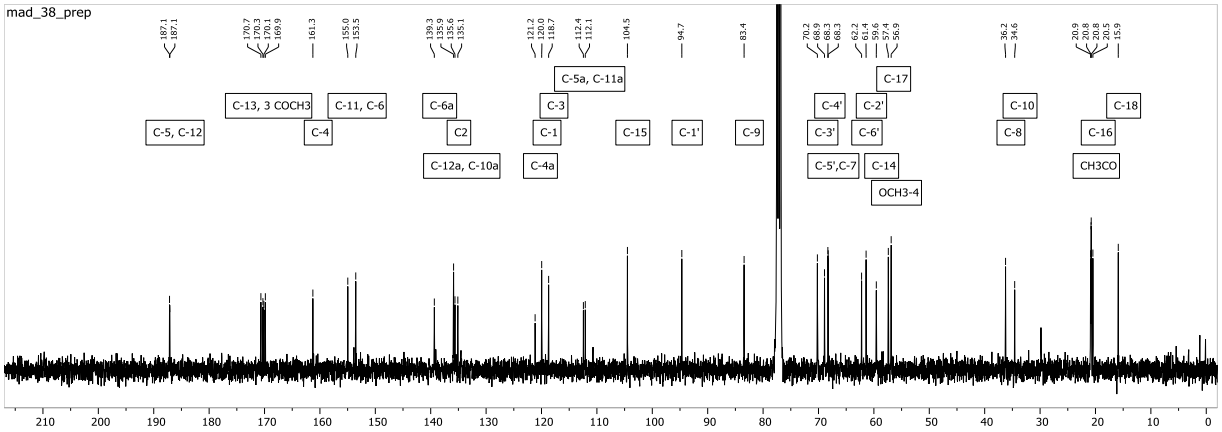


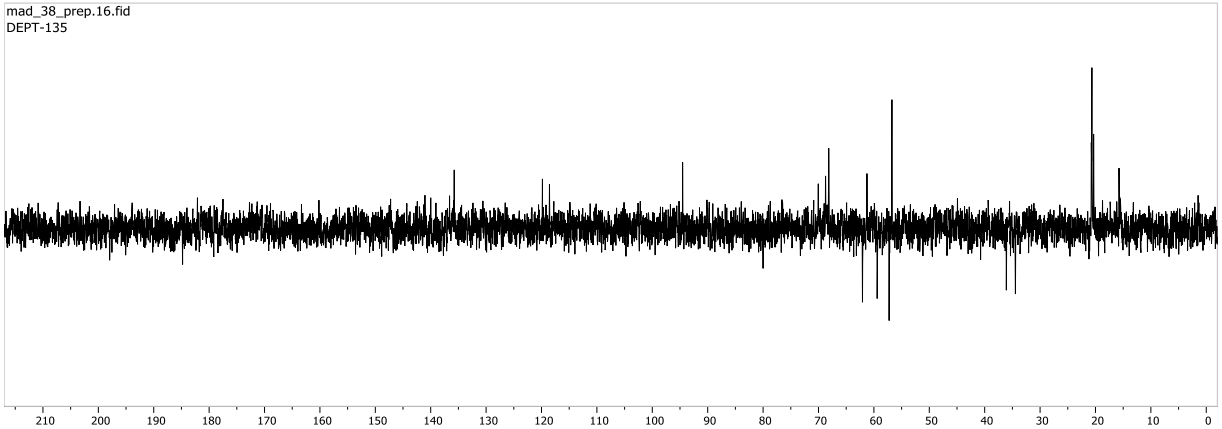
Meas. m/z	#	Ion Formula	m/z	err [ppm]	err [mDa]	mSigma	rdb	N-Rule	e⁻ Conf
422.226764	1	C18H37N3NaO3Si2	422.226566	0.5	0.2	4.4	3.5	ok	even

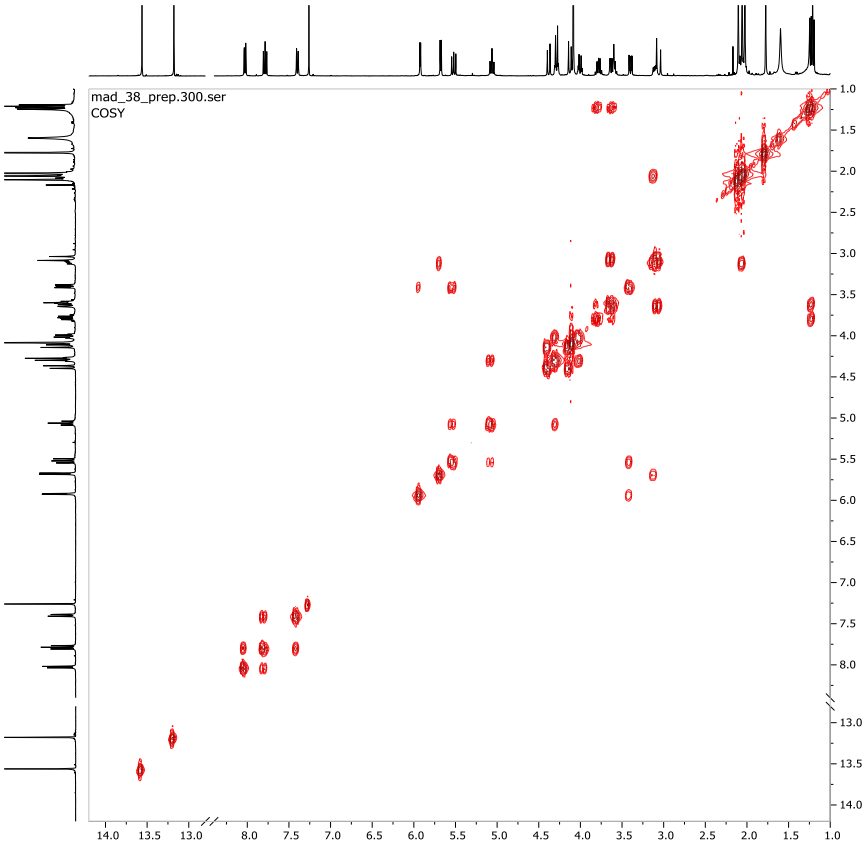
APPENDIX 33. Compound **45**

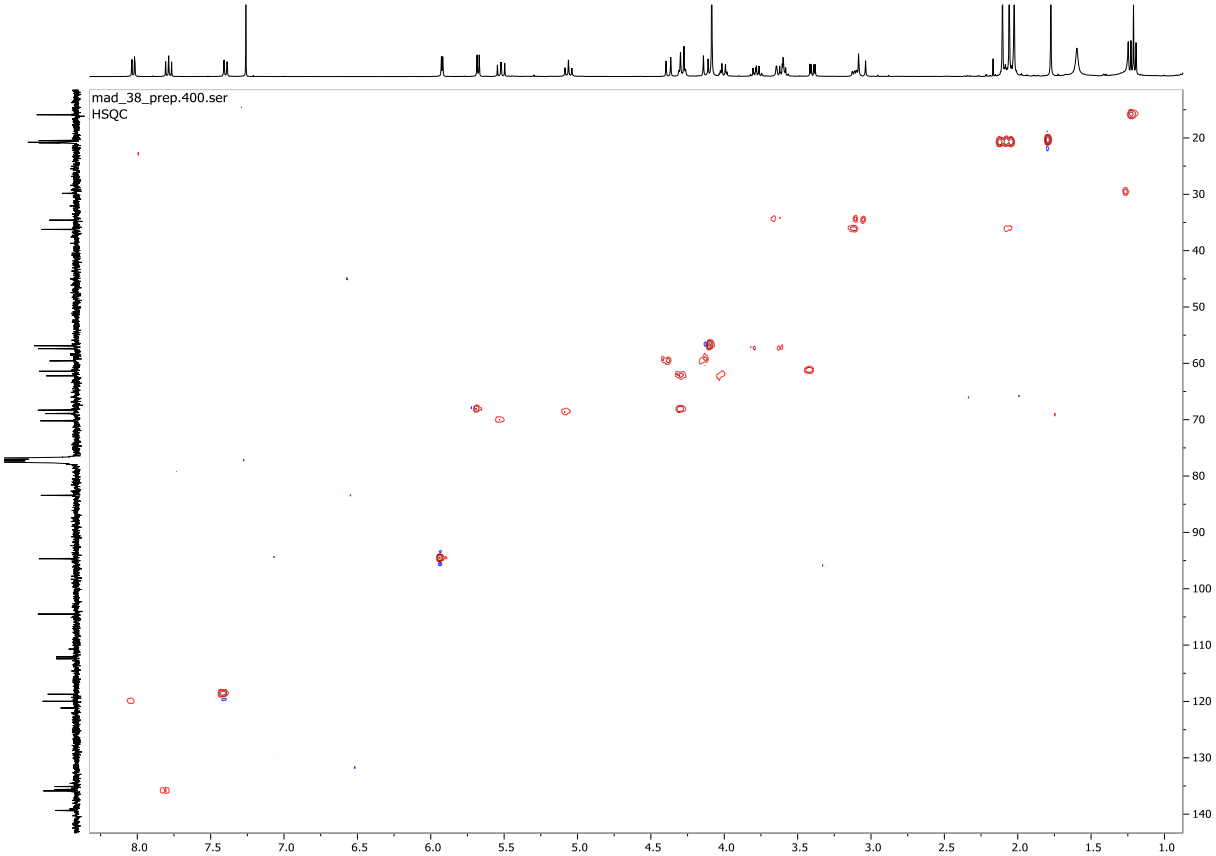








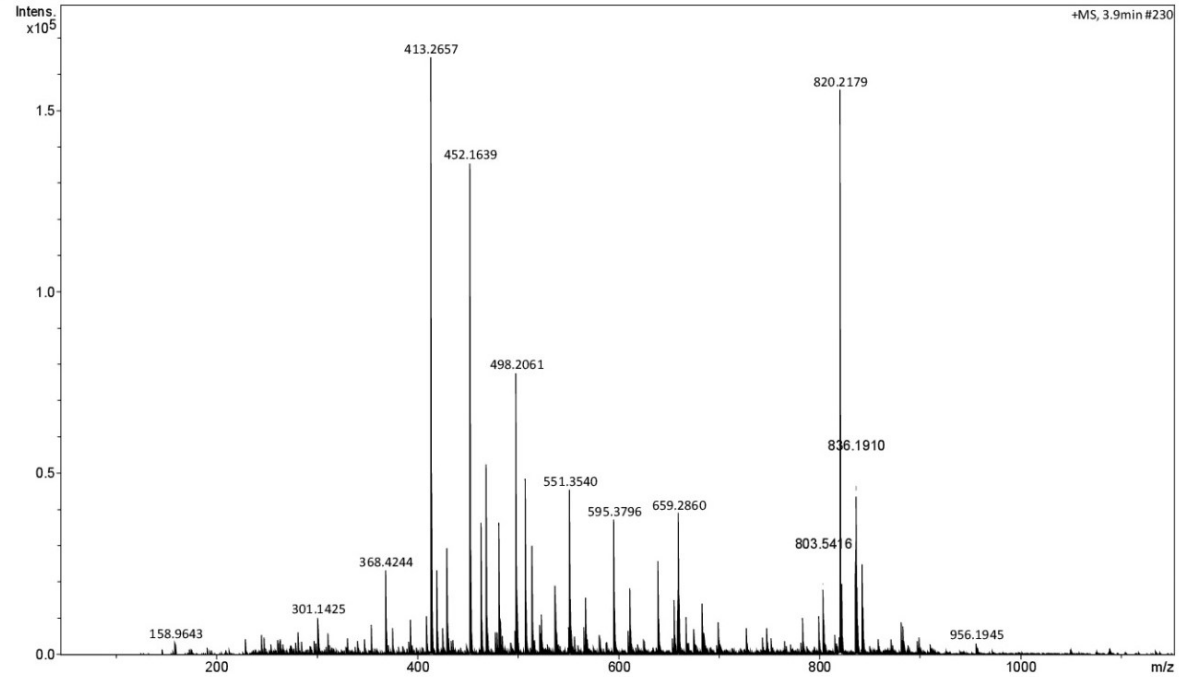

mad_38_prep.300.ser
COSY


mad_38_prep.400.ser
HSQC

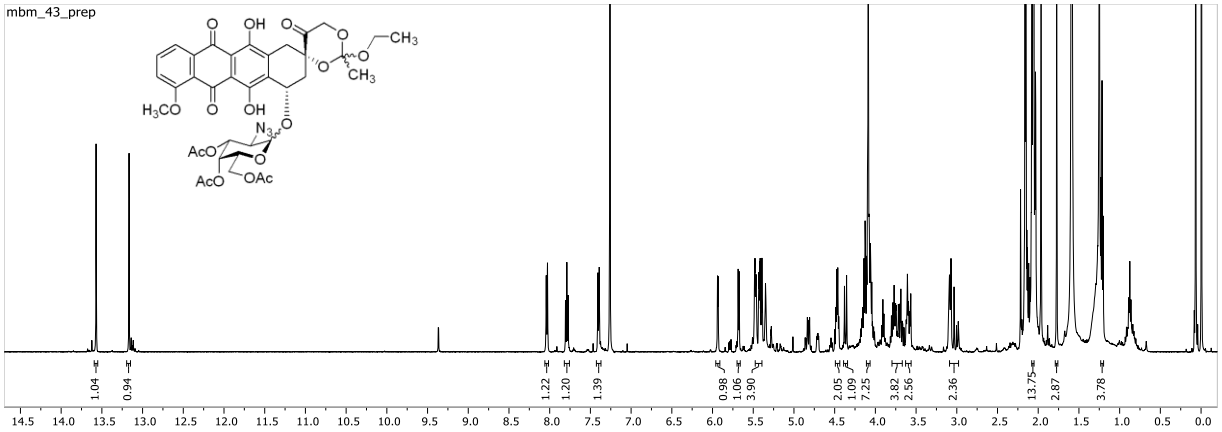
Generic Display Report

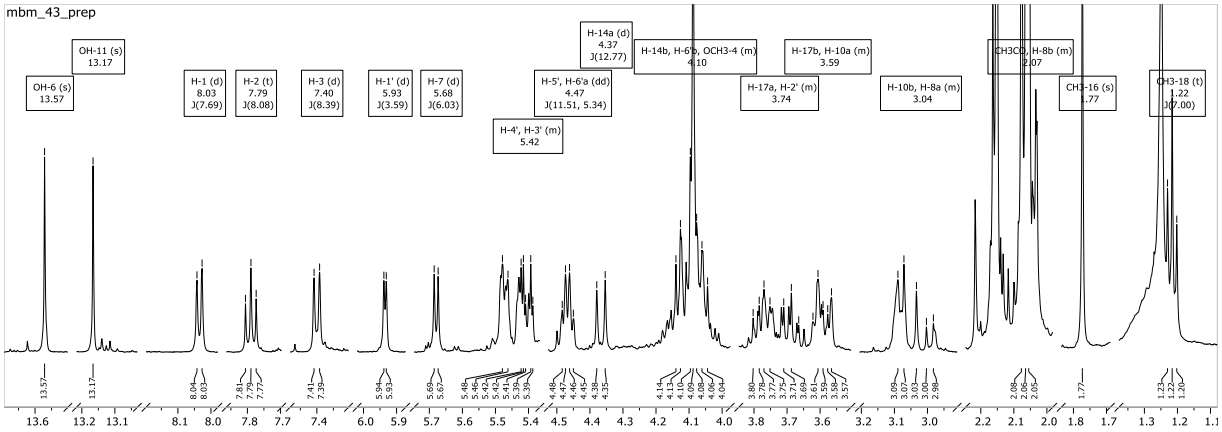
Analysis Info

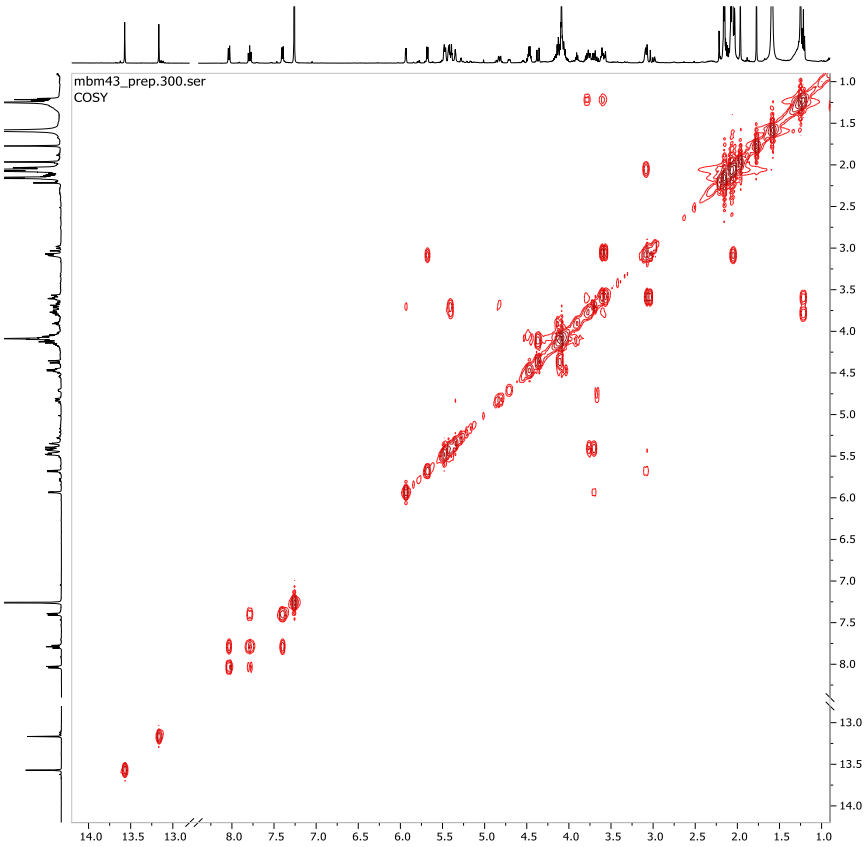
Analysis Name	D:\Data\PROF. IVONE\MAD_38_POS.d	Acquisition Date	12/20/2016 3:14:52 PM
Method	Tune_Low_Tomaz_Pos_1300u_Willian 1.m	Operator	BDAL@DE
Sample Name	MAD_38_POS	Instrument	micrOTOF-Q II
Comment			



APPENDIX 34. Compound **46**



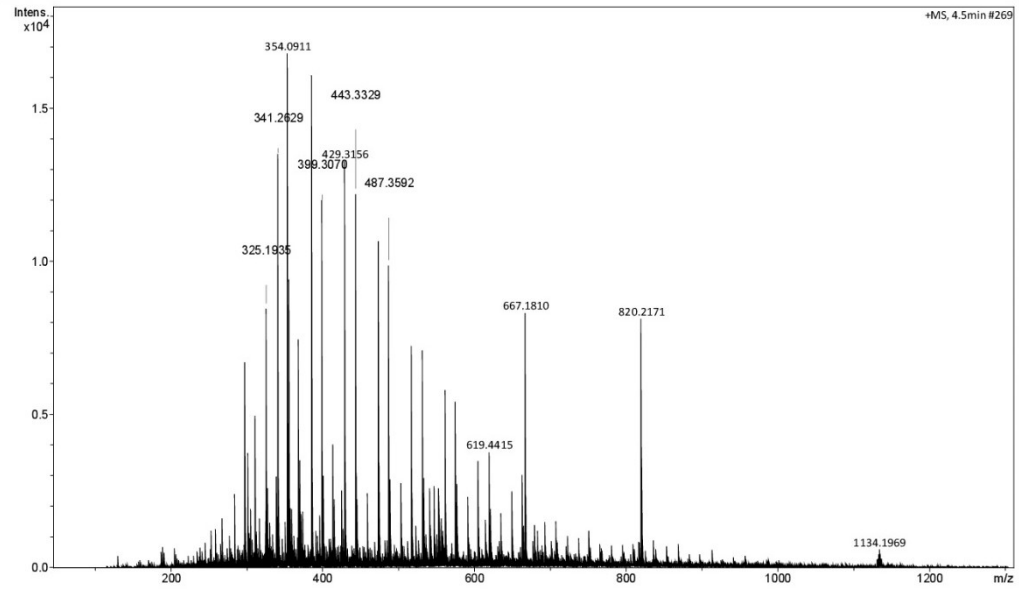




Generic Display Report

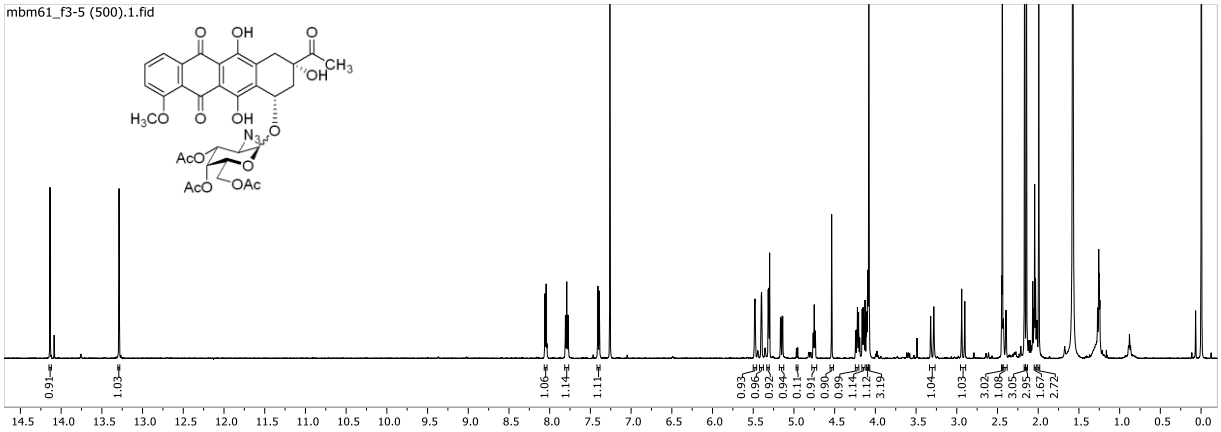
Analysis Info

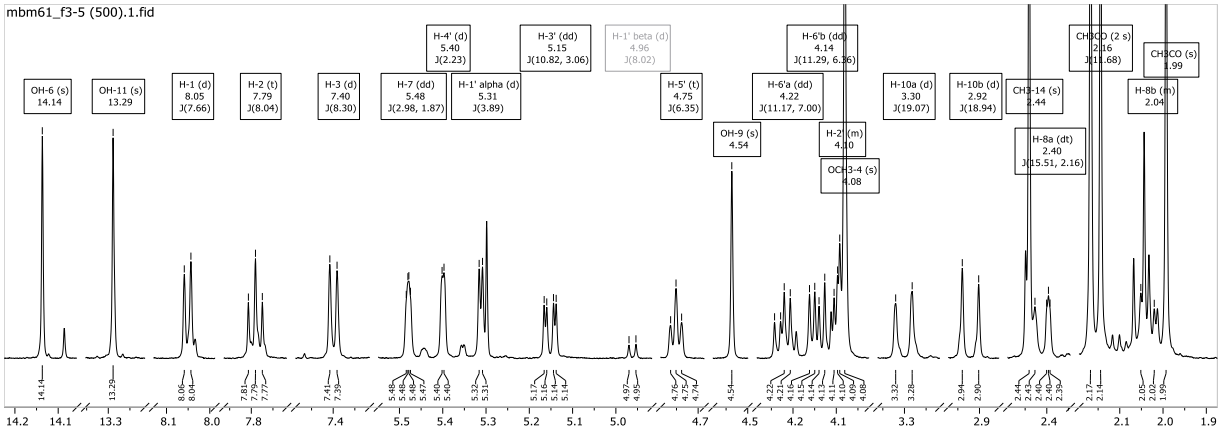
Analysis Name	D:\Data\PROF. IVONE\MBM_43_POS.d	Acquisition Date	12/20/2016 3:09:33 PM
Method	Tune_Low_Tomaz_Pos_1300u_Willian 1.m	Operator	BDAL@DE
Sample Name	MBM_43_POS	Instrument	micrOTOF-Q II
Comment			

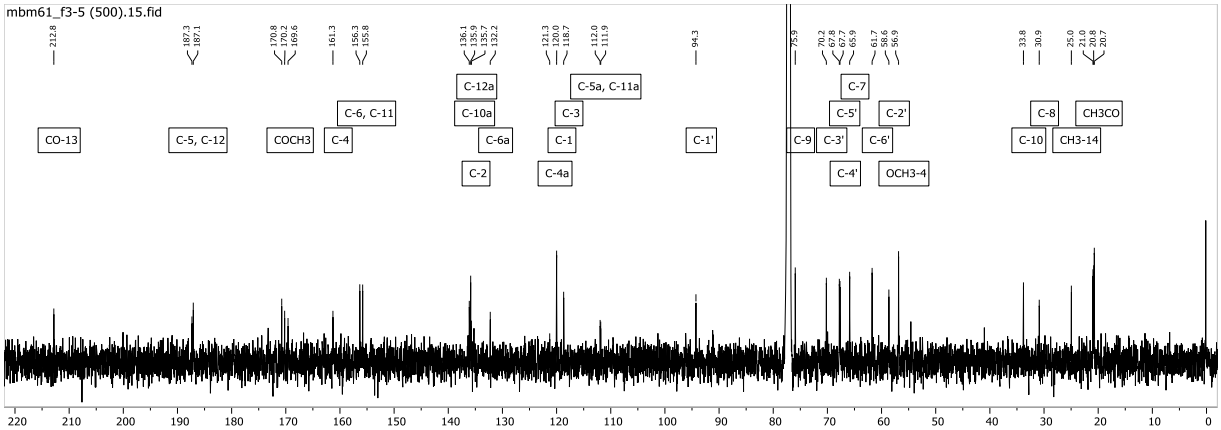


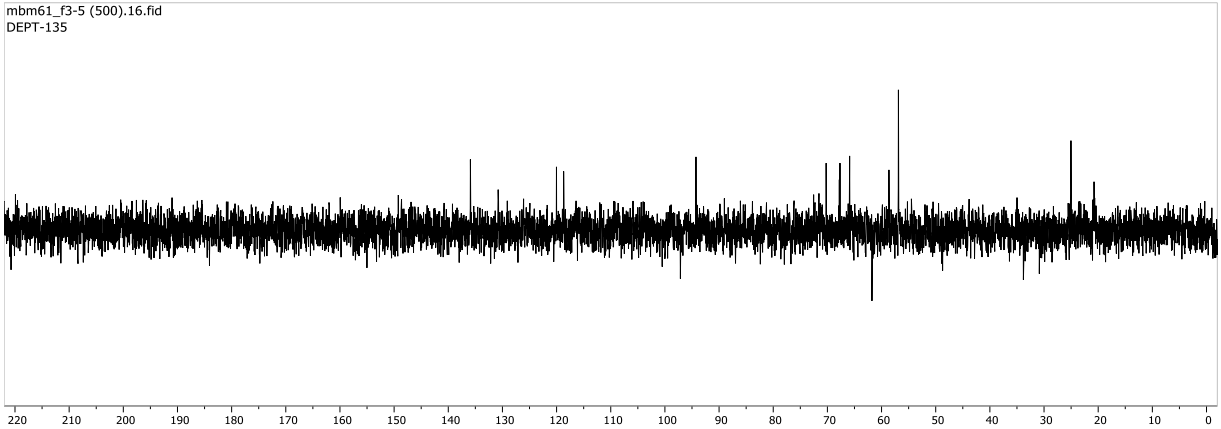
Bruker Compass DataAnalysis 4.3 printed: 1/10/2017 4:37:40 PM by: BDAL@DE Page 1 of 1

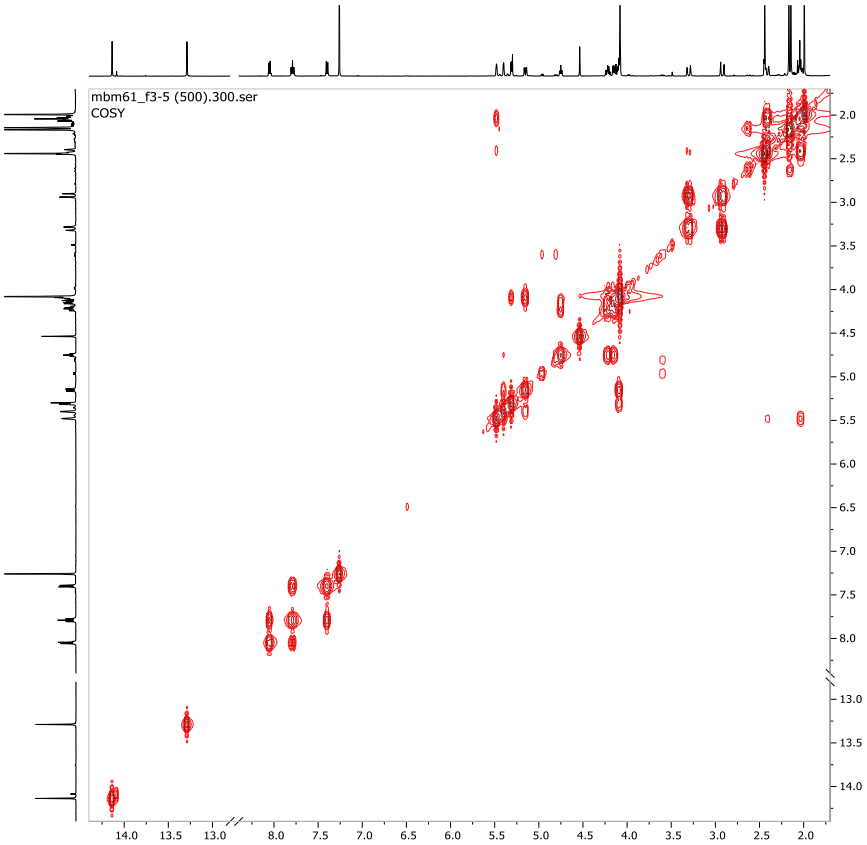
APPENDIX 35. Compound **47**

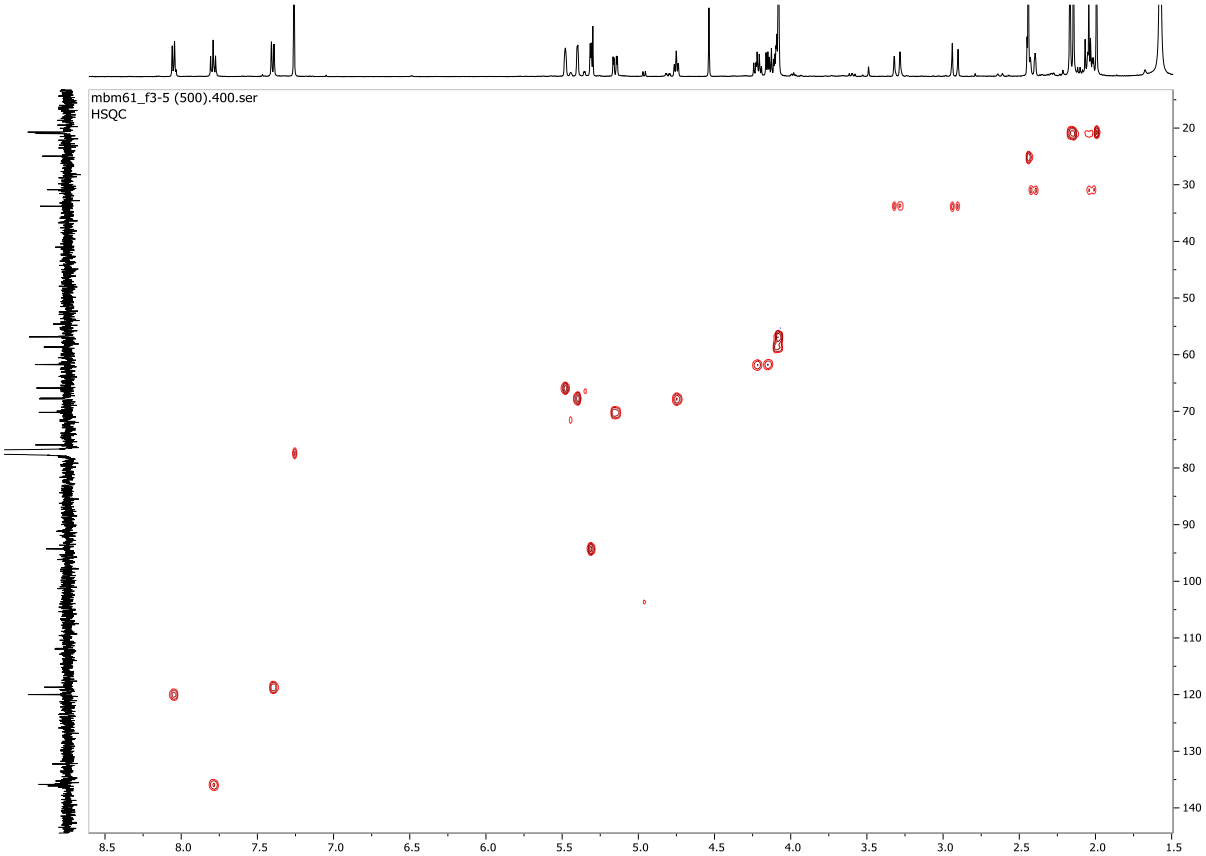








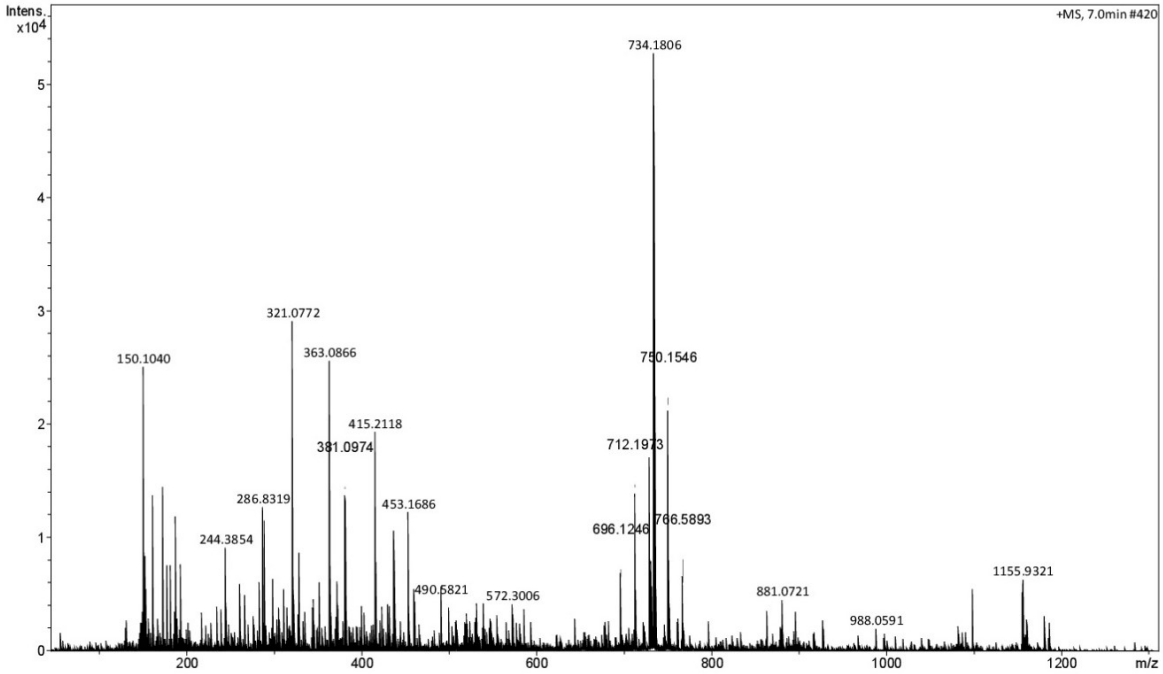

mbm61_f3-5 (500).300.ser
COSY


mbm61_f3-5 (500).400.ser
HSQC

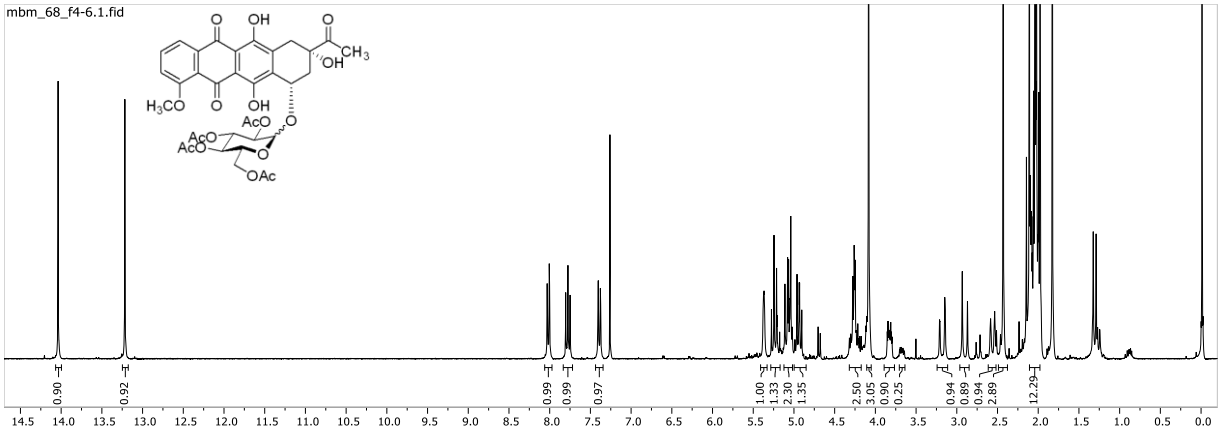
Generic Display Report

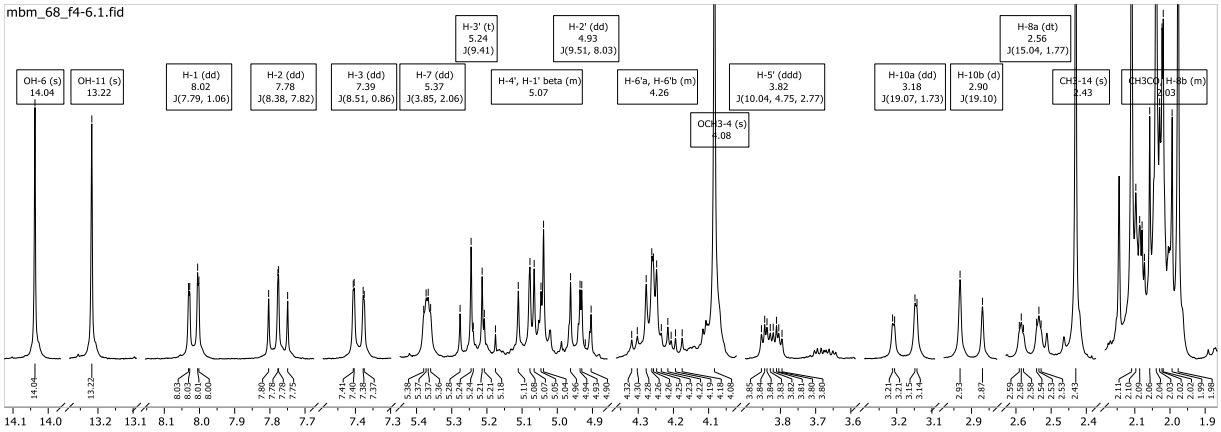
Analysis Info

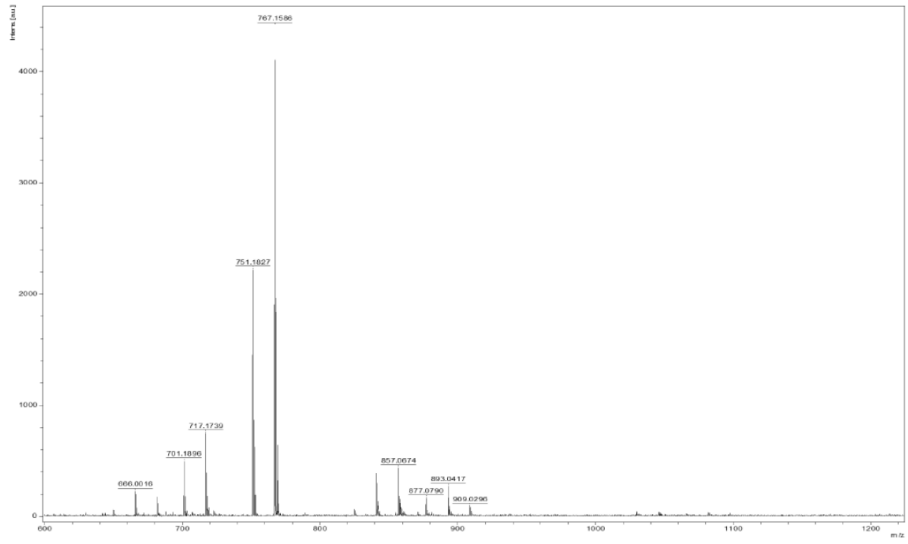
Analysis Name	D:\Data\IVONE\MBM - 61_f 3-5_POSITIVO.d	Acquisition Date	4/13/2018 2:39:33 PM
Method	tune_low_DANIEL 1300 daltons_POS.m	Operator	BD@AL
Sample Name	MBM - 61_f 3-5_POSITIVO	Instrument	micrOTOF
Comment			



APPENDIX 36. Compound **48**







Target

Target type	0209519
Target serial number	0005277
Position	E7

Laser

Laser beam attenuation	85
Laser beam focus	64
Laser repetition rate	1000 Hz
Number of shots	500

Spectrometer

positive voltage polarity	POS
PIE delay	100 ns
Ion source voltage 1	25 kV
Ion source voltage 2	22.35 kV
Lens voltage	7.75 kV
Linear detector voltage	2.645 kV
Deflection on	
Deflection mass	
MSMS parent mass	
LIFT voltage 1	
LIFT voltage 2	
LIFT 1 Pulser time depending on the parent mass	
LIFT 2 Pulser time	
Reflector voltage 1	26.6 kV
Reflector voltage 2	13.3 kV
Reflector detector volt.	2.305 kV

Instrument

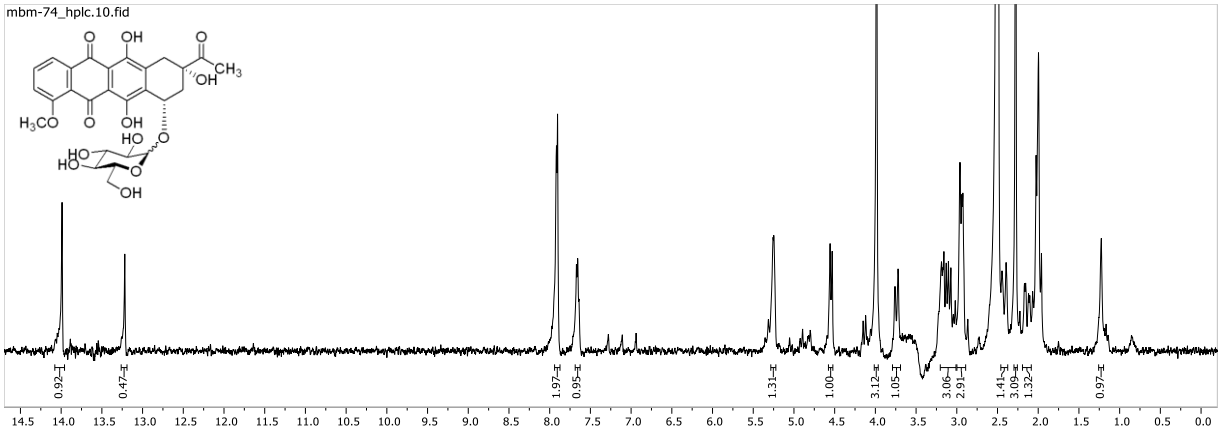
Instrument type	ultraflexTOF/TOF
Instrument serial	259901.00326
Name of computer	FLEX-PC
Operator ID or name	BDAL@DE
flexControl version	flexControl 3.4.135.0
flexAnalysis version	

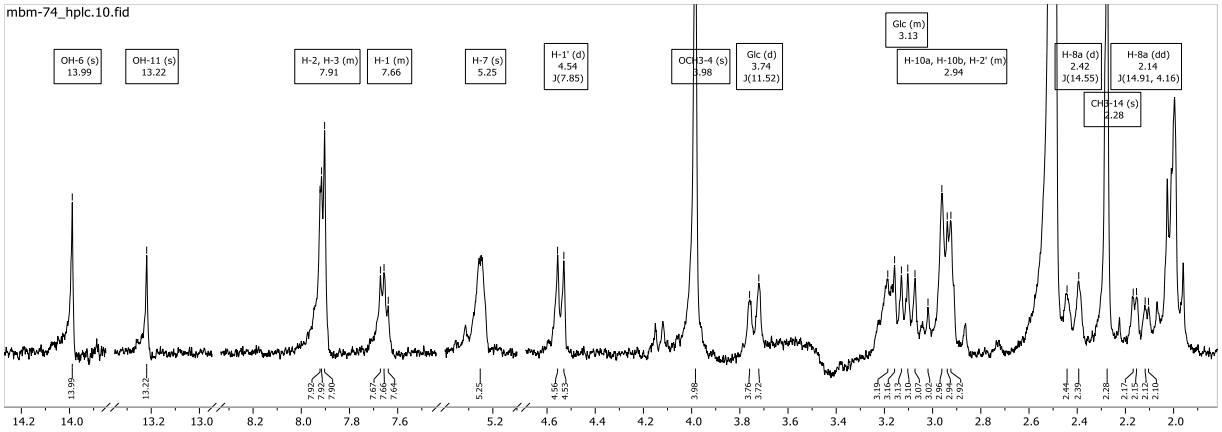
Date of Acquisition 2018-04-20T17:43:01.095-03:00 printed: 4/24/2018 6:31:30 PM
Acquisition method D:\Methods\flexControlMethods\RP_700-3500_Da.par
Processing method
File Name D:\Data\Data\MaristelaBMT\MBM-68_f4-6_v3\0_E7\1

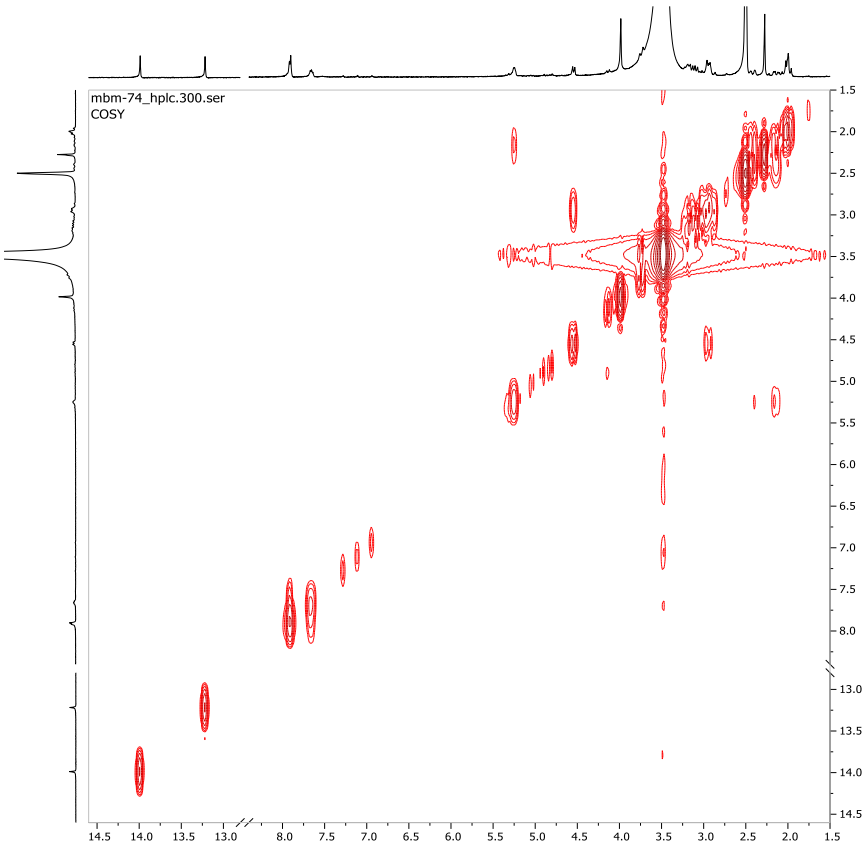
Performed by	Viewed by
Date / Sign	Date / Sign

Bruker Daltonics

APPENDIX 37. Compound **49**



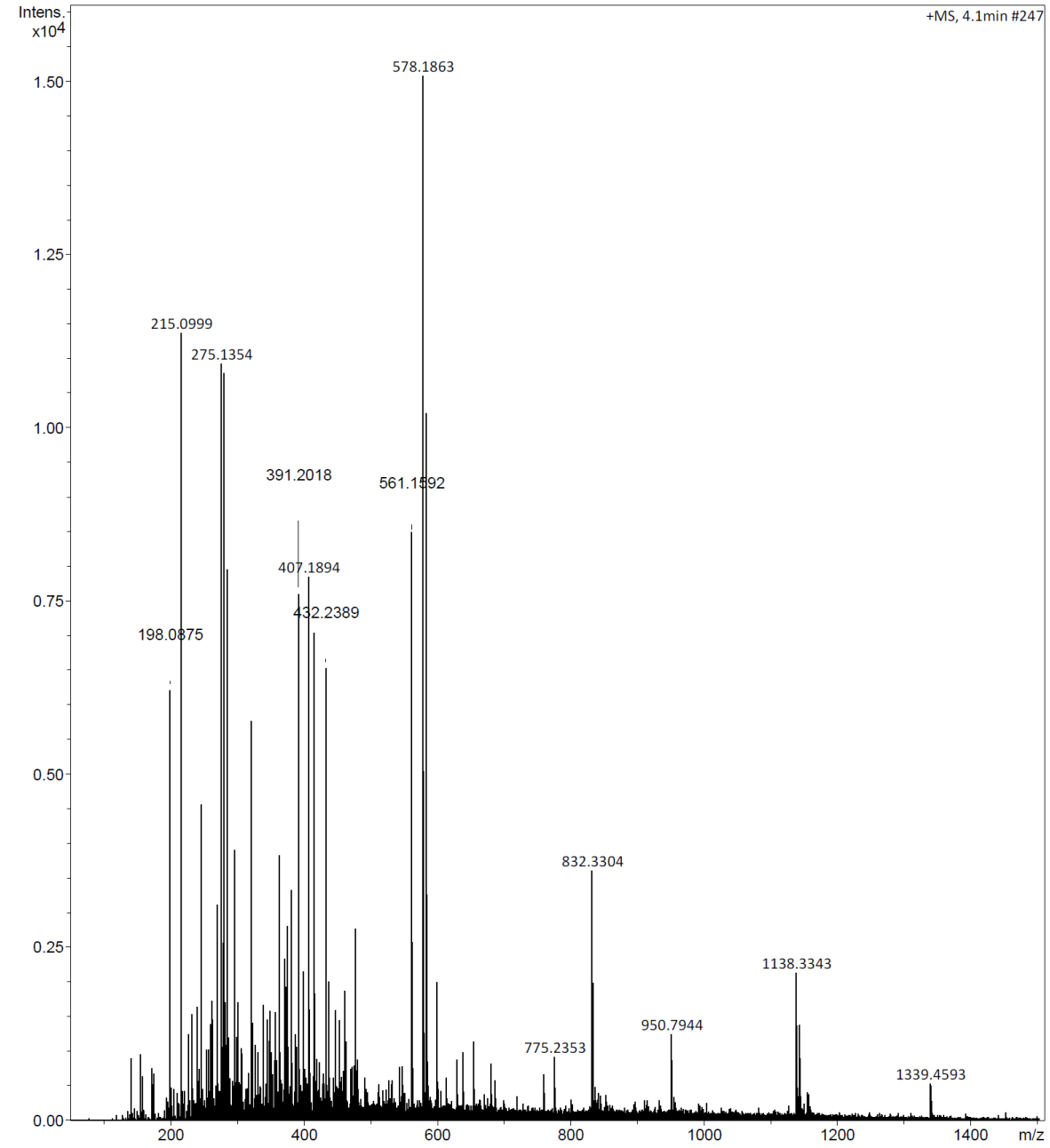




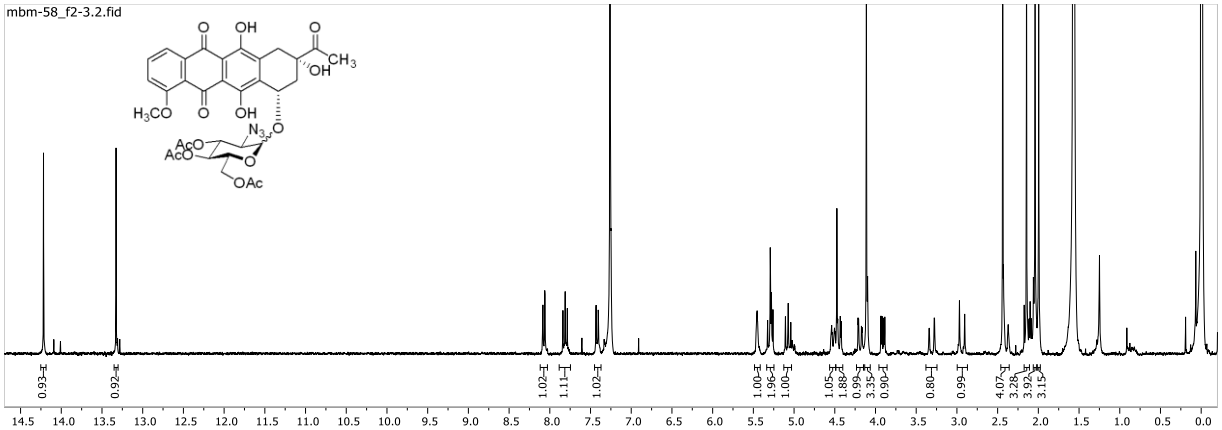
Generic Display Report

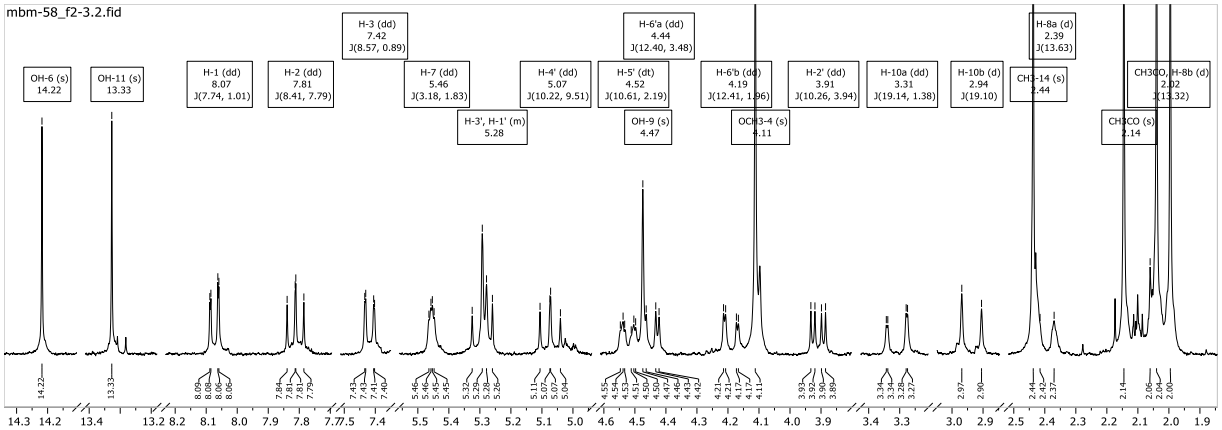
Analysis Info

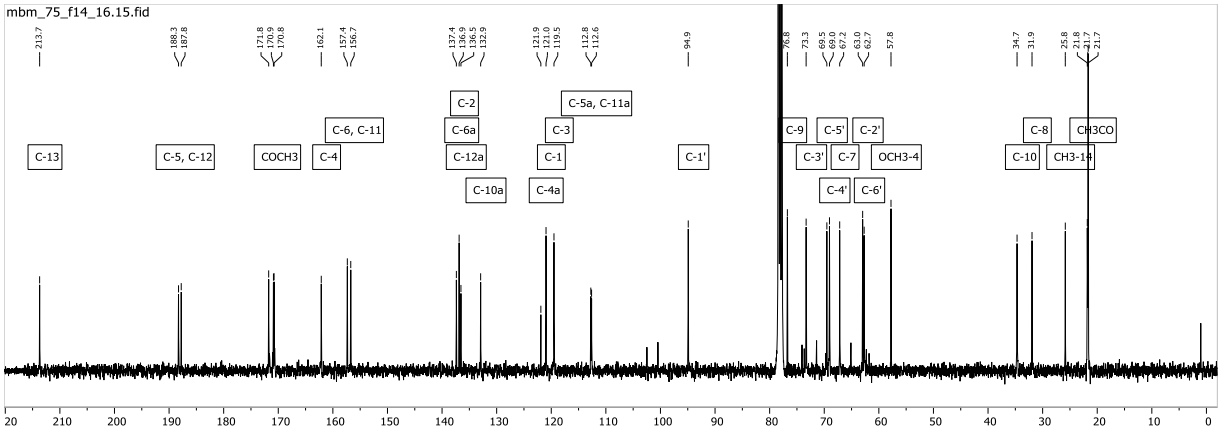
Analysis Name	D:\Data\PROF. IVONE\MBM-74_f 14-15_POS.d	Acquisition Date	7/2/2018 3:55:28 PM
Method	Tune_Low_Tomaz_Pos_1000.m	Operator	TOMAZ
Sample Name	MBM-74_f 14-15_POS	Instrument	micrOTOF-Q
Comment			

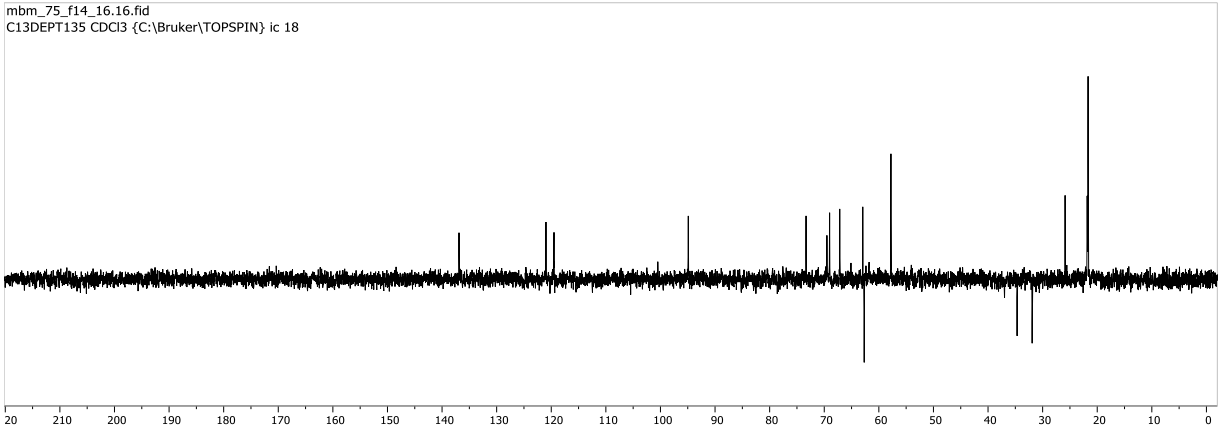


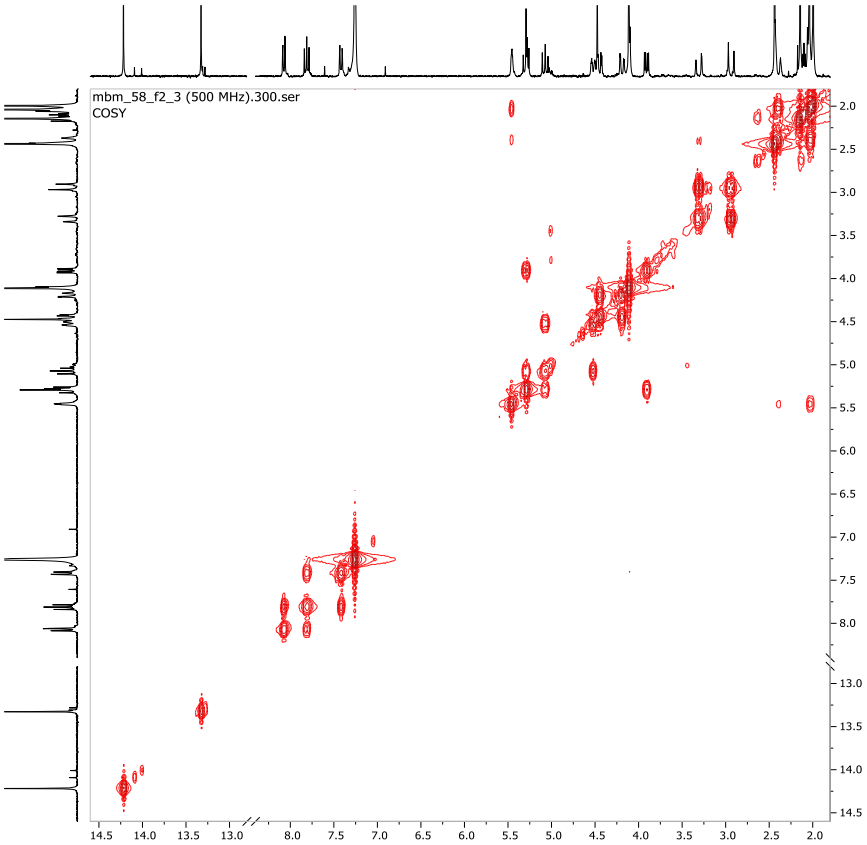
APPENDIX 38. Compound **50**

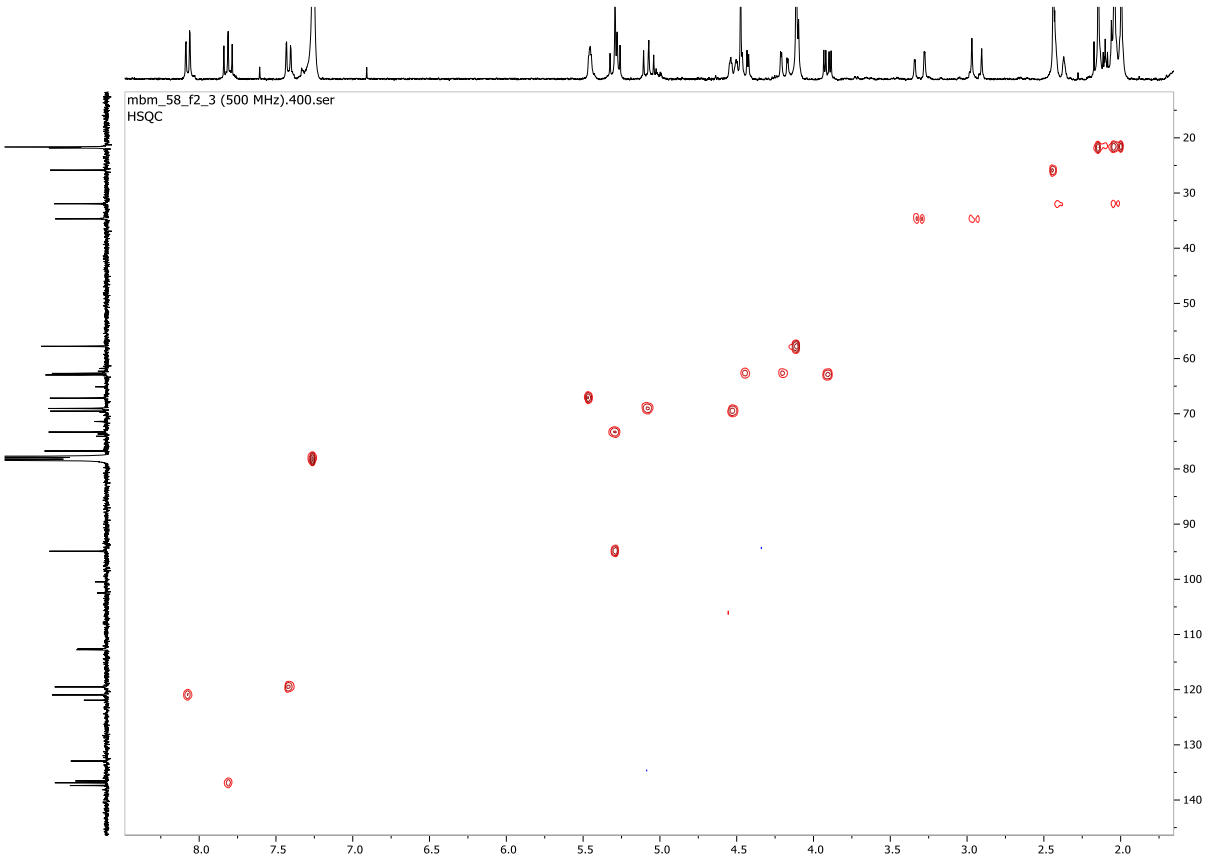








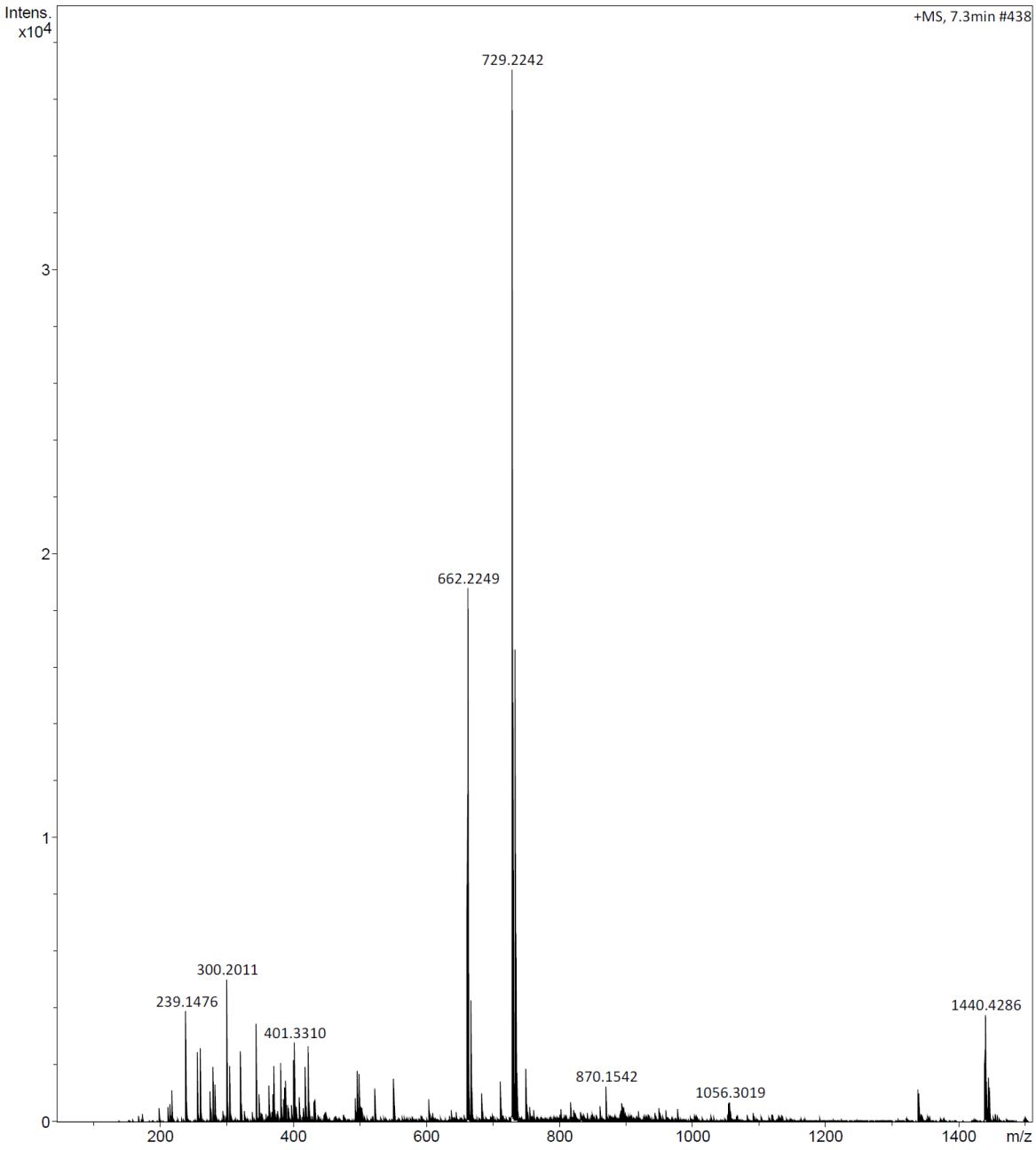

mbm_58_f2_3 (500 MHz).300.ser
COSY


mbm_58_f2_3 (500 MHz).400.ser
HSQC

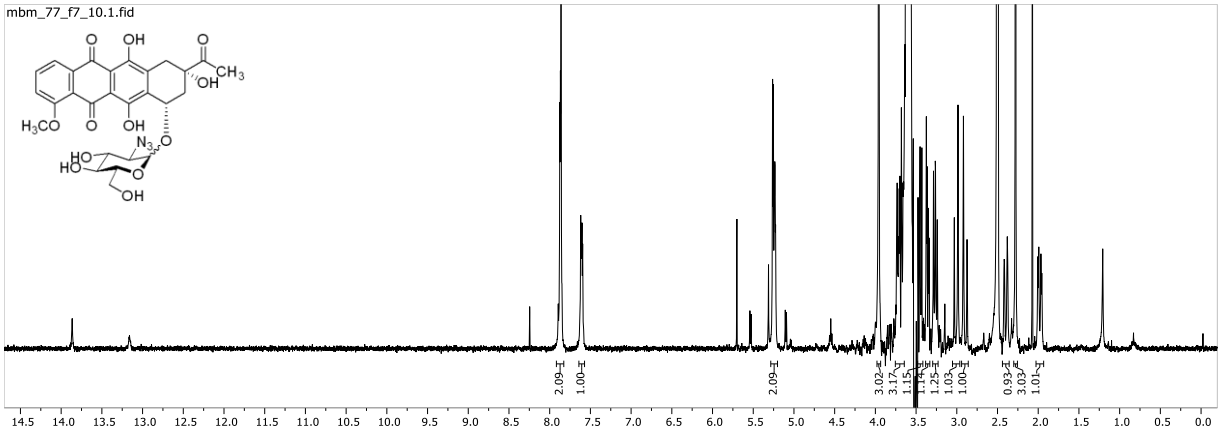
Generic Display Report

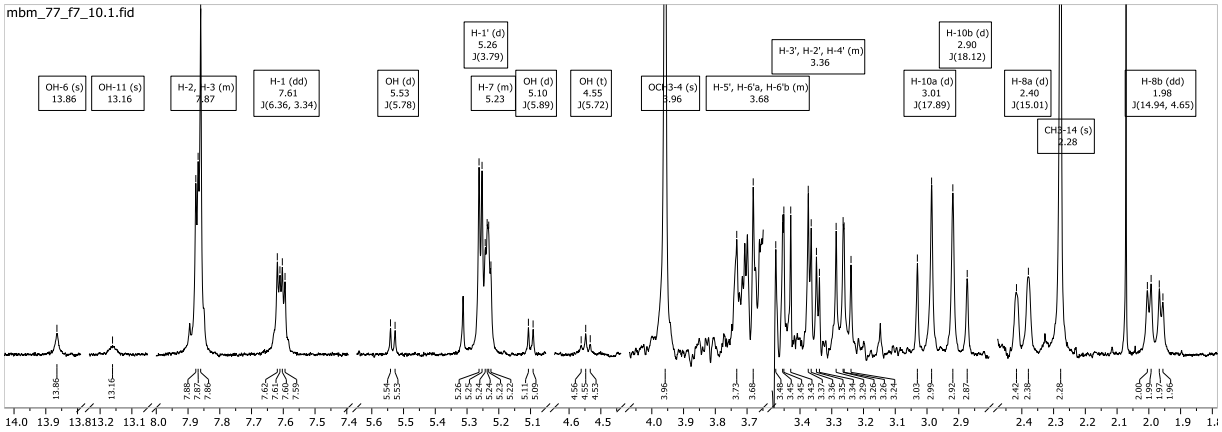
Analysis Info

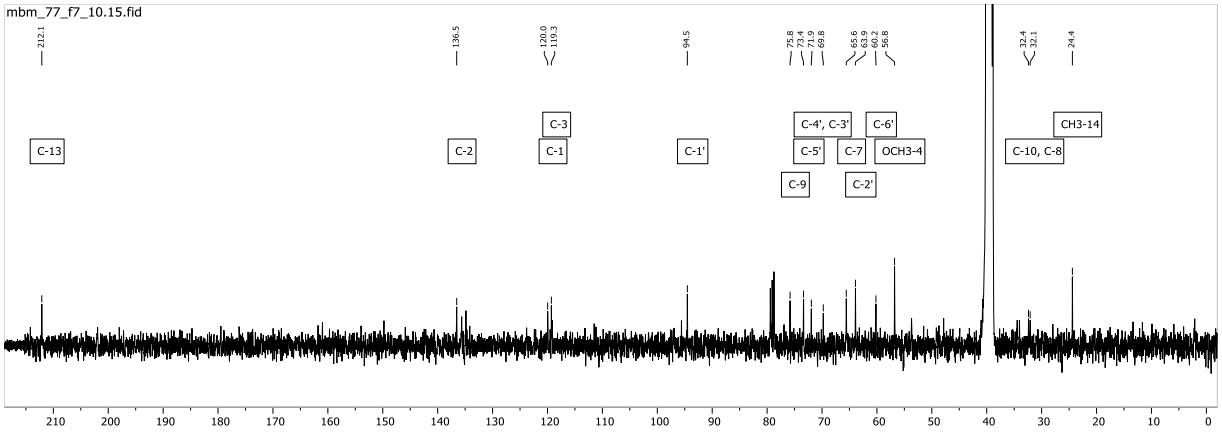
Analysis Name	D:\Data\PROF. IVONE\MBM-75_f 13-16_POS.d	Acquisition Date	7/2/2018 4:11:47 PM
Method	Tune_Low_Tomaz_Pos_1000.m	Operator	TOMAZ
Sample Name	MBM-75_f 13-16_POS	Instrument	micrOTOF-Q
Comment			

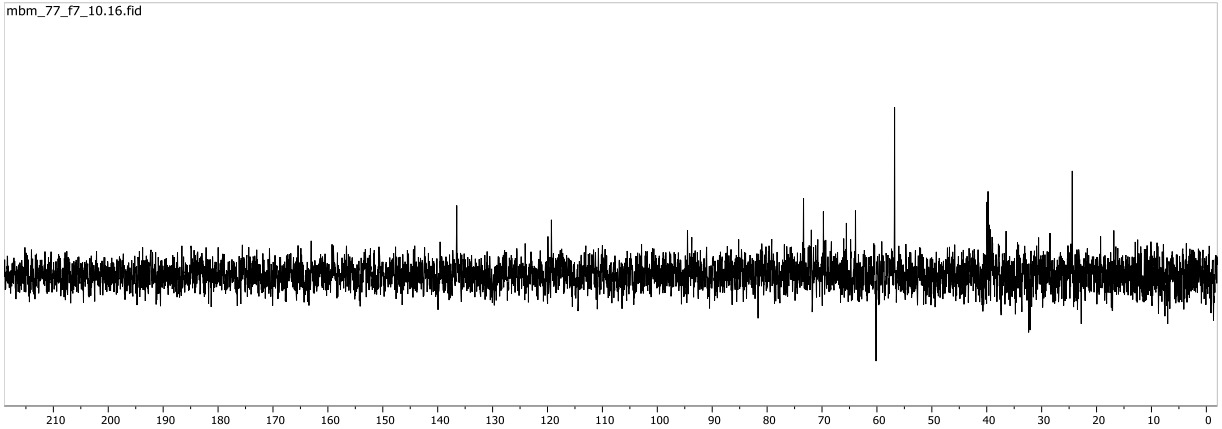


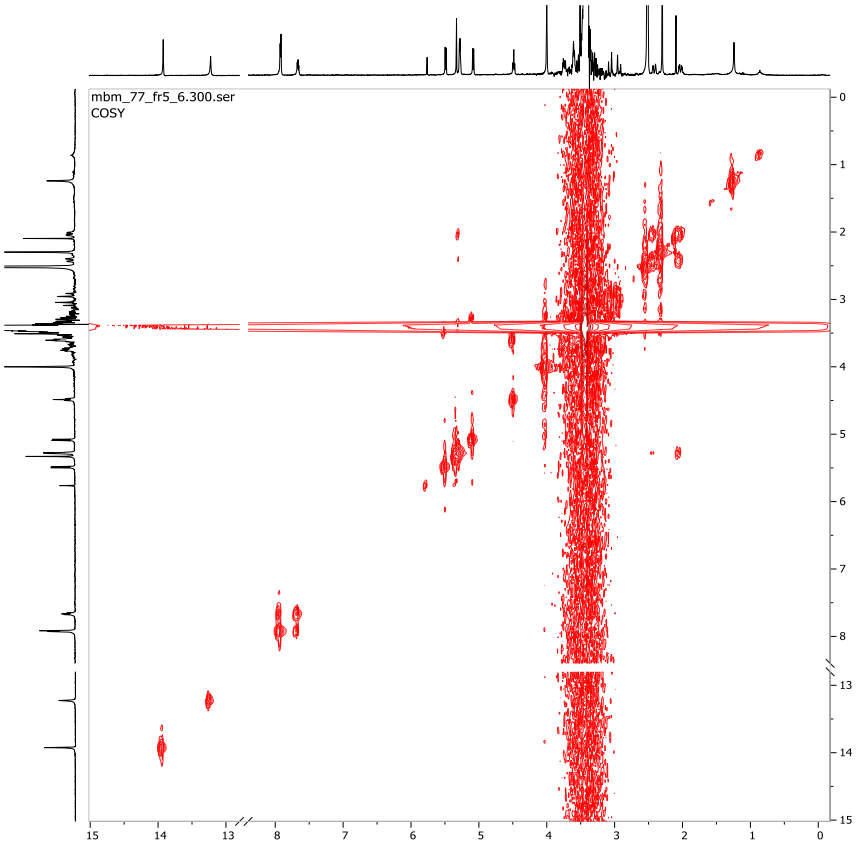
APPENDIX 39. Compound **51**

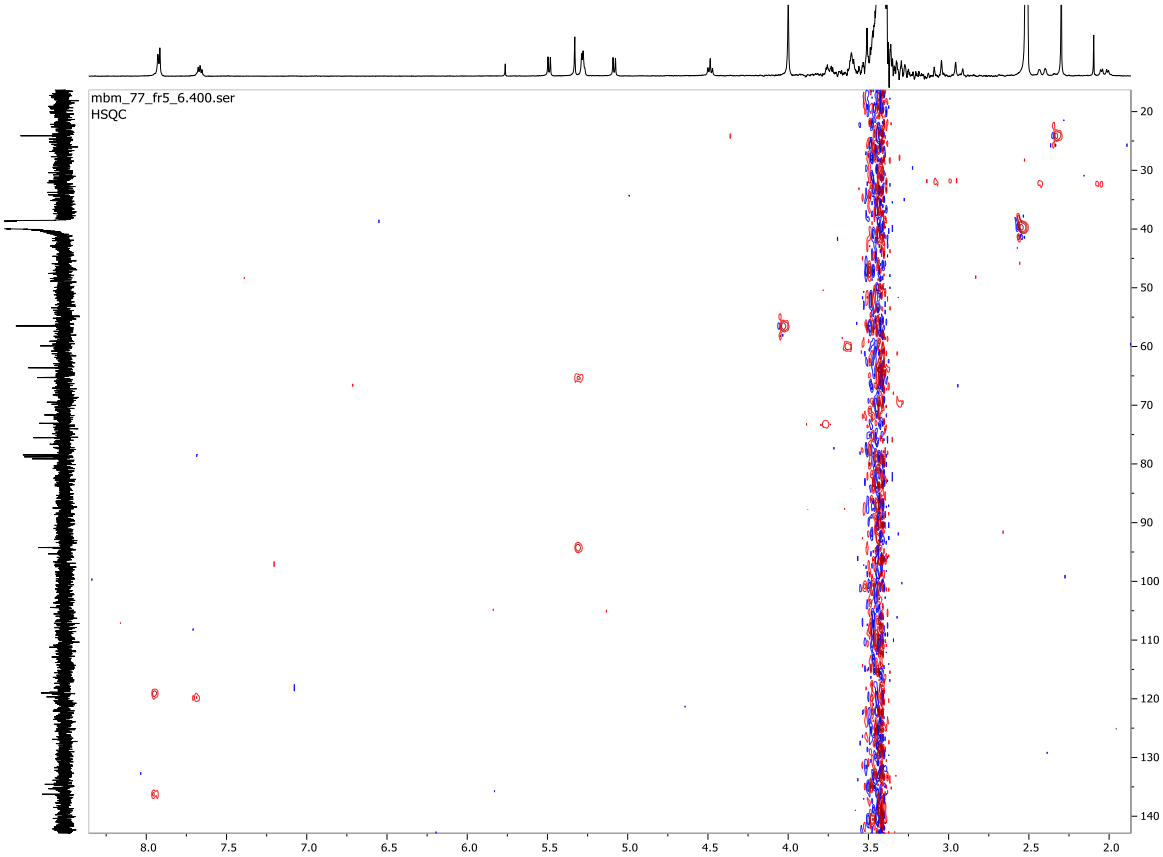








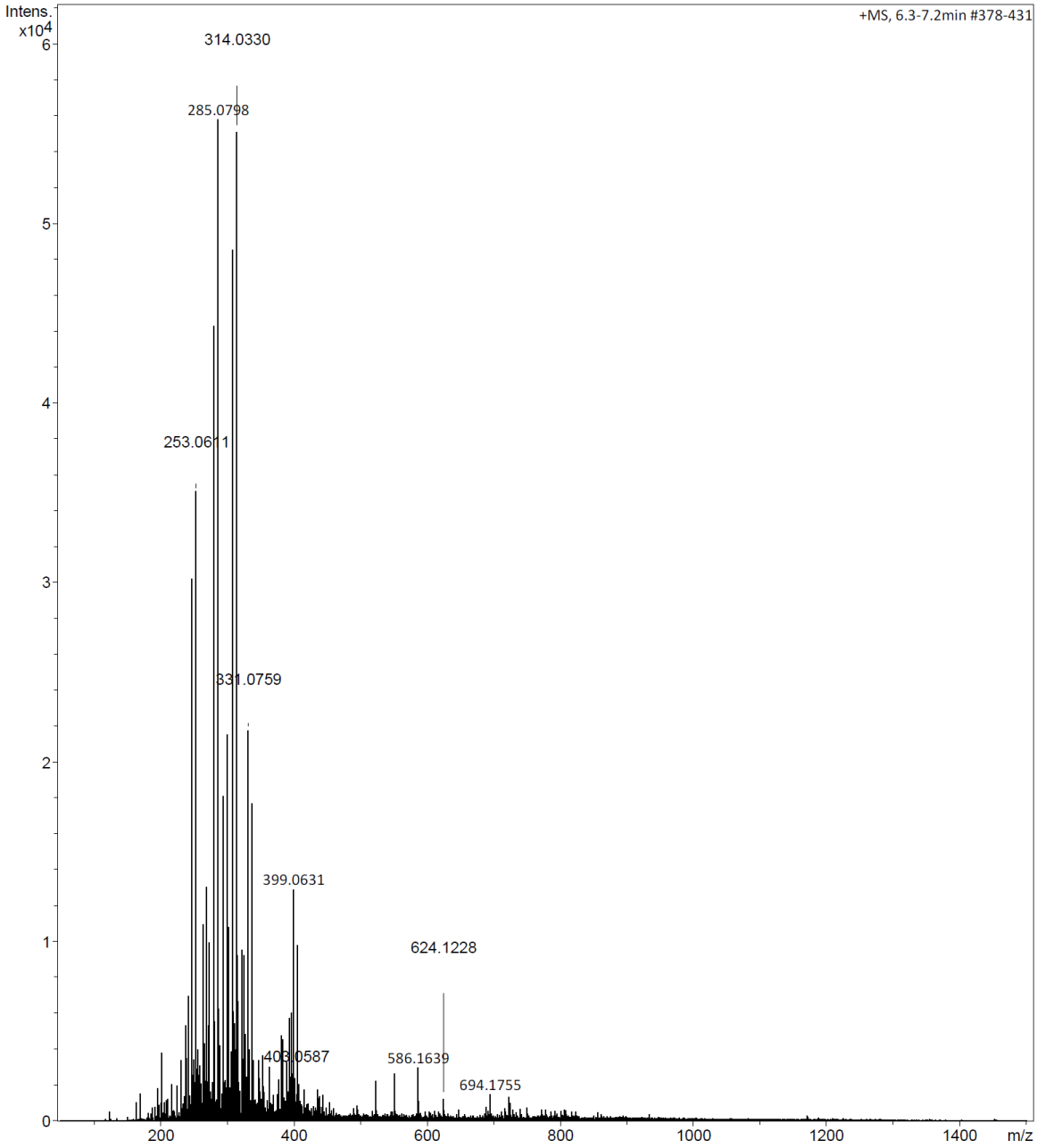

mbm_77_fr5_6.300.ser
COSY


mbm_77_fr5_6.400.ser
HSQC

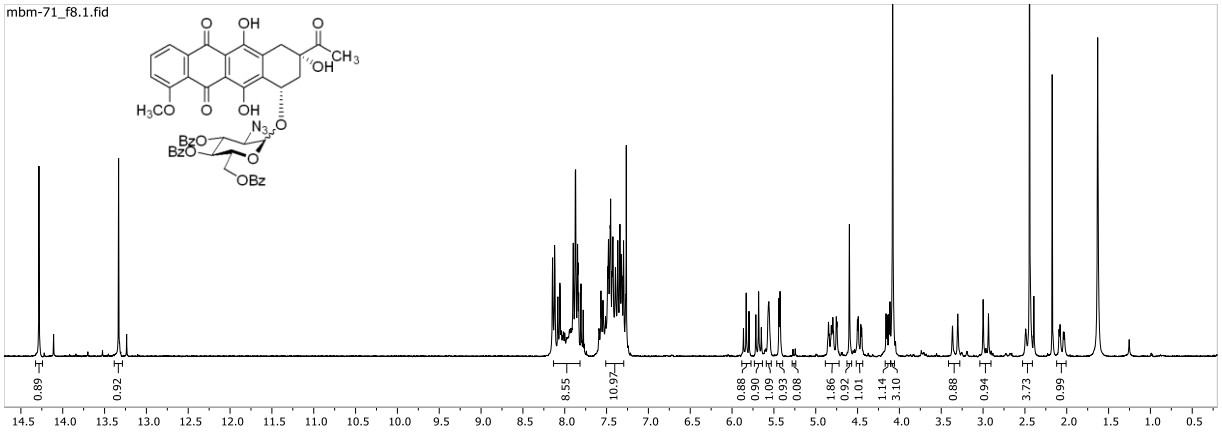
Generic Display Report

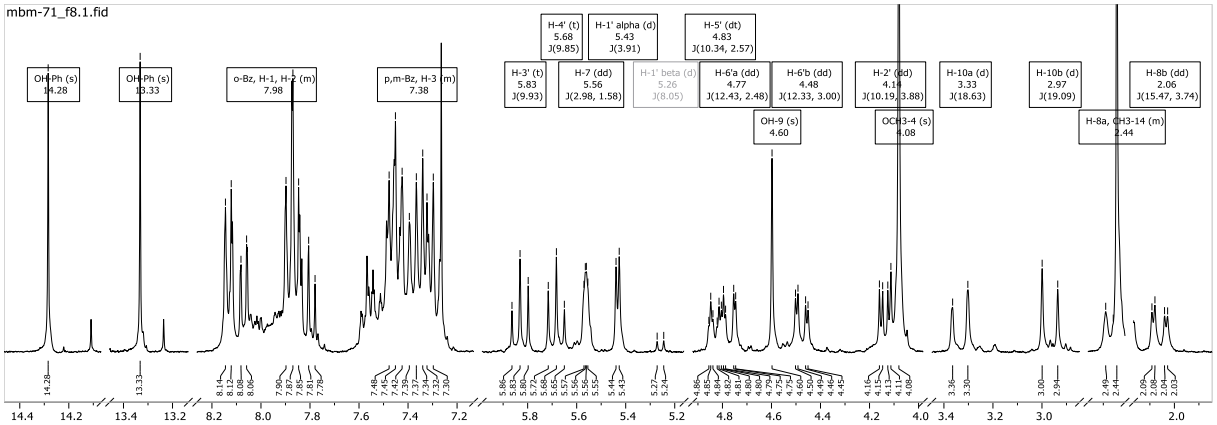
Analysis Info

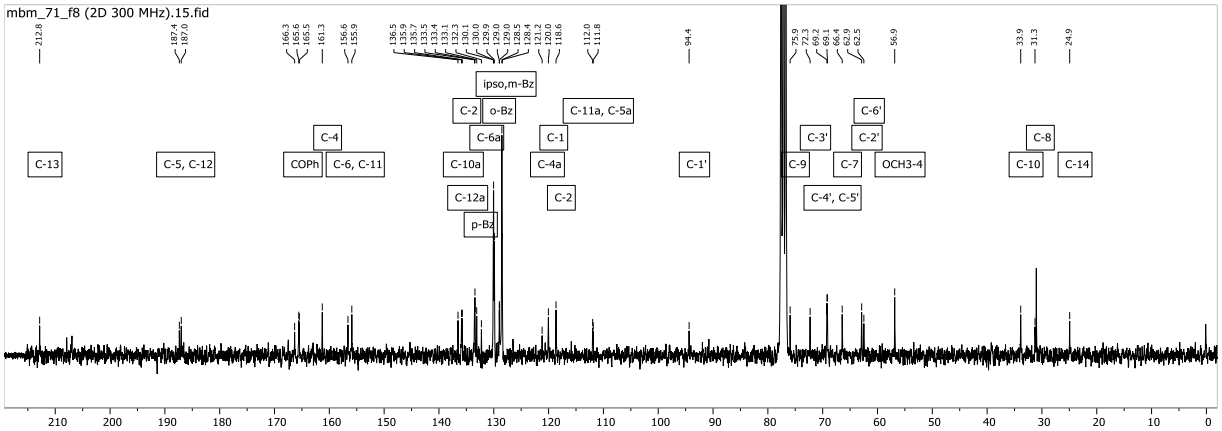
Analysis Name	D:\Data\PROF. IVONE\MBM-77_f 7-10_POS.d	Acquisition Date	7/2/2018 4:22:25 PM
Method	Tune_Low_Tomaz_Pos_1000.m	Operator	TOMAZ
Sample Name	MBM-77_f 7-10_POS	Instrument	micrOTOF-Q
Comment			

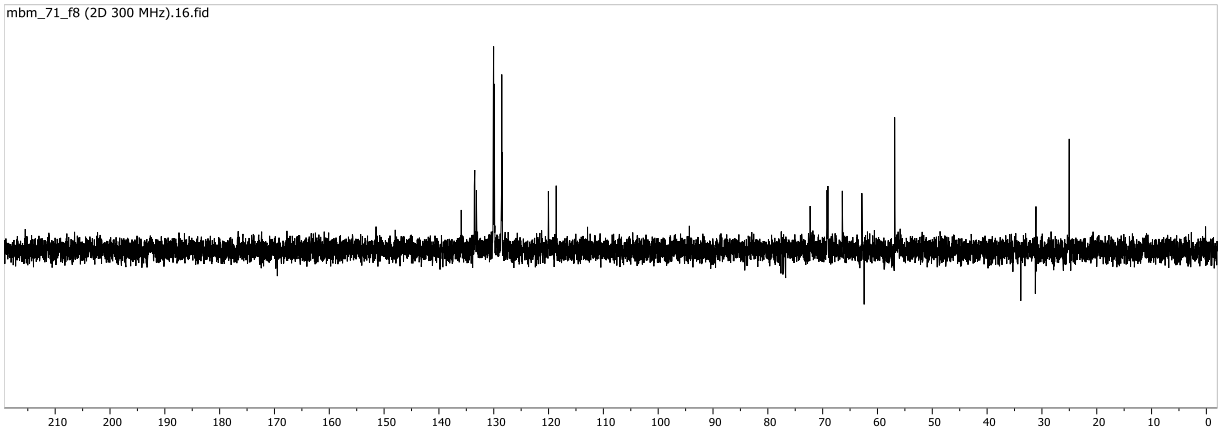


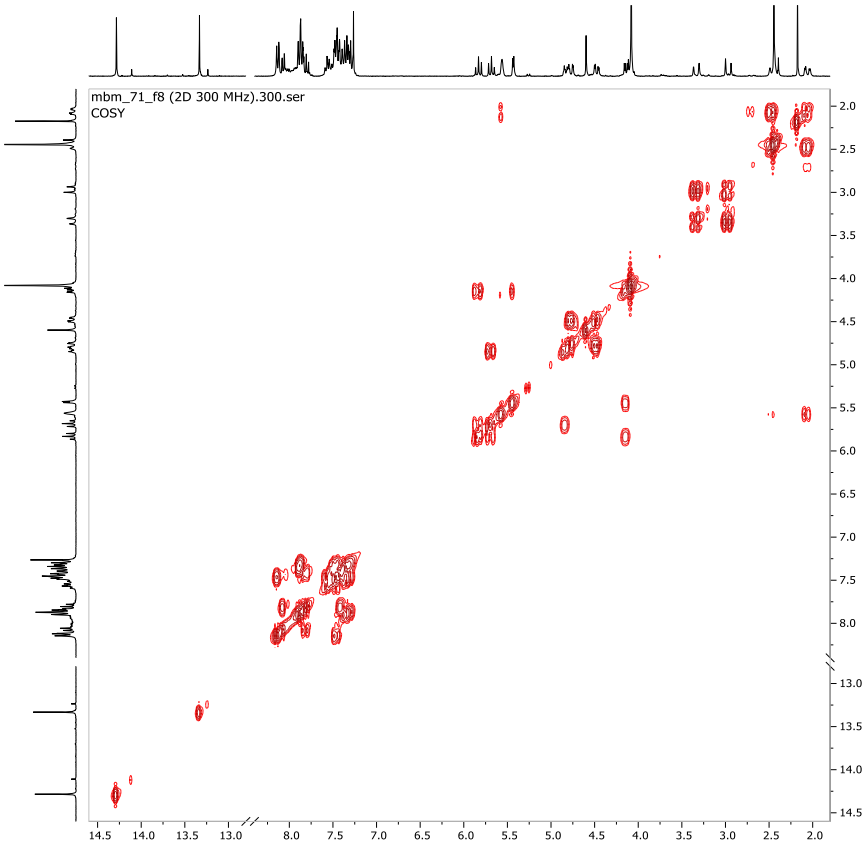
APPENDIX 40. Compound **52**

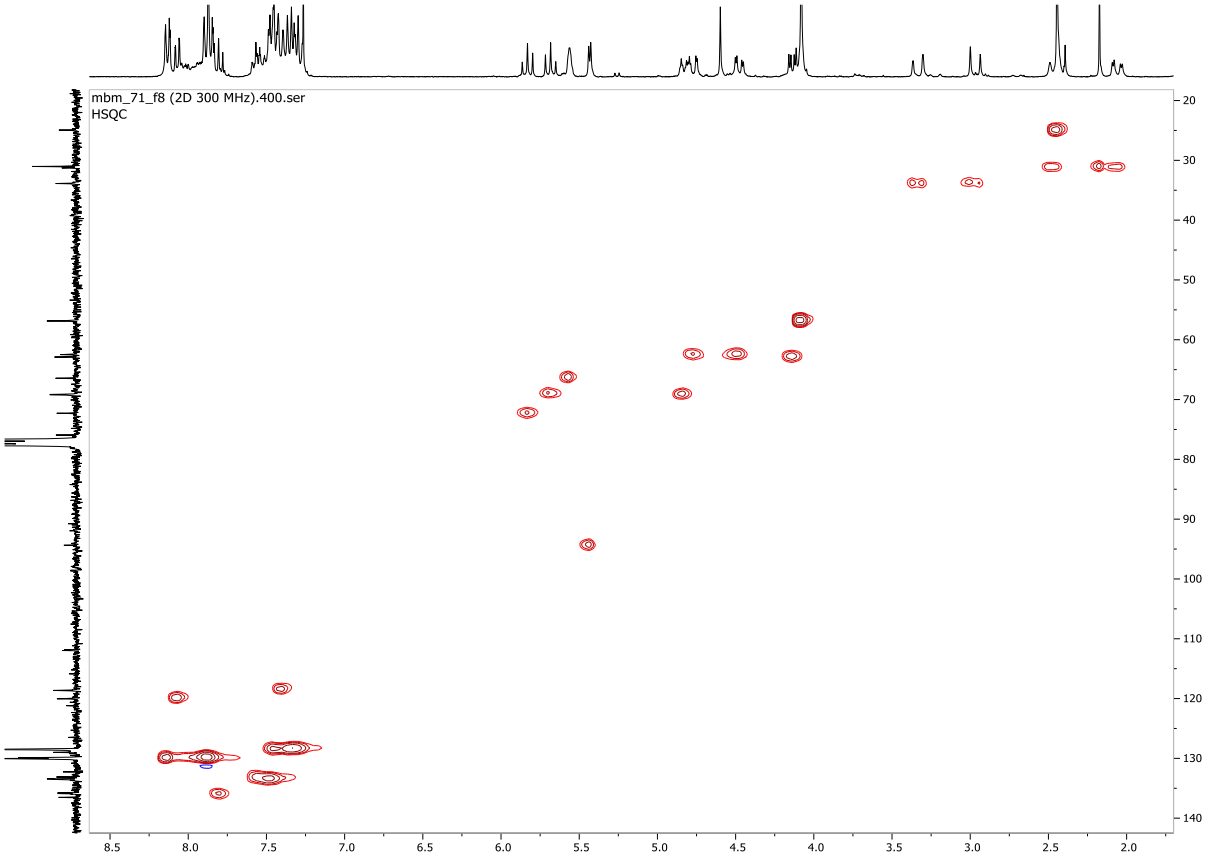








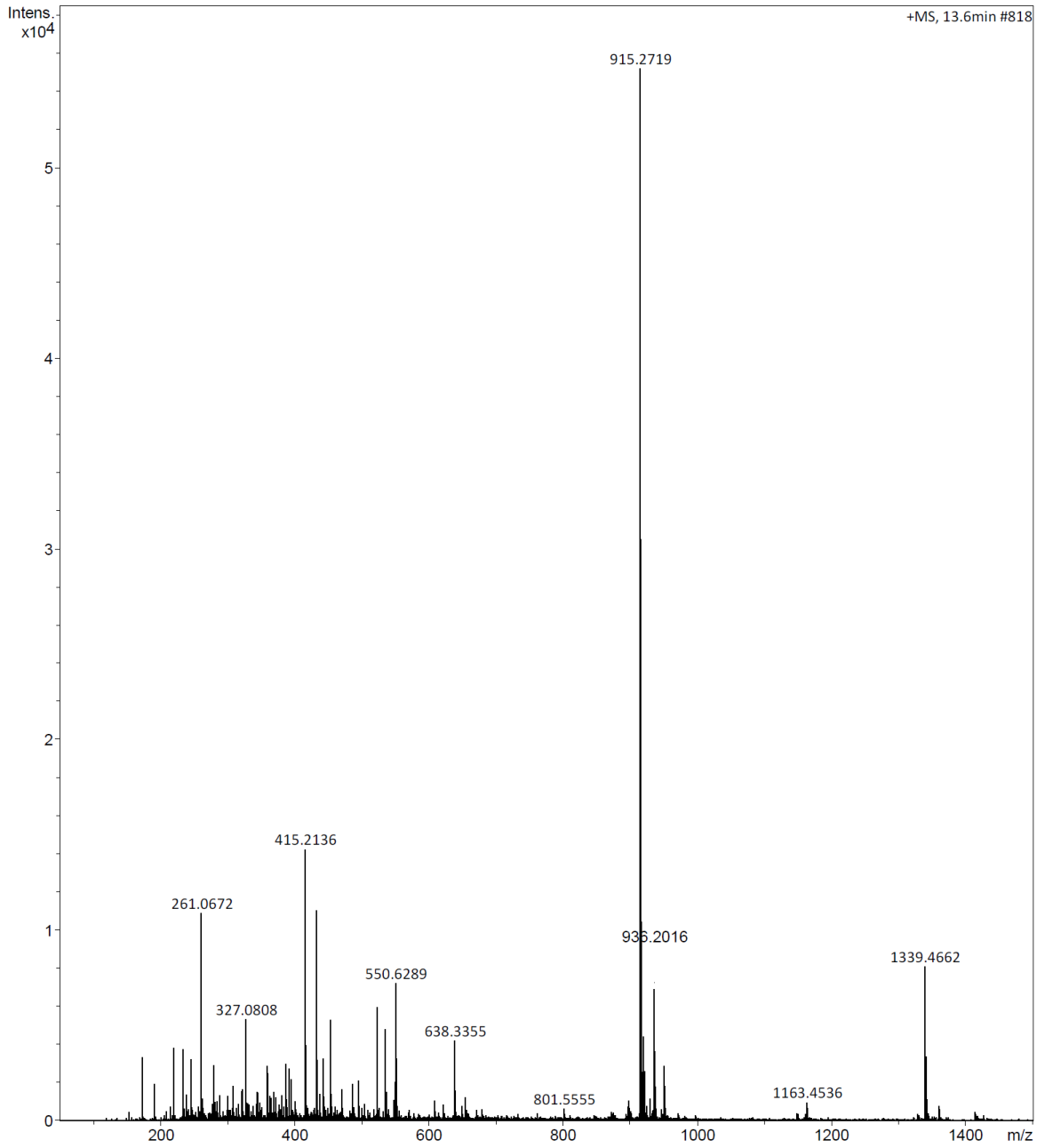

mbm_71_f8 (2D 300 MHz).300.ser
COSY


mbm_71_f8 (2D 300 MHz).400.ser
HSQC

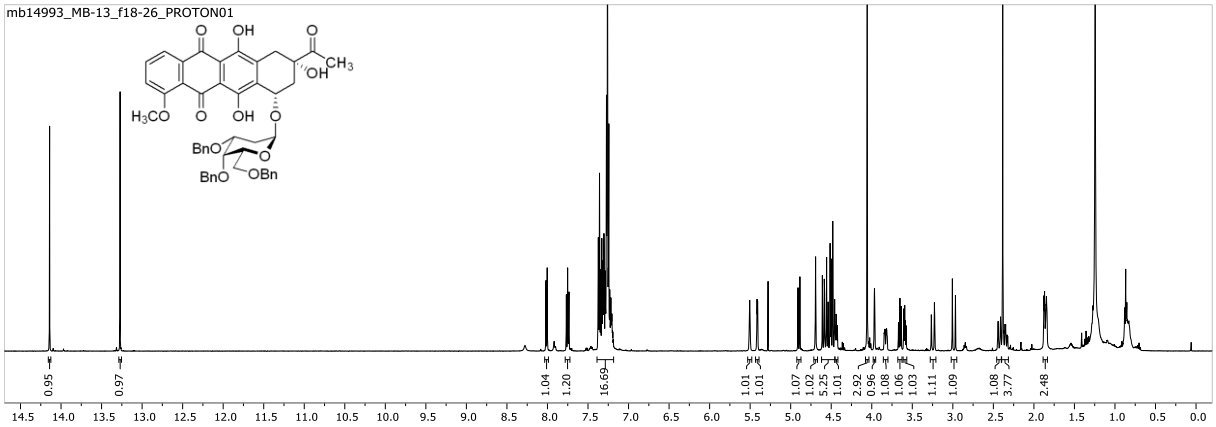
Generic Display Report

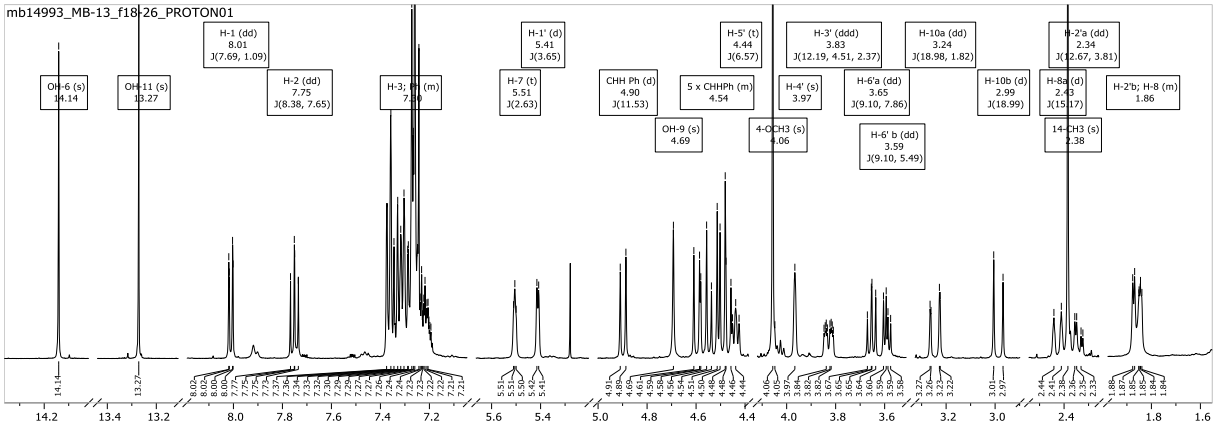
Analysis Info

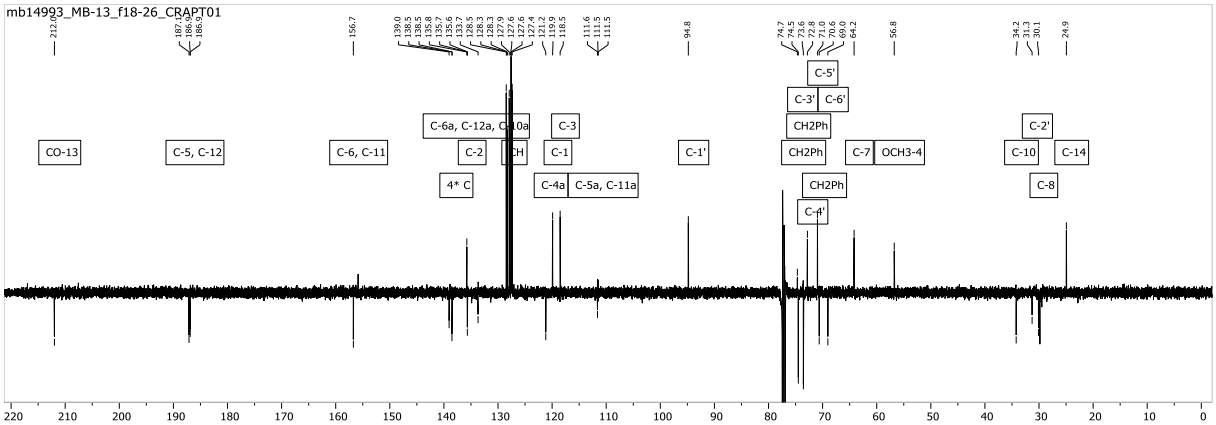
Analysis Name	D:\Data\PROF. IVONE\MBM_71_f 8_POS.d	Acquisition Date	7/2/2018 3:00:19 PM
Method	Tune_Low_Tomaz_Pos_1000.m	Operator	TOMAZ
Sample Name	MBM_71_f 8_POS	Instrument	micrOTOF-Q
Comment			

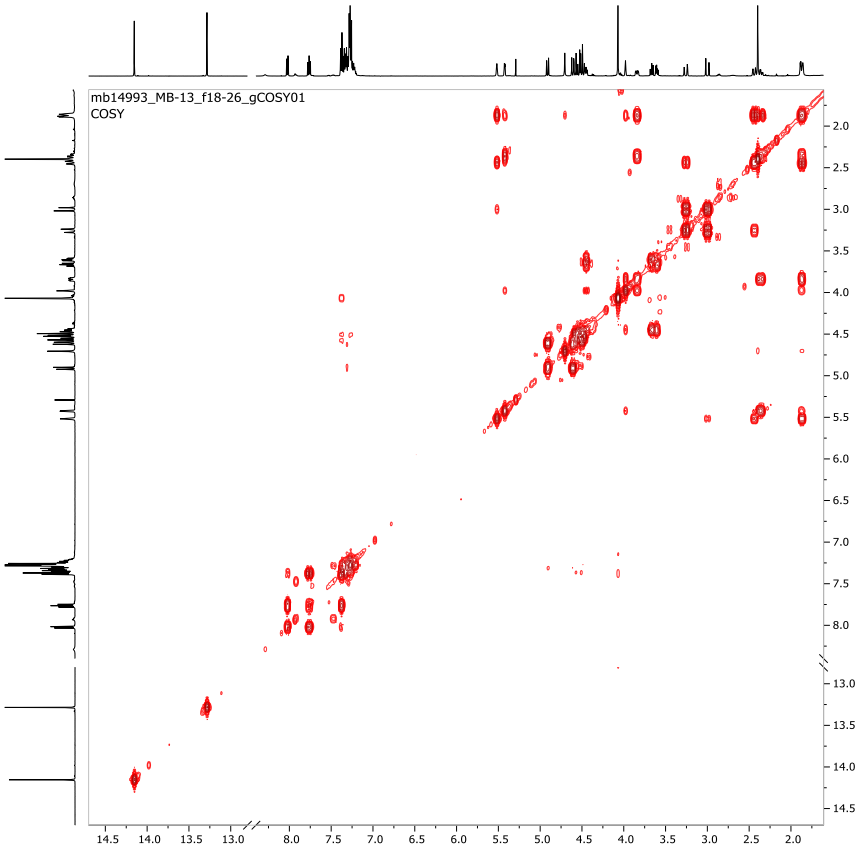


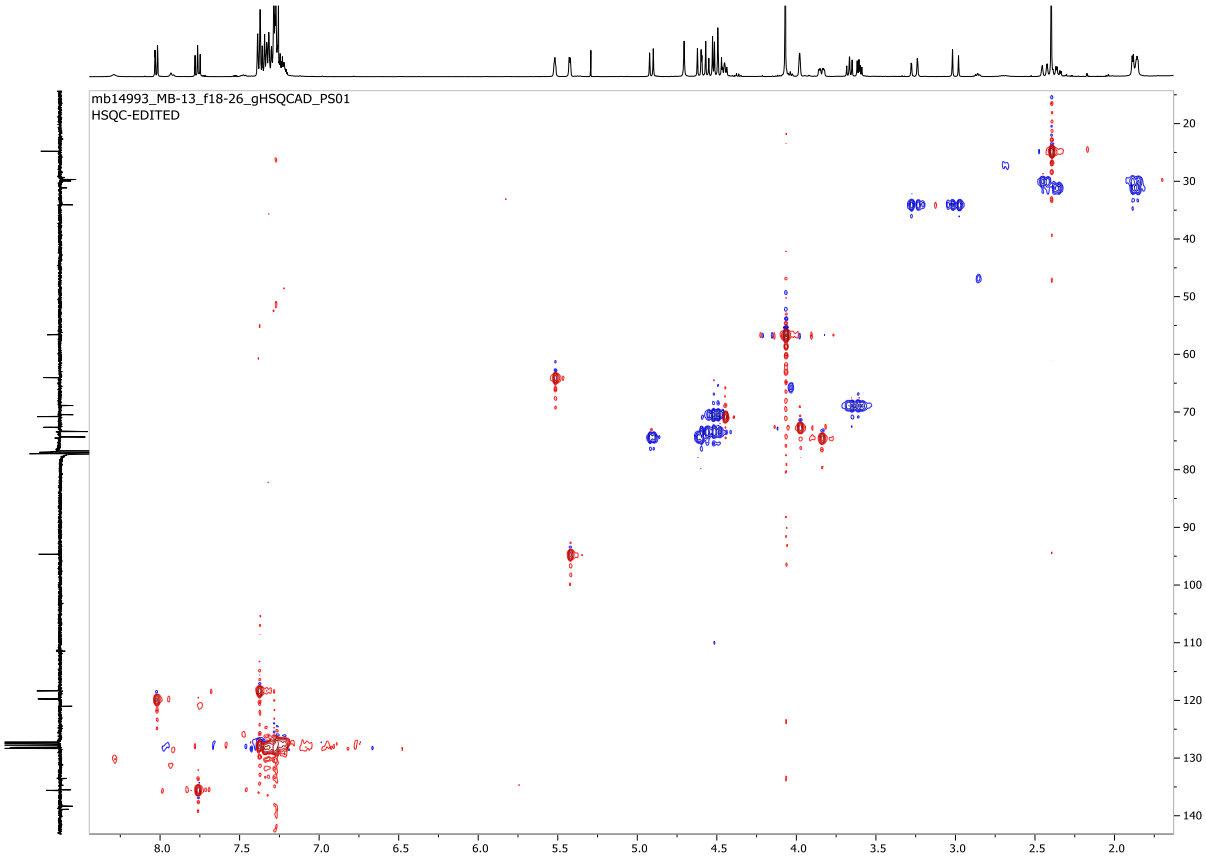
APPENDIX 41. Compound **53α**







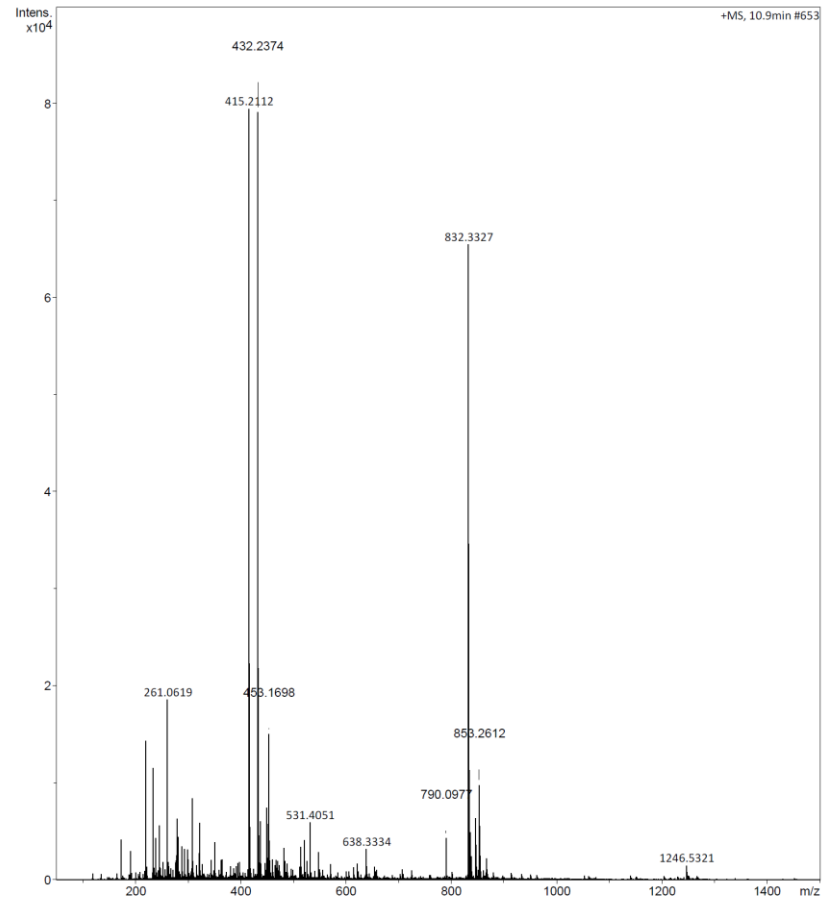

mb14993_MB-13_f18-26_gCOSY01
COSY


mb14993_MB-13_f18-26_gHSQCAD_PS01
HSQC-EDITED

Generic Display Report

Analysis Info

Analysis Name	D:\Data\PROF. IVONE\MB-13_f 27-47_POS.d	Acquisition Date	7/2/2018 3:18:19 PM
Method	Tune_Low_Tomaz_Pos_1000.m	Operator	TOMAZ
Sample Name	MB-13_f 27-47_POS	Instrument	micrOTOF-Q
Comment			



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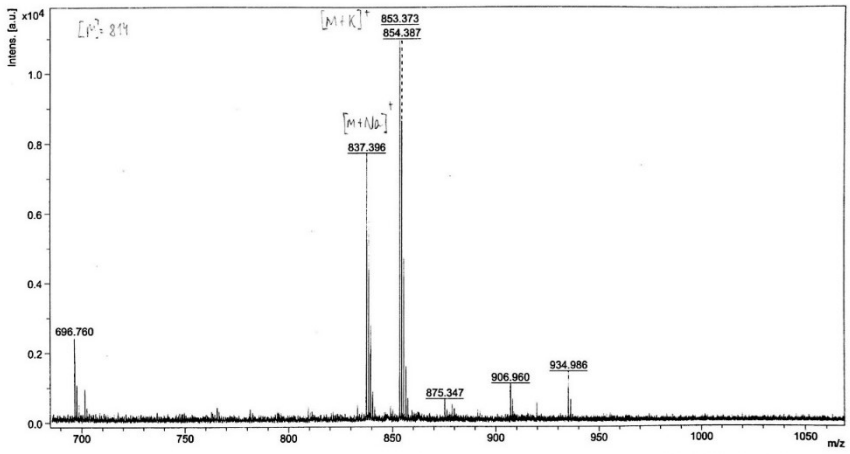
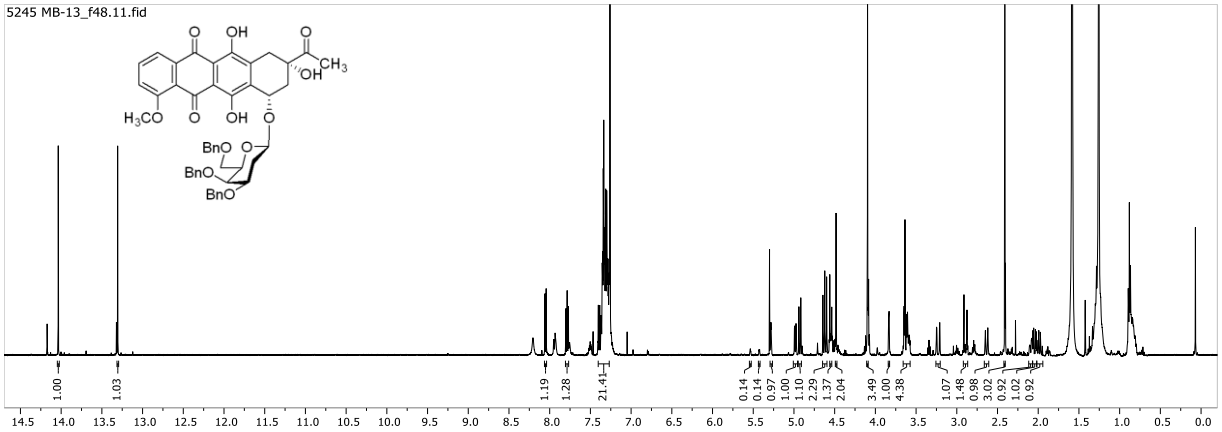
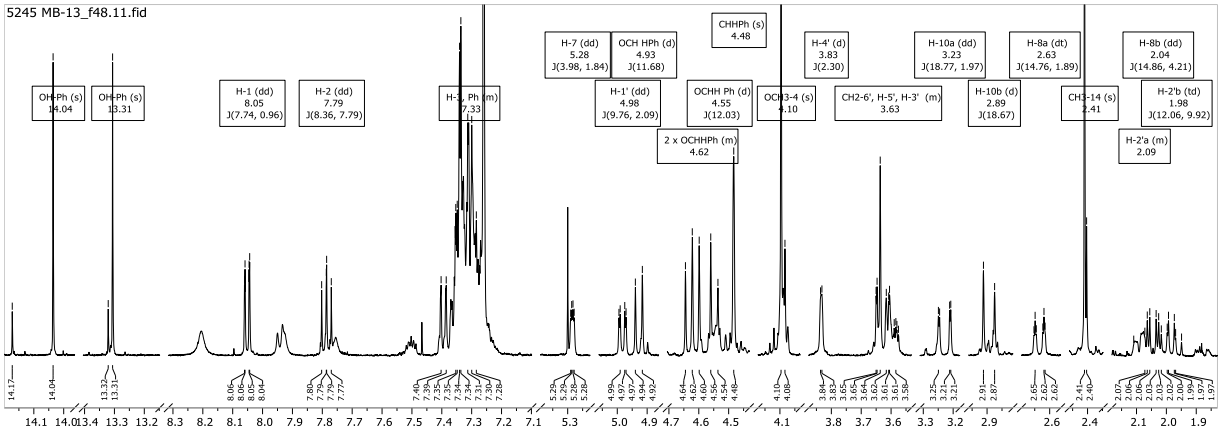
Analysis Name	170502-mb-mb13-f18-26\0_F19\1	*User*	bdal
Comment 1		*Instrument*	FLEX-PC
Method	D:\Methods\flexControlMethods\RP_700-3500_Da.par		MALDI MASS SPECTRUM REPORT

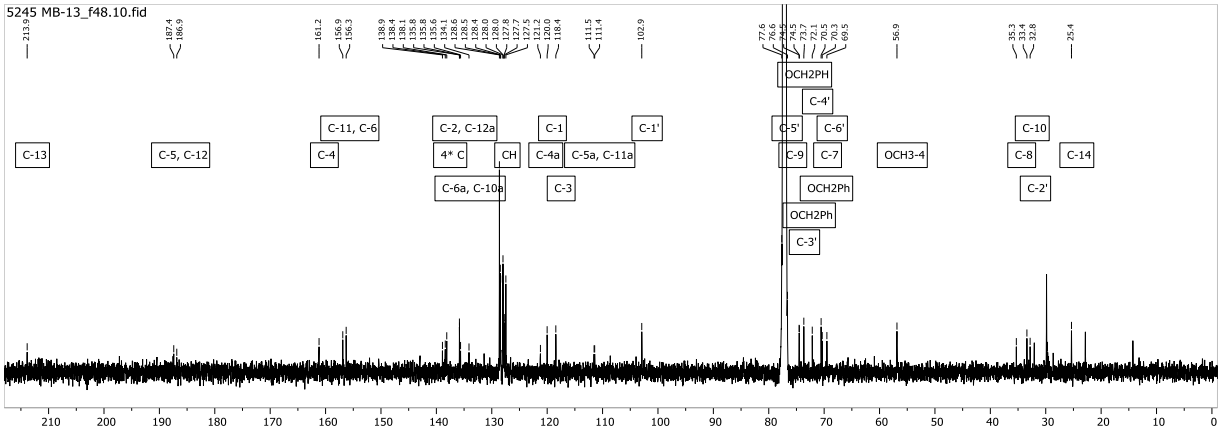


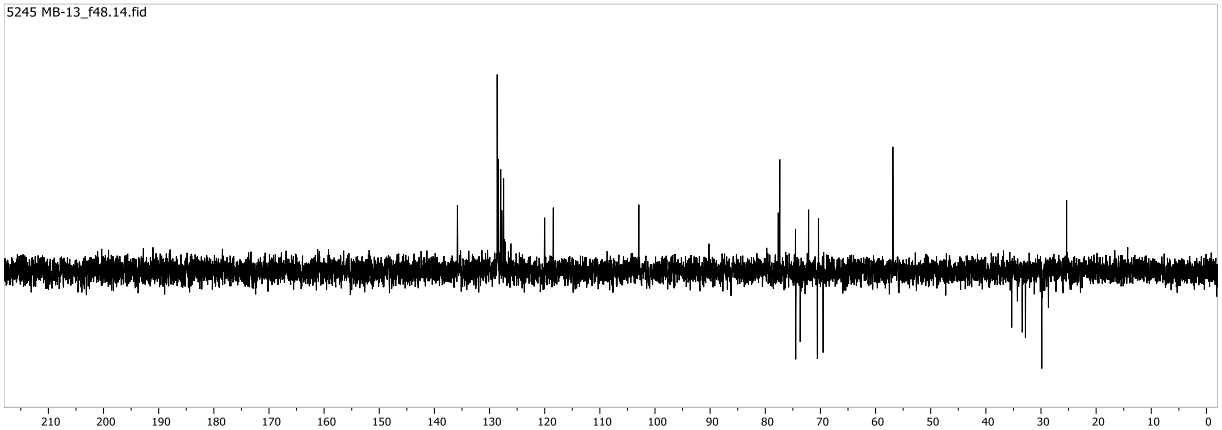
Bruker Daltonics flexAnalysis printed: 02/05/17 14:12:23

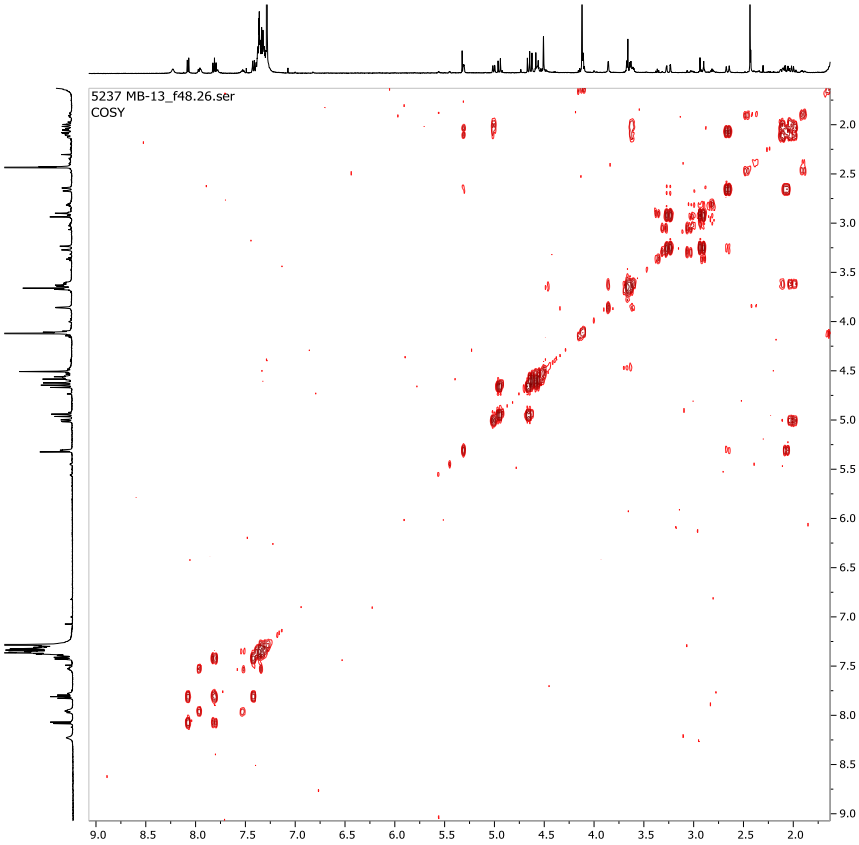
APPENDIX 42. Compound **53β**

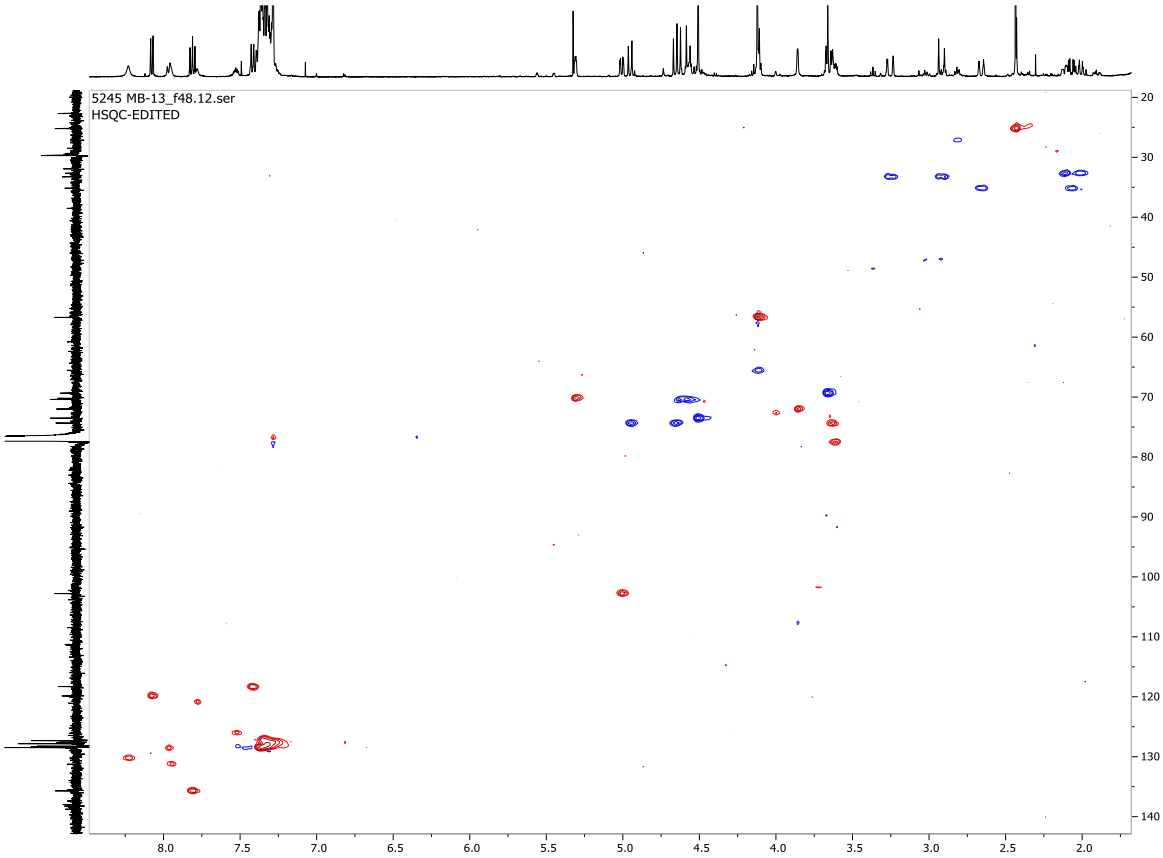








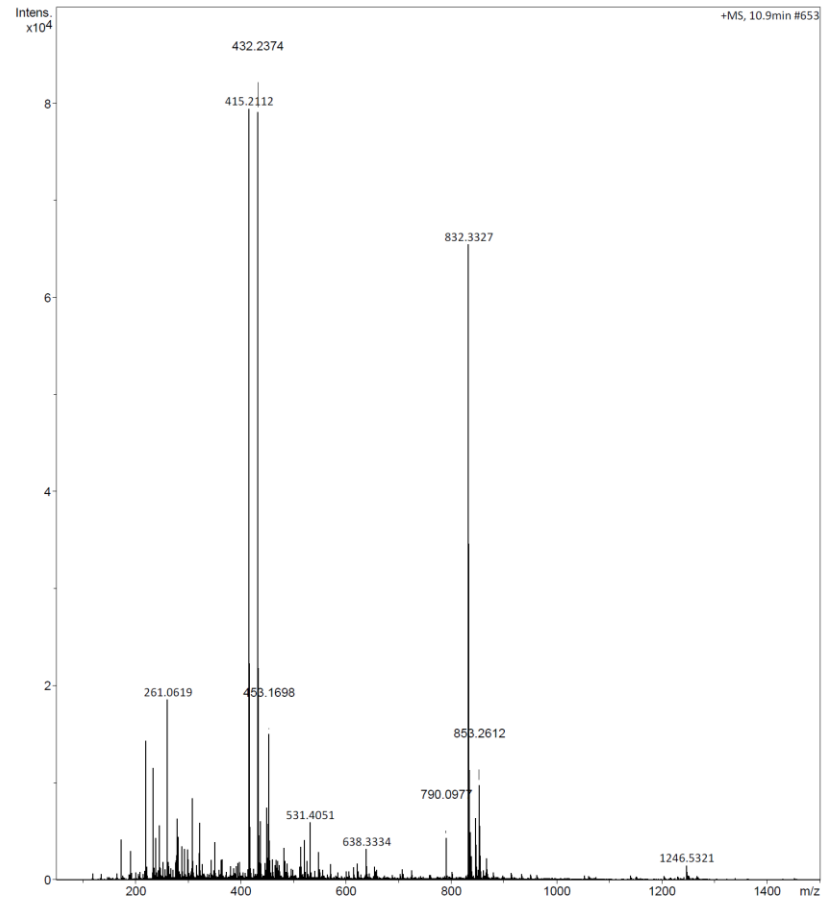

5237 MB-13_f48.26.ser
COSY
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0


5245 MB-13_f48.12.ser
HSQC-EDITED
20 30 40 50 60 70 80 90 100 110 120 130 140
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0

Generic Display Report

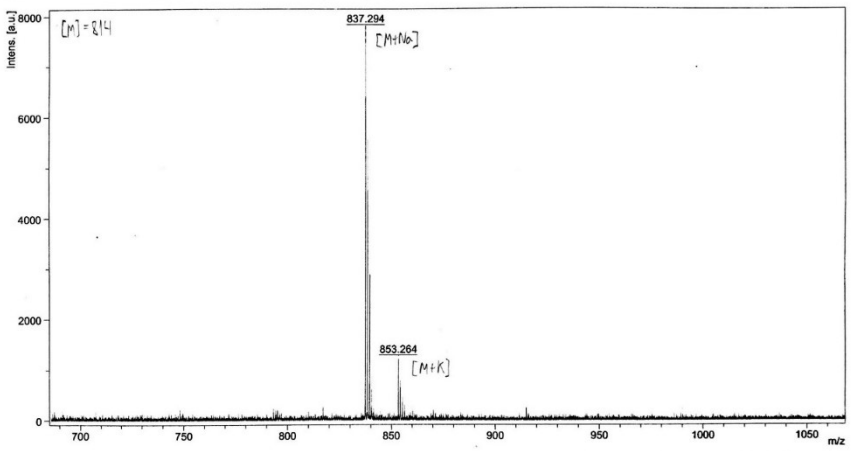
Analysis Info

Analysis Name	D:\Data\PROF. IVONE\MB-13_f 27-47_POS.d	Acquisition Date	7/2/2018 3:18:19 PM
Method	Tune_Low_Tomaz_Pos_1000.m	Operator	TOMAZ
Sample Name	MB-13_f 27-47_POS	Instrument	micrOTOF-Q
Comment			



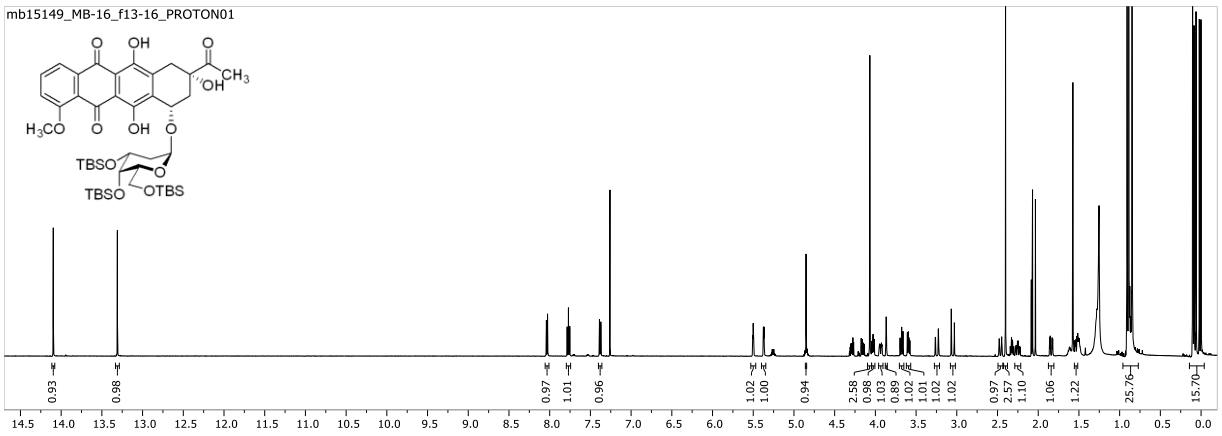
Bruker Compass DataAnalysis 4.3 printed: 11/6/2018 9:12:54 AM by: TOMAZ Page 1 of 1

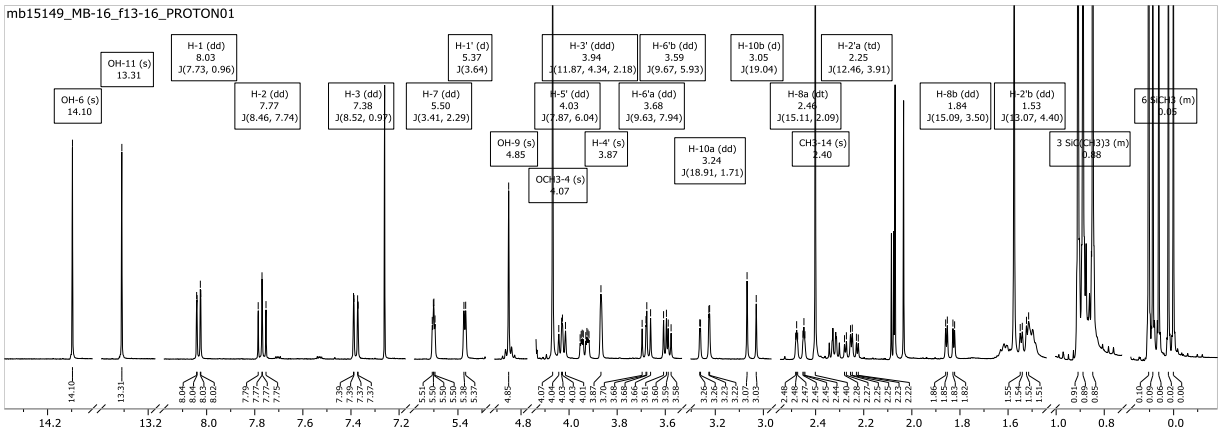
Analysis Name	170502-mb-mb13-f48\0_F13\1	*User*	bdal
Comment 1		*Instrument*	FLEX-PC
Method	D:\Methods\flexControlMethods\RP_700-3500_Da.par		MALDI MASS SPECTRUM REPORT

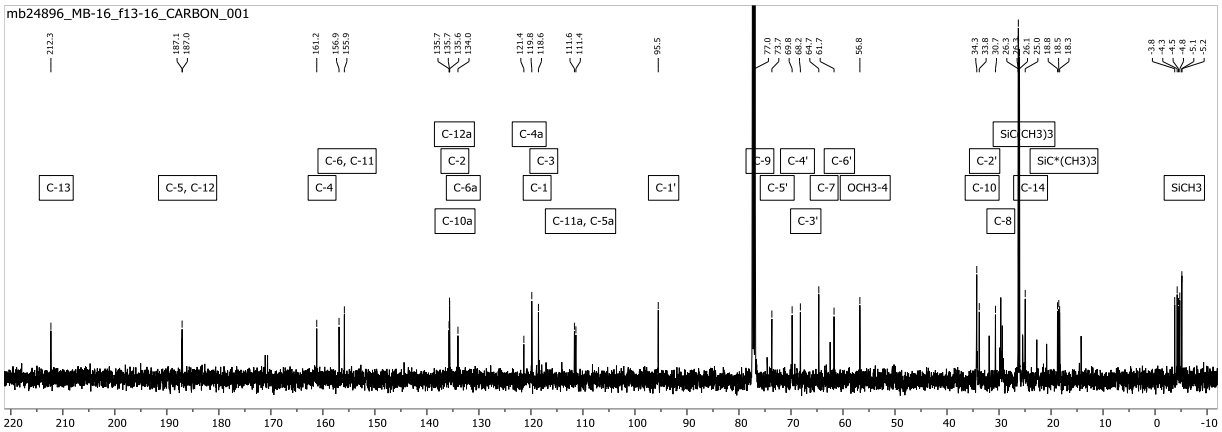


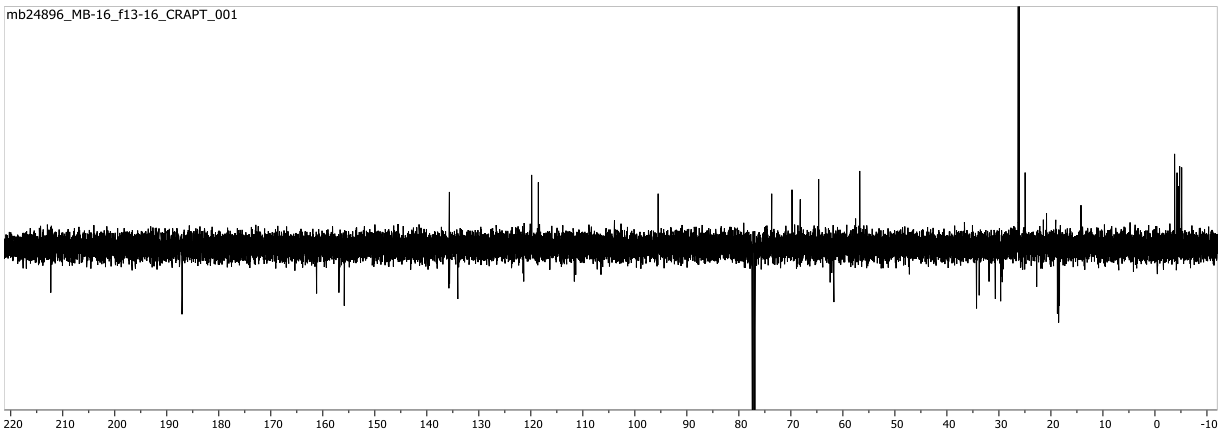
Bruker Daltonics flexAnalysis printed: 02/05/17 14:18:44

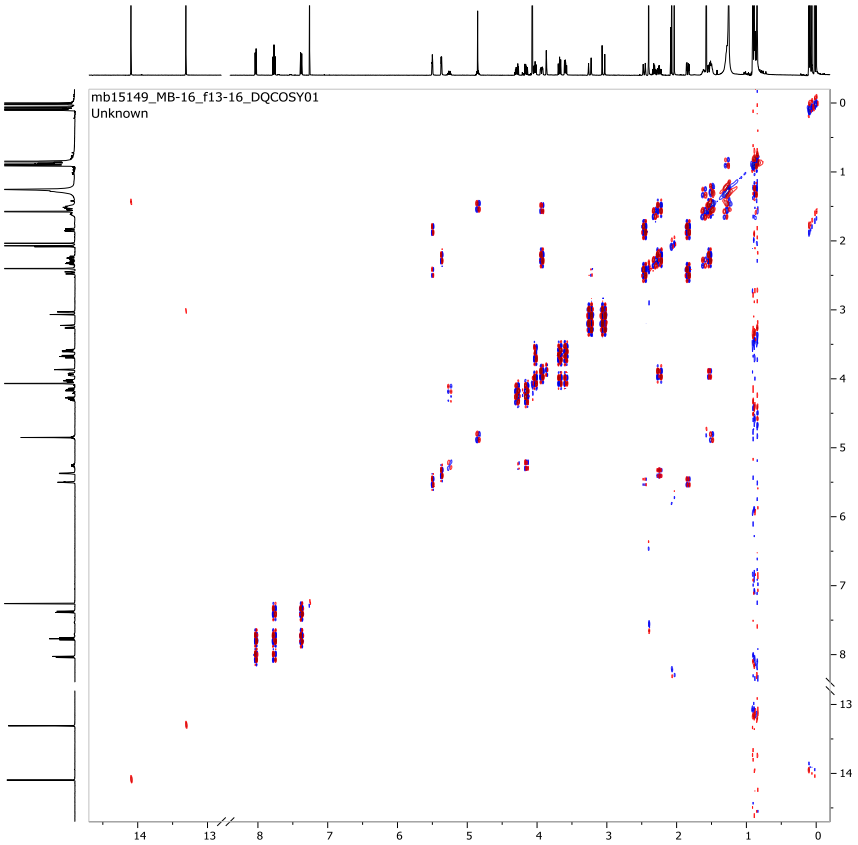
APPENDIX 43. Compound **54α**

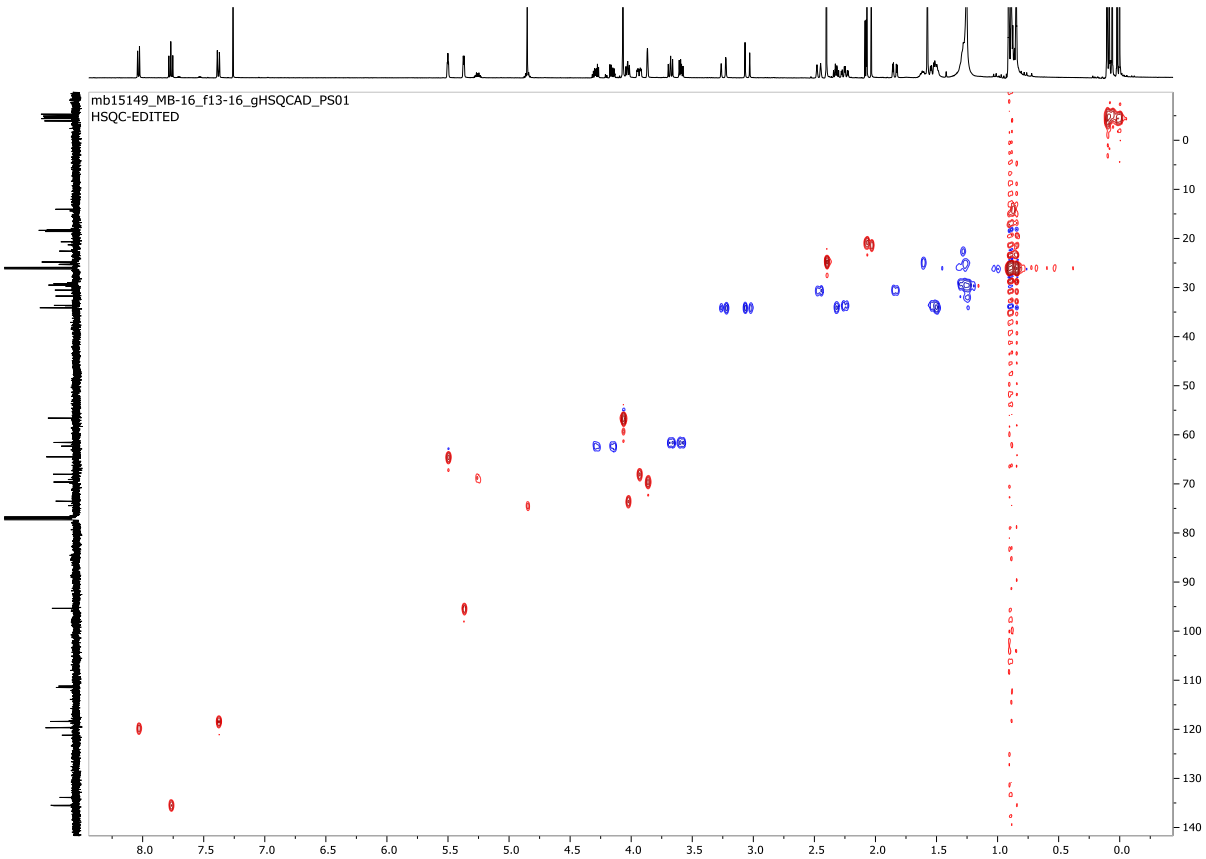









mb15149_MB-16_f13-16_DQCOSY01
Unknown

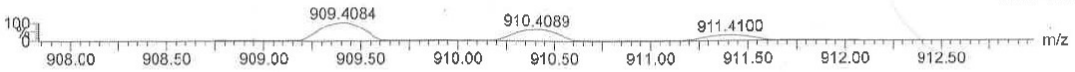

mb15149_MB-16_f13-16_gHSQCAD_PS01
HSQC-EDITED

ental Composition Report **Page 1**

itiple Mass Analysis: 2 mass(es) processed
erance = 5.0 PPM / DBE: min = -1.5, max = 50.0
ement prediction: Off

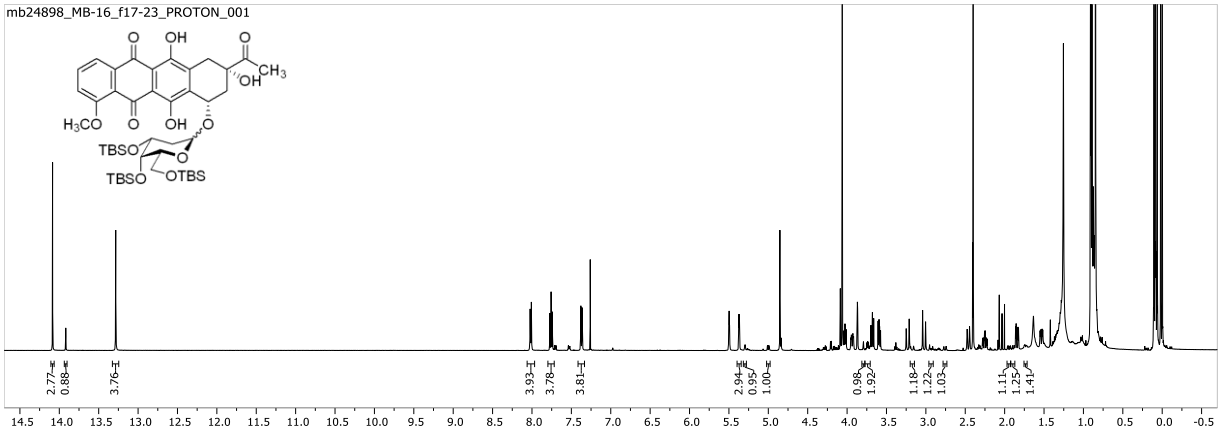
Monoisotopic Mass, Even Electron Ions
52 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-100 O: 11-13 Na: 1-1 Si: 3-3
CG-DB-15707 25 (0.440) Sm (SG, 1x10.00); Cm (2:57)
TOF MS ES+

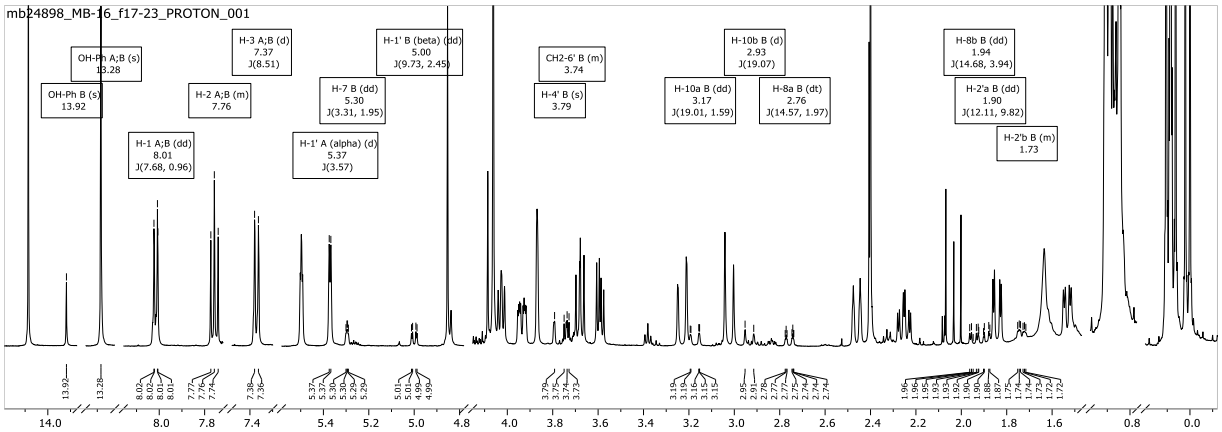
1.33e+006



Minimum:	99.20				-1.5	
Maximum:	100.00		5.0	5.0	50.0	
Mass	RA	Calc. Mass	mDa	PPM	DBE	Formula
909.4084	100.00	909.4073	1.1	1.2	13.5	C45 H70 O12 Na Si3
909.4216	99.70	---				

APPENDIX 44. Compound **54α/β**





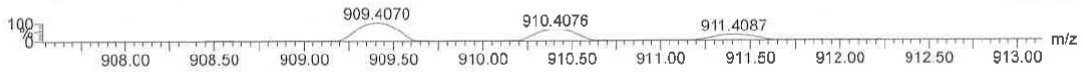
ntal Composition Report

Page 1

iple Mass Analysis: 2 mass(es) processed
rance = 5.0 PPM / DBE: min = -1.5, max = 50.0
ment prediction: Off

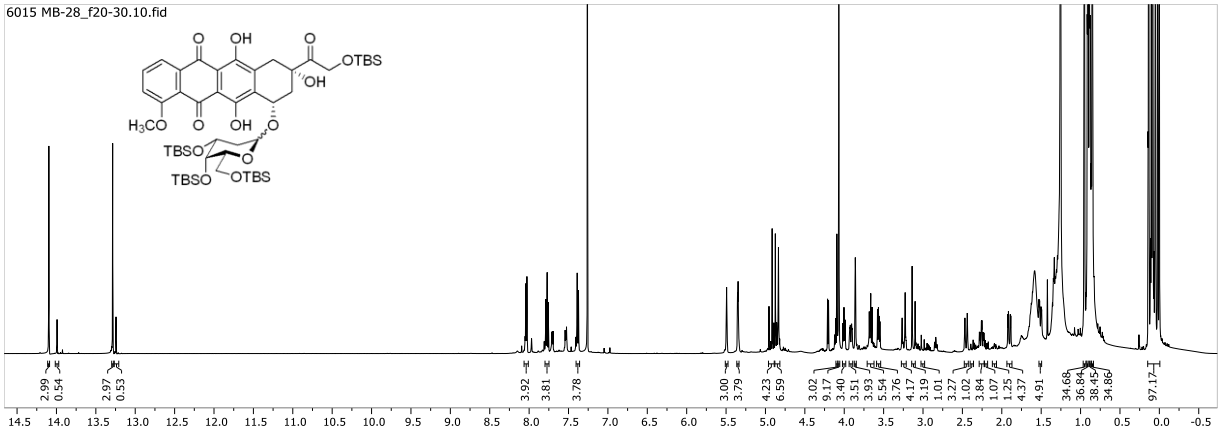
Monoisotopic Mass, Even Electron Ions
52 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-100 O: 11-13 Na: 1-1 Si: 3-3
CG-DB-15708 3 (0.068) Sm (SG, 1x10.00); Cm (3:58)
TOF MS ES+

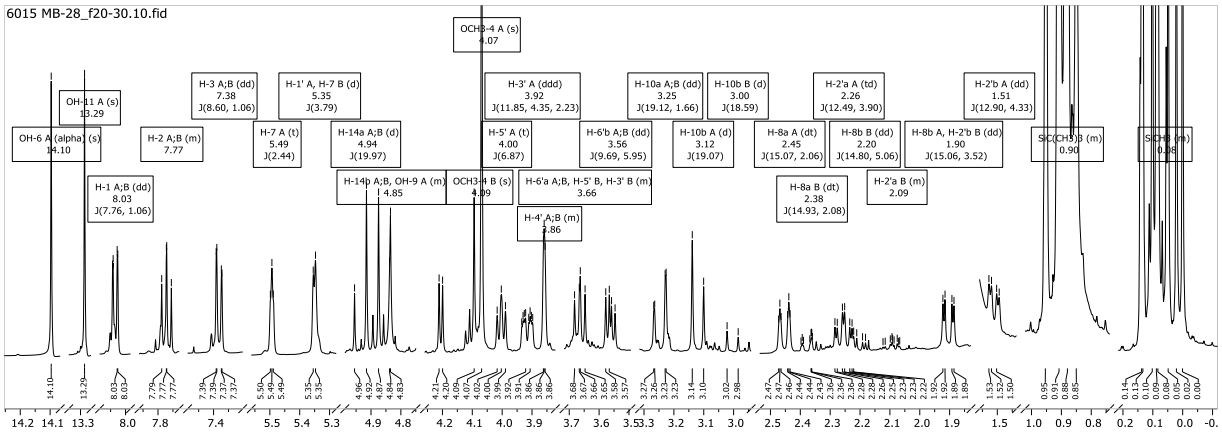
4.59e+005

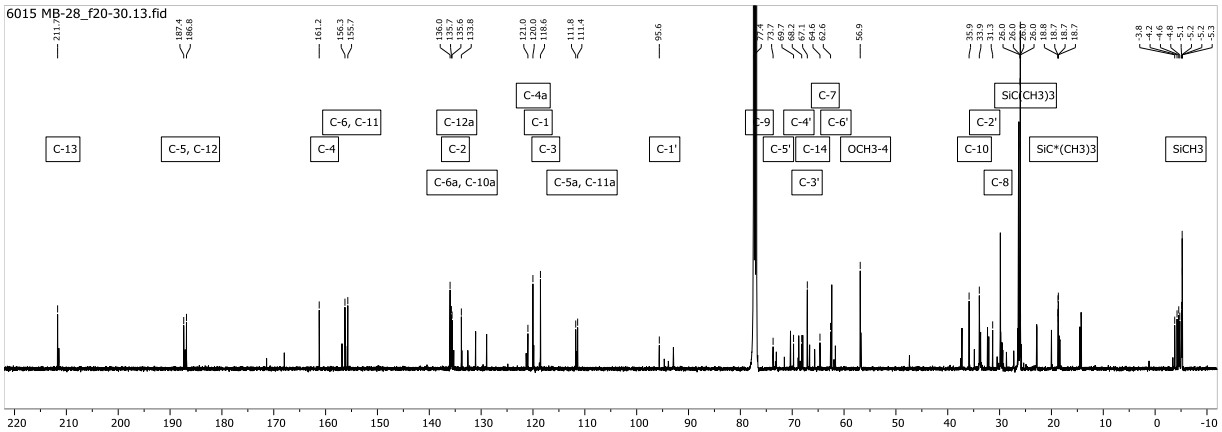


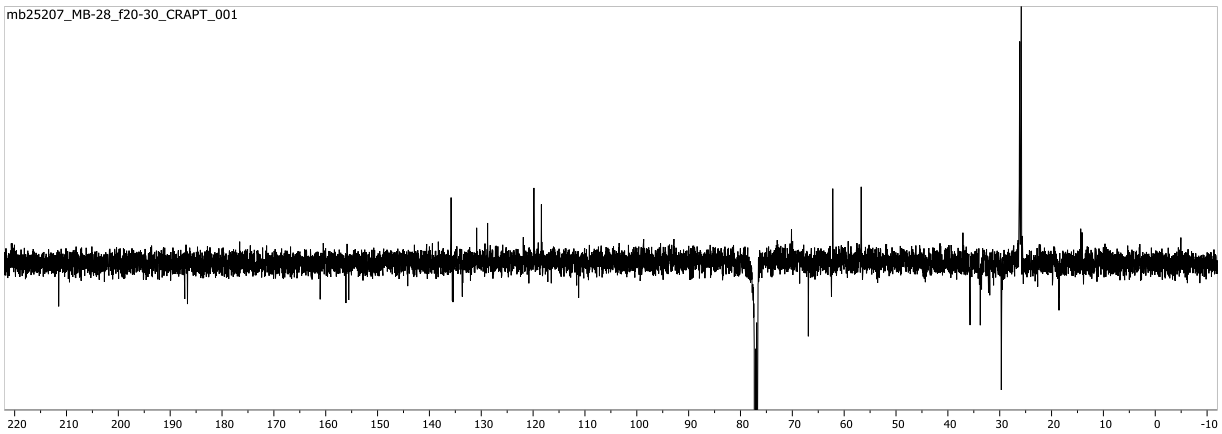
Minimum:	99.20				-1.5	
Maximum:	100.00		5.0	5.0	50.0	
Mass	RA	Calc. Mass	mDa	PPM	DBE	Formula
909.4070	100.00	909.4073	-0.3	-0.3	13.5	C45 H70 O12 Na Si3
909.4202	99.93	---				

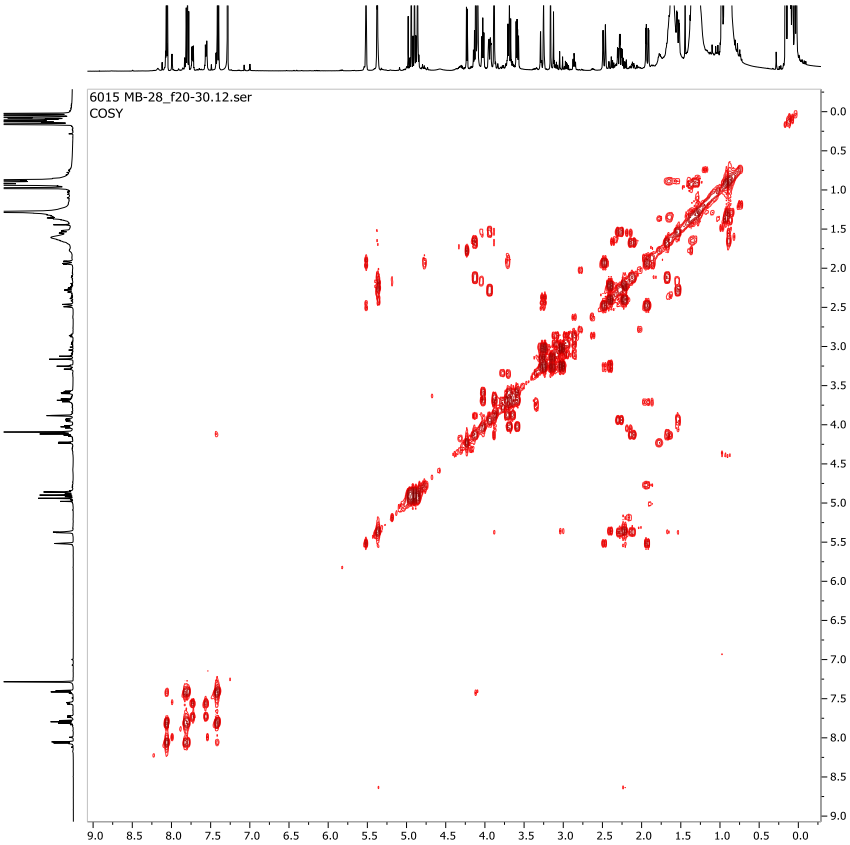
APPENDIX 45. Compound **55**

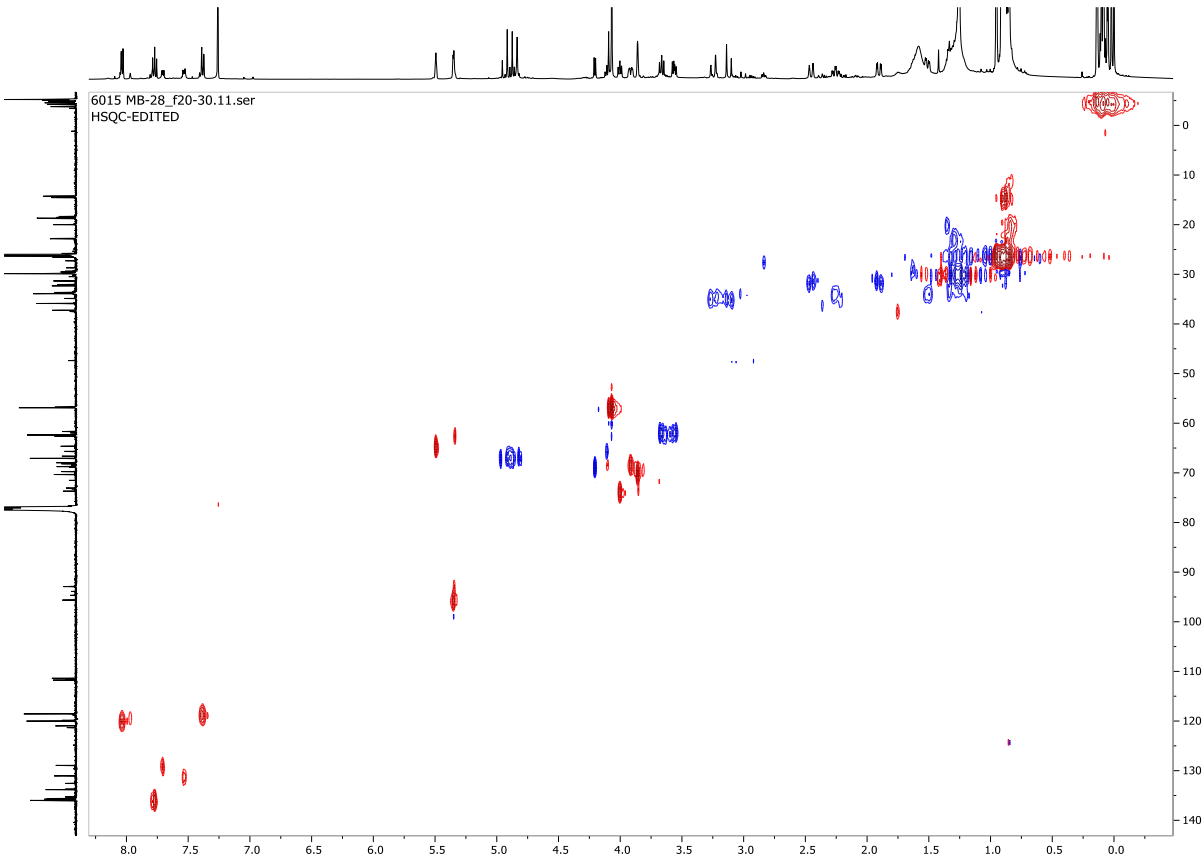


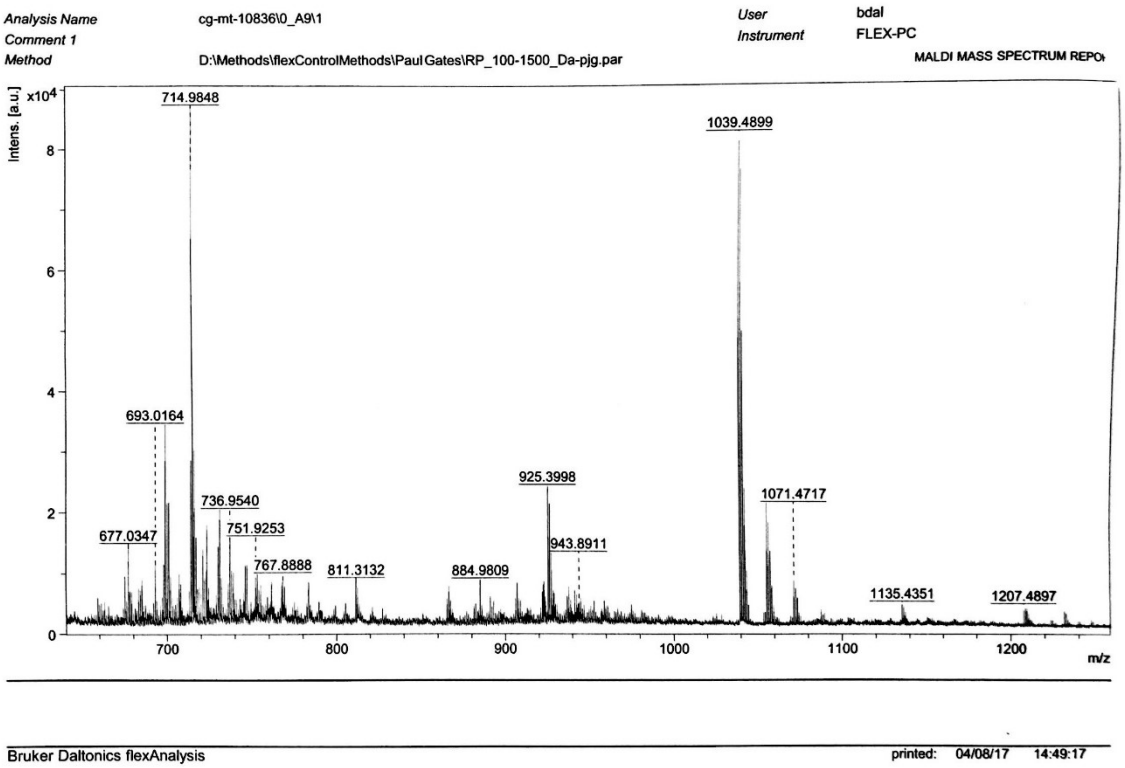







6015 MB-28_f20-30.12.ser
COSY


6015 MB-28_f20-30.11.ser
HSQC-EDITED



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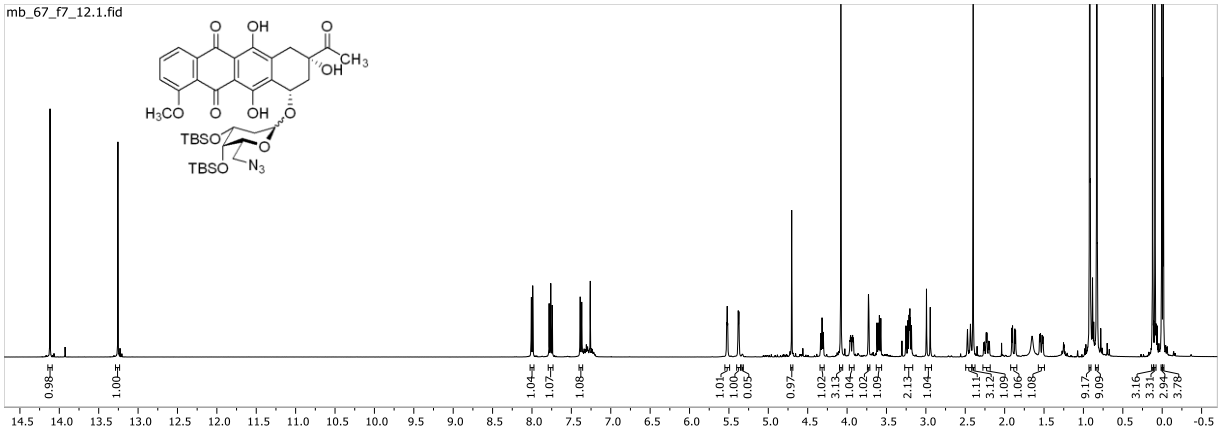
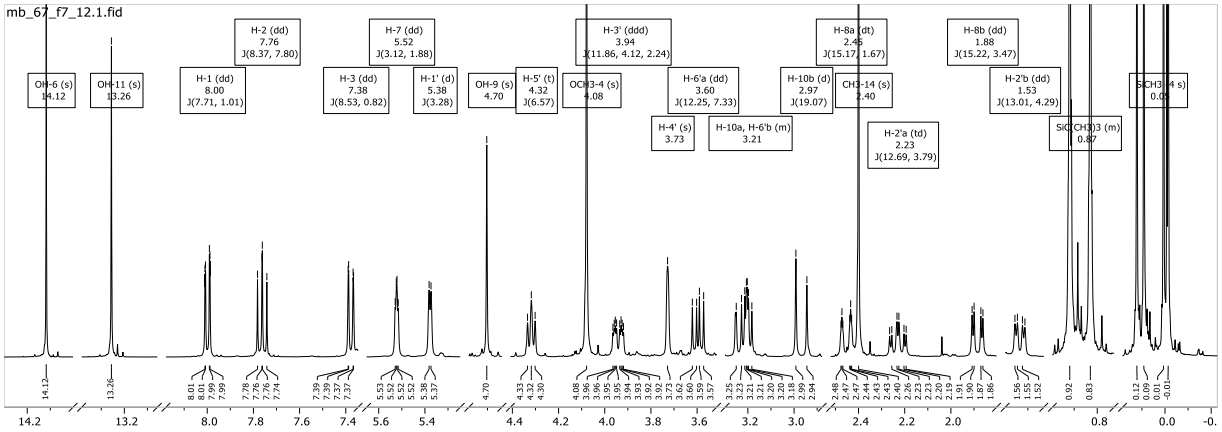
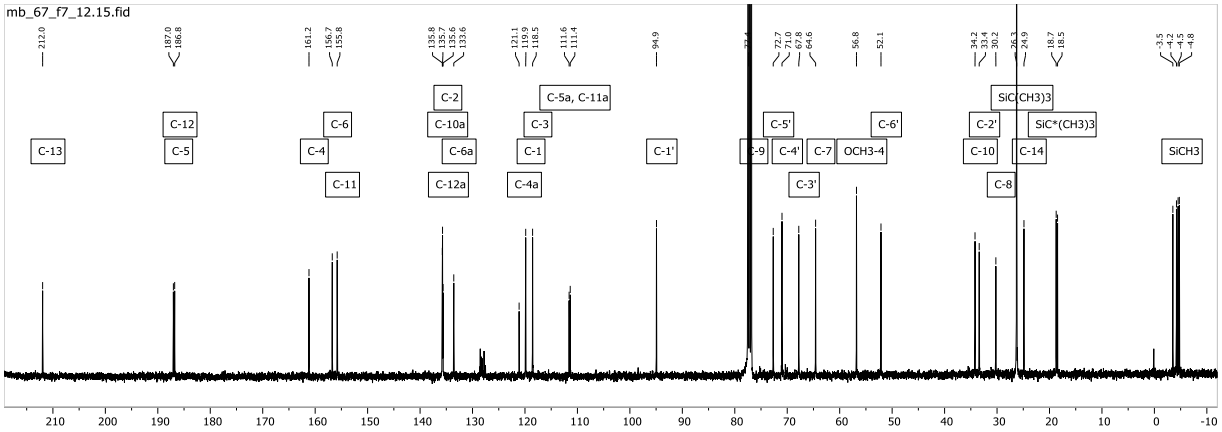
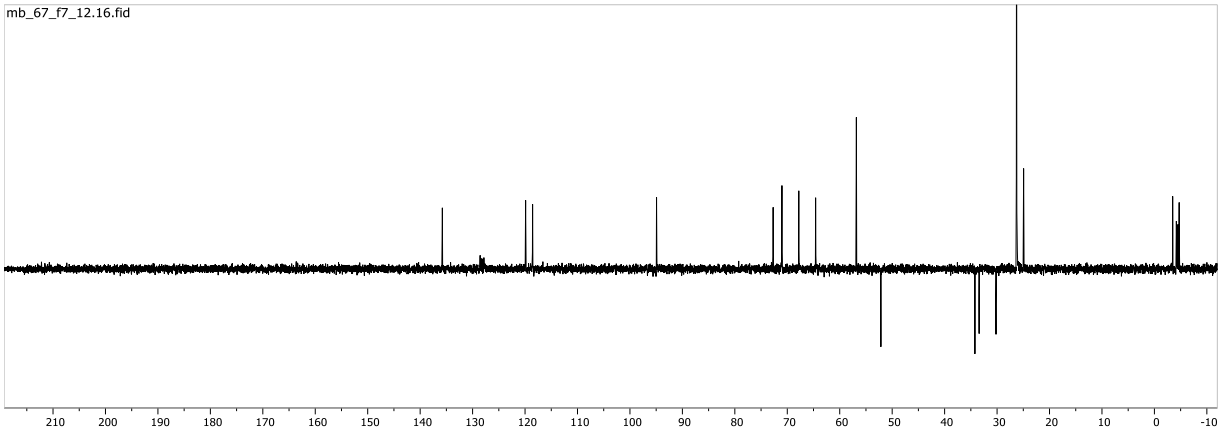
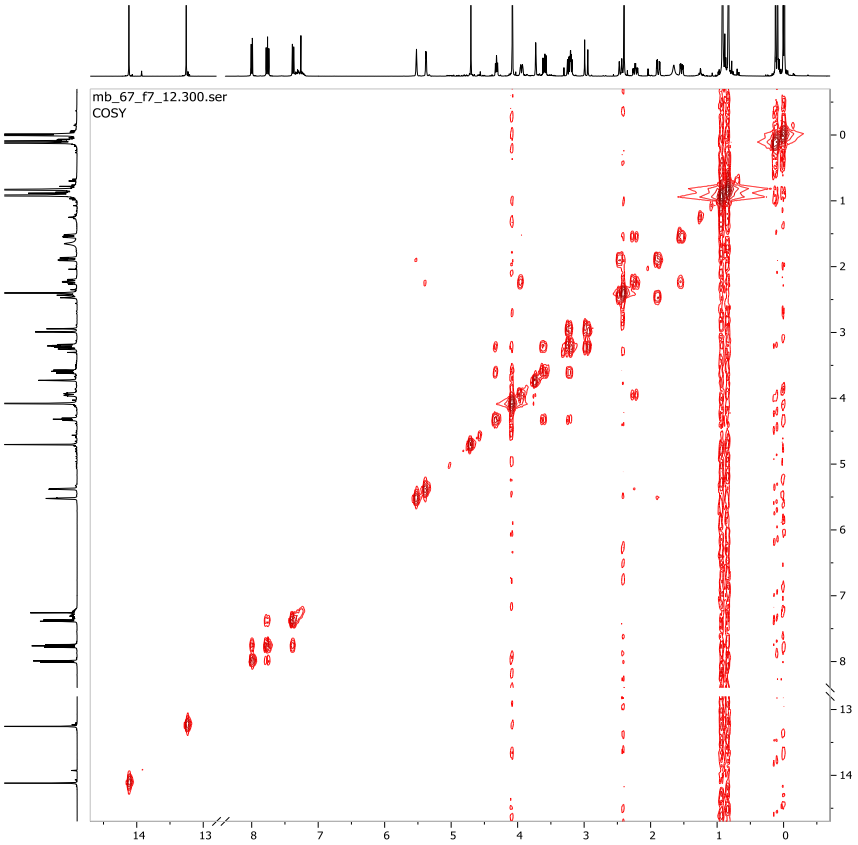
APPENDIX 46. Compound **56**







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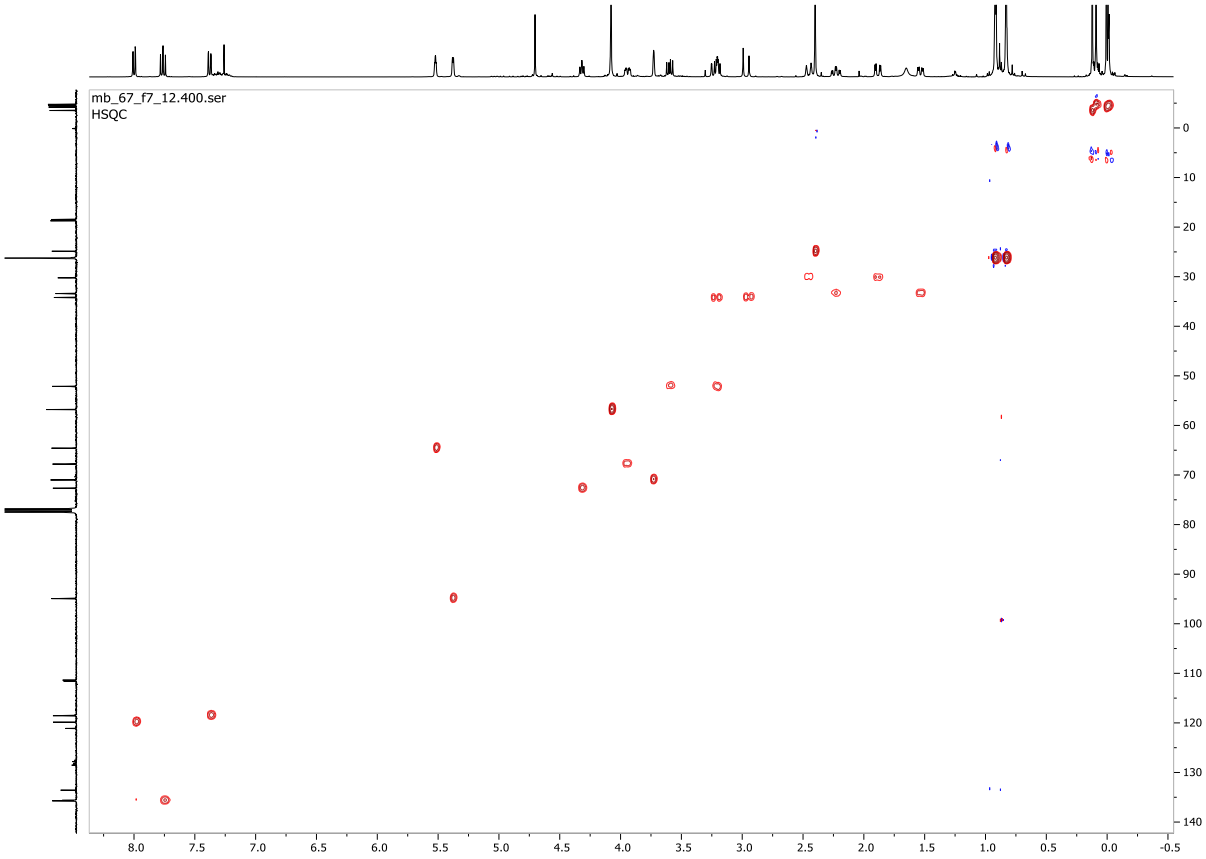
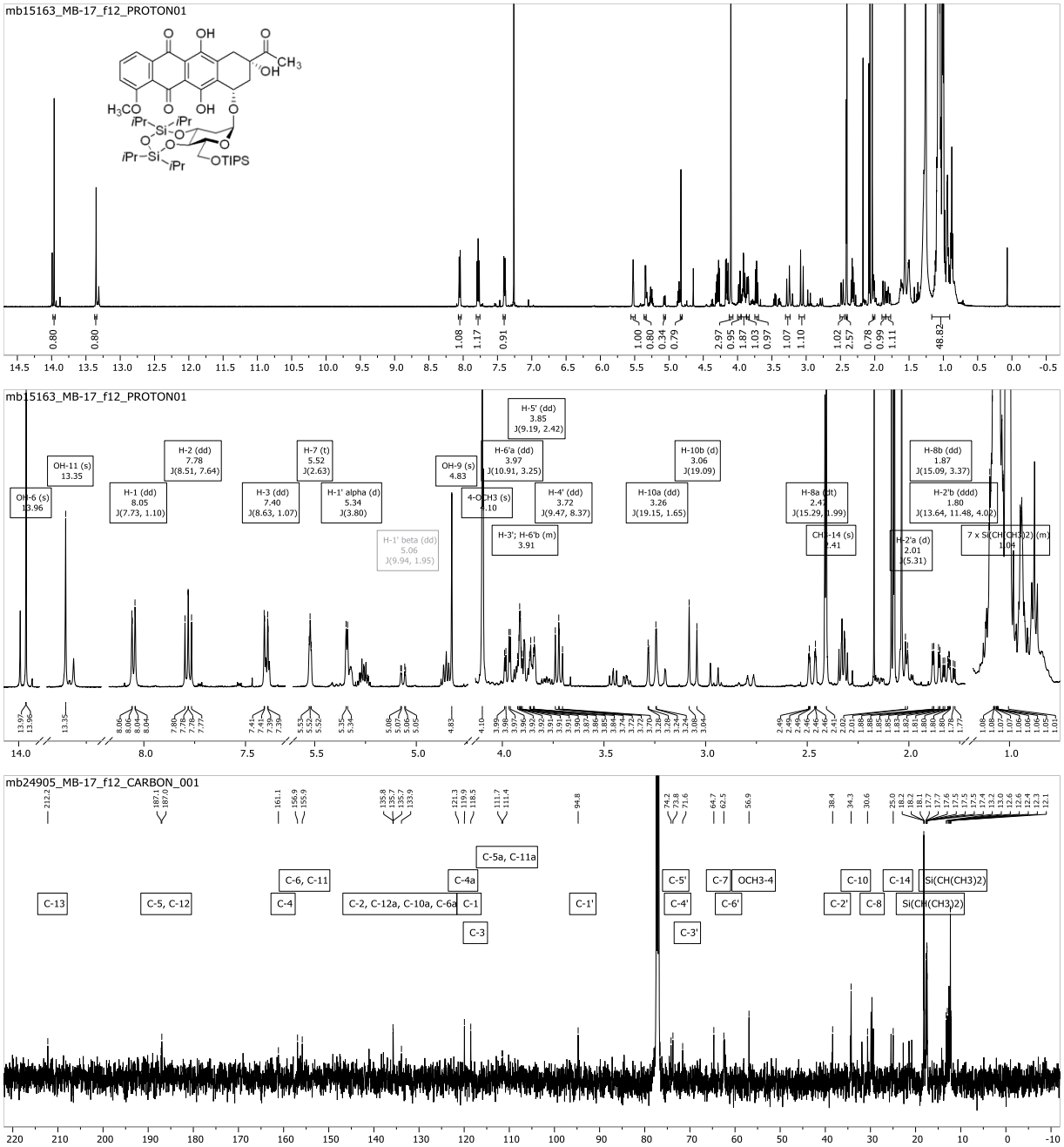
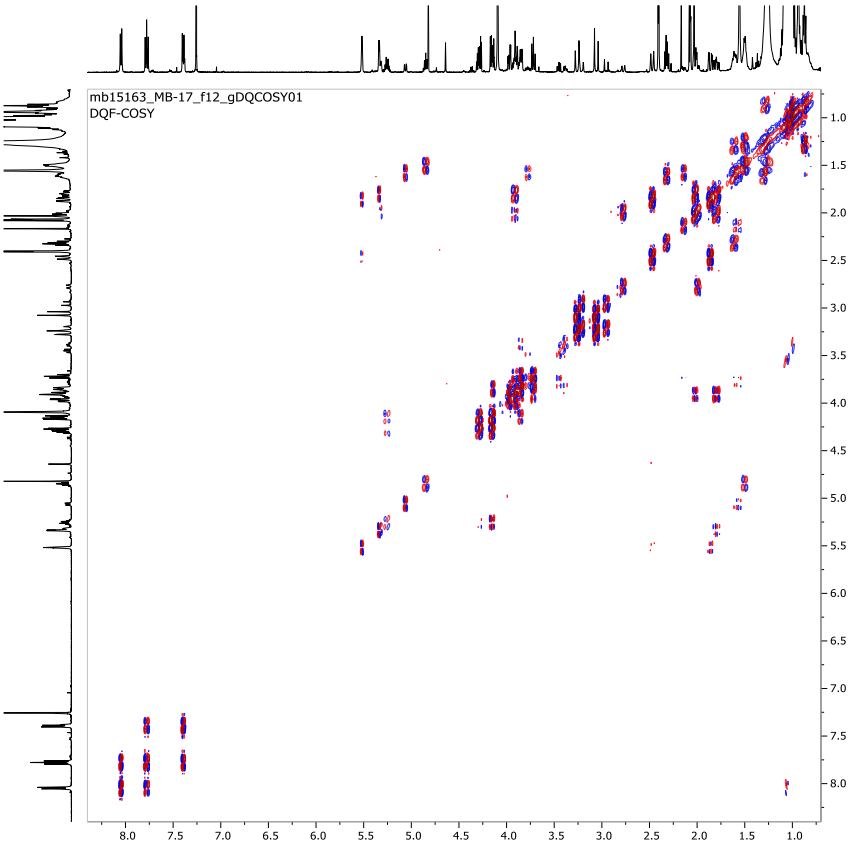
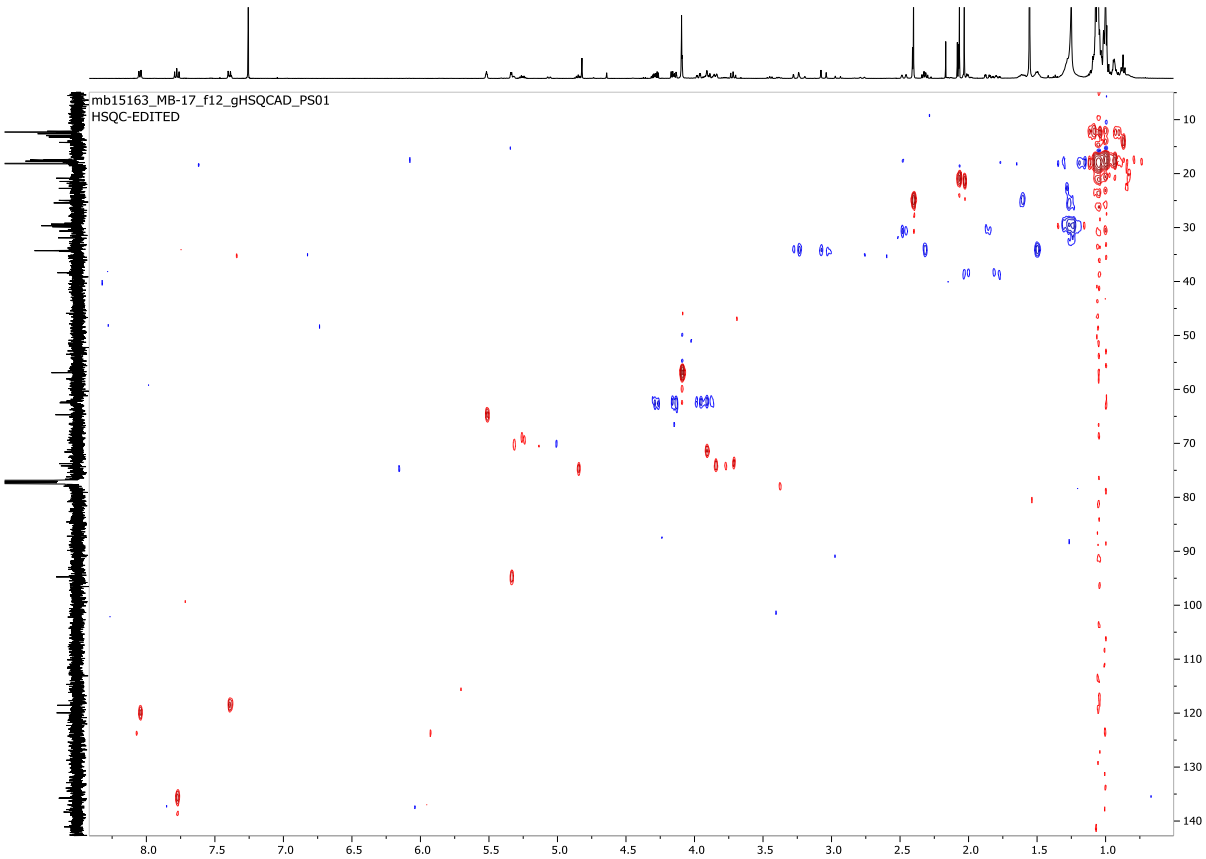
COSY


mb_67_f7_12.400.ser
HSQC

APPENDIX 47. Compound **57α**




mb15163_MB-17_f12_gDQCOSY01
DQF-COSY

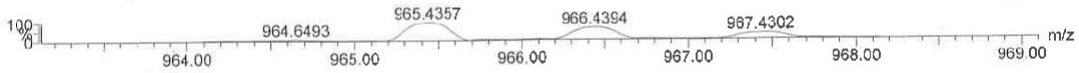

mb15163_MB-17_f12_gHSQCAD_PS01
HSQC-EDITED

ital Composition Report Page 1

ple Mass Analysis: 3 mass(es) processed
rance = 5.0 PPM / DBE: min = -1.5, max = 50.0
ment prediction: Off

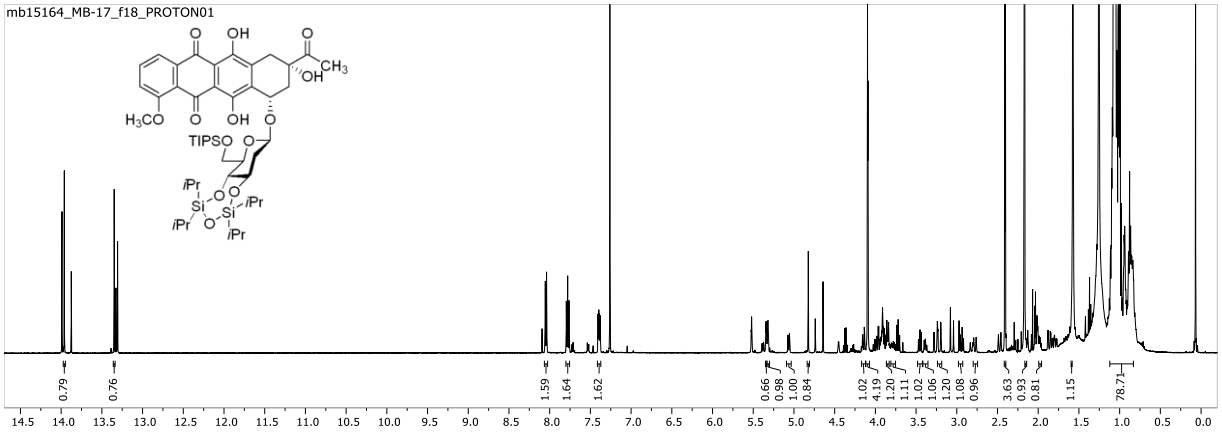
onoisotopic Mass, Even Electron Ions
78 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-100 O: 11-13 Na: 1-1 Si: 3-3
CG-DB-15709 11 (0.203) Sm (SG, 1x10.00); Cm (3:58)
TOF MS ES+

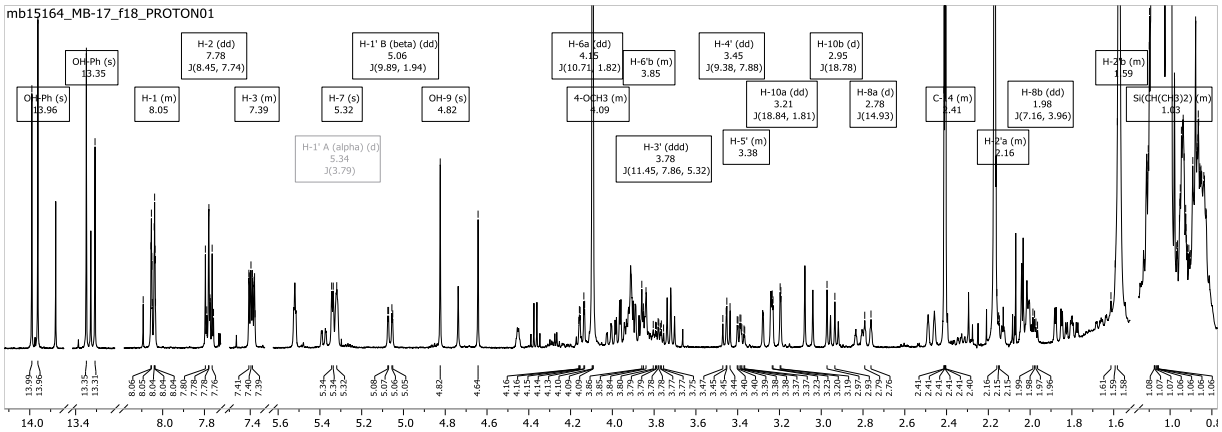
5.31e+005

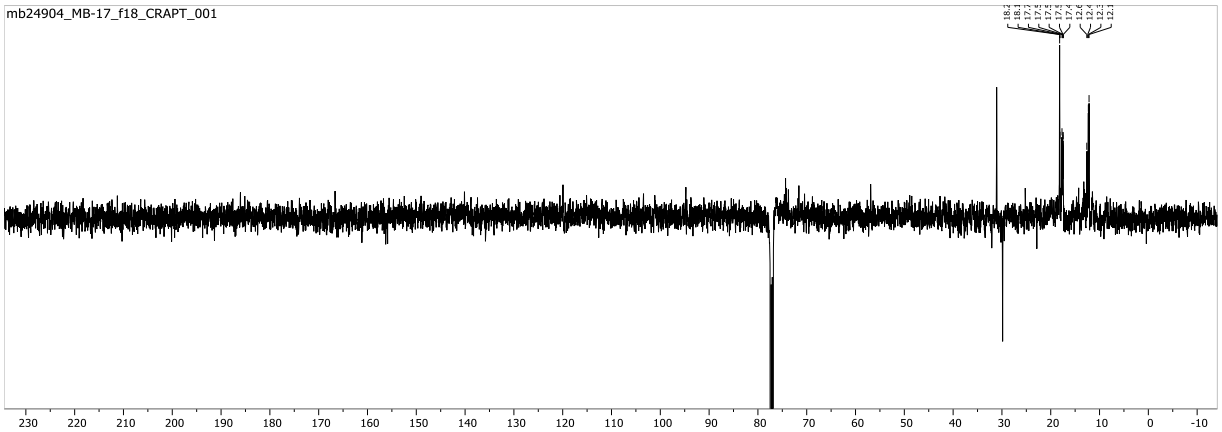


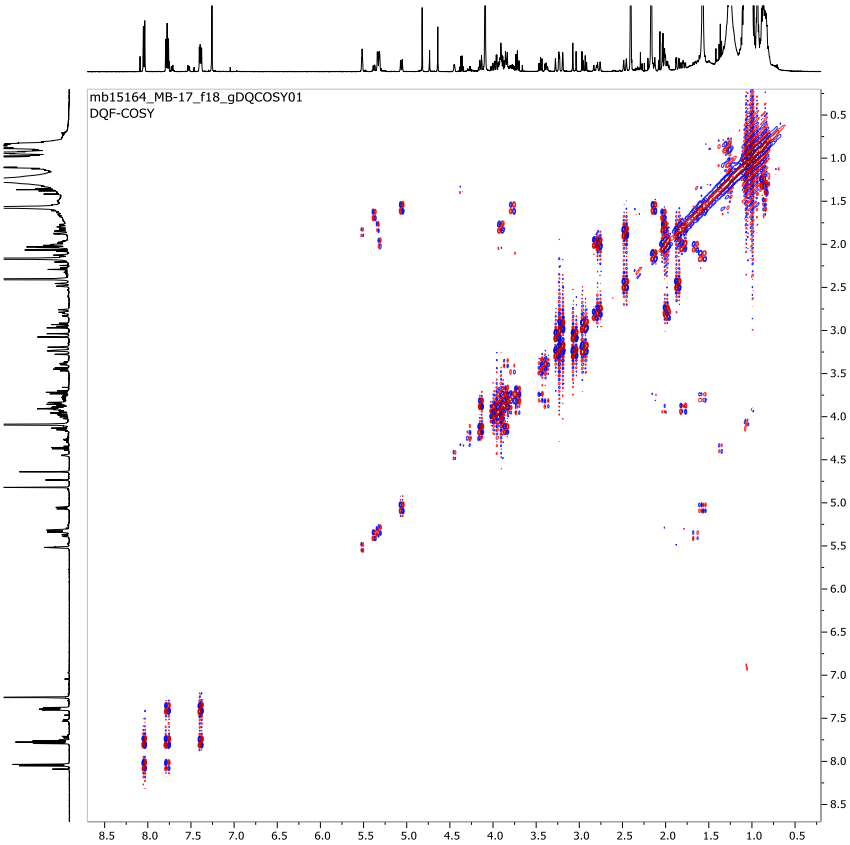
Minimum:	99.20				-1.5	
Maximum:	100.00		5.0	5.0	50.0	
Mass	RA	Calc. Mass	mDa	PPM	DBE	Formula
965.4221	99.53	---				
965.4357	100.00	965.4335	2.2	2.3	14.5	C48 H74 O13 Na Si3
965.4492	99.77	---				

APPENDIX 48. Compound **57β**





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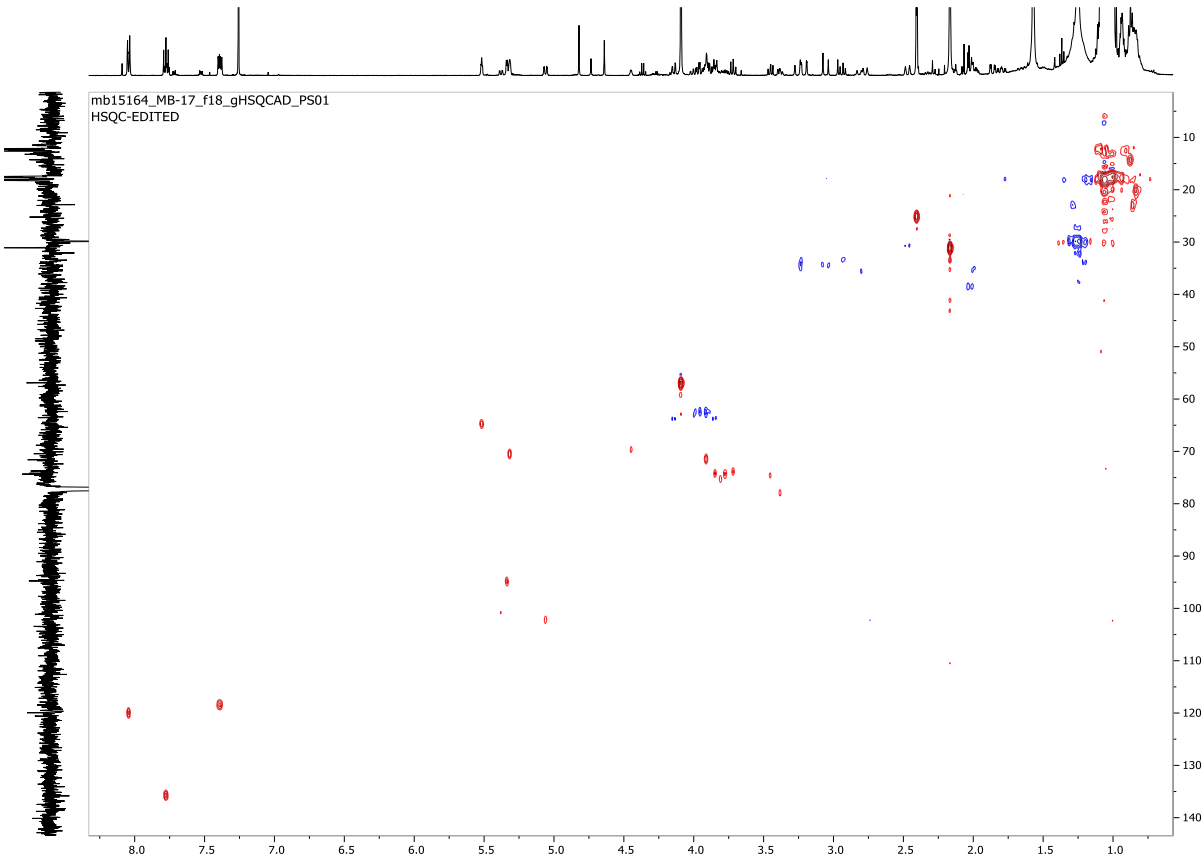
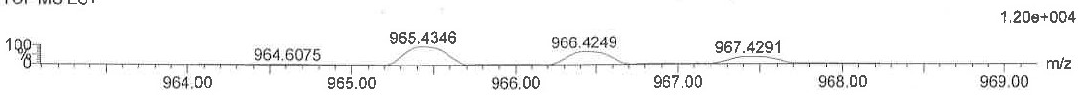
DQF-COSY


mb15164_MB-17_f18_gHSQCAD_PS01
HSQC-EDITED

tal Composition Report **Page 1**

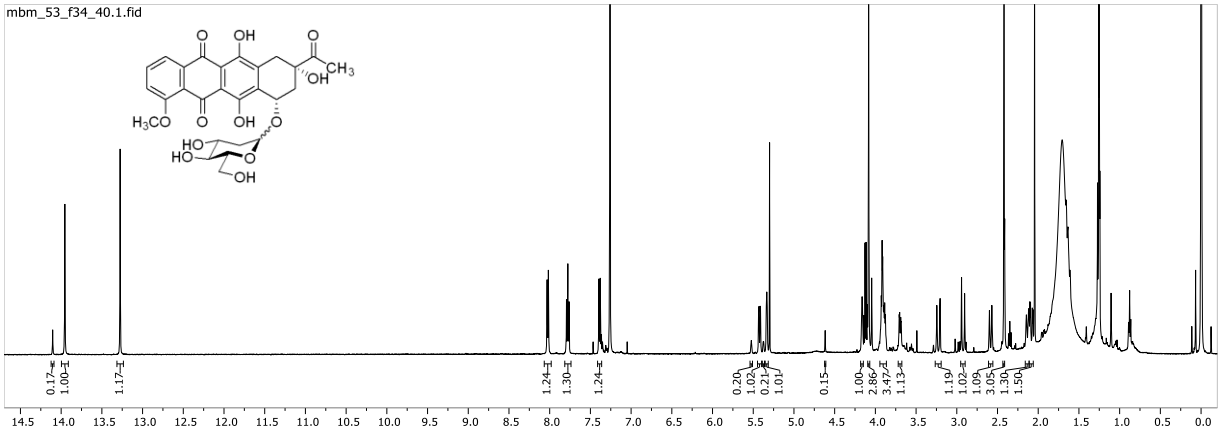
ple Mass Analysis: 4 mass(es) processed
rance = 5.0 PPM / DBE: min = -1.5, max = 50.0
ment prediction: Off

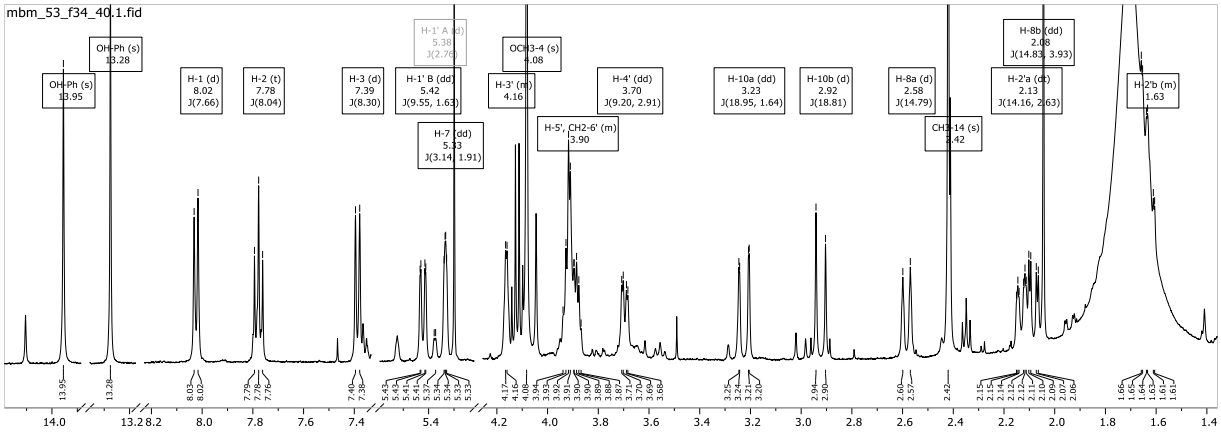
Monoisotopic Mass, Even Electron Ions
104 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-100 O: 11-13 Na: 1-1 Si: 3-3
CG-DB-15710 13 (0.237) Sm (SG, 1x10.00); Cm (8:58)
TOF MS ES+

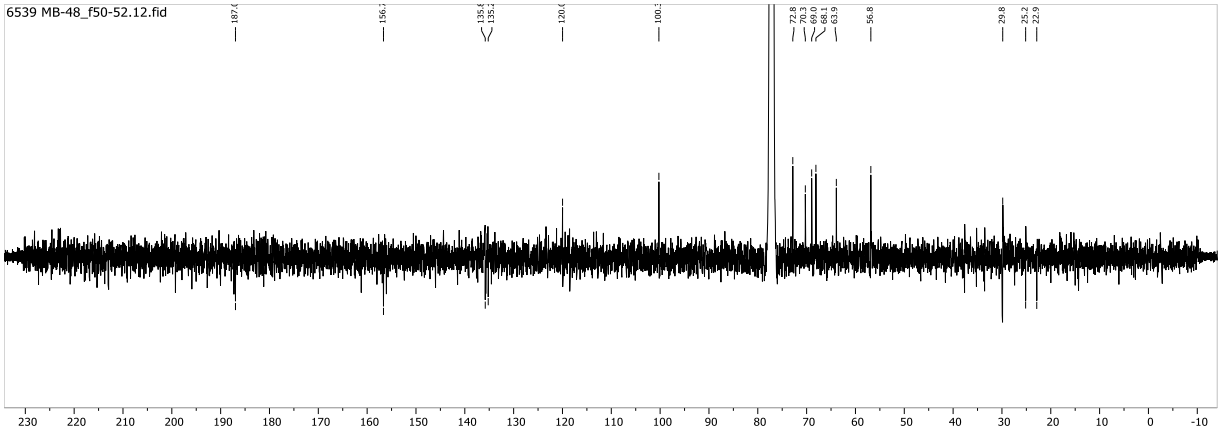


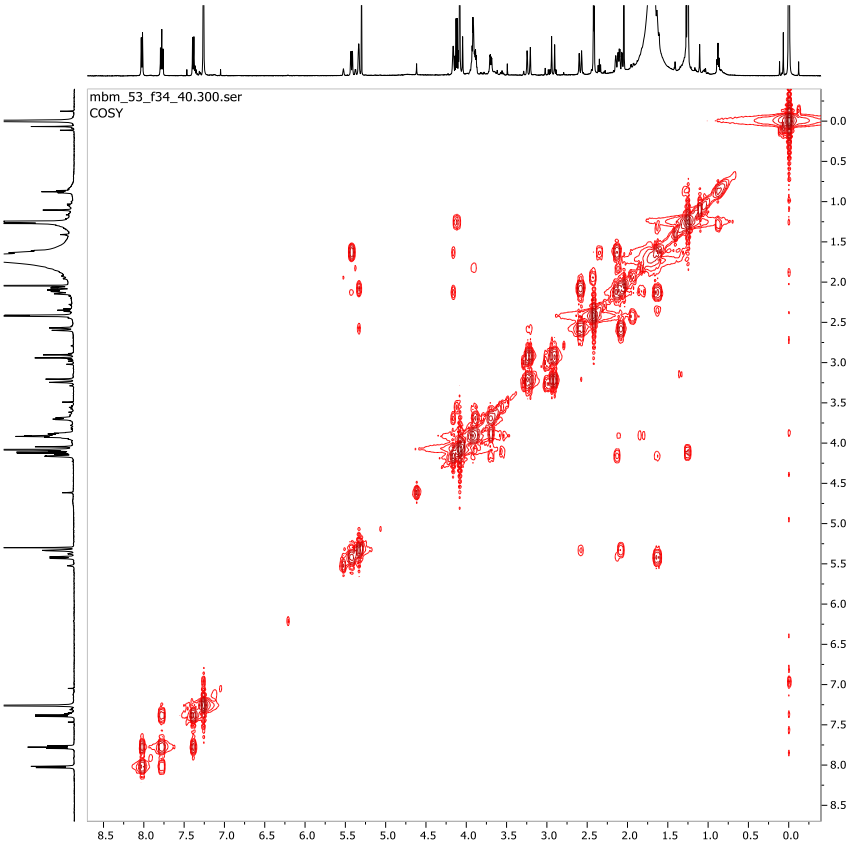
Minimum:	99.20				-1.5	
Maximum:	100.00		5.0	5.0	50.0	
Mass	RA	Calc. Mass	mDa	PPM	DBE	Formula
965.4075	99.42	---				
965.4211	99.92	---				
965.4346	100.00	965.4335	1.1	1.1	14.5	C48 H74 O13 Na Si3
965.4482	99.58	---				

APPENDIX 49. Compound **58**





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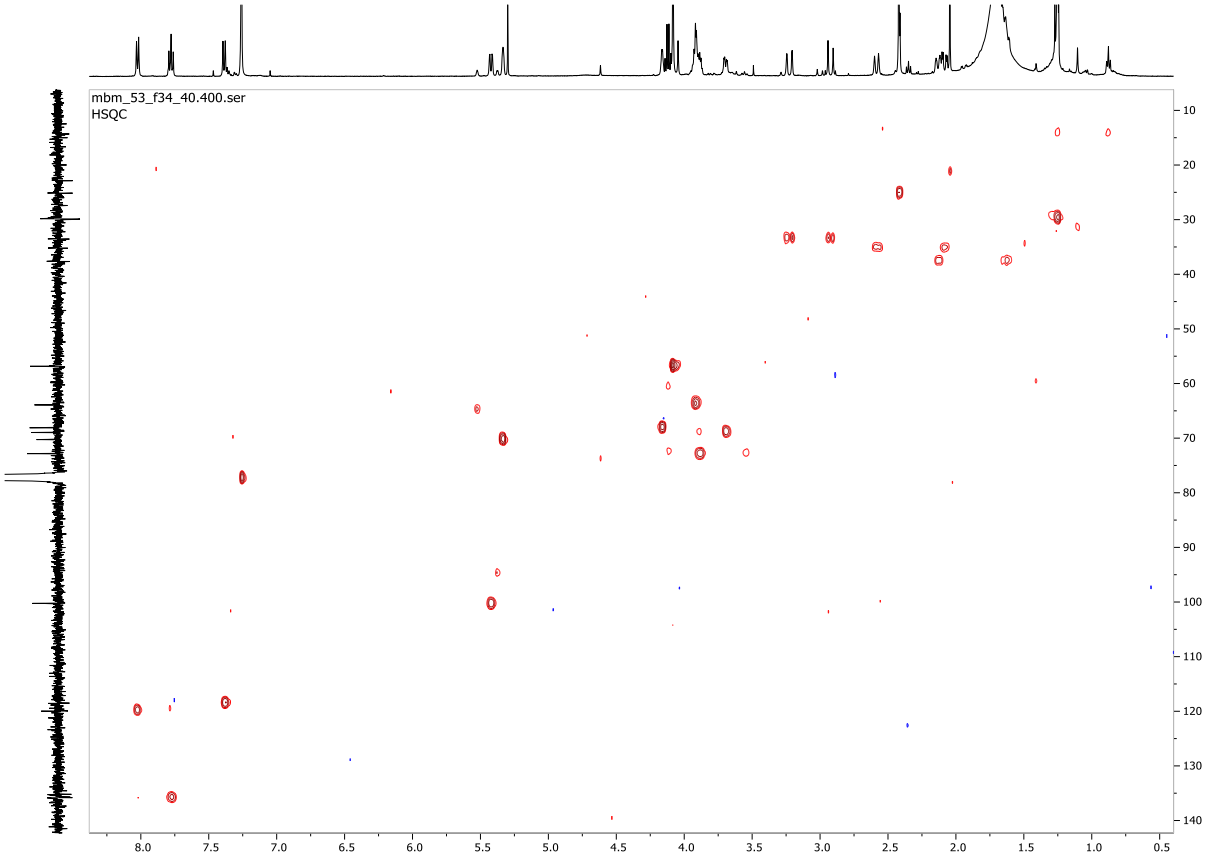
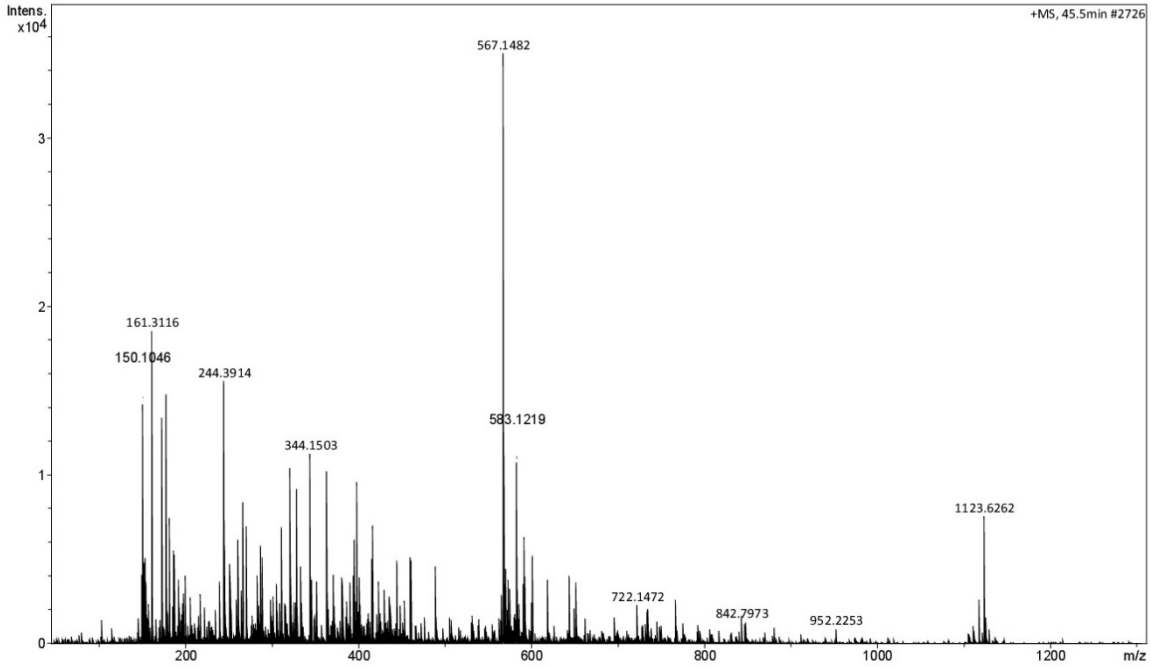
COSY


mbm_53_f34_40.400.ser
HSQC

Generic Display Report

Analysis Info

Analysis Name	D:\Data\IVONE\MBM - 53-f 34-40_POS.d	Acquisition Date	4/13/2018 3:04:06 PM
Method	tune_low_DANIEL 1300 daltons_POS.m	Operator	BD@AL
Sample Name	MBM - 53-f 34-40_POS	Instrument	micrOTOF
Comment			



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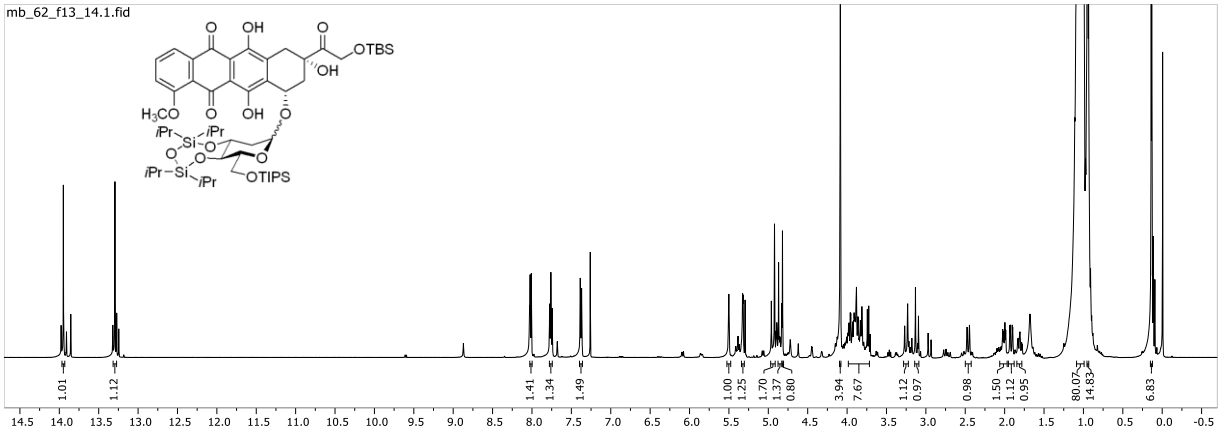
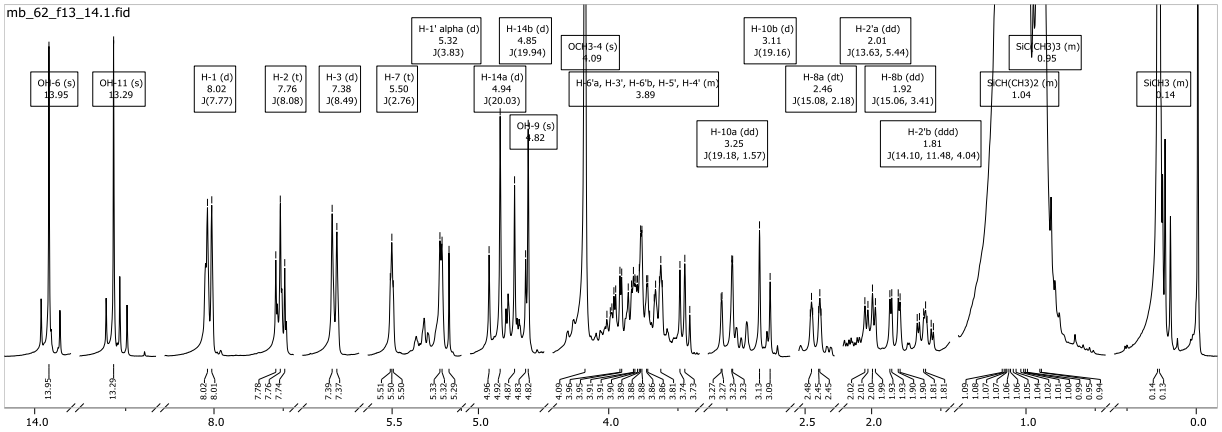
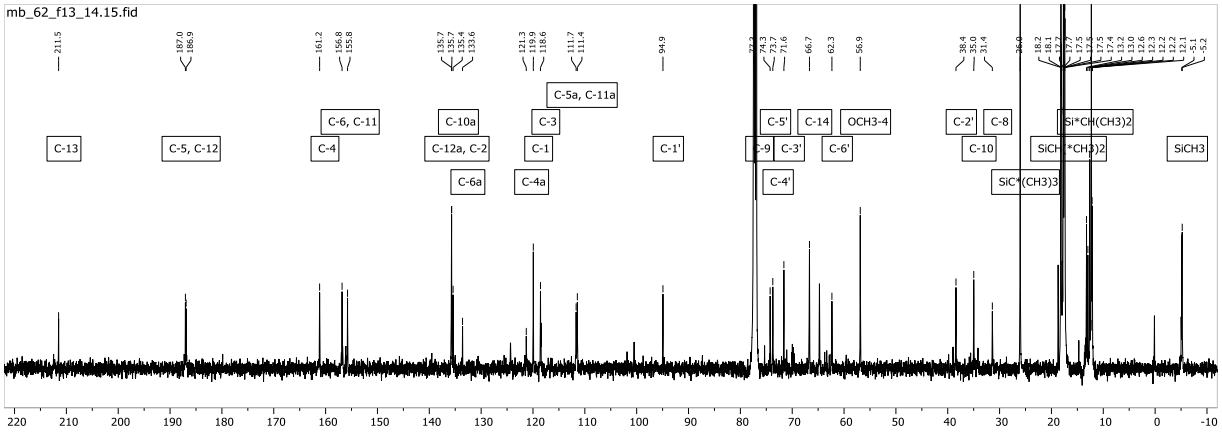
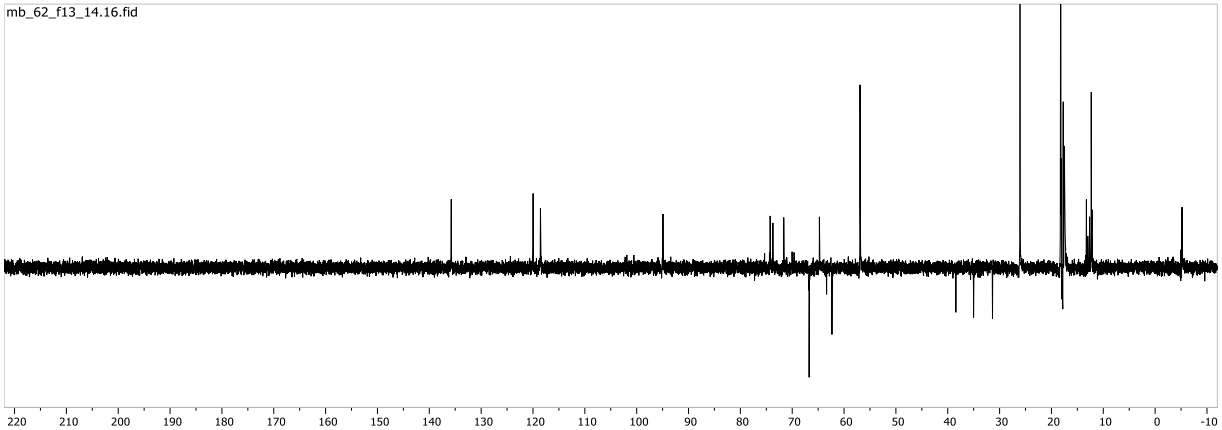
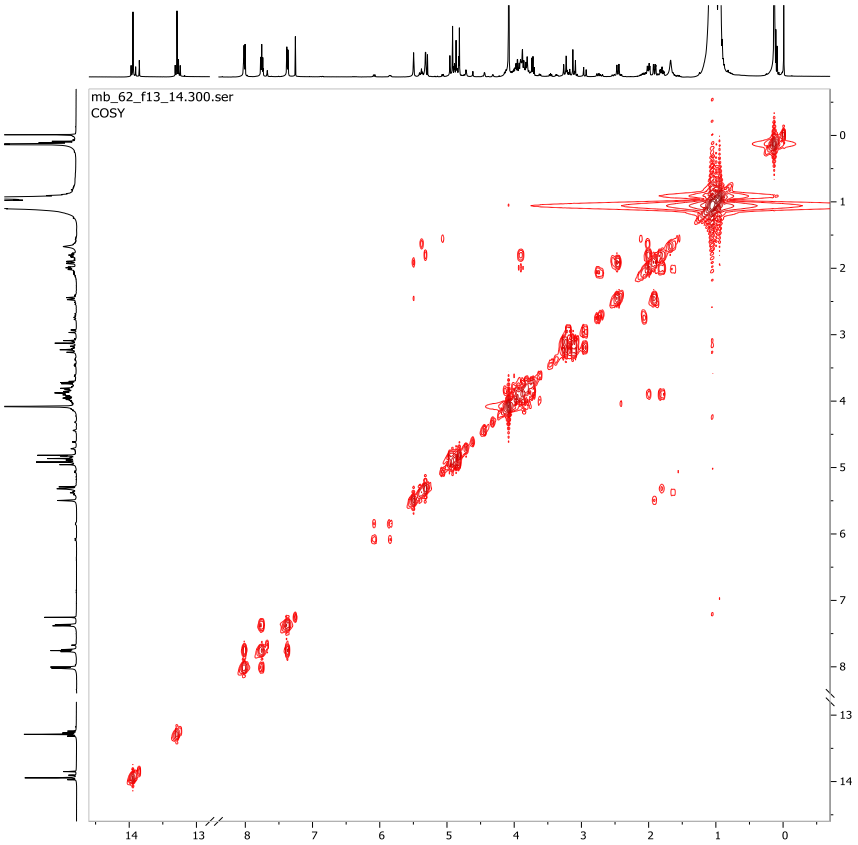
APPENDIX 50. Compound **59**

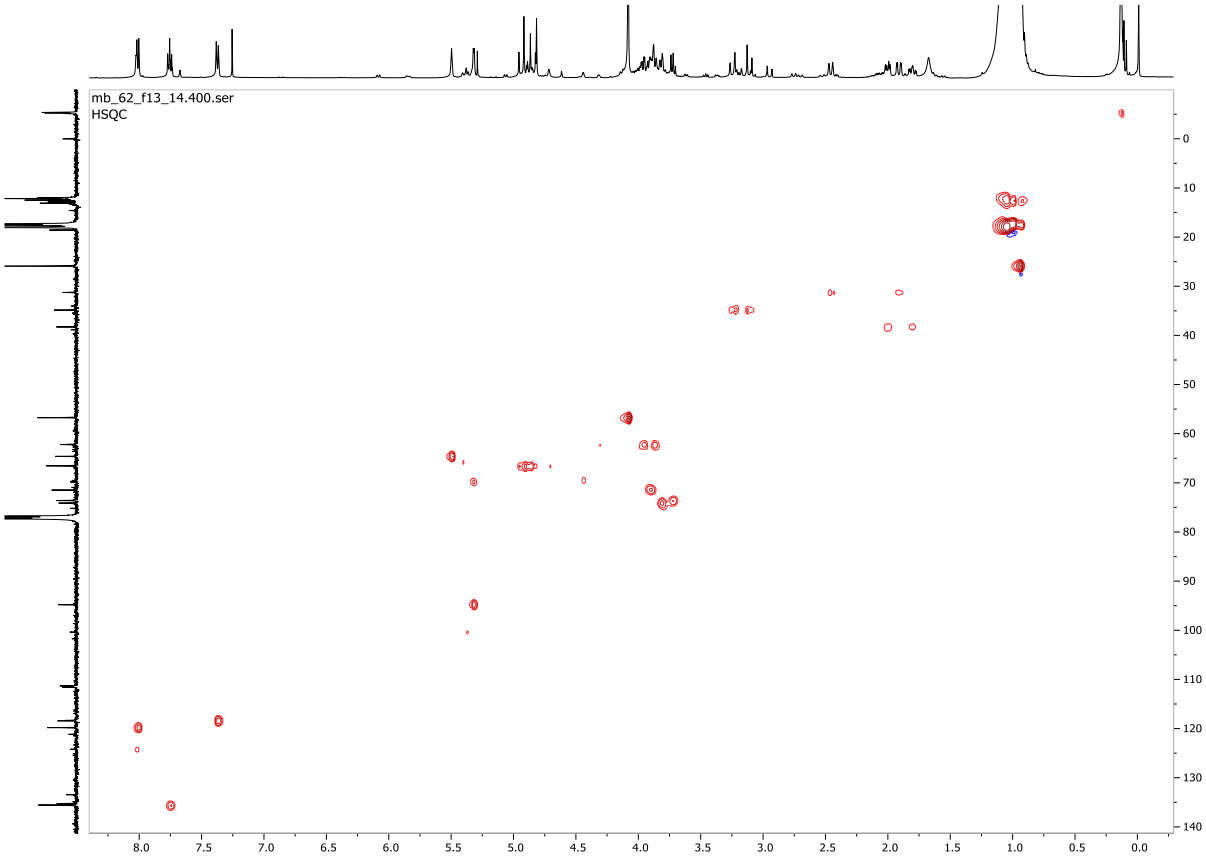




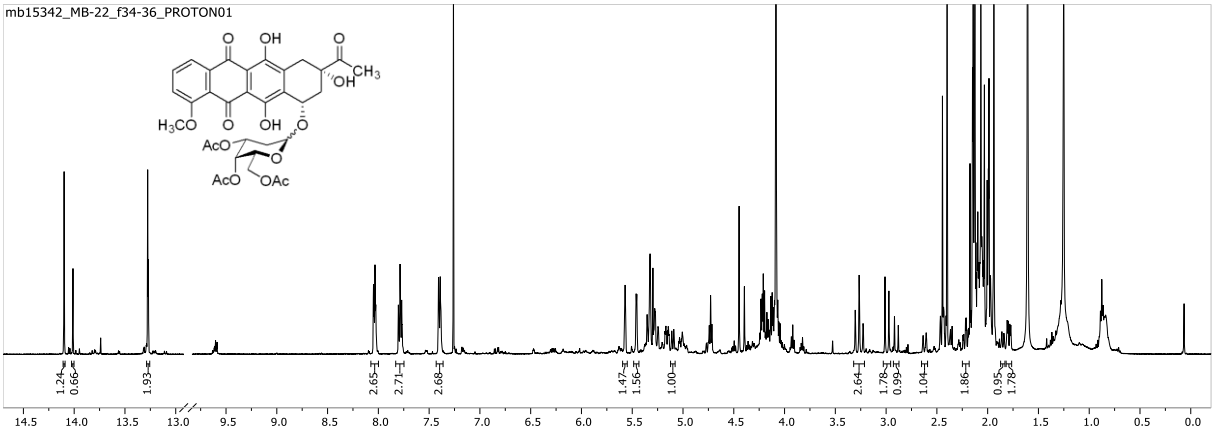


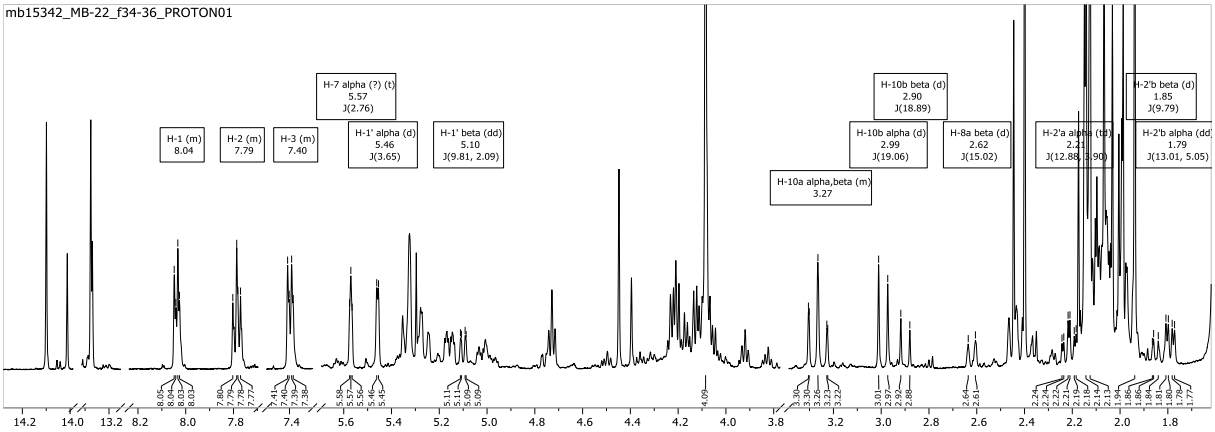


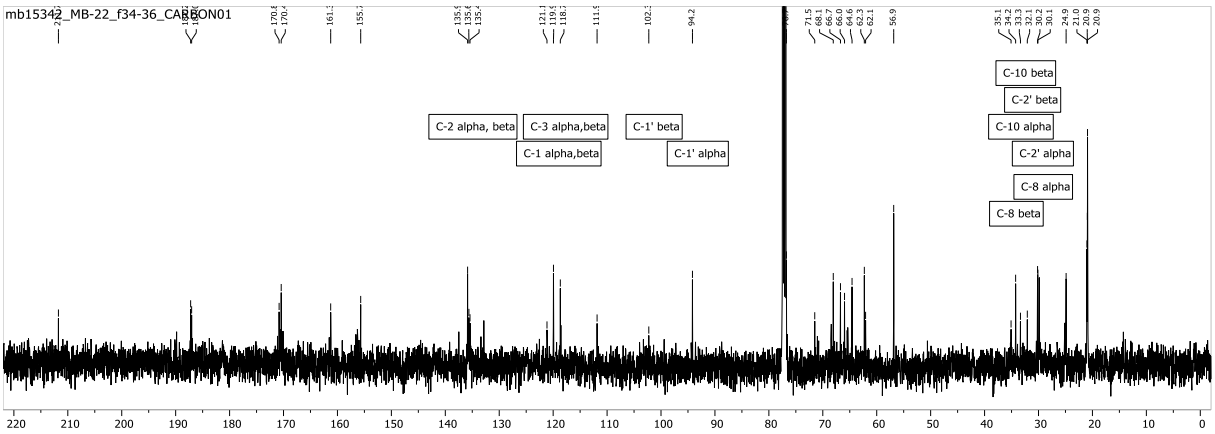

mb_62_f13_14.300.ser
COSY

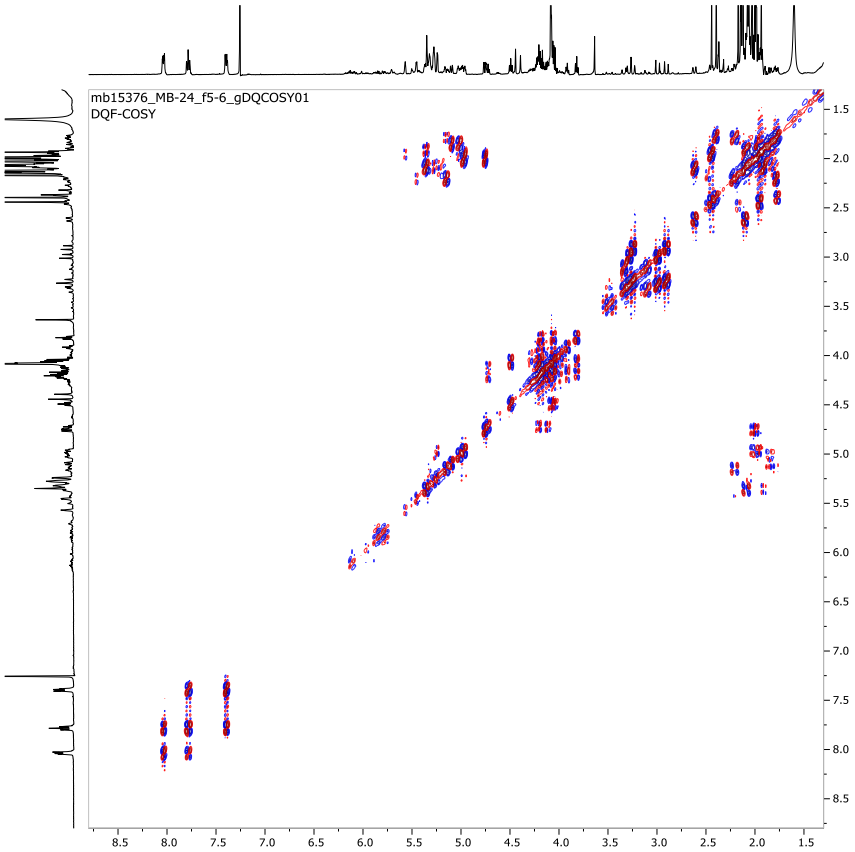

mb_62_f13_14.400.ser
HSQC

APPENDIX 51. Compound **60**





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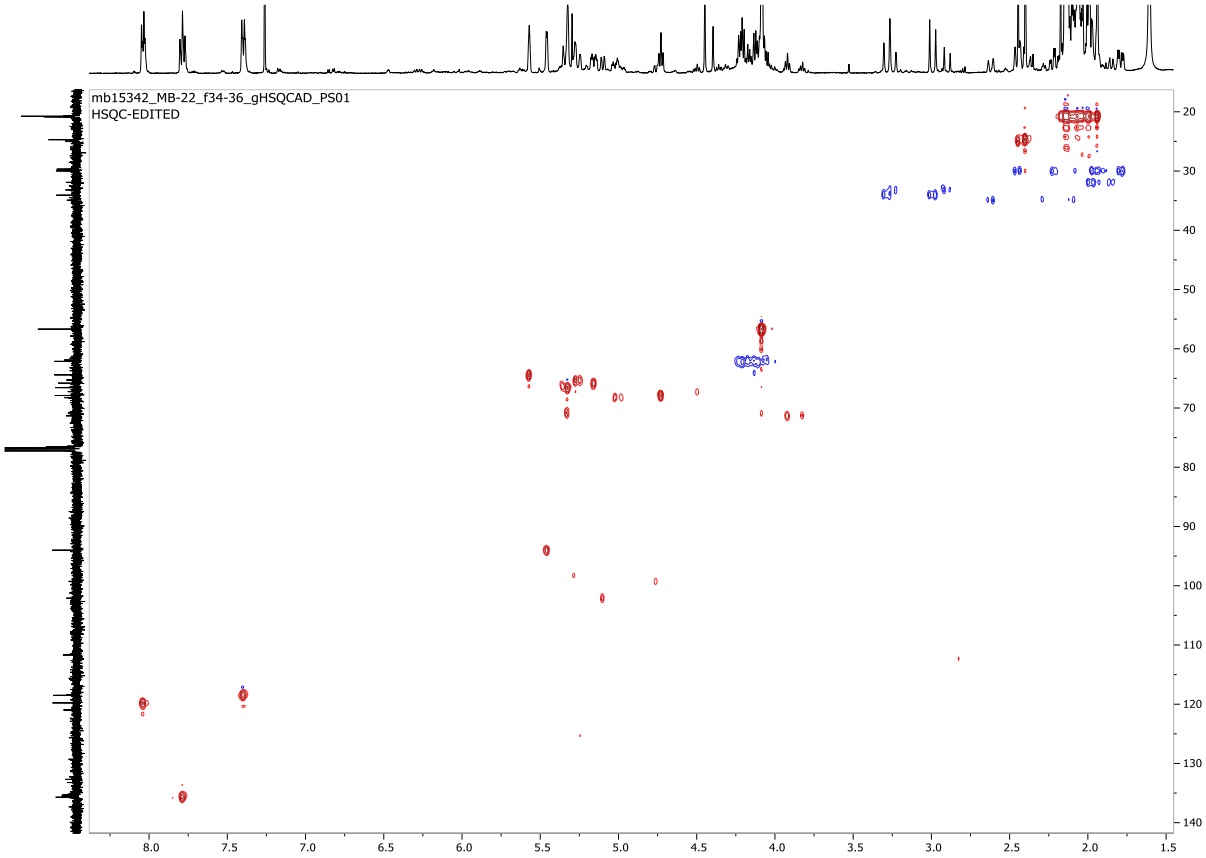
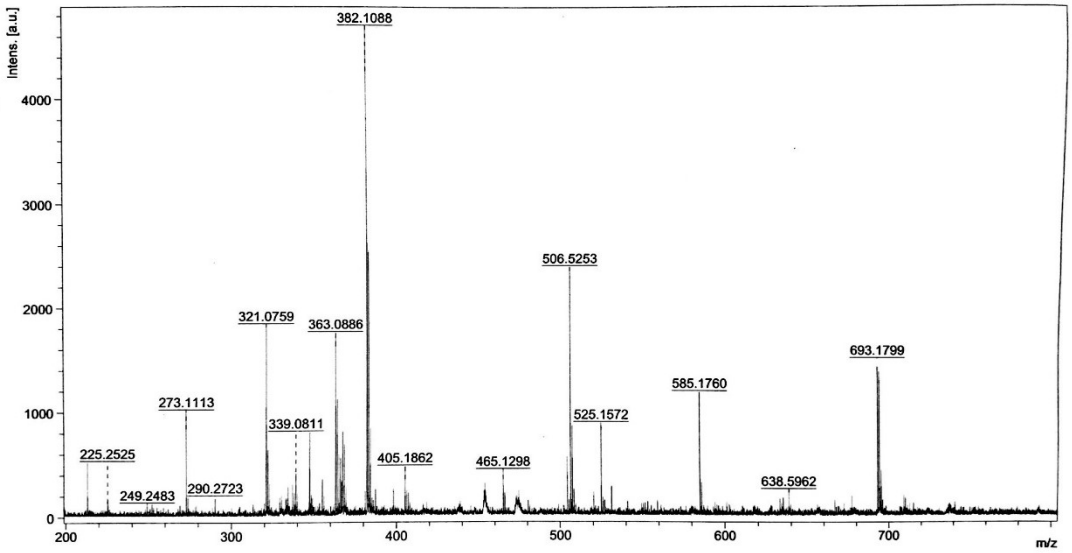
DQF-COSY

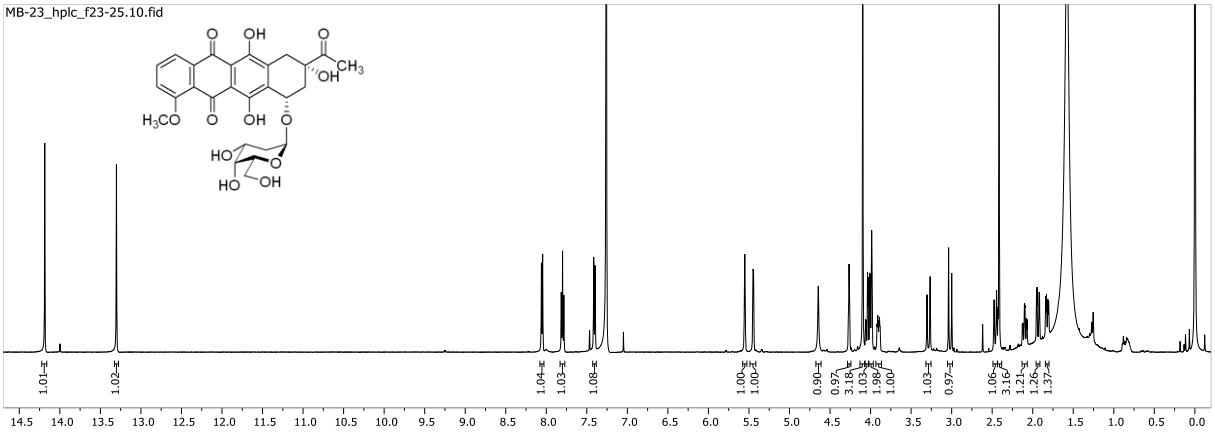

mb15342_MB-22_f34-36_gHSQCAD_PS01
HSQC-EDITED

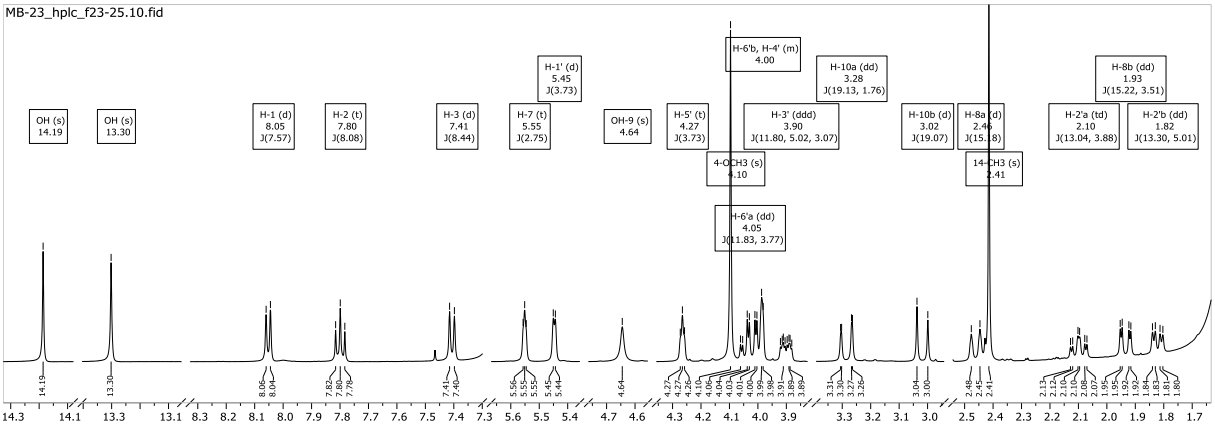
Analysis Name	cg-mt-10878\0_B22\1	*User*	bdal
Comment 1		*Instrument*	FLEX-PC
Method	D:\Methods\flexControlMethods\Paul Gates\RP_100-1500_Da-pjg.par		MALDI MASS SPECTRUM REPORT

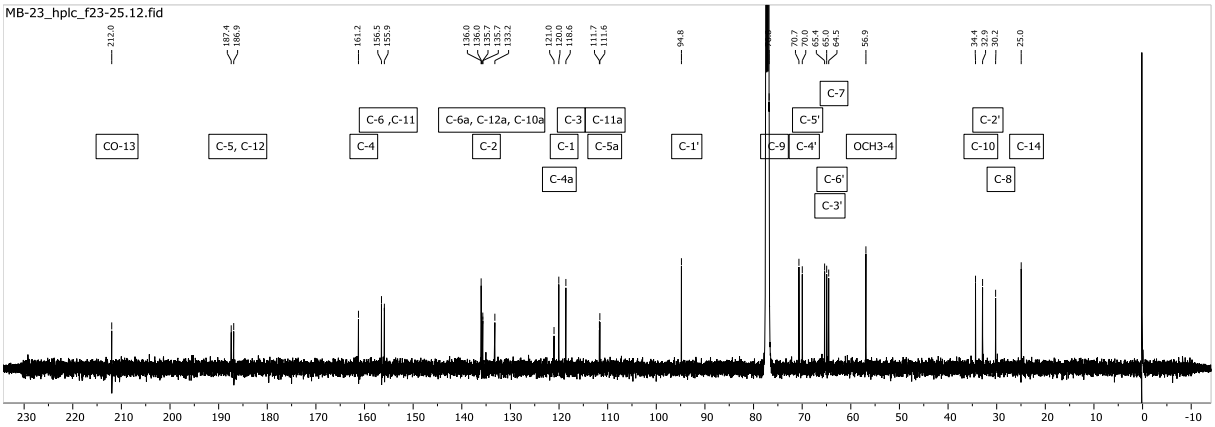


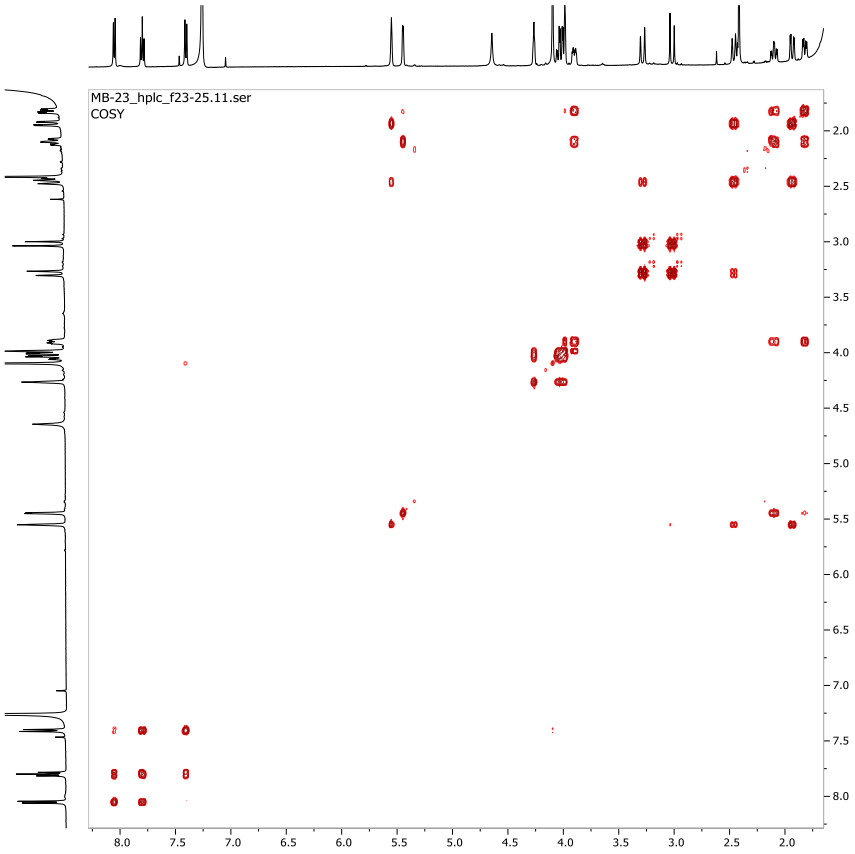
Bruker Daltonics flexAnalysis printed: 21/08/17 12:58:03

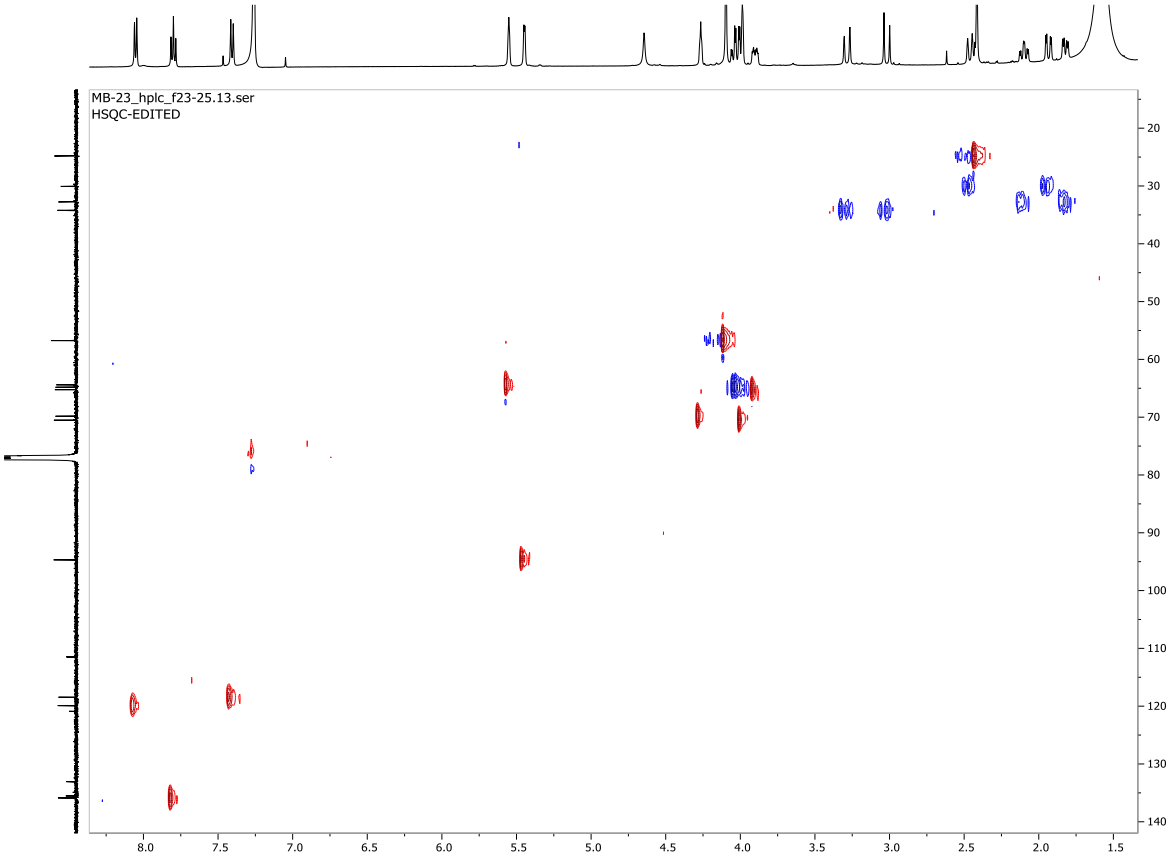
APPENDIX 52. Compound **61α**

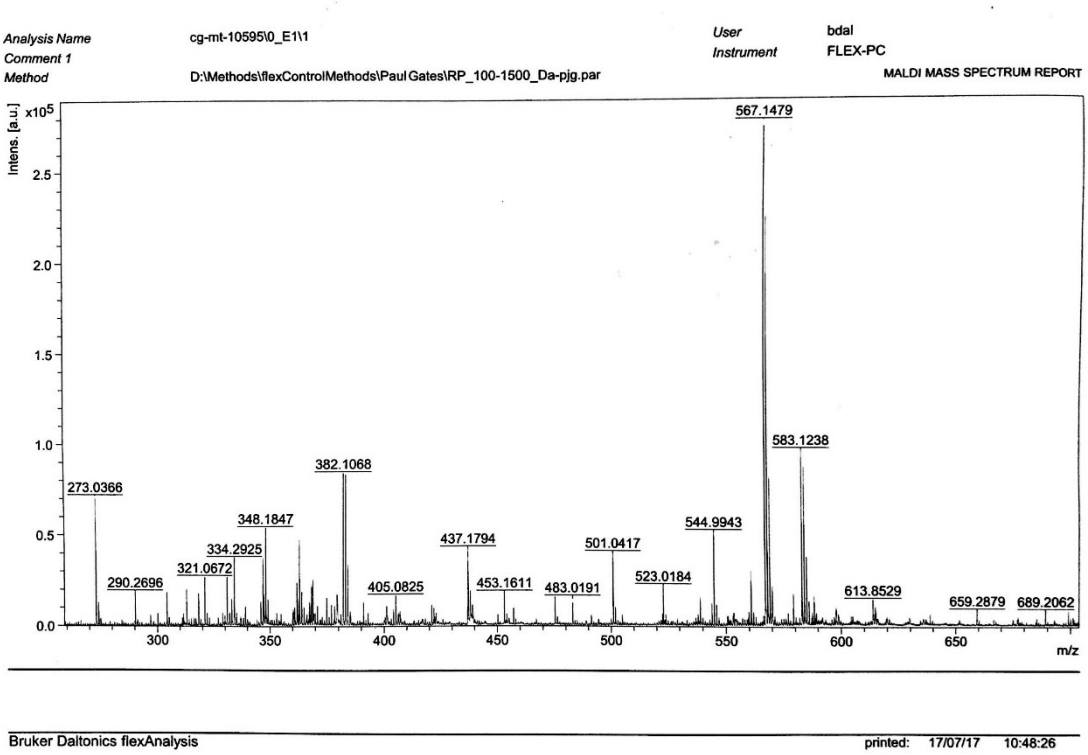







MB-23_hplc_f23-25.11.ser
COSY


MB-23_hplc_f23-25.13.ser
HSQC-EDITED



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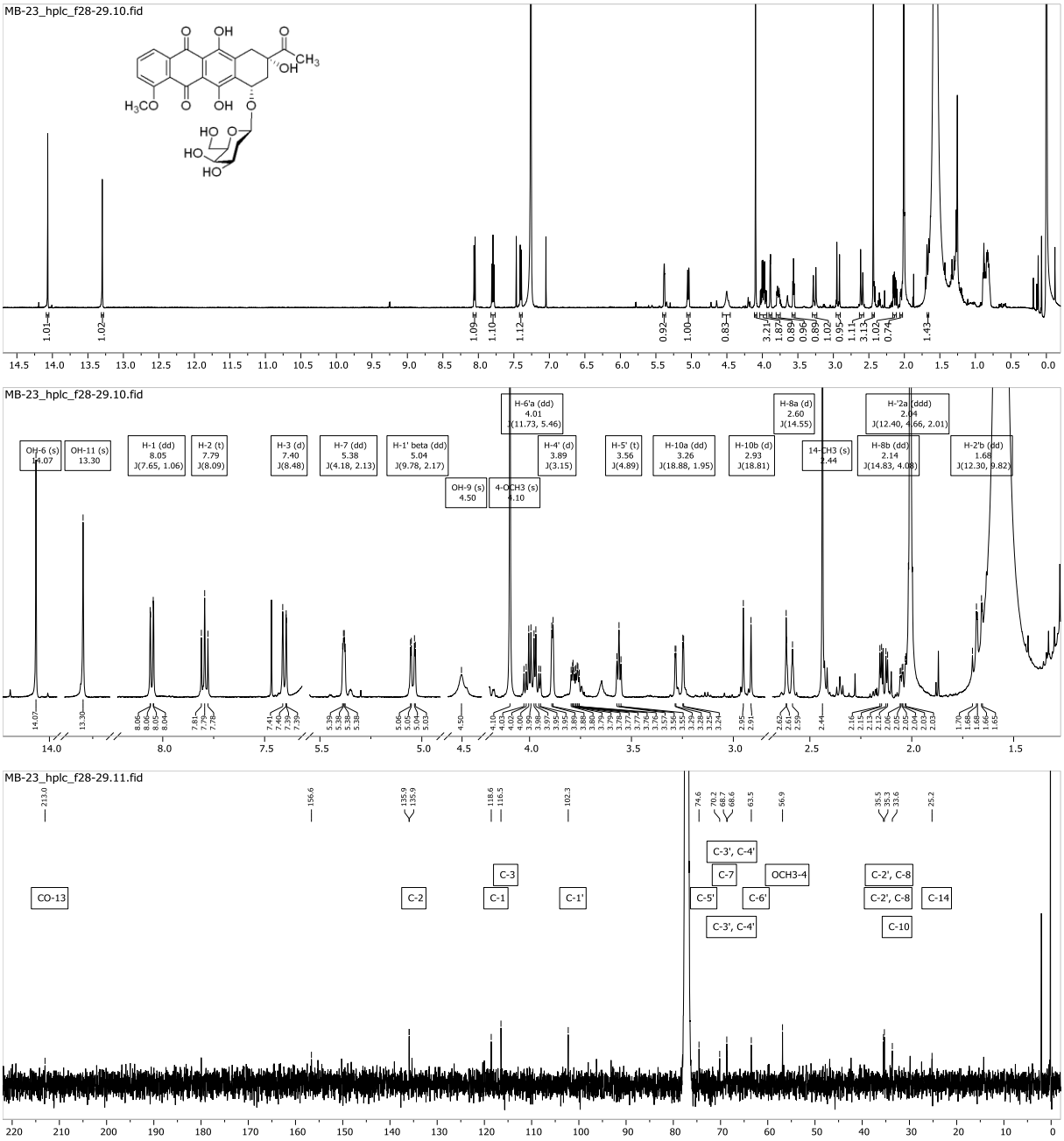
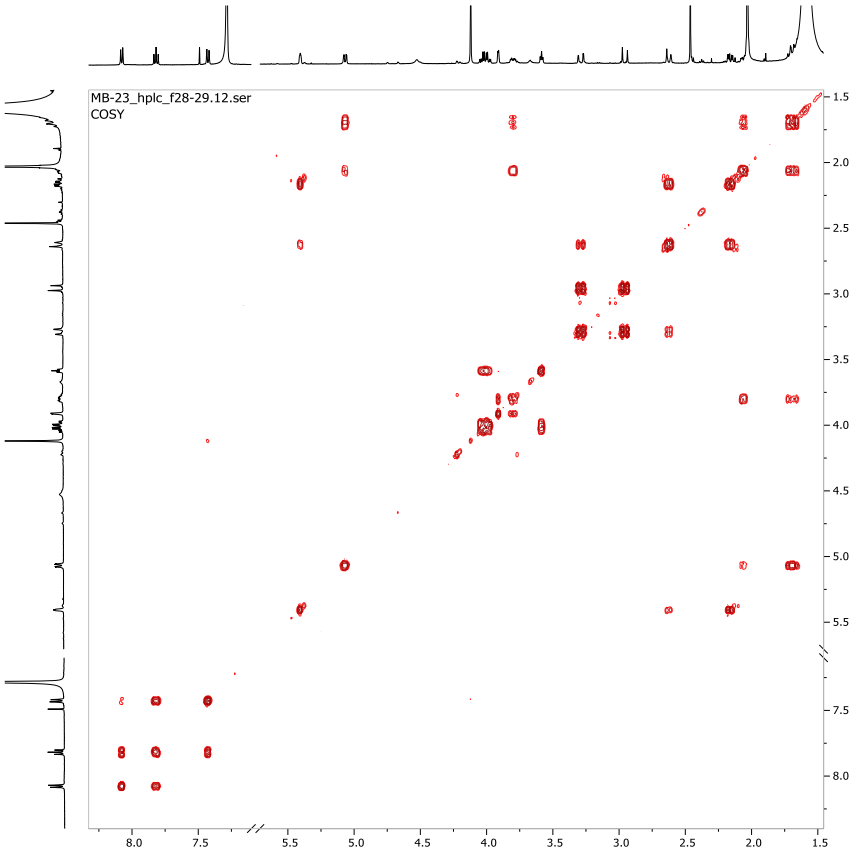
APPENDIX 53. Compound **61β**




MB-23_hplc_f28-29.12.ser
COSY
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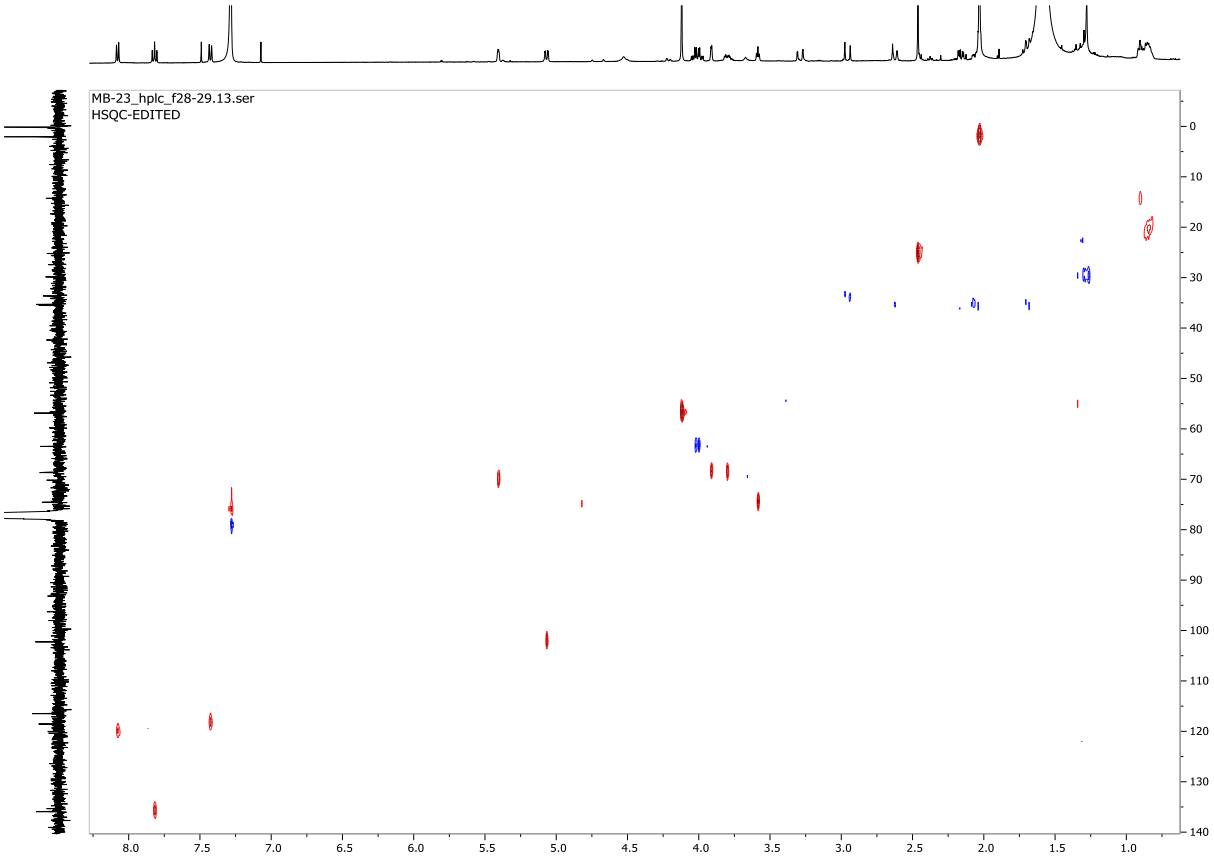
7.5
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5


MB-23_hplc_f28-29.13.ser
HSQC-EDITED
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0
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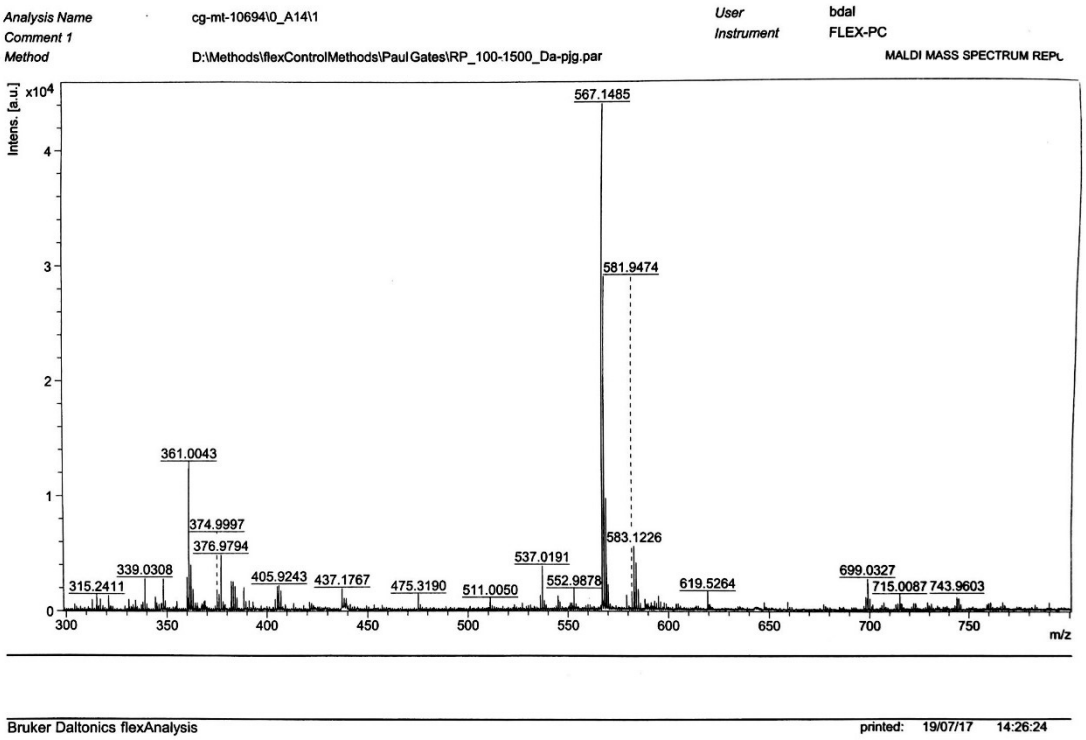
20
30
40
50
60
70
80
90
100
110
120
130
140



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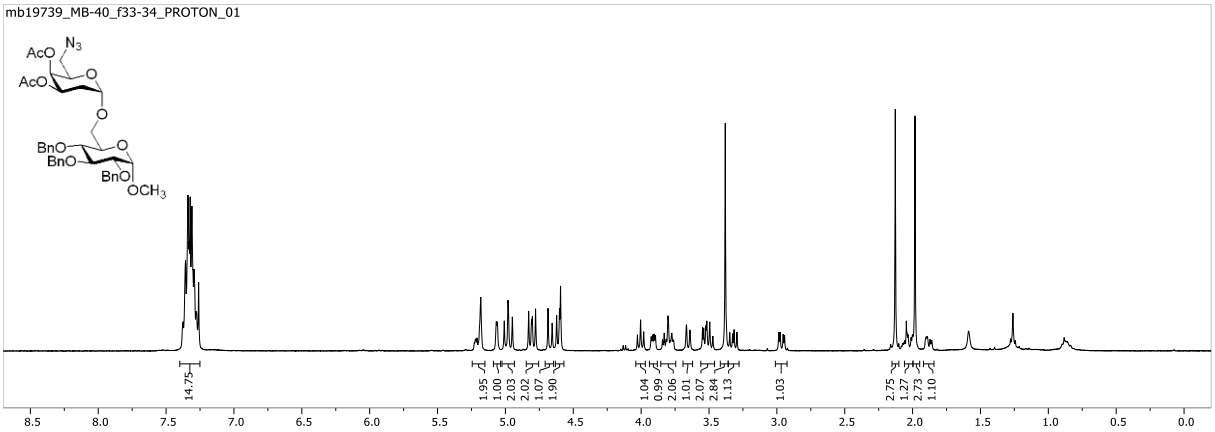
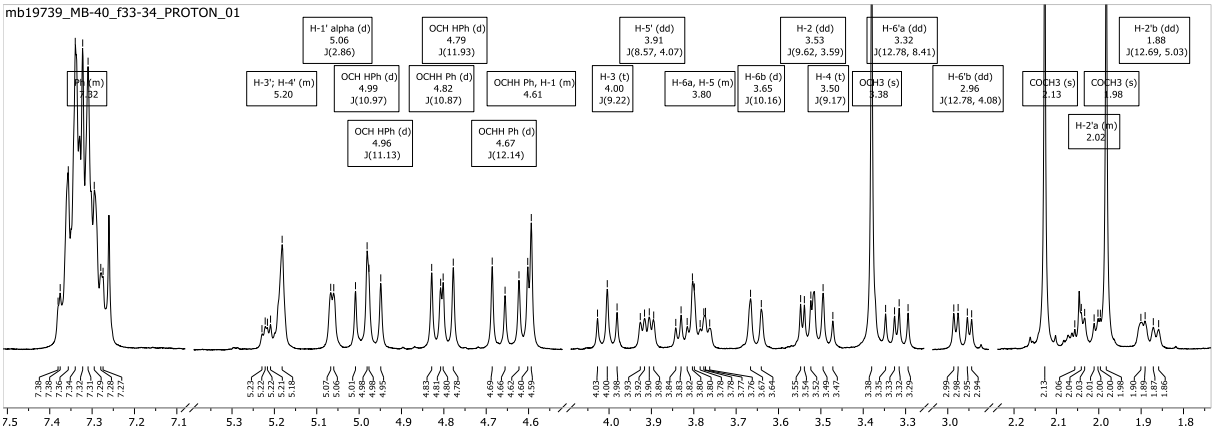
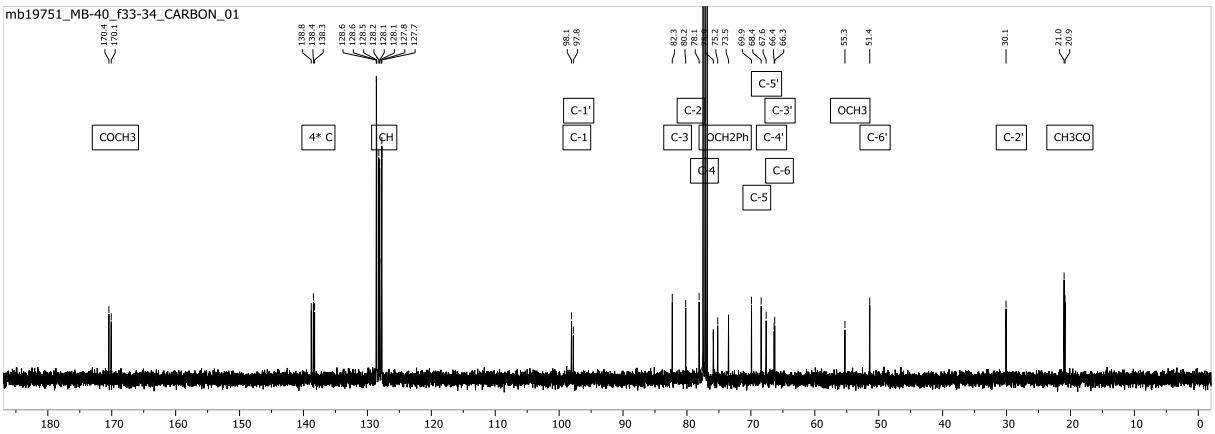
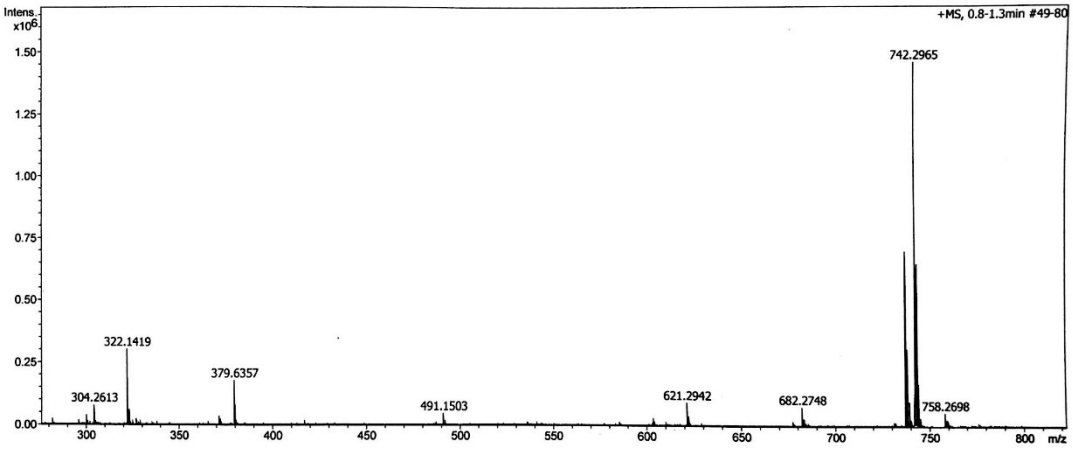
APPENDIX 54. Compound **62**





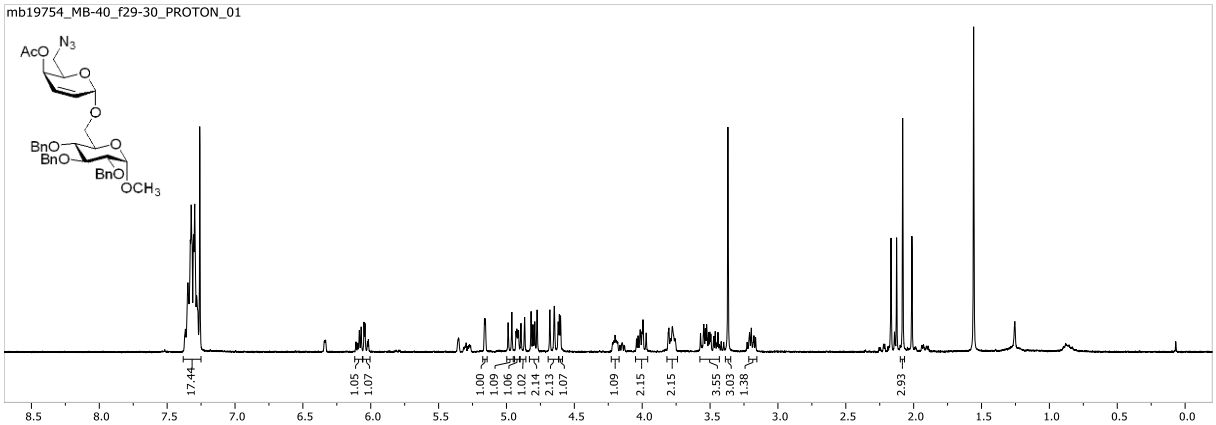


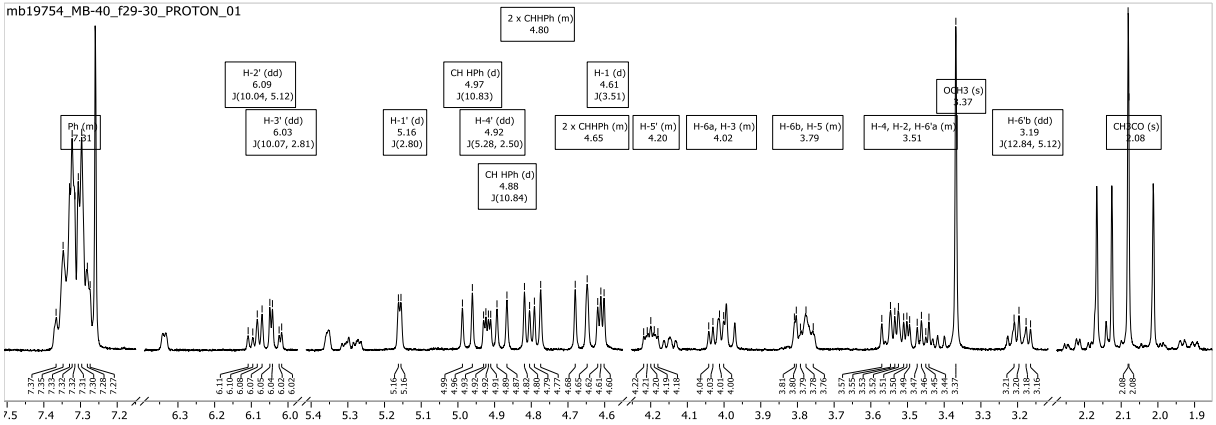
Analysis Name	D:\Data\msservice\cg-mb-11701.d		
Method	Verify Compound by Loop MS Positive.m	Operator	MS Service
Sample Name	cg-mb-11701_13887	Instrument	micrOTOF 213750.10405
Comment	Test sample 1		
Source Type	ESI	Ion Polarity	Positive

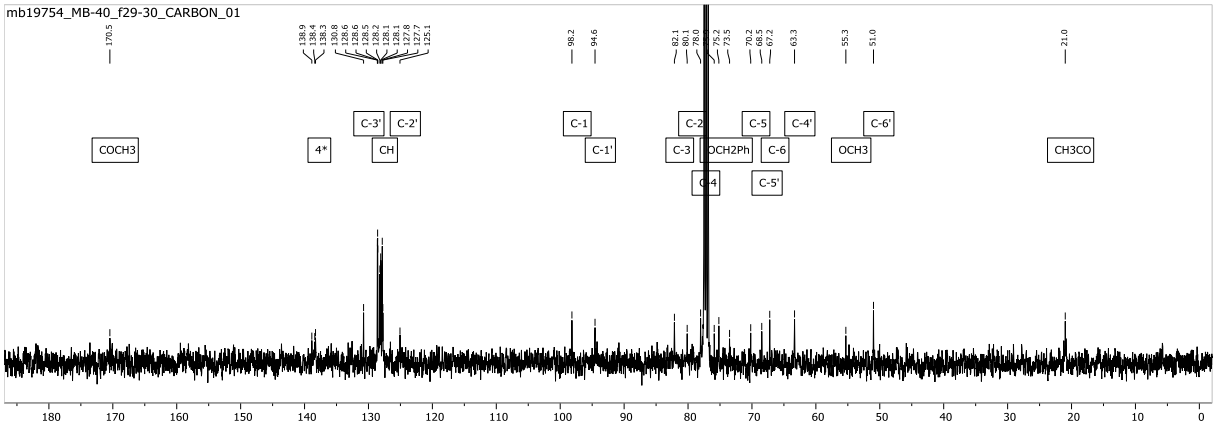


Meas. m/z	#	Ion Formula	m/z	err [ppm]	err [mDa]	mSigma	rdb	N-Rule	e⁻ Conf
742.296541	1	C38H45N3NaO11	742.294630	-2.6	-1.9	6.1	17.5	ok	even

APPENDIX 55. Compound **63**

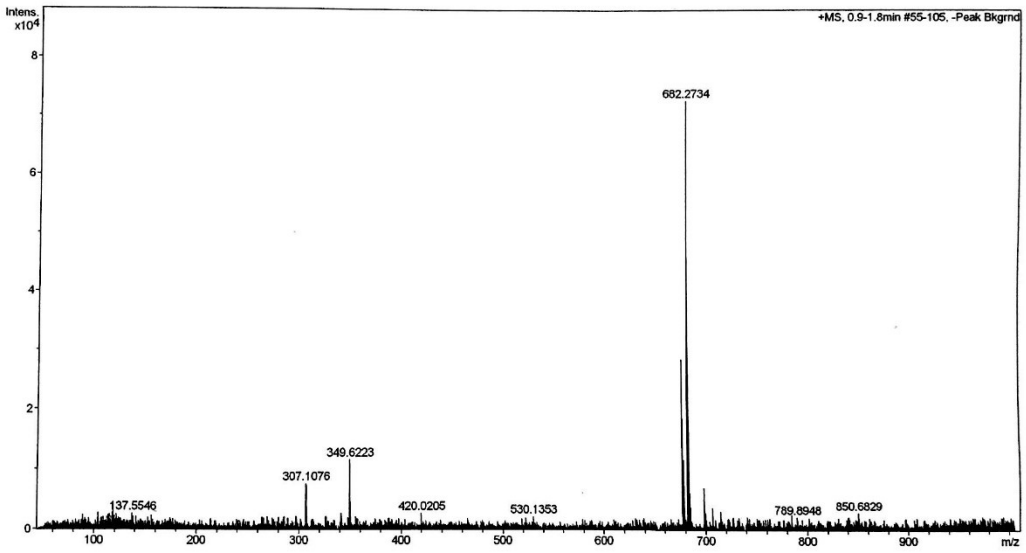






Bruker OpenAccess micrOTOF - Compound Verification Report

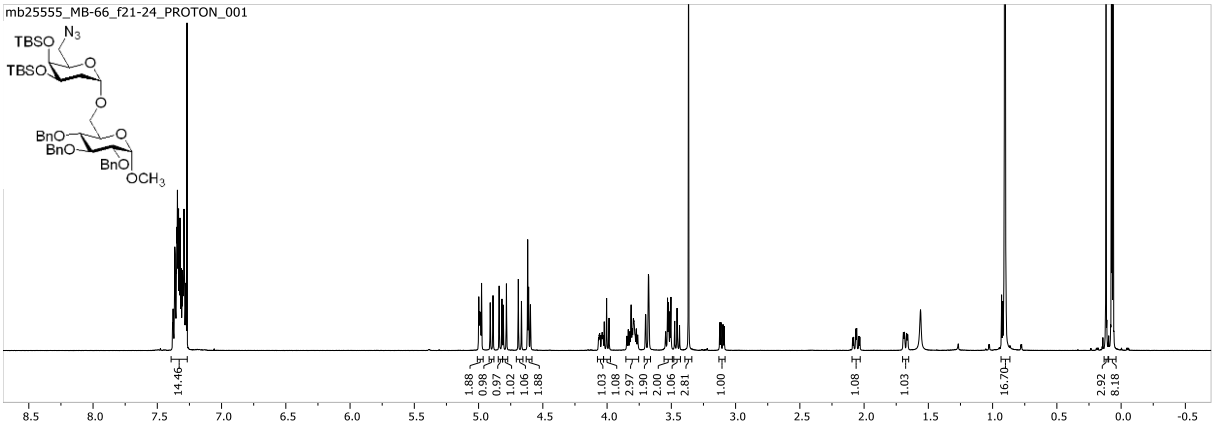
Cmpd 5, 1.0 min

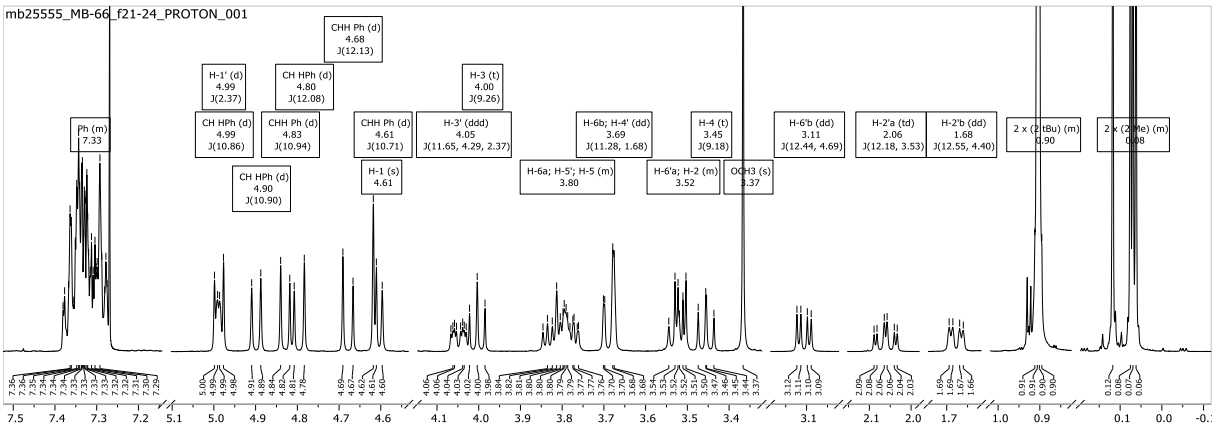


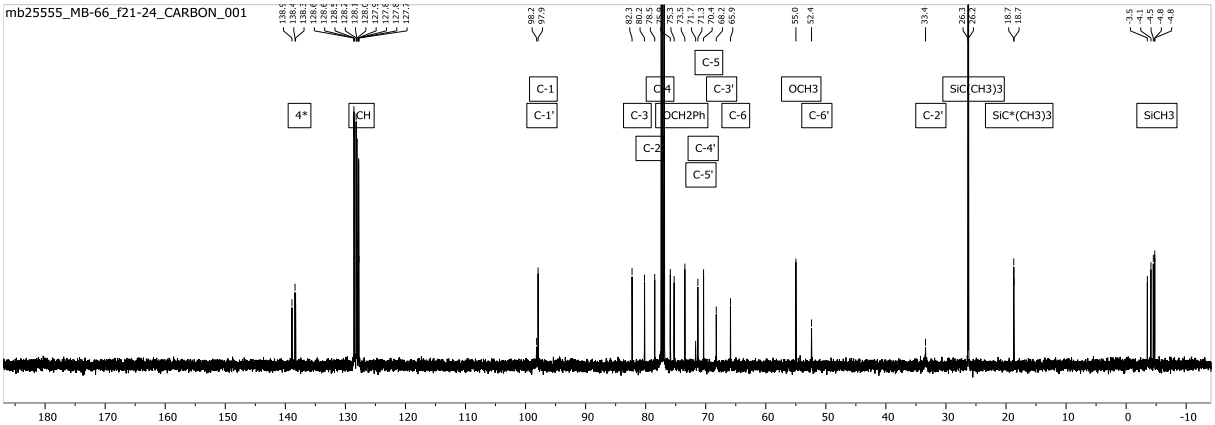
Bruker Compass DataAnalysis 4.1 printed: 10/12/2017 12:33:01 PM Page 6 of 6

Analysis Name D:\Data\msservice\cg-mt-11737_13936_4_01_13637.d

APPENDIX 56. Compound **64**







Analysis Name	cg-db-16142\0_D16\1	*User*	bdal
Comment 1		*Instrument*	FLEX-PC
Method	D:\Methods\flexControl\Methods\Paul Gates\RP_100-1500_Da-pjg.par		MALDI MASS SPECTRUM REPORT