Precursor-Directed Biosynthesis of Novel Jadomycins and Expansion of the Jadomycin Library

by

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DALHOUSIE UNIVERSITY

DEPARTMENT OF CHEMISTRY

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ABSTRACT

Jadomycins are secondary metabolites produced by *Streptomyces venezuelae* ISP5230 VS1099 in response to conditions of stress such as heat or ethanol shock. They have been shown to exhibit antibiotic and anticancer activity. Unique structural features of the jadomycins include a rare 2,6-dideoxysugar, L-digitoxose, and an oxazolone ring with an amino acid component. Previous studies have revealed that jadomycin derivatives can be produced by altering the amino acid in *S. venezuelae* ISP5230 VS1099 culture media which becomes incorporated into the oxazolone ring. One jadomycin from a proteogenic amino acid and three new jadomycins from non-proteogenic amino acids have been successfully produced on a large scale (4 mg/L to 12 mg/L, 2 L) and characterized using mass spectrometry, infrared spectroscopy, UV-visible spectroscopy, and nuclear magnetic resonance spectroscopy. One of these contains a terminal alkyne functionality and has been used in cycloaddition reactions with various azides to produce a library of triazole-containing jadomycins. This is the first report of jadomycin derivatives generated via chemical modification of the jadomycin scaffold post-biosynthesis. All new jadomycins produced are currently under investigation for their biological activities.

LIST OF ABBREVIATIONS AND SYMBOLS USED

^{13}C	carbon-13 isotope
^{1}H	proton
$^{1}J_{C1,H1}$	single bond coupling constant between carbon 1 and proton 1of a sugar
Abs_{256}	absorbance at 526 nm
Abs_{600}	absorbance at 600 nm
Ac_2O	acetic anhydride
AcOH	acetic acid
BBO	broadband observe
Boc-	tert-butoxycarbonyl- (protecting group)
$CaCl_2$	calcium chloride
cat.	catalytic amounts
$CDCl_3$	deuterated chloroform
$CHCl_3$	chloroform
COSY	correlation spectroscopy
CuAAc	copper(I)-catalyzed azide-alkyne cycloaddition
$CuSO_4 \cdot 5H_2O$	copper sulfate pentahydrate
CV	column volume
d	doublet
D_2O	deuterium oxide
DCM	dichloromethane
dH_2O	distilled water
DIPEA	diisopropylethylamine
DMF	*N*,*N*-dimethylformamide
EC_{50}	concentration at which half the maximum response is observed
EPI-MS	enhanced product ion mass spectrometry
eq./equiv.	molar equivalent
ESI	electrospray ionization
EtOAc	ethyl acetate
EtOH	ethanol
$FeSO_4 \cdot 7H_2O$	ferrous sulfate heptahydrate
Glc	glucose

H_3BO_3	boric acid
HCl	hydrochloric acid
HETCOR	heteronuclear correlation
HMBC	heteronuclear multiple bond coherence
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
HSQC	heteronuclear single quantum coherence
Hz	hertz
IR	infrared spectroscopy
J	couling constant
K_2CO_3	potassium carbonate
KBr	potassium bromide
$KHSO_4$	potassium hydrogen sulfate
$KMnO_4$	potassium permanganate
LRMS	low resolution mass spectrometry
m	multiplet
m/z	mass per charge
Man	mannose
MeOD	deuterated methanol
MeOH	methanol
MeONa	sodium methoxide
$MgSO_4$	magnesium sulfate
$MnSO_4 \cdot 4H_2O$	manganese sulfate tetrahydrate
MS/MS	tandem mass spectrometry
MSM	mineral salt medium
MTD	maximum tolerated dose
MTT	(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (dye)
MYM	maltose, yeast extract, malt extract (medium)
Na_2SO_4	sodium sulfate
NaCl	sodium chloride
NaH	sodium hydride
NaN_3	sodium azide
NaOH	sodium hydroxide
NCI	National Cancer Institute

$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$	ammonium heptamolybdate tetrahydrate
NMR	nuclear magnetic resonance
NMR-3	Nuclear Magnetic Resonance Research Resource
nOe	nuclear Overhauser effect
NRC-IMB	National Research Council Canada Institute for Marine Biosciences
OD_{600}	optical density measured at 600 nm
*O*PS	*O*-propargyl-L-serine
p	pentet
PBr_3	phosphorus tribromide
pK_a	acid dissociation constant
ppm	part per million
prep	preparatory
q	quartet
Q1	first quadrupole
R_f	retention factor
Rha	rhamnose
rpm	revolutions per minute
RT	room temperature
s	singlet
SAR	structure-activity relationship
S_N2	substitution nucleophilic bimolecular
t	triplet
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TOCSY	total correlation spectroscopy
UV-Vis	ultraviolet-visible spectroscopy
$ZnSO_4\cdot 7H_2O$	zinc sulfate heptahydrate
α-	alpha- (configuration)
β-	beta- (configuration)
δ	chemical shift
ε	molar extinction coefficient
λ_{max}	wavelength at which an absorbance maximum is observed
ν	vibrational frequency

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CHAPTER 1 INTRODUCTION

Many drugs in clinical use today have their origins in natural products. For example, penicillin, salicylic acid, and morphine, drugs commonly used to treat infection or inflammation and its associated pain, were all derived from natural sources. Penicillin was discovered from a species of mold fungi, while salicylic acid and morphine were found and extracted from plants.[1] With the huge variety of chemistry that occurs in nature, it is not surprising that some of the compounds generated display medicinal potential for humans as curative or preventative agents. In some cases, the medicinal relevance to humans is coincidental. For example, salicylic acid acts as a hormone in its natural plant sources but exhibits analgesic effects in humans.[2] In other cases, the medicinal relevance is the direct result of evolution. Many examples exist where evolution and competition in nature have led to the production of toxic chemicals that defend against other forms of life (e.g. a snake's venom), and some of these compounds have been discovered and exploited by humans for medicinal purposes. At the microbial level, many bacteria and fungi have evolved entire biochemical pathways specific to warding off competing species. These pathways are collectively referred to as secondary metabolic pathways and their products, secondary metabolites, have been largely exploited by man for their microbial toxicities achieved over generations and generations of evolution.

Secondary metabolites are compounds produced by microbes that play an important role in the pharmaceutical industry. Having evolved as chemical defense mechanisms for these organisms, they are produced in conditions of stress, which trigger secondary metabolism, to kill or deter competing species. They give the organisms which produce them a competitive edge as they are toxic to other microbes and thus antibiotic in nature. Man has discovered several of these naturally produced microbial toxins and developed them as pharmaceutical antibiotics used to fight infection. Penicillin, a group of antibiotics derived from secondary metabolites of the mold fungus *Penicillium chrysogenum*, is an example.[1] Many antibiotic drugs used today are derived from secondary metabolites produced by bacteria, and most of them are produced by one genus of bacteria: *Streptomyces*.[3,4]

Streptomyces, a genus of soil bacteria, is the source of many antibiotic pharmaceutical products on the market today. For example, neomycin (**1**), vancomycin (**2**), tetracycline (**3**), and chloramphenicol (**4**) are derived from *S. fradiae*, *S. orientalis*, *S. rimosus*, and *S. venezuelae*,

respectively (Figure 1).[4,5] *S. venezuelae* is also responsible for the production of a largely unexplored class of molecules with antibacterial activity: the jadomycins.

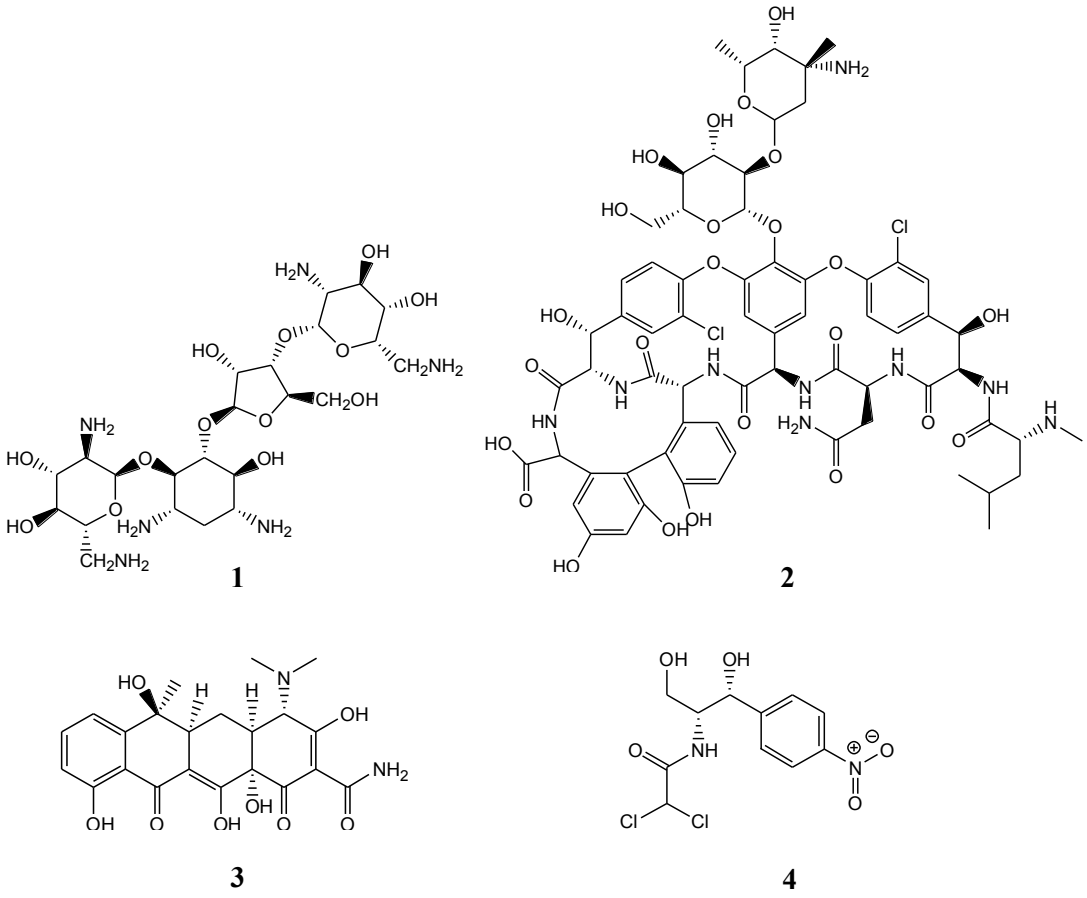

Figure 1. Examples of clinical antibiotics produced by *Streptomyces*: neomycin (**1**), produced by *S. fradiae*; vancomycin (**2**), produced by *S. orientalis*; tetracycline (**3**), produced by *S. rimosus*; and chloramphenicol (**4**), produced by *S. venezuelae*.

Jadomycins are brightly coloured compounds produced by *S. venezuelae* ISP5230 that display promising antibiotic and anticancer activities. They were the first class of secondary metabolites to be isolated from *S. venezuelae* ISP5230, the strain that produces chloramphenicol, when subjected to environmental stress.[6,7] Their intense colour is due to the presence of multiple aromatic rings. As seen in the general structure of jadomycin (**5**) shown below, jadomycins are large, complex molecules (Figure 2). They contain five fused rings, A through E, one of which (E) is an oxazolone ring containing an amino acid side chain. The oxazolone ring contains two chiral carbon atoms, one where the B ring connects to the oxazolone and the other where the side chain connects to the oxazolone. Jadomycins also contain a sugar functionality, L-digitoxose, which is unique for its 2,6-dideoxy substitution pattern. Altogether, the jadomycins have a variety of

lipophilic and hydrophilic moieties and molecular weights greater than five hundred. Preliminary screening assays using several bacterial strains and cancer cell lines have revealed the jadomycins to be active against Gram-positive bacteria and to have anticancer activity.[6,8]

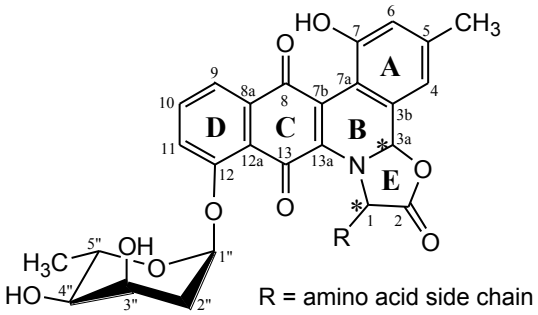

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Figure 2. General structure of jadomycin. Structural features include four aromatic rings (A-D), an oxazolone ring (E) with variable R-group, and an L-digitoxose group. The atoms are numbered according to the numbering scheme used for all jadomycins. The stereocenters of the oxazolone ring are indicated with asterisks.

Jadomycins belong to the class of antibiotics known as angucyclines, which are a large group of secondary metabolites with a common structural backbone and biosynthetic origin.[9] The term angucycline refers to the angular assembly of the four-ring frame of the aglycone moeity of these molecules. This common structural feature can be seen in jadomycin (**5**, Figure 2) and in structurally-related analogues such as kinamycin F (**6**), landomycin C (**7**), hedamycin (**8**), gilvocarcin V (**9**), and urdamycin B (**10**; Figure 3).

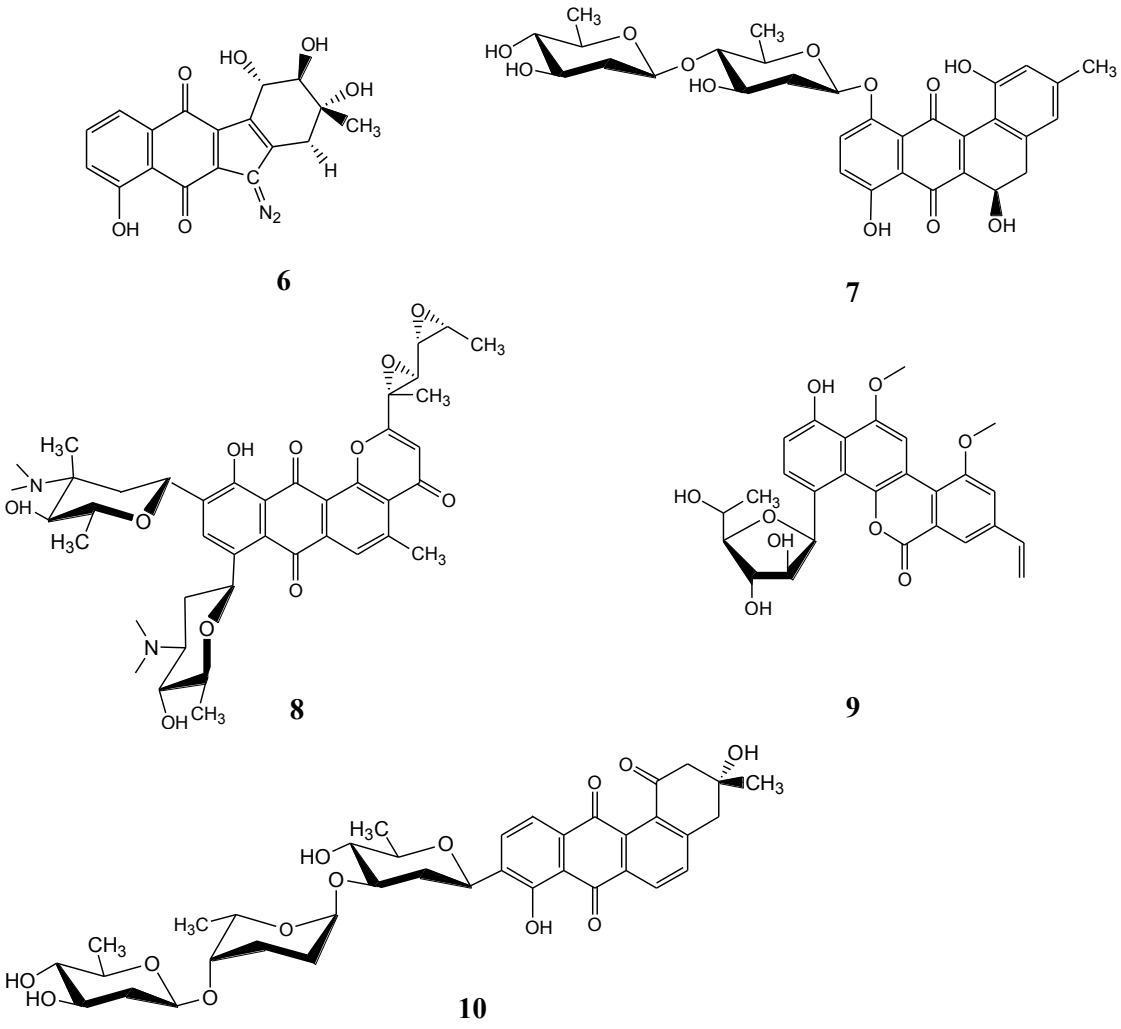


Figure 3. Structures of some angucycline group antibiotics: kinamycin F (**6**), landomycin C (**7**), hedamycin (**8**), gilvocarcin V (**9**), and urdamycin B (**10**).

1.1 Jadomycin Biosynthesis

Jadomycins are secondary metabolites produced by *S. venezuelae* ISP5230 and, as such, are synthesized by enzymes that make up the secondary metabolic pathway of the organism. The enzymes involved in this biosynthetic pathway are coded for by genes which are found in clusters along the *S. venezuelae* ISP5230 chromosome (Figure 4), and the entire pathway is induced by some sort of stress, like heat or ethanol shock. The particular roles of many of these genes were deduced by Dr. Leo Vining, Dept. of Biology, Dalhousie University, and his coworkers. Numerous sequence homology and gene knock-out studies were conducted to identify genes and enzymes

involved in jadomycin biosynthesis and assign their individual functions.[10-18] A number of genes were identified, as well as their corresponding proteins which were named JadA, JadB, JadC, and so on. Some of these proteins act as enzymes which catalyze particular chemical steps in the biosynthetic pathway, while others have regulatory functions. There are also proteins whose precise functions are still unknown, for example JadX which is not essential for jadomycin biosynthesis but ensures complete conversion of the aglycone to the glycosylated product.[16] A brief overview of the proposed jadomycin biosynthetic pathway and the enzymatic steps involved is shown below (Scheme 1).

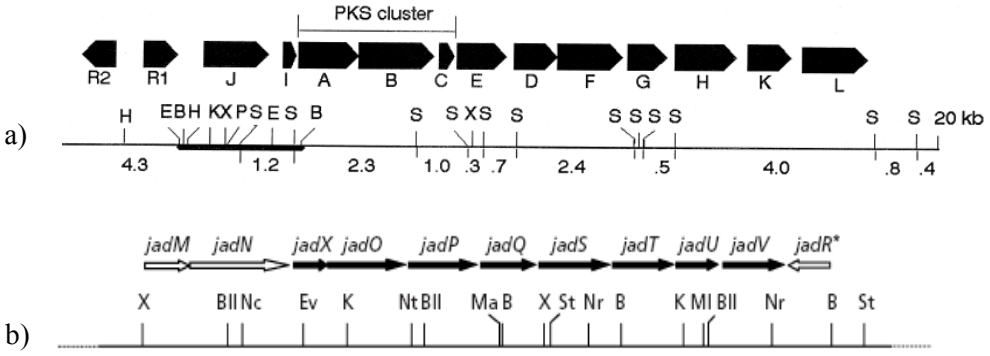
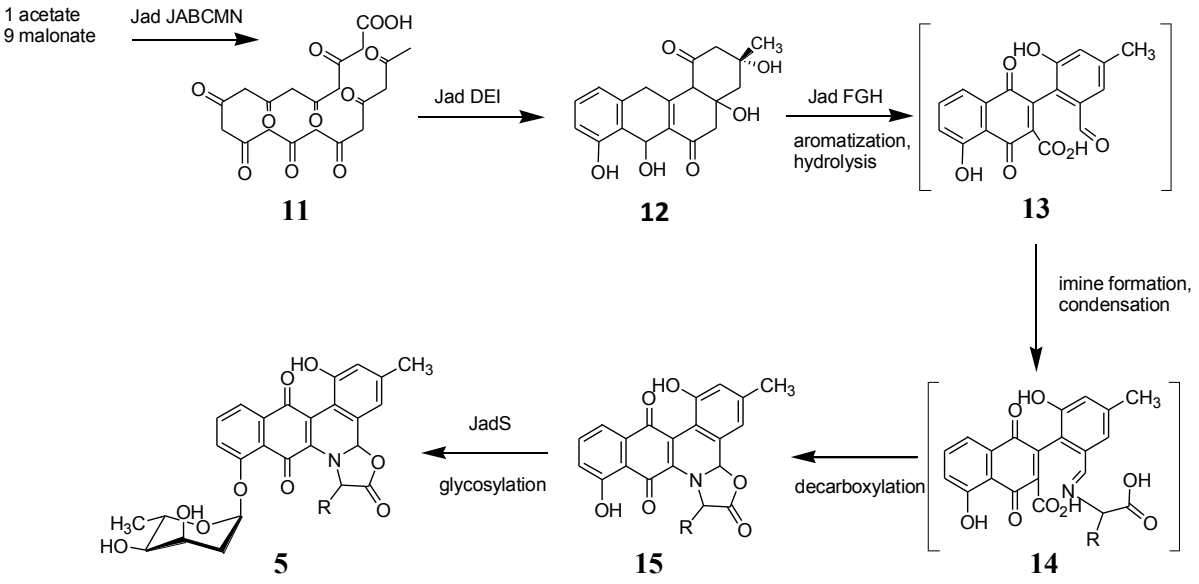


Figure 4. Segments of the *S. venezuelae* ISP5230 chromosome showing genes involved in jadomycin biosynthesis:[13,16] a) genes involved in biosynthesis of the polyaromatic backbone, and b) genes involved in biosynthesis of the dideoxysugar unit.



Scheme 1. Proposed biosynthetic pathway for jadomycin production. The enzymes involved in each step are indicated above the arrows.

As seen above, the biosynthesis begins from one malonate and nine acetate molecules. Initially, JadA, JadB, and JadC, which make up the polyketide synthase cluster, combine these substrates into a polyketide chain (**11**). Next, JadD, JadE, and JadI, reform this chain into rings, generating compound **12**.[19] Then, Jad F,G and H are responsible for aromatization and hydrolysis to produce a reactive aldehyde intermediate (**13**). An amino acid reacts with this intermediate to produce an imine (**14**), which undergoes ring closure and decarboxylation to give the aglycone form of jadomycin (**15**). It is glycosylated at position C12, specifically by the glycosyltransferase JadS, but JadO through JadV are all involved in producing the activated sugar.[15,16]

From this overview, it is apparent that the genetic studies accomplished by Vining and coworkers have revealed a number of genes which code for enzymes that are involved in particular steps in jadomycin biosynthesis. However, these studies have failed to reveal any candidate genes for enzymes that catalyze the amino acid incorporation. Because of this, it was proposed that this particular step is chemical, rather than enzymatic, and that the amino acid is incorporated spontaneously.

1.2 Jadomycin Derivatization and Activities

The first jadomycin produced, isolated, and characterized was Jadomycin A (**16**), the aglycone form of Jadomycin B (**17**) which was also produced and isolated as the main component in the mixture (Figure 5).[6,7] The conditions for the production of this jadomycin by *S. venezuelae* ISP5230 were as follows: the bacteria were grown on a minimal galactose-isoleucine medium and heat-shocked to induce the secondary metabolic pathway. Looking at the structure of the resulting jadomycin, one can see that its R group at position C1 is the side chain of isoleucine, the amino acid that was present in the growth medium. Isoleucine was the only amino acid present in the growth medium and furthermore the only nitrogen source. Therefore, the jadomycin's oxazolone ring with isoleucine side chain was unquestionably the result of the bacteria's incorporation of isoleucine from the environment. This immediately suggested a relatively easy method for making derivatives of this jadomycin: simply varying the amino acid used in the culture medium.[7,9]

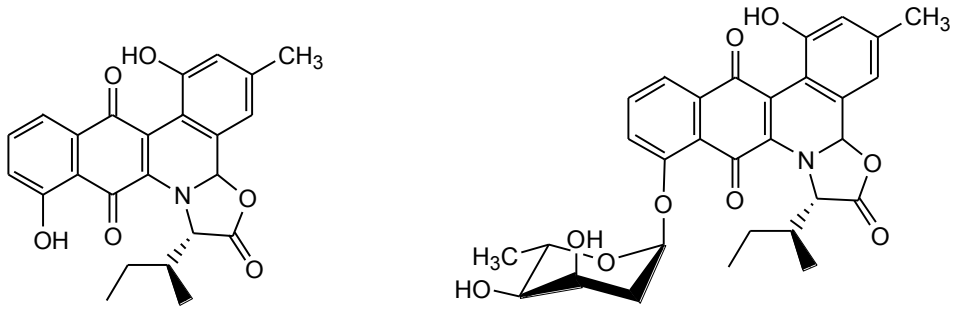
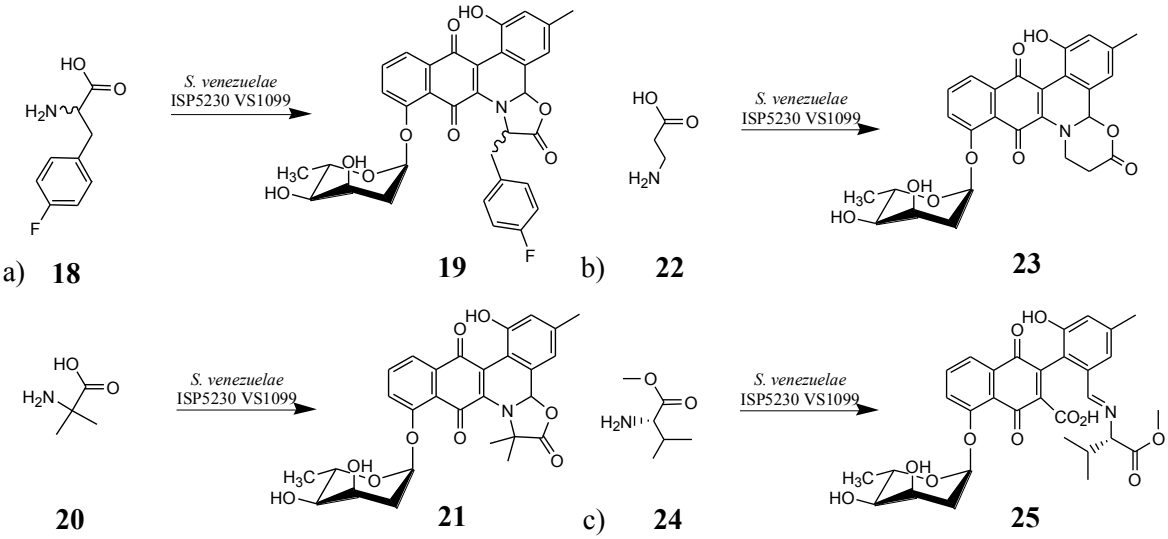


Figure 5. Structures of the first jadomycins isolated: Jadomycin A (**16**); and Jadomycin B (**17**), the glycosylated form of Jadomycin B. The oxazolone rings directly reflect the structure of isoleucine, the amino acid used in the growth medium.

Some preliminary experiments varying the amino acid in *S. venezuelae* ISP5230 culture medium[20,21] prompted the Jakeman group to further explore the hypothesis that jadomycin derivatives could be made by using different amino acids as the sole nitrogen sources in *S. venezuelae* ISP5230 growth media. Jakeman *et al.*[22] first examined the effects of alternate stress inducers, carbon sources, phosphate concentrations, and other components of the growth medium to render production of the secondary metabolite more efficient. Their improved method consisted of, among other things, ethanol rather than heat shock, a glucose rather than galactose carbon source, and the VS1099 strain of *S. venezuelae* ISP5230 which gave 5-10 times more jadomycin than the wild type.[23] Jakeman *et al.* then developed methods for monitoring jadomycin production during incubation and analyzing the jadomycin product after collection. These protocols rely on UV-visible and mass spectrometric analyses, respectively. The levels of natural product production are measured spectrophotometrically at one of the jadomycin λ_{max} values, and expected breakdown patterns observed via MS/MS are used to confirm the structure of the product.[22,24] With reliable production and analytical methods in place, the Jakeman group initiated a survey of different amino acids as the sole nitrogen sources in jadomycin growth media.

Nineteen of the twenty natural amino acids were successfully incorporated into jadomycin products, with only L-proline yielding ambiguous results.[24] A number of non-proteogenic amino acids were also explored.[25] The D-forms of some natural amino acids were successfully incorporated, as well as a few chemically synthesized amino acid derivatives including fluorinated (**18**) and alpha-methyl (**20**) amino acids (Scheme 2a). A beta-amino acid, β-alanine (**22**), was tested and yielded a jadomycin product with a 6-membered oxazinane rather than 5-membered oxazolone ring (**23**; Scheme 2b). NMR analysis was used to confirm this structure. An amino acid methyl ester, L-valine methyl ester (**24**), was used and, as expected, yielded no jadomycins; rather,

it yielded a precursor whose mass spectrometric data were consistent with the structure of the proposed aldimine intermediate (shown in Scheme 1; **25**; Scheme 2c). Equimolar amounts of different amino acids with differing stereochemistry were also used, and the jadomycin products isolated were also present in equimolar amounts, as determined by NMR spectroscopy.[26] The aglycone forms of the jadomycin derivatives were also observed throughout these numerous amino acid altering growth experiments.



Scheme 2. Examples of non-proteogenic amino acids used in *S. venezuelae* ISP5230 VS1099 culture media and their jadomycin products: a) DL-4-fluorophenylalanine (**18**) and 2-aminoisobutyric acid (**20**) yielded the expected derivatives (**19**, **21**); b) β-alanine (**22**) afforded a jadomycin derivative with a 6-membered oxazinane ring (**23**); and c) L-valine methyl ester (**24**) afforded the aldimine intermediate (**25**) of the proposed biosynthetic pathway.

Several of the amino acid altering experiments conducted by the Jakeman group (described above) have served to support portions of the proposed biosynthetic pathway as well as deduce new information about jadomycin biosynthesis. For example, the mere observation of various aglycone jadomycin analogues supports the hypothesis that the amino acid is incorporated before the sugar group is added by the enzyme JadS. The methyl ester provided straightforward evidence for the existence of an aldimine intermediate, as the amino acid could not be incorporated into an oxazolone ring and the biosynthetic pathway was stopped at this point, resulting in a build-up of the aldimine metabolite. The methyl ester experiments also suggested that JadS does not require an oxazolone ring in its substrate, as both aglycone and glycosylated forms of the aldimine intermediate were observed. Finally, these experiments strongly support a chemical rather than enzymatic amino acid incorporation step.

If amino acid incorporation into an oxazolone ring was not spontaneous, *i.e.* if there was an enzyme responsible, one would expect a certain degree of specificity in the substrates accepted, but this was not observed. L-Amino acids and D-amino acids were both incorporated with no general bias observed in the yields. Even fluorinated amino acids and alpha-methyl amino acids were incorporated. Even more supportive of a chemical rather than enzymatic amino acid incorporation was the production of a 6-membered oxazinane ring from a beta-amino acid. Probably most persuasive of all were the experiments in which the ratios of two different amino acids in the same medium were conserved in the corresponding jadomycin analogues. Altogether, these results are supportive of a chemical rather than enzymatic process.

Jakeman *et al.* demonstrated production of novel jadomycins via precursor-directed biosynthesis by simply altering the amino acid present in *S. venezuelae* ISP5230 VS1099 growth medium. This is a facile way to obtain new secondary metabolites with predictable structures and has already provided a variety of compounds. As mentioned, nineteen proteogenic amino acids, several D-amino acids, beta-amino acids, and other synthesized amino acids derivatives have been successfully incorporated into jadomycin analogues. The result has been a library of jadomycins with subtle structural changes. As structural alterations can affect biological properties, this library holds the potential to express a range of biological activities. Biological testing has been done on the majority of these molecules with the goal of elucidating a structure-activity relationship (SAR) for jadomycins and identifying SAR patterns that can give insight into which alterations are beneficial or detrimental for potential drug development.

Growth inhibition data from studies on human breast ductal carcinoma cell lines have revealed some preliminary SAR trends. For instance, glycosylated jadomycins demonstrated EC_{50} values ten-fold lower than their aglycone forms (unpublished data), suggesting that they would act as more potent drugs and are therefore more desirable targets. Also, jadomycins with small polar side chains were found to be the most potent, followed by those with nonpolar aliphatic side chains, then aromatic side chains, and finally a 6-membered oxazinane ring analogue which was the least effective.[26] The incorporation of L- versus D- amino acids did not have a significant effect on potency. Thus, the cytotoxicity appears to be strongly affected by the composition of the side chain but not by its stereochemistry. In addition to these general trends, one particular jadomycin derivative, Jadomycin L from L-leucine (**26**, Figure 6), showed promising results. It demonstrated high levels of activity against a number of cancer cell lines at the National Cancer Institute (NCI) and is a candidate for further studies.

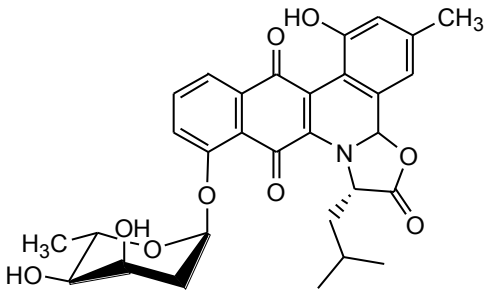


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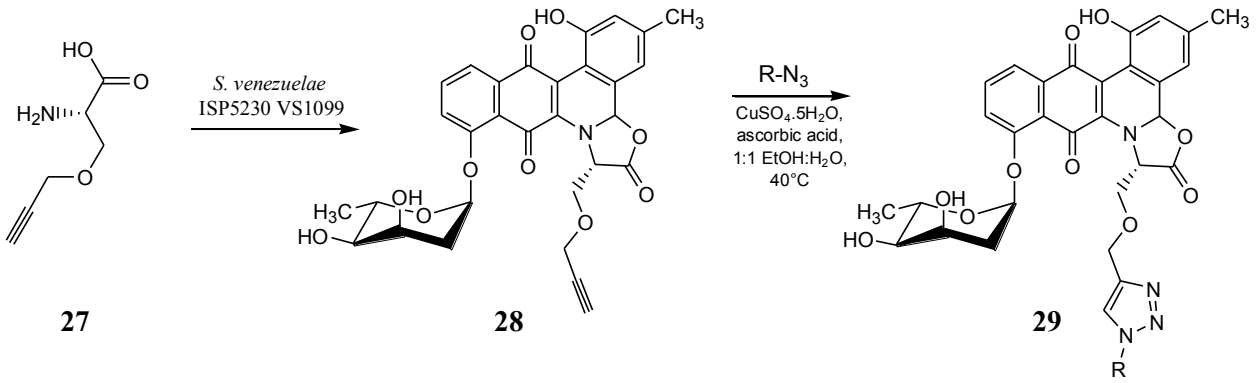
Figure 6. The structure of Jadomycin L (**26**), the derivative generated from *S. venezuelae* ISP5230 VS1099 in L-leucine culture medium.

An SAR profile for jadomycins is under construction as the Jakeman group continues to make jadomycin derivatives. It is evident that one way to fine-tune the biological properties of these potential drug molecules is to fine-tune their structural features. The goal of this project is to generate novel jadomycins, purify, characterize, and assess these new compounds.

1.3 Project goals

One specific group of derivatives to explore is those resembling the L-leucine jadomycin analogue. This compound passed through preliminary screening phases at the NCI, indicating that it shows promise as a drug candidate. It passed the 60-cell line screening and acute toxicity assays, and the NCI requested further material (100 mg) for solid tumour efficacy studies. The production of more Jadomycin L is essential for its continued testing, and related novel derivatives with structurally similar side chains should also be produced.

Another specific class of jadomycins to explore includes jadomycins with triazole rings on the variable side chain at position C1. Jadomycins with this functionality may have enhanced biological activities as there are many reports of triazoles with good antifungal, antibacterial, antiviral, and anticancer activities.[27-32] The triazole functionality may be introduced using the Copper(I)-catalyzed Azide-Alkyne Cycloaddition (CuAAC), a variation of the Huisgen 1,3-dipolar cycloaddition which unites organic azides and terminal alkynes to give 1,4-disubstituted 1,2,3-triazole products.[33,34] This would entail producing a jadomycin derivative with a terminal alkyne functionality (**28**) and reacting it with various azides to make a library of triazole jadomycins (**29**) from just one isolated jadomycin. This plan is outlined below (Scheme 3), where a proposed L-serine derivative (**27**) acts as the terminal alkyne-containing nitrogen source.



27 **28** **29**

Scheme 3. Proposed route for the production of a library of triazole jadomycins. A terminal alkyne functionalized jadomycin derivative (**28**) is produced using *O*-propargyl-L-serine (**27**) as the sole nitrogen source in *S. venezuelae* ISP5230 VS1099 growths and reacted with a library of azides using CuAAC to produce 1,4-disubstituted triazole jadomycins (**29**).

Producing a library of triazole derivatives in this way would constitute a new route to jadomycin derivatives and would be beneficial over the traditional method because it requires the large-scale production and intensive purification of only one jadomycin derivative (**28**). Producing and purifying jadomycin derivatives are time-consuming and challenging tasks, so minimizing repetition of these steps would make the entire library-constructing process more efficient. Once the terminal alkyne-containing secondary metabolite (**28**) is isolated and purified, it could be reacted with different azides using straightforward stoichiometric chemistry and the products purified using standard chemical work-up techniques. Also, a variety of azides can be synthesized straightforwardly from bromide precursors,[35,36] a factor that should contribute to the overall efficiency of this proposed expansion of the jadomycin library.

The goals of this project, specifically, are to:

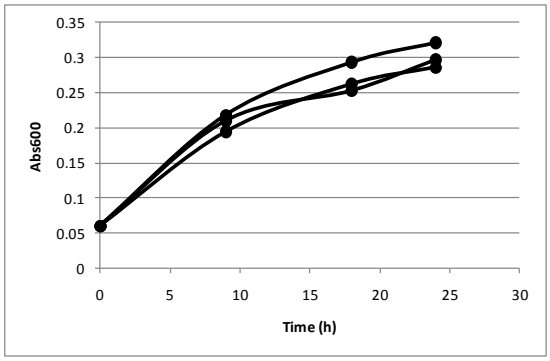
1. Produce and purify Jadomycin L (**26**) and other related jadomycin derivatives using L-leucine and other amino acids with short, alkyl side chains;
2. Synthesize an amino acid derivative containing a terminal alkyne functionality (**27**);
3. Carry out *S. venezuelae* ISP5230 VS1099 culture growths with the synthesized amino acid derivative (**27**) to produce the corresponding jadomycin derivative (**28**);
4. Purify and quantify new terminal alkyne jadomycin (**28**);
5. Synthesize a number of azides and react them with the purified jadomycin to produce a library of triazole jadomycins;
6. Characterize all novel jadomycins by mass spectrometry and NMR spectroscopy;
7. Assess biological activity of all novel jadomycins.

CHAPTER 2 RESULTS AND DISCUSSION

2.1 Production of Jadomycins L, DNV, and DNL

Excerpts of this section were taken from Jakeman, D; Dupuis, S; Graham, C. *Pure Appl. Chem.* **2009**, 81, 1041-1050.

Jadomycin L (**26**) was produced by *S. venezuelae* ISP5230 VS1099 grown in minimal culture media (4 x 2 L) with L-leucine as the sole nitrogen source and ethanol-shocked to induce secondary metabolism, as described previously.[22] The cultures were monitored spectrophotometrically using a method previously shown to give accurate measurements of cellular growth and natural product production (Figure 7).[20,22]

a) 

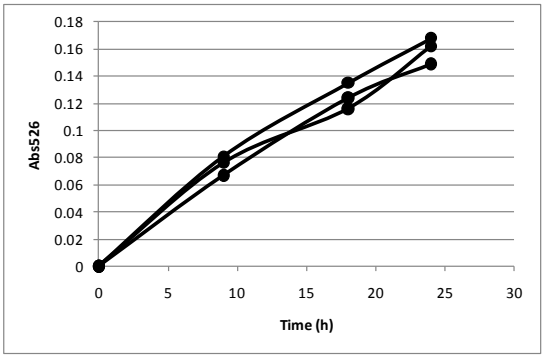
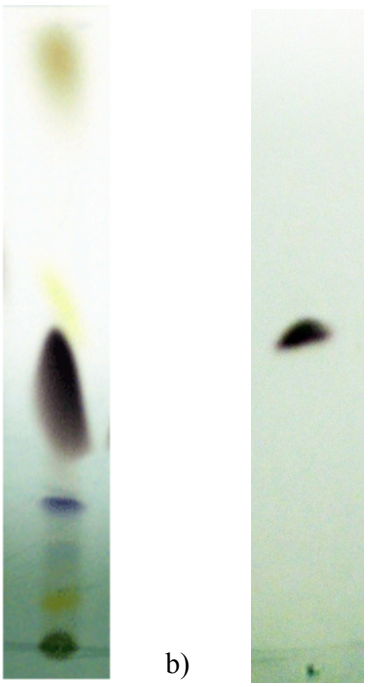
b) 

Figure 7. Absorbance plots for *S. venezuelae* ISP5230 VS1099 grown in 2 L L-leucine production media. a) Abs_{600} indicates bacterial growth. b) Abs_{526} indicates natural product production. The three overlaid curves represent aliquots taken from three different flasks.

After the incubation period, media were filtered, passed through a reversed-phase C18 column, and the crude product collected (718 mg). The natural product was purified via column chromatography (95 mg, 12 mg/L; Figure 8) and characterized using UV-visible spectroscopy (UV-Vis), infrared spectroscopy (IR), low resolution mass spectrometry (LRMS), high resolution mass spectrometry (HRMS), and nuclear magnetic resonance (NMR) spectroscopy. Specifically, an enhanced product ion mass spectrum (EPI-MS) was used to observe the fragmentations characteristic of jadomycin derivatives, as reported previously.[24] Under an EPI scan in positive mode, the predicted breakdown pattern was observed: the parent ion at 550 $[M+H]^+$, fragmentation of the glycosidic linkage to the aglycone ion at 420 $[M+H-130]^+$, and cleavage of the oxazolone ring in the aglycone to the phenanthroviridin ion at 306 $[M+H-244]^+$ (Figure 9). For



a) b)

characterization by NMR spectroscopy, ^{1}H-NMR, ^{1}H-^{1}H COSY, 1D TOCSY, and 1D nOe experiments were used to assign protons and distinguish diastereomers, and HSQC and HMBC were used to assign ^{13}C resonances. Purified **26** was a mixture of diastereomers 3a*S* and 3a*R* (Figure 10), the major diastereomer being 3a*S* (1.22:1).

Figure 8. TLC (10:90 MeOH:DCM) of Jadomycin L (**26**) before (a) and after (b) purification.

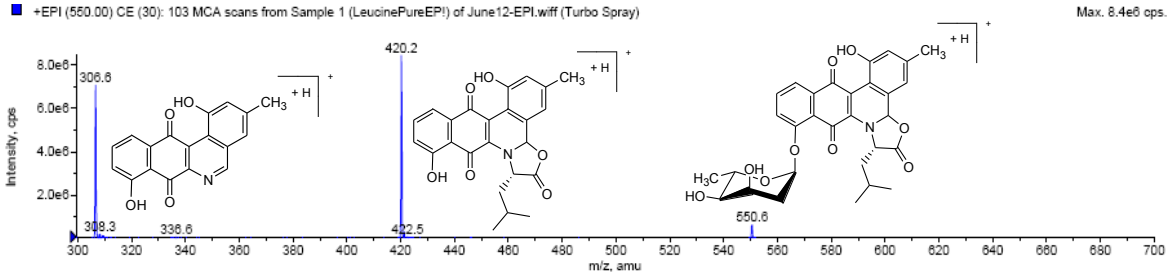
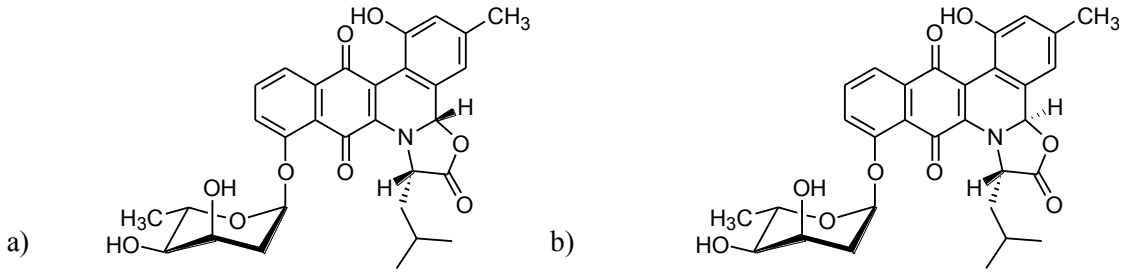


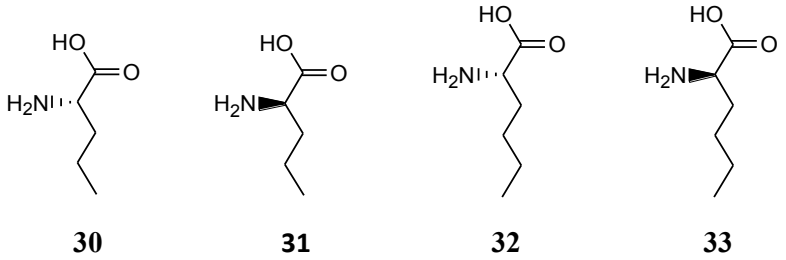
Figure 9. Fragmentation observed from an EPI-MS scan of **26**. The parent jadomycin ion $[M+H]^{+}$ loses its L-digitoxose sugar group to form the aglycone ion $[M+H-130]^{+}$, whose oxazolone ring breaks down to give the phenanthroviridin ion $[M+H-224]^{+}$.



a) b)

Figure 10. The two diastereomeric forms of **26**: a) the 3a*S* diastereomer, and b) the 3a*R* diastereomer.

Non-proteogenic amino acids L-norvaline (**30**), D-norvaline (**31**), L-norleucine (**32**), and D-norleucine (**33**) (Figure 11) were each used as the sole nitrogen source in small-scale *S. venezuelae* ISP5230 VS1099 culture growths (3 x 25 mL), as described previously.[22] The cultures containing D-amino acids showed production of jadomycin derivatives over time, while those containing L-amino acids did not (Figure 12). The result observed from the absorbance plots was confirmed by EPI-MS: the expected breakdown patterns were observed in the culture media containing the D-amino acids (Figure 13) but were not seen in the corresponding L-amino acid cultures. The reason for this lack of jadomycin production in the L-amino acid cultures is unknown. The bacteria grew better in the L-amino acid culture media (based on Abs_{600} readings), but failed to produce the corresponding secondary metabolite. One possible explanation is that the straight-chained L-amino acids mimic a polyketide-related intermediate, causing inhibition of one of the early Jad enzymes in the jadomycin biosynthetic pathway.



30 **31** **32** **33**

Figure 11. Structures of four non-proteogenic amino acid derivatives used in *S. venezuelae* ISP5230 VS1099 growths for jadomycin production: L-norvaline (**30**), D-norvaline (**31**), L-norleucine (**32**), and D-norleucine (**33**).

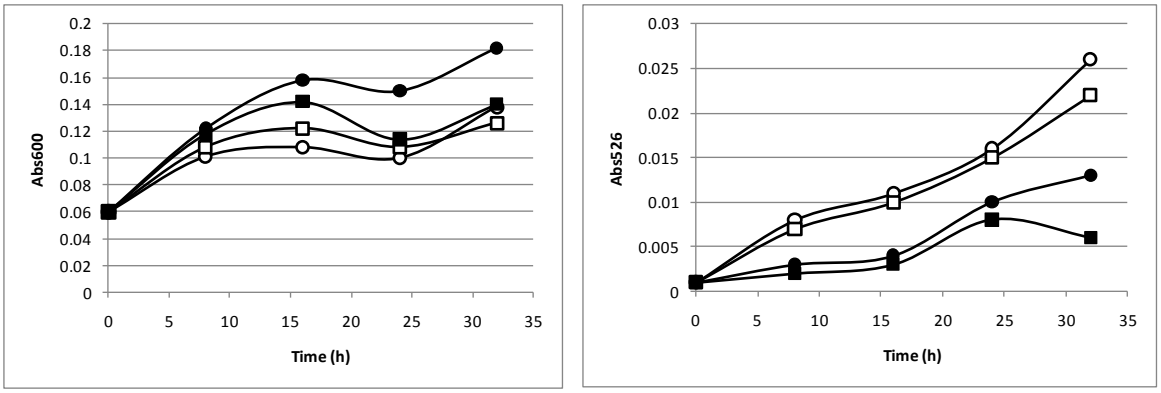


Figure 12. Absorbance plots from *S. venezuelae* ISP5230 VS1099 growths using L-norvaline (■), D-norvaline (□), L-norleucine (●), and D-norleucine (○). Culture media containing D-norvaline and D-norleucine showed increases in Abs_{526} characteristic of jadomycin production while those containing L-norvaline and L-norleucine did not.

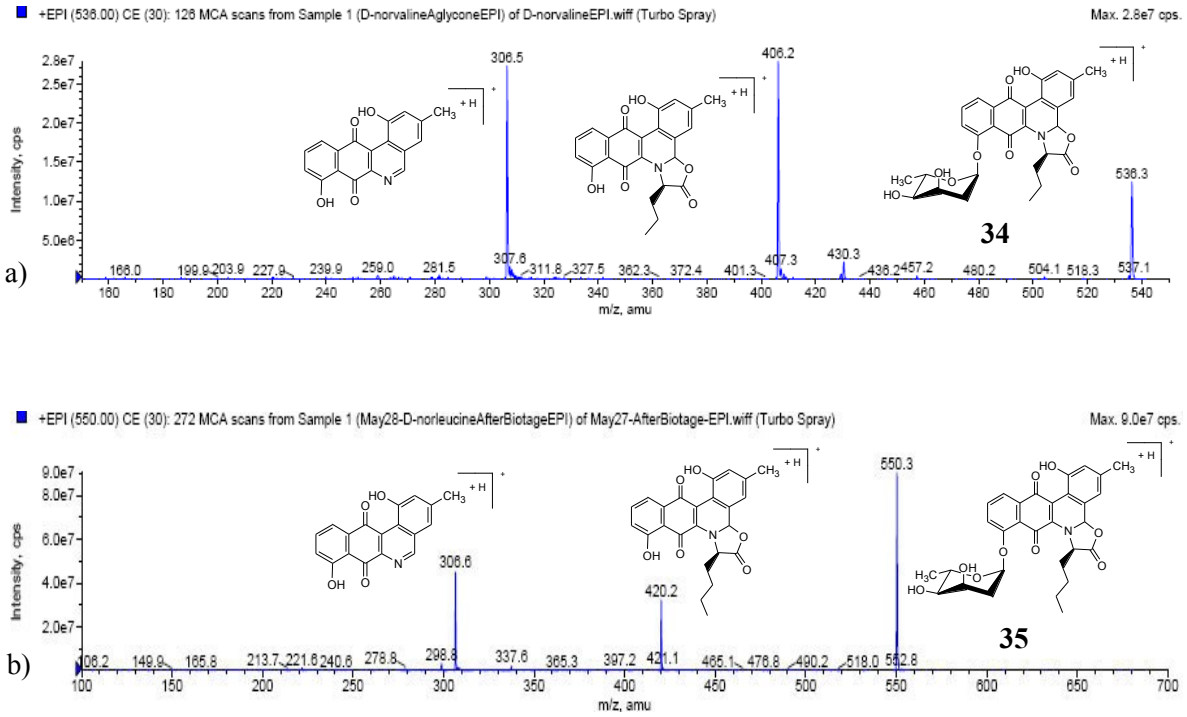


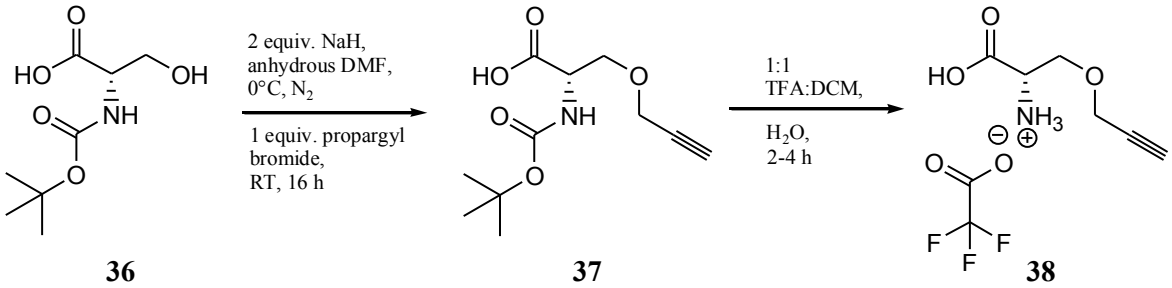
Figure 13. Fragmentations of Jadomycin DNV (**34**, a) and Jadomycin DNL (**35**, b) observed by EPI-MS. The expected jadomycin, aglycone, and phenanthroviridin ions are observed.

Jadomycin DNV and Jadomycin DNL productions were scaled up to 2 L culture growths and monitored spectrophotometrically. The products were captured using a reversed-phased C18 column (56 mg **34**, 84 mg **35**) and purified via column chromatography (8.5 mg, 4.3 mg/L **34**; 17 mg, 8.5 mg/L **35**). The natural products were characterized using UV-Vis, IR, LRMS, HRMS, and NMR. Fragmentations observed by EPI-MS were as shown in Figure 13. Signals in the ^{1}H-NMR spectra were assigned using COSY, 1D TOCSY, and 1D nOe NMR, and carbons assigned via HSQC and HMBC NMR. Jadomycins DNV and DNL were purified as diastereomeric mixtures, with 3a*S* in majority 1.67:1 and 1.65:1, respectively.

The diastereomeric ratios observed for the jadomycin derivatives described here (Jadomycins L, DNV, and DNL) are consistent with those reported for other jadomycin derivatives. Structurally related jadomycin derivatives Jadomycin B (with L-leucine side chain; Figure 6), Jadomycin V (with L-valine side chain), and Jadomycin DV (with D-valine side chain) have diastereomeric ratios of 1.86:1, 1.91:1, and 0.43:1, respectively.[26] Ratios of 1.22:1 (Jadomycin L), 1.67:1 (Jadomycin DNV), and 1.65:1 (Jadomycin DNL) compare well with these. These derivatives all have aliphatic side chains composed of three or four carbon atoms, a commonality that suggests they would have similar conformational characteristics.

2.2 Synthesis of a Non-Proteogenic Amino Acid, *O*-Propargyl-L-Serine

Towards the production of a jadomycin derivative with a terminal alkyne functionality, synthetic efforts were focused on generating an amino acid derivative with a terminal alkyne functionality, specifically *O*-propargyl-L-serine (**27**), an L-serine derivative that could be generated via alkylation of the hydroxyl group. Synthesis of **38**, a salt form of **27**, was attempted according to the scheme below (Scheme 4), which was previously used by former group member Alfred Rolle (Dalhousie chemistry project 2007, unpublished).



36 **37** **38**

Scheme 4. A proposed synthesis of *O*-propargyl-L-serine trifluoroacetate salt (**38**) from *N*-Boc protected L-serine (**36**).

Using the reaction conditions outlined above,[37] **37** was obtained in poor yields (<10%). In an attempt to improve the yield, a variety of conditions were explored for the first reaction in the above scheme (Table 1). This involved repeating the reaction several times, varying factors such as temperature, duration of the reaction, and the base used (entries 2-13, Table 1). It was observed that the reaction yielded a mixture of products when sodium hydride (NaH) was used as the base but a single product when diisopropylethylamine (DIPEA) was used (entries 3&4, Table 1). Based on this finding, DIPEA was selected as the base for the reaction and other conditions were varied until a set of reaction conditions were reached that yielded one pure product in high yield (entry 9, Table 1; 2 g scale).

Table 1. Reaction conditions attempted for the first reaction in Scheme 4. Yield, purity, and major product are indicated for each set of conditions.

Entry	Solvent (DMF / DCM)	Base (NaH / DIPEA)	Propargyl Bromide	Temp. (°C)	Duration (h)	Yield (%)	Pure? (Y/N)	Major Product (Ester/Ether/Bis)
1	DMF	2 equiv. NaH	1.2 equiv.	RT	16	6	N	Ether
2	DCM	3 equiv. DIPEA	1.2 equiv.	RT	56	25	N	Ester
3	DMF	2 equiv. NaH	1.2 equiv.	30	43	5	N	Ester & Ether
4	DCM	3 equiv. DIPEA	1.2 equiv.	30	44-55	22-55	Y	Ester
5	DCM	3 equiv. DIPEA	2 equiv.	30	22	50	Y	Ester
6	DCM	excess DIPEA	excess	30	22	50-72	Y	Ester
7	DCM	excess DIPEA	excess	30-40	79-118	86-95	N	Ester
8	Minimal DCM	excess DIPEA	excess	30-40	30	86	N	Ester
9	Minimal DMF	excess DIPEA	excess	40	41-124	80-90	Y	Ester
10	Minimal DMF	2 equiv. NaH	excess	40	48	60	N	Ester & Bis
11	Minimal DMF	2 equiv. NaH	3 equiv.	0	73	54	N	Ester & Bis
12	Minimal DMF	2 equiv. NaH	1 equiv.	0	3	21	Y	Ether
13	DMF	3 equiv. NaH	1 equiv.	0	1-2	66-86	Y	Ether

The Boc protecting group was removed according to Scheme 4 (59% yield, 650 mg scale, over 24 h), and the salt was used as the sole nitrogen source in 3 x 25 mL *S. venezuelae* ISP5230 VS1099 growths for jadomycin production. While the control growths, 3 x 25 mL culture media containing L-leucine as the sole nitrogen source, successfully produced jadomycin, the cultures with the L-serine derivative did not (Figure 14). The increase in absorbance at 526 nm characteristic of jadomycin-producing cultures was not observed, and no signals corresponding to the mass of the expected jadomycin derivative or any of its byproducts could be found by mass spectrometry. Considering a wide variety of jadomycin derivatives have been made via incorporation and biosynthesis by *S. venezuelae* ISP5230 VS1099 of a wide variety of amino acids and amino acid derivatives,[24-26] the lack of incorporation observed here suggested the structure of the L-serine derivative synthesized was different from the target derivative (**38**) shown in Scheme 4.

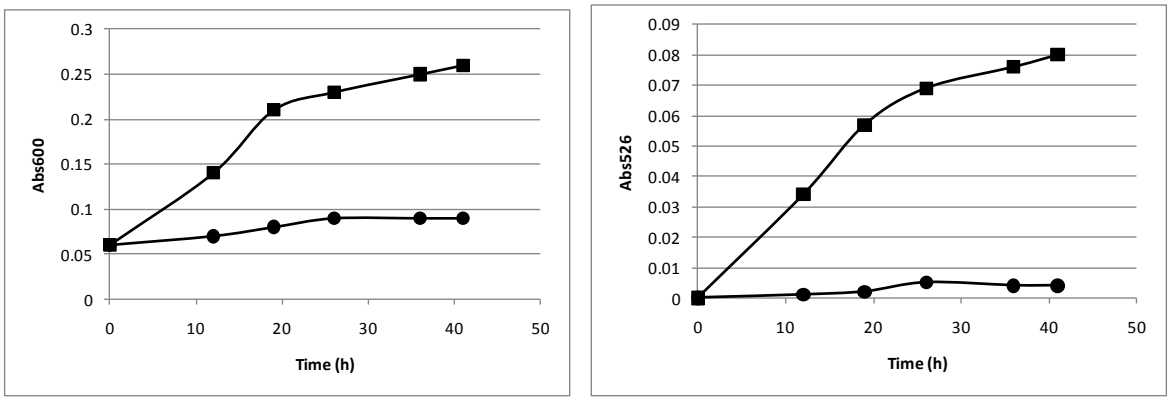


Figure 14. Absorbance plots of 25 mL *S. venezuelae* ISP5230 VS1099 cultures containing the synthesized trifluoroacetate (TFA) salt (●) or L-leucine (■) as the sole nitrogen source. The cultures with the synthesized TFA salt show little growth and no jadomycin production.

IR and HMBC NMR spectra were recorded on the TFA salt (Appendix A). The ν = 1740 cm^{-1} was too high for an acid and suggested the presence of an ester functionality. In the HMBC, a significant correlation was observed between the methylene unit from the propargyl functionality and the carboxyl carbon, while no correlation was observed between the propargyl methylene protons and the beta-carbon of the amino acid. These data concluded that the TFA salt generated was the ester, rather than the ether, product (Figure 15).

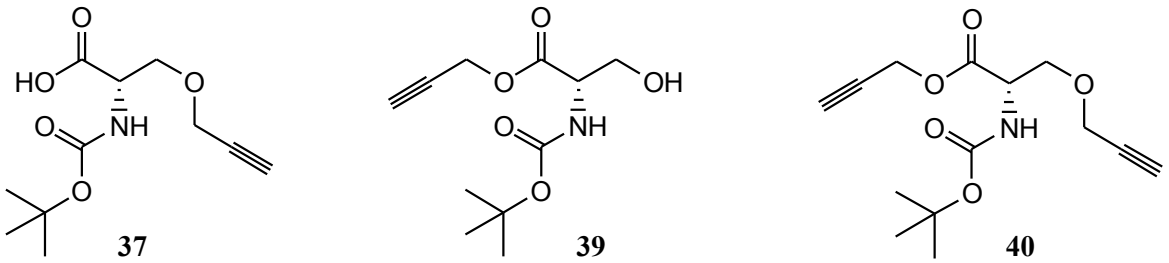
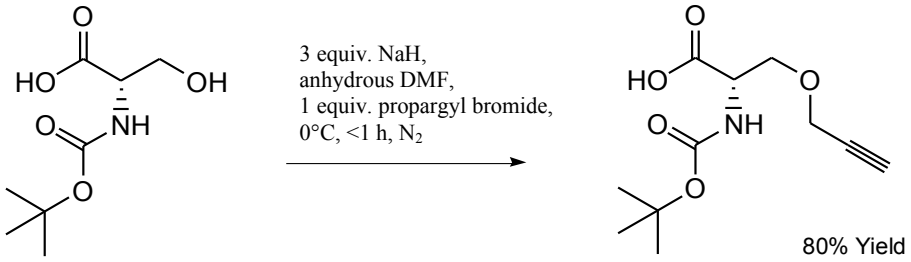


Figure 15. The three different products observed from the reaction of *N*-Boc-L-serine with propargyl bromide: *N*-Boc-*O*-propargyl-L-serine (**37**); *N*-Boc-L-serine propargyl ester (**39**); and bis-propargyl-*N*-Boc-L-serine (**40**). All three compounds have been characterized by MS and NMR (Appendix A).

The first reaction in the synthetic scheme, alkylation of the hydroxyl group of *N*-Boc-L-serine with propargyl bromide, was revisited using NaH, the stronger base which when used previously had yielded a mixture of products. The compounds produced from this reaction were isolated by flash chromatography using a normal phase silica column and their structures determined by MS and NMR spectroscopy (^{1}H, COSY, ^{13}C, HSQC, HMBC; Appendix A; Figure 15). The reaction generated three products: the ether (**37**), the ester (**39**), and the bis-propargylated

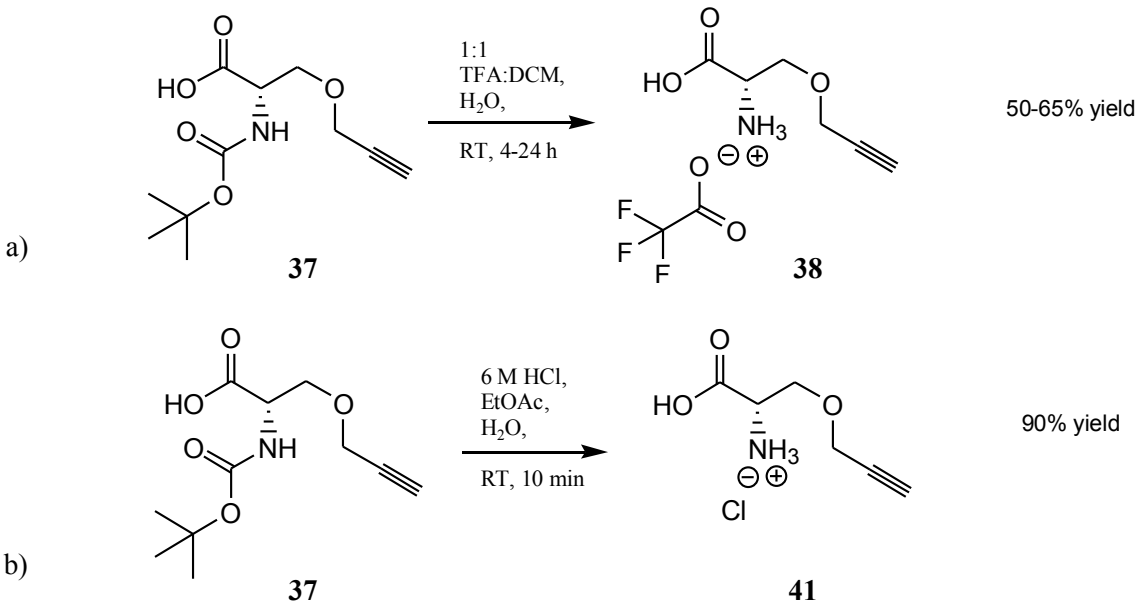
(**40**) serine derivatives. Clearly, there were both S_N2 substitutions and esterifications taking place, and NaH was needed in order for S_N2 to occur. Esterification was able to proceed when DIPEA was used because the pK_a`s of carboxylic acids are low (~2-3), allowing for easy deprotonation to form the carboxylate ion which can then act as a nucleophile. The hydroxyl group was unable to act as a nucleophile until it was deprotonated, and it could not be deprotonated by DIPEA because its pK_a was too high (~15 for primary alcohols). NaH was a strong enough base to deprotonate the hydroxyl proton, but it also deprotonated the carboxylic acid proton and the initial reaction conditions favoured esterification. The reaction conditions were altered to reduce or prevent esterification, namely by decreasing temperature and duration of the reaction, until a set of conditions was found that yielded the pure ether product (**37**) in high yield (entry 13, Table 1). The optimal conditions for the selective formation of **37** in high yield were the following: at 0°C in an ice bath, a nitrogen atmosphere, 1 equivalent *N*-Boc-L-serine, dry DMF, 3 equivalents NaH, and 1.1 equivalents propargyl bromide, stirred <1 h (Scheme 5).



36 **37**

Scheme 5. Optimal reaction conditions found for the synthesis of **37**.

Deprotection of the *N*-Boc protecting group with TFA was achieved using the conditions outlined in Scheme 4[37] in 50-65% yield (4.2 g scale). Due to the low yields and long reaction times (4-24 h), an alternative deprotection method was explored (Scheme 6). Using HCl and EtOAc in place of TFA and DCM, the reaction was complete in ten minutes and the product, a hydrochloride salt of **27** (**41**), was obtained in 90% yield (7.4 g scale).



Scheme 6. Alternative deprotection methods used to produce *O*-propargyl-L-serine salts. The reaction yielding the TFA salt (**38**; a) has longer reaction times and lower yield than that yielding the HCl salt (**41**; b).

2.3 Incorporation of *O*-Propargyl-L-Serine into a Jadomycin Derivative

The incorporation of **27** into a jadomycin derivative by *S. venezuelae* ISP5230 VS1099 was initially explored using the trifluoroacetate salt (**38**) as the sole nitrogen source in small-scale growths (25 mL culture media) as described previously, [22] except that the concentration of the amino acid was varied to determine the most efficient concentration of this particular amino acid derivative for jadomycin production. Considering 60 mM is the typical amino acid concentration used for jadomycin production, [22] concentrations of 15 mM, 30 mM, 45 mM, and 60 mM **38** were evaluated (Figure 16).

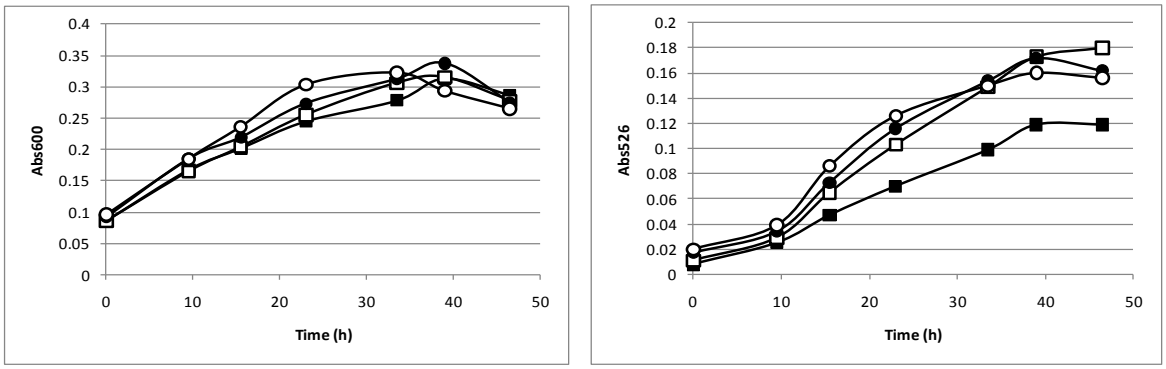


Figure 16. Absorbance plots from *S. venezuelae* ISP5230 VS1099 growths using **38** at concentrations of 15 mM (■), 30 mM (□), 45 mM (●), and 60 mM (○).

All four cultures grew comparably, based on OD_{600} measurements. However, similar Abs_{526} values observed at 30 mM, 45 mM, and 60 mM suggested similar production levels of secondary metabolites at these three concentrations, potentially indicating that a more dilute amino acid culture could be utilized. Comparative NMR data from the four cultures supported the notion that 30 mM **38** is the most efficient concentration for jadomycin production of the four explored here (Appendix B). Using 30 mM rather than 60 mM, twice the volume of culture medium could be produced from the same amount of amino acid.

When HCl and EtOAc were found to be more effective Boc-deprotection conditions than TFA and DCM and **41** subsequently replaced **38** as the salt form of **27** being synthesized, the experiment varying the amino acid concentration in small-scale jadomycin production growths (25 mL culture media) was repeated (Figure 17). The results resembled those obtained when **38** was used, based on absorbance measurements, therefore 30 mM was deemed a suitable concentration of **41** to use in *S. venezuelae* ISP5230 VS1099 culture media for jadomycin production.

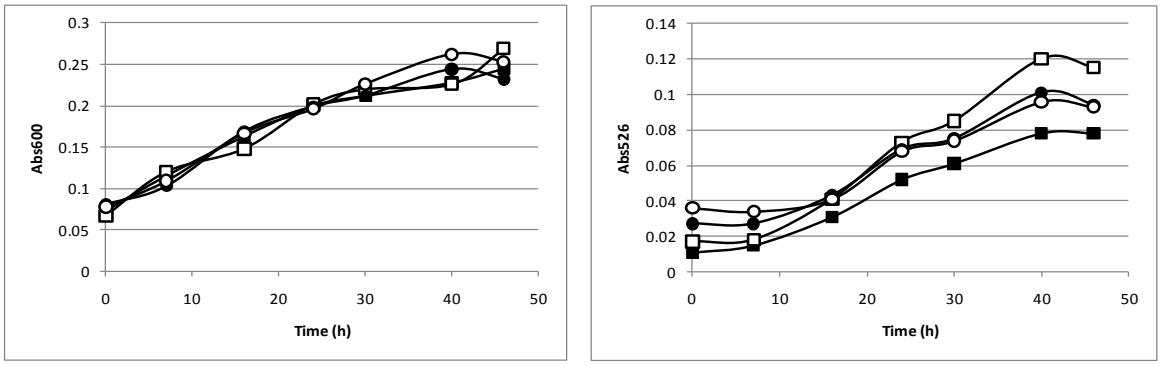


Figure 17. Absorbance plots from *S. venezuelae* ISP5230 VS1099 growths using **41** at concentrations of 15 mM (■), 30 mM (□), 45 mM (●), and 60 mM (○).

After optimizing the concentration of **41** for production media, production of Jadomycin *O*PS (**28**) was scaled up to 2 L culture growths and monitored spectrophotometrically (Figure 18). Levels of secondary metabolite production were not as high as those in the L-leucine controls, but variation in the colour intensity of culture media with different amino acids has been reported previously.[20] Crude product was obtained using a reversed-phased C18 column (120 mg). Attempts made to purify the jadomycin derivative by column chromatography were unsuccessful. Normal phase columns (both silica- and alumina-based) resulted in degradation of the compound, while reversed-phase columns were ineffective at separating impurities from the natural product, even at low gradients and flow rates. The natural product was thus purified by preparative TLC (10:90 MeOH:DCM; 12 mg/L).

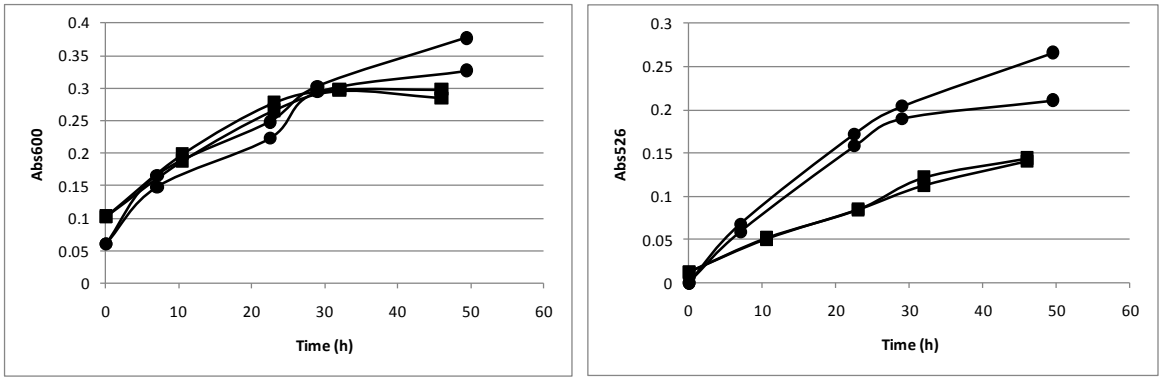
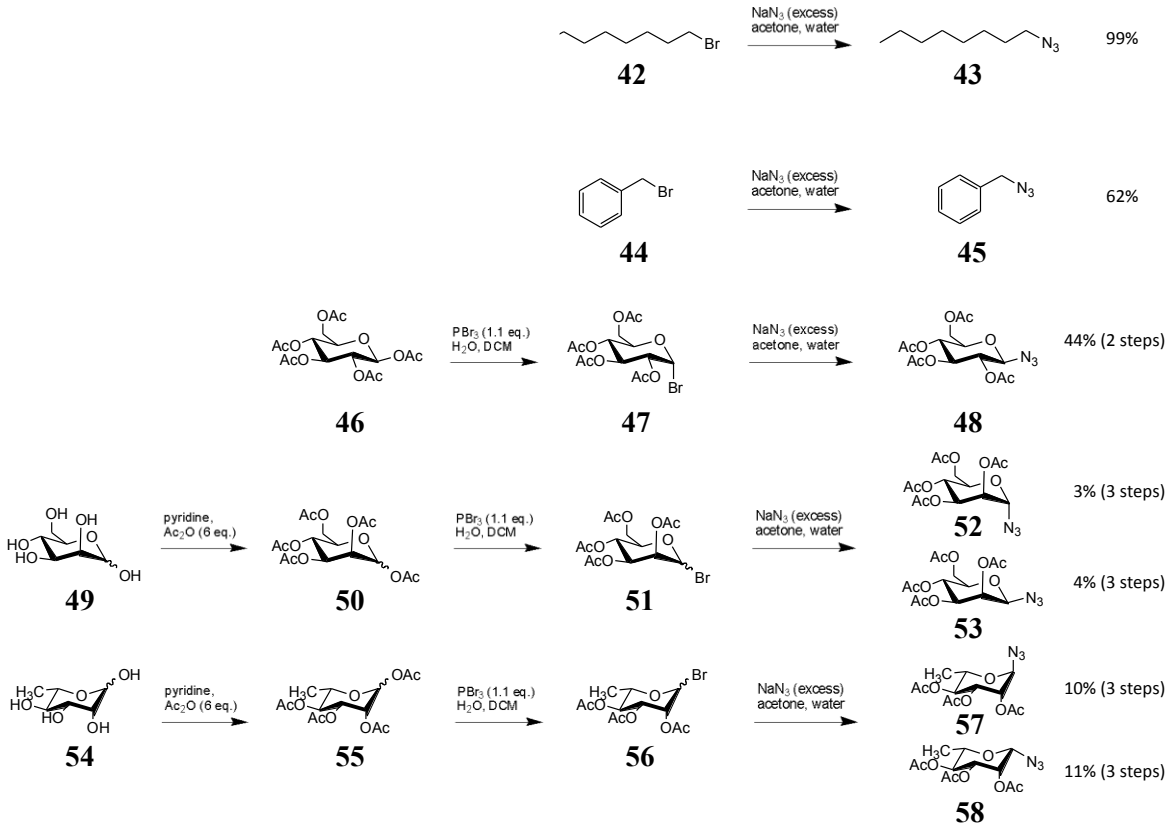


Figure 18. Absorbance plots for 2 L *S. venezuelae* ISP5230 VS1099 cultures containing 30 mM **41** (■) or 60 mM L-leucine (●) as the sole nitrogen source. The two overlaid curves represent aliquots taken from two different flasks.

The purified jadomycin derivative was characterized by UV-Vis, IR, LRMS, HRMS, and NMR. The EPI-MS fragmentation observed showed the expected masses for the jadomycin (562 m/z), aglycone (432 m/z), and phenanthroviridin (306 m/z) ions. The accurate mass was as anticipated. ^{1}H, COSY, and 1D nOe NMR were used to assign protons, and HSQC and HMBC to assign ^{13}C resonances. The diastereomeric ratio between the 3a*S* and 3a*R* isomers was 3.20:1. This is not at all comparable to the diastereomeric ratio observed for Jadomycin S, the serine-incorporated derivative which exists almost exclusively in the 3a*R* configuration.[26] However, this ratio is similar to that observed for Jadomycin M, the methionine derivative (1.70:1 3a*S*:3a*R*). The side chain of serine is a hydroxymethyl group, whereas the methionine side chain is an unbranched five-atom alkyl chain containing an internal sulfur atom. The *O*-propargyl-L-serine side chain is also an unbranched five-atom alkyl chain containing an internal Group 6 heteroatom, therefore the methionine derivative (Jadomycin M) is more structurally similar to **28** than the serine derivative (Jadomycin S) and a better comparison for configurational trends.

2.4 Preparation of Azides for Cycloadditions

For the CuAAC reactions, alkyl, aryl, and sugar azides were synthesized (Scheme 7). A total of seven azides were produced, and all were generated by reacting their corresponding bromides with excess sodium azide in water and acetone (1:1).[35,36] The alkyl and aryl azides (**43** and **45**) were synthesized directly from their bromide precursors, as benzyl bromide and octyl bromide are readily available starting materials. Glycosyl-1-bromides are unstable and required synthesis immediately prior to azide formation. The sugars' hydroxyl groups first required protection before any chemistry. D-Mannose (**49**) and L-rhamnose (**54**) were thus acetylated, while β-D-glucose pentacetate (**46**) was purchased. Selective bromination at C1 was then achieved using phosphorus tribromide,[38] and the azide formation reactions immediately followed.



Scheme 7. Syntheses of seven azides and their yields.

As seen above, compound **48**, synthesized from an anomerically pure sugar starting material, was the only product formed from the sodium azide reaction. In contrast, pairs of azide products (**52**&**53**, **57**&**58**) were obtained from those reactions whose sugar starting materials

existed as anomeric mixtures. The azide anomers were distinctly visible by TLC (R_f = 0.65 (**52**) and 0.60 (**53**), 0.76 (**57**) and 0.70 (**58**), 50:50 EtOAc:Hexanes), and were separated by flash chromatography (10:90 EtOAc:Hexanes). The stereochemistry of each anomer was assigned by measuring the direct-bond coupling constant for C1 and H1 ($^1J_{C1,H1}$) using HETCOR NMR (Figure 19). $^1J_{C1,H1}$ measured 170 Hz for α-sugar azides (**52**, **57**) and 158 Hz for β-sugar azides (**48**, **53**, **58**). These values are consistent with published values.[39-41]

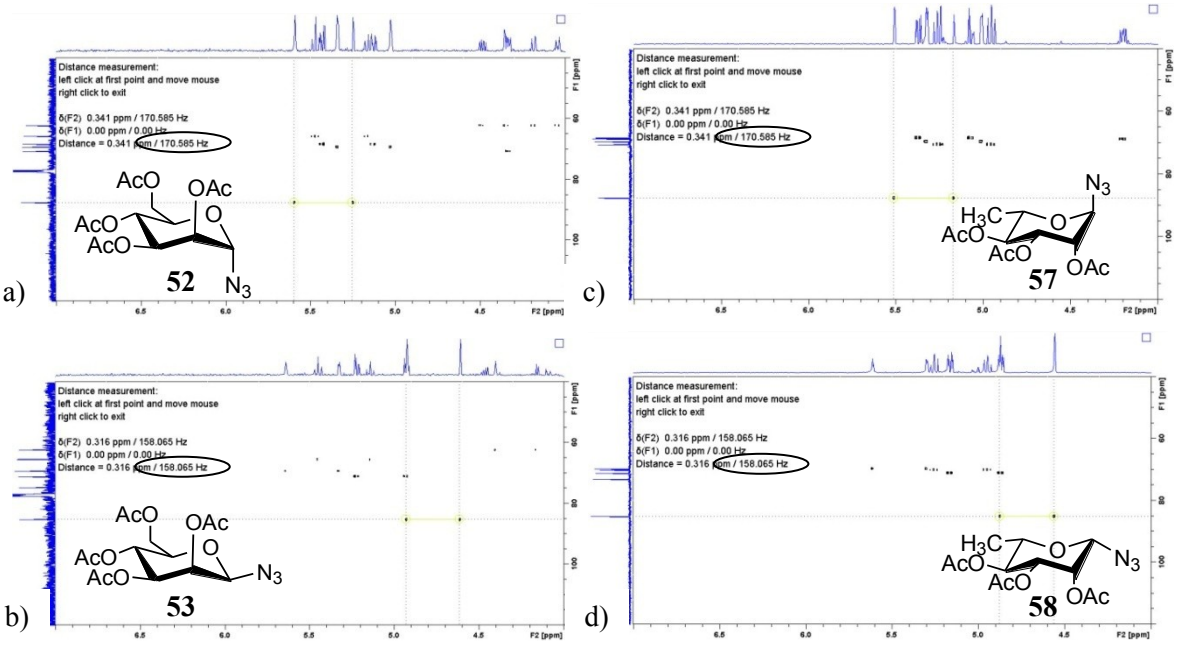
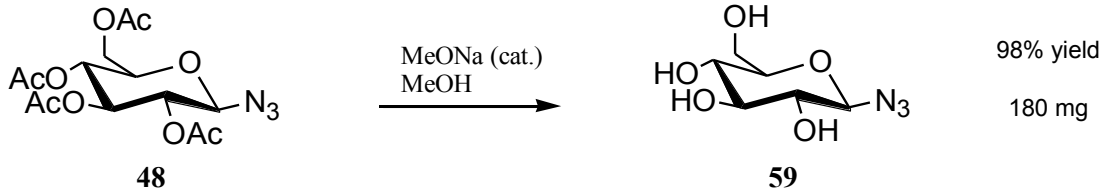


Figure 19. HETCOR NMR spectra of **52** (a), **53** (b), **57** (c), and **58** (d), showing the C1-H1 coupling constants indicating α or β stereochemistry. **52** and **57** had $^1J_{C1,H1}$ = 170 Hz, revealing them as α-sugar azides; **53** and **58** had $^1J_{C1,H1}$ = 158 Hz, revealing them as β-sugar azides.

A deprotected sugar azide was also synthesized for use in 1,3-dipolar cycloaddition reactions (Scheme 8). Compound **48** was deprotected using catalytic amounts of sodium methoxide in methanol to give **59** in 98% yield.

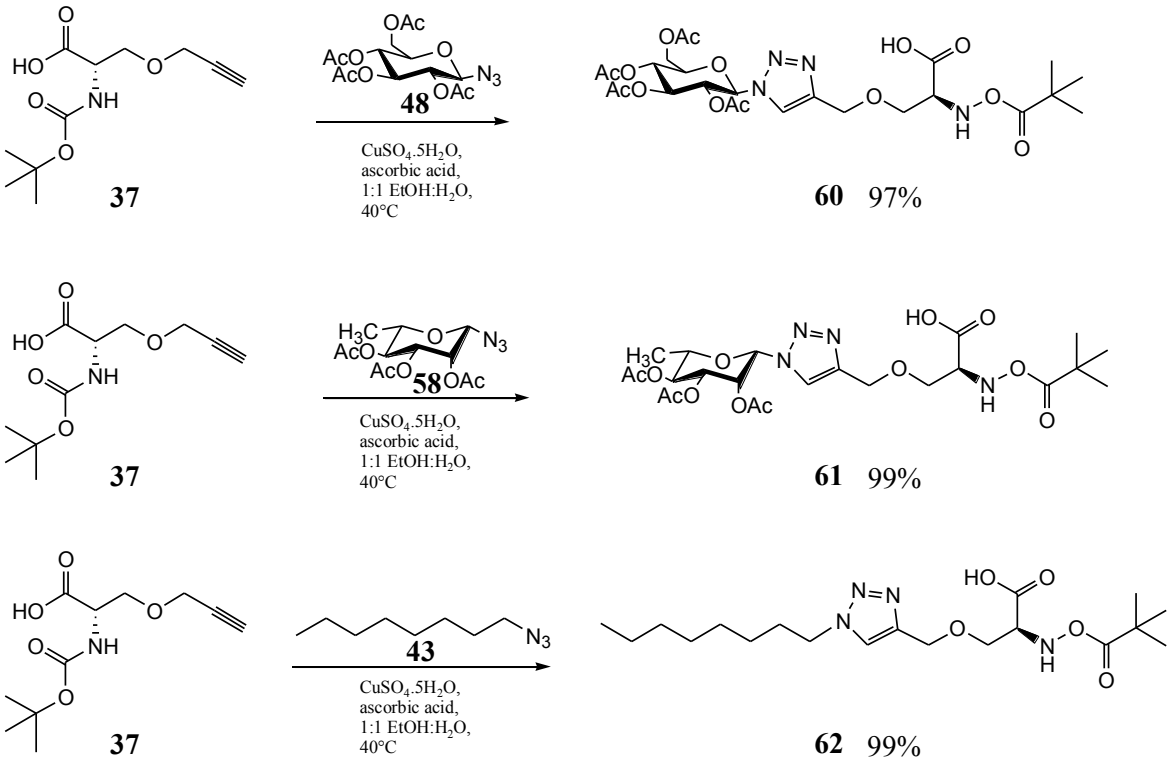


48 **59**

Scheme 8. Generation of a deprotected sugar azide, β-D-glucopyranosyl azide (**59**), via acetyl deprotection using NaOMe (cat.) in MeOH.

2.5 Cycloadditions Using *O*-Propargyl L-Serine

As a prelude to jadomycin CuAAC reactions with the above azides, reactions were conducted using the alkyne-functionalized L-serine derivative **37** and a selection of the synthesized azides (Scheme 9). The azides tested consisted of two acetyl-protected sugar azides (**48** and **58**) and the alkyl azide **43**. The reaction conditions used involved catalytic amounts of copper sulfate pentahydrate and L-ascorbic acid which allow for selective formation of the 1,4-disubstituted triazole over the 1,5-regioisomer.[33,34] The triazole products from these three reactions were generated exclusively as 1,4-regioisomers and in 95-100% yield, as expected. This confirmed the robustness of the reaction conditions chosen and suggested similar reactions using jadomycin **28** would likely give the anticipated products.



Scheme 9. CuAAC reactions carried out using alkyne **37** and three azides: a) **48**; b) **58**; c) **43**. All three reactions yielded the corresponding 1,4-disubstituted triazoles in 97-99% yields. ·

The cycloadditions were carried out using stoichiometric equivalents of reagents, as described, [34,42] except for the reaction using **43** which is volatile and was used in excess and then removed *in vacuo*. When stoichiometric equivalents were used, the products obtained (**60** and **61**) were 90-95% pure with only miniscule amounts of leftover sugar azide impurities. Efforts made to purify the these triazole products revealed the sugar triazoles are labile to silica- and alumina-based

normal phase columns and are not separated from their sugar azide impurities when reversed-phased columns are used, even at low gradients and flow rates. Prep TLC (100% EtOAc) successfully separated sugar triazole and sugar azide without causing degradation of the sugar triazole.

2.6 Cycloadditions Using Jadomycin *O*PS

With a set of 1,3-dipolar cycloaddition reaction conditions and a method of triazole purification established, formation of triazole jadomycins was attempted by reacting **28** with the library of azides synthesized previously (Scheme 7). To avoid running twice as many prep TLC plates and losing significant amounts of material, **28** was used in its crude form. The cycloaddition reactions were monitored by TLC analyses (10:90 MeOH:DCM), which in every case showed quantitative conversion of starting material to product (Figure 20; Appendix C). The change in R_f value observed for the deep purple band indicated conversion of jadomycin **28** to a triazole jadomycin. Interestingly, a change in R_f value was also observed for the dark green band, indicating reaction of the aglycone form of **28** with the azide present to form the aglycone triazole product. The triazole jadomycins generated from the cycloaddition reactions were purified by prep TLC (10:90 MeOH:DCM) and their structures confirmed by MS and NMR. A library of triazole jadomycins was thus successfully generated (Scheme 10).

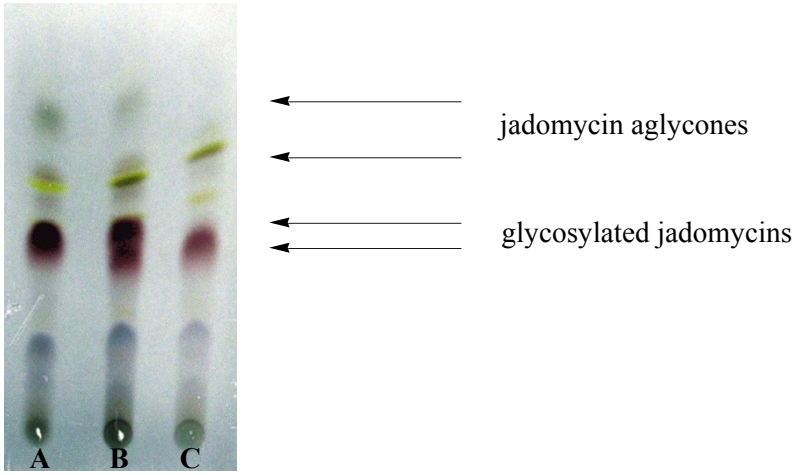
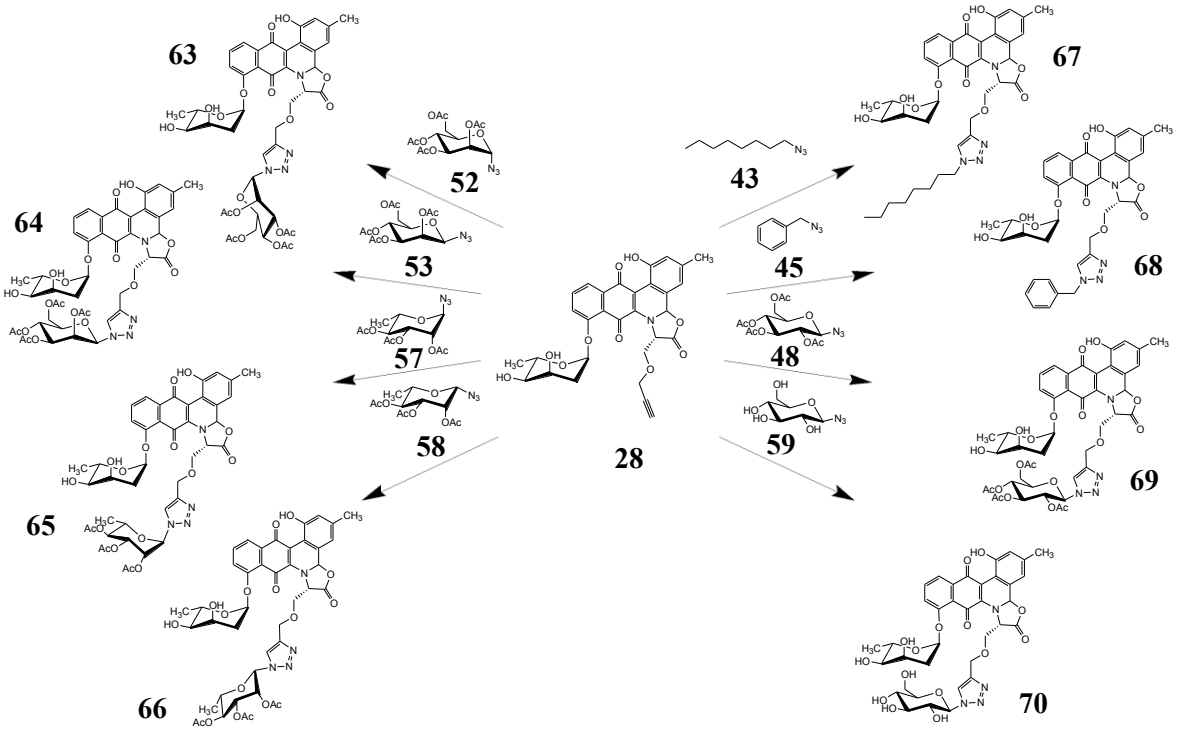


Figure 20. Example of a TLC analysis for the reaction of **28** with an azide to produce a triazole jadomycin. TLC (10:90 MeOH:DCM) was used to monitor the reaction of **28** with **53**: lane A = crude **28**; lane B = cospot; lane C = reaction mixture. The aglycone form of **28** can be seen in the crude starting material (lanes A&B). The plate shows complete conversion of **28** and aglycone **28** to triazole products.



Scheme 10. Generation of eight jadomycin triazoles (**63**-**70**) from **28** via CuAAC reactions.

One of the cycloaddition reactions depicted in the scheme above was conducted slightly differently: the reaction of **28** with the deprotected sugar azide **59** to produce triazole **70**. This reaction was performed using pure **28** (5.5 mg), as it was suspected that the product, containing two deprotected sugar functionalities, may be water-soluble. Complete conversion was seen by TLC (10:90 MeOH:DCM), and the product was aqueous-soluble. Reversed-phase flash chromatography was used to remove copper, ascorbic acid, and excess sugar azide from the crude product 9.0 mg). However, the resulting material (4.0 mg) contained an unknown impurity visible by NMR. Prep TLC (10:90 MeOH:DCM) was then employed, but the impurity was still abundant in the extracted product (0.7 mg). This triazole jadomycin was therefore characterized by MS only while all others were characterized by MS, IR, UV-Vis, and NMR (Appendix D).

The EPI-MS analysis of each product (**63-70**) showed the expected fragmentations: $[\text{jadomycin+H}]^+$, $[\text{aglycone+H}]^+$, and $[\text{phenanthroviridin+H}]^+$. The accurate masses were as expected. The ^{1}H-NMR spectrum of each purified product when compared to that of the starting material **28** showed the expected differences: the signals for the terminal proton of the propargyl functionality were no longer seen, and new singlets corresponding to the triazole ring proton were observed in the aromatic region (Figure 21; Appendix D). New proton signals were also observed

for the functionality that was present in the azide and became attached to the jadomycin via the triazole ring (Appendix D).

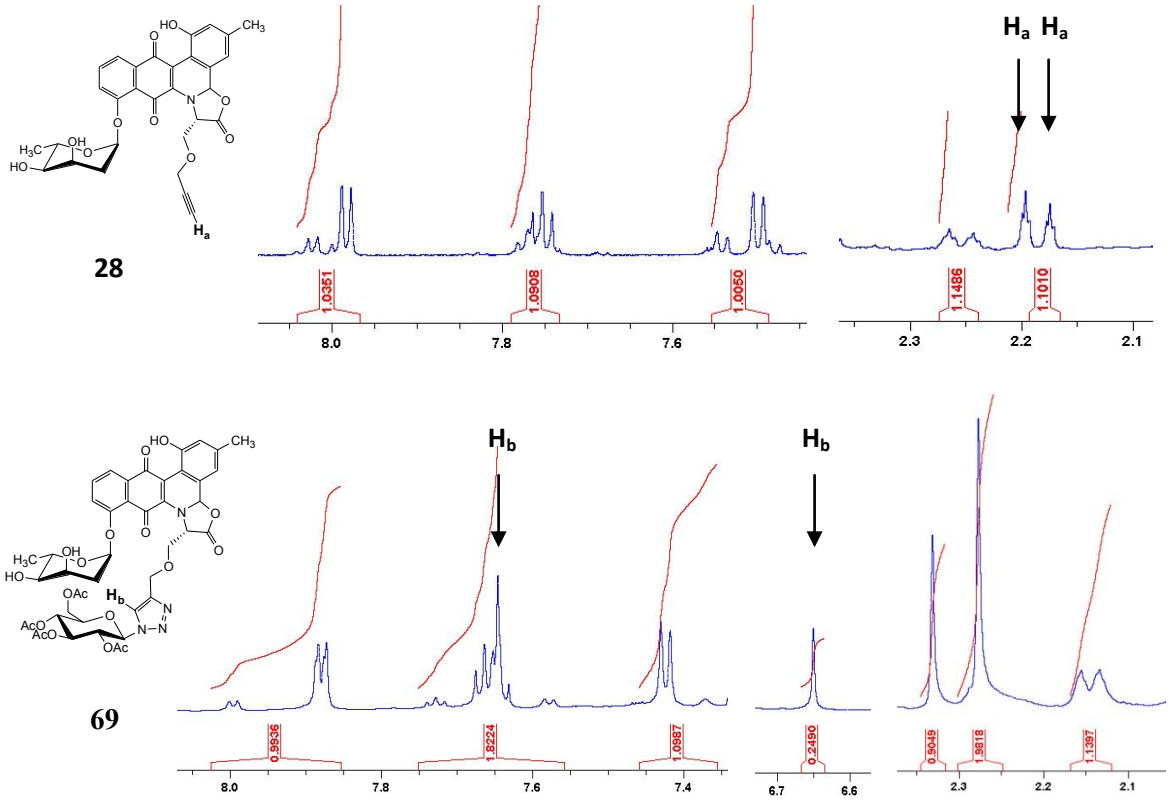


Figure 21. Selected differences in the ^{1}H-NMR spectra of **28** and an example of a triazole jadomycin product, **69**. The propargyl C≡CH signals (2.18 ppm, 2.20 ppm; $\mathbf{H_a}$) have disappeared, and the triazole C=CH-N signals have appeared (6.65 ppm, 7.65 ppm; $\mathbf{H_b}$).

In general, the diastereomeric proton signals of jadomycins have very small differences in chemical shifts (<0.1 ppm);[26] for example, see the selected portions of the ^{1}H-NMR spectrum of **28** shown above (Figure 21). However, the triazole proton signals for the 3a*S* and 3a*R* diastereomers of the triazole jadomycin **69** are separated by a large shift (1.0 ppm; Figure 21). This large difference in chemical shifts was observed in the ^{1}H-NMR spectra of all the triazole jadomycins generated from compound **28** (**63-69**). This phenomenon is likely due to the different spatial arrangements of the triazole moiety permitted by the 3a*S* and 3a*R* conformations. In the 3a*S* conformation, the triazole moiety could potentially stack on top of the oxazoline or one of the aromatic rings and hydrogen bond with the oxygen or nitrogen atoms present there. Hydrogen bonding with these electronegative atoms would cause a downfield chemical shift and is a potential explanation for the downfield chemical shifts observed for the 3a*S* triazole protons of triazole jadomycins **63-69**. In the 3a*R* conformation, however, the location of proton 3a may prevent the

triazole moiety from coming into close proximity with the oxazoline or aromatic rings and prevent the corresponding hydrogen bonding interactions.

Because the triazole jadomycins were all derived from jadomycin **28** via cycloaddition reactions at C3 and C4, the diastereomeric ratio of **23** was conserved among all triazoles jadomycins (**63-69**; Table 2). Slight discrepancies in the diastereomeric ratios of the triazole jadomycins compared to **28** can be attributed to loss of material during purification by prep TLC and experimental error in NMR sample preparation.

Table 2. Diastereomeric ratios of all new jadomycin derivatives and selected jadomycin derivatives produced previously.

Jadomycin Derivative	**Ratio of Diastereomers (3a*S*:3a*R*)**
Jadomycin L (**26**)	44:36
Jadomycin DNV (**34**)	55:33
Jadomycin DNL (**35**)	56:34
Jadomycin *O*PS (**28**)	64:20
63	41:35
64	65:22
65	52:30
66	63:20
67	55:35
68	60:36
69	60:31
Jadomycin B (**17**)	65:35
Jadomycin V	66:34
Jadomycin DV	70:30
Jadomycin S	<5:>95
Jadomycin T	<5:>95
Jadomycin M	63:37

Quantitation of **28** in the crude material used in the cycloaddition reactions and subsequent determination of cycloaddition reaction yields were attempted using UV-Vis and NMR experiments. UV-Vis spectra were recorded for standards of pure **28** in methanol (1 μg/mL, 5 μg/mL, 10 μg/mL, 20 μg/mL, 40 μg/mL, 60 μg/mL, 80 μg/mL, and 100 μg/mL) and for a sample of crude **28** (10 μg/mL). Six λ_{max} values were used to generate standard curves and pinpoint the concentration of pure **28** in the crude material (Figure 22). Five of the six plots generated had consistent data, therefore the sixth plot was discarded and the quantitation achieved as an average of the remaining five. Using the UV-Vis method, the crude material was determined to be 64% pure **28** by mass.

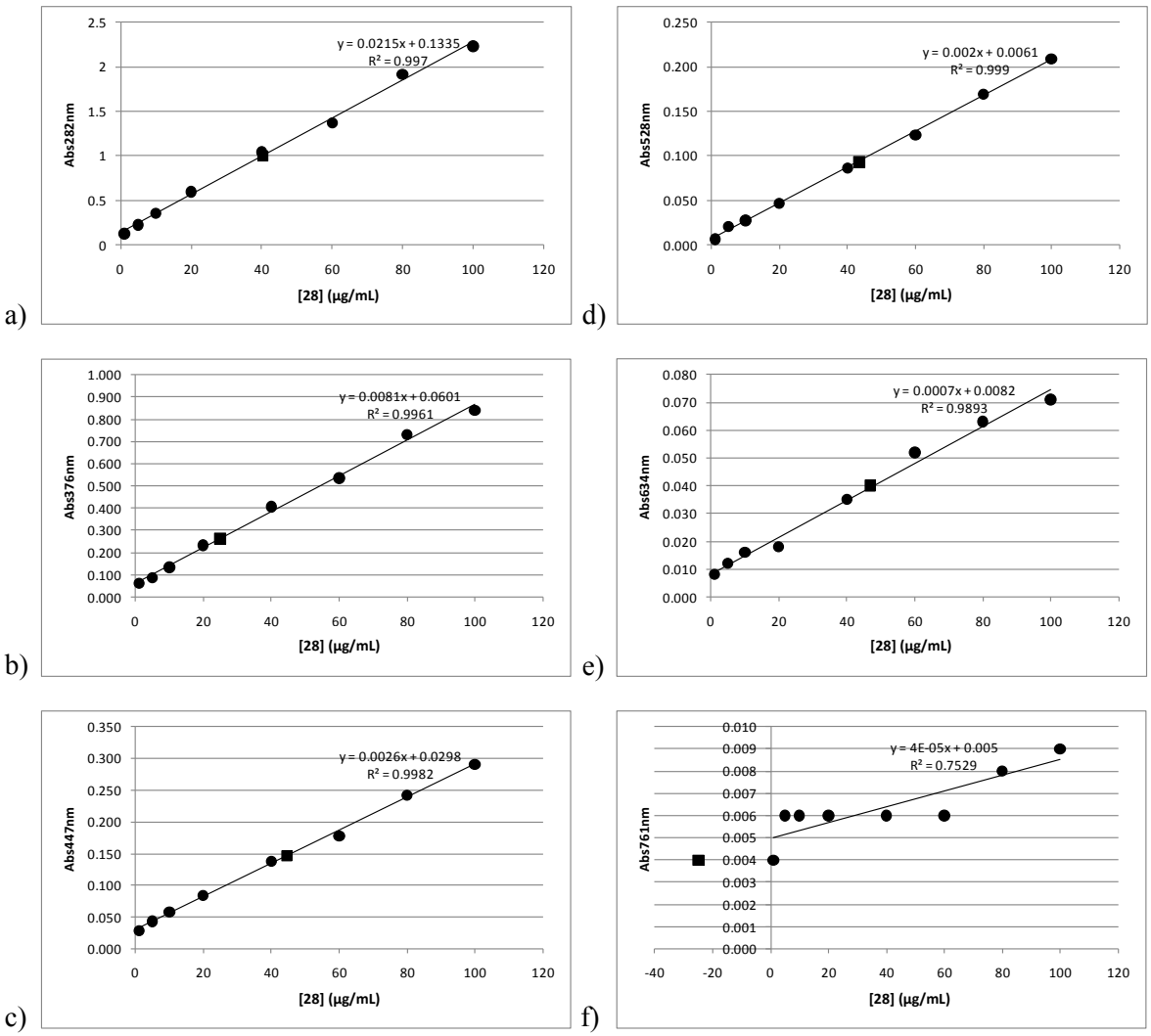


Figure 22. Absorbance plots generated from six λ_{max} values of **28**: a) 282 nm; b) 376 nm; c) 447 nm; d) 528 nm; e) 634 nm; and f) 761 nm. The curves were generated using standard solutions of pure **28** in MeOH (●), and the concentration of crude **28** determined by interpolation (■). Plot f was discarded when calculating the average concentration of crude **28**, as 761 nm did not appear to be a valid λ_{max} for measuring concentrations. The concentrations of crude **28** was calculated as the average of those found in plots a-e.

An external standard NMR experiment was used to confirm the value determined by UV-Vis. This consisted of running a ^{1}H-NMR of a standard of caffeine (**71**; 432 µM) in D_2O and using the integration to quantify a sample of crude **28** (25 mg/mL) in methanol-d_3. Chloroform could not be used because although pure **28** is soluble in this solvent, the crude material is not. It is instead soluble in methanol, however using methanol-d_4 as the NMR solvent would result in deuterium-exchange between MeOD and the phenolic protons of the jadomycin (7-OH, see Figure 5). These proton signals, which appear in the upfield region (10.0 ppm -10.5 ppm; Appenix D) far away from any other proton signals, would not be seen. Methanol-d_3 was therefore selected as the NMR

solvent for this quantitation experiment so that the isolated 7-OH signals could be used for integration.

The ^{1}H-NMR spectra of the caffeine and jadomycin samples were obtained over 16 scans, and a methyl proton signal in the caffeine spectrum was integrated to 3.0 (Figure 23). In the jadomycin spectrum, the 7-OH signals could not be unambiguously identified as their chemical shifts in methanol-d_3 were different from those in chloroform-d. Also, the integration values of these signals would not be reliable because water was present in the sample and water protons would exchange with the 7-OH protons in solution. For these reasons, alternative proton signals in the jadomycin spectrum were used for integration. These were located in regions with considerable overlap, therefore several signals (protons 11, 6, 4, 3a, 5-CH_3) were integrated and an average determined for the value of one jadomycin proton (18x that of a caffeine proton). Having concluded that the jadomycin present in the crude sample was 18x more concentrated than the caffeine standard, the concentration of pure **28** in the crude sample was calculated (18 x 0.432 mM = 7.8 mM, ie. 4.8 mg/mL rather than 25 mg/mL). This concentration indicated that the crude sample was 19% pure **23** by mass.

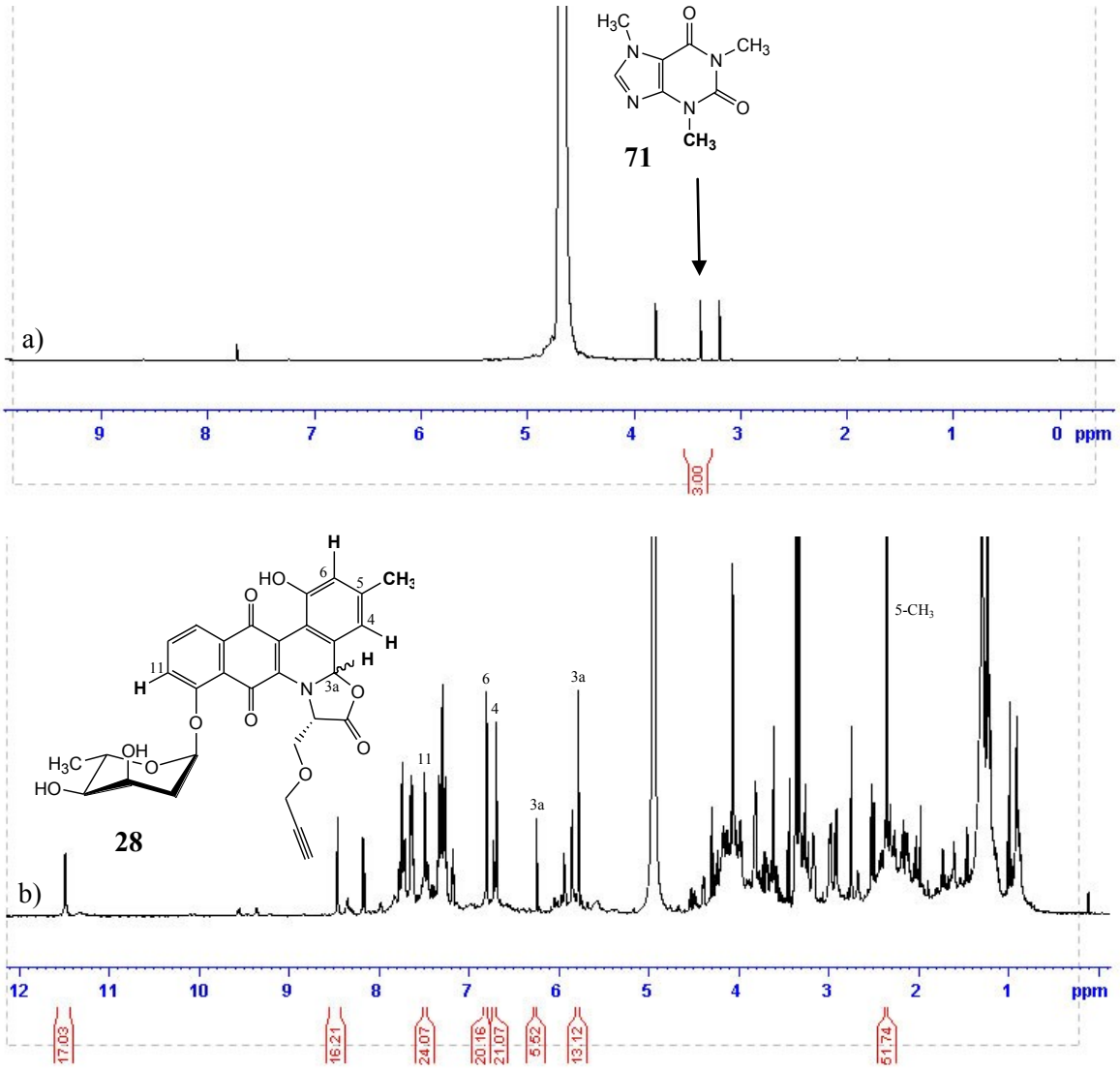


Figure 23. [1]H-NMR spectra (700 MHz, 16 scans) from an external standard NMR experiment used to quantify the amount of pure **28** in a sample of crude **28**: a) a caffeine (**71**) standard (432 μM) in D_2O, and b) a sample of crude **28** (25 mg/mL) in methanol-d_3. The structures indicate the proton signals integrated. Comparison of the integration values in each spectrum reveals that the abundance of compound **28** in sample b is 18x that of caffeine in sample a.

The two methods used to quantitate the amount of pure **28** in the crude material used for cycloadditions reactions yielded significantly different results. According to UV-Vis, the crude material is 64% pure by mass, but according to NMR, the material is 19% pure by mass. UV-Vis is regarded as an extremely accurate technique for measuring concentrations of dilute solutions. However, impurities in the crude sample absorbing light within the range of 200 nm – 800 nm could have contributed to the absorbance values obtained for crude **28**, causing the concentration of pure **28** in the sample to appear higher than it actually is. Integration of protons via NMR spectroscopy, on the other hand, measures the concentration of a specific molecule of interest. This

method has its downsides too, however: inorganic salts can cause peak broadening and contribute to integration values, making concentrations seem higher than they actually are; residual solvent can exchange with certain protons of the molecule of interest; and deuterated solvents can cause degradation of the molecule under investigation, lowering apparent concentrations. Since the values for purity of crude **28** obtained by these methods are so drastically different, and both methods have their advantages and disadvantages, neither value can be identified as the true measure of purity at this time.

2.7 Biological Activities of Jadomycins

All purified jadomycin derivatives (**26**, **28**, **34**, **35**, **63-69**) are currently being tested by collaborators for their antibiotic and anticancer activities. The jadomycins are being studied for their activities against four strains of Gram-positive bacteria and four strains of Gram-negative bacteria by Dr. Sue Douglas, NRC-IMB, Halifax, Nova Scotia. These compounds, as well as the non-jadomycin triazoles (**60-62**), are also currently under investigation for their anticancer activities in 60-cell line screening assays at the National Cancer Institute (NCI), Rockville, Maryland.

One jadomycin derivative in particular, Jadomycin L (**26**), has already been tested by both collaborators. At the NRC-IMB, Jadomycin L was screened against eleven strains of pathogen microorganisms, as described previously.[43] In this study, Jadomycin L was one of the most active compounds of eleven jadomycin derivatives tested, all of which displayed greater activities against Gram-positive microorganisms than Gram-negative microorganisms.

Jadomycin L has also been through multiple phases of testing at the NCI. It was first studied using the 60-cell line screening assays mentioned above. These assays consist of testing a potential anticancer agent against 60 human tumour cell lines representing leukemia, melanoma, and cancers of the lung, brain, colon, ovary, breast, prostate, and kidney (information from the NCI website: http://dctd.cancer.gov/ProgramPages/dtp/tools_drug_discovery.htm). Jadomycin L was initially assayed at one dose (10 μM) and showed growth inhibition relative to the no-drug control in 39 of the 60 cell lines (Appendix E). Jadomcyin L was then assayed at five doses (10 nM, 100 nM, 1 μM, 10 μM, and 100 μM), showing growth inhibition of 54 out of 60 cell lines at the highest concentration tested (100 μM; Appendix E). After showing significant growth inhibition in the 1-dose and 5-dose *in vitro* screens, Jadomycin L was selected for *in vivo* testing.

The maximum tolerated dose (MTD) was determined using acute toxicity studies where nontumoured mice were injected with single doses of Jadomycin L and monitored over two weeks (Appendix E). This value was then used to determine high ([MTDx1.5]/4) and low (0.67 x high dose) doses of Jadomycin L for use in hollow fiber assays in mice. The hollow fiber assays involved growing tumour cells inside biocompatible fiber cells,[44] implanting them into mice intraperitoneally and subcutaneously, waiting three to four days, and then treating the mice with low and high doses of Jadomycin L daily for four days. Twelve tumour cell lines were used, each delivered intraperitoneally and subcutaneously at low and high doses for a total of 48 (12 cell lines x 2 implantation sites x 2 doses) experiments. After treatment with Jadomycin L, the fibers were collected and the percent net tumour growth was measured using an MTT assay[45] and compared to the percent net growth in the no-drug controls. Each experiment that caused a 50% or greater reduction in net tumour growth counted as a positive result and scored two points, for a maximum total of 96 (48 experiments x 2 points) points. Two of 48 experiments caused 50% or greater reduction in net tumour growth, for a total hollow fiber assay score of 4 out of 96 for Jadomycin L (Appendix E). In order to be considered for xenograft testing, the next phase of *in vivo* testing, a compound must have a final score of 20 out of 96, or a score of 8 out of 48 for the subcutaneous tumour experiments. Therefore, the level of antitumour activity observed for Jadomycin L was too low for further investigation by NCI.

The other jadomycins produced in this report will be tested according to the same order of assays described for Jadomycin L, [46] if they demonstrate sufficient growth inhibition in the initial single dose 60-cell line screen. In the condition that one or more of these jadomycins is sufficiently active in the hollow fiber assays, it may be selected for subsequent testing by the NCI in xenograft assays and surpass the biological activity of Jadomycin L.

CHAPTER 3 CONCLUSIONS AND FUTURE WORK

3.1 Conclusions

Jadomycin derivatives were produced as described previously[22] by growing *S. venezuelae* ISP5230 VS1099 in minimal media using the amino acid of interest as the sole nitrogen source and ethanol-shocking to induce secondary metabolite production. Amino acids L-leucine, D-norvaline, and D-norleucine were used as the sole nitrogen sources in large-scale (2 L) growths to yield the corresponding jadomycin derivatives in yields of 12.0 mg/L, 4.3 mg/L, and 8.5 mg/L, respectively. Growths using L-norvaline and L-norleucine as the sole nitrogen sources did not produce any jadomycin derivatives, an interesting result that stands in contrast to previous experiments in which both D- and L- forms of amino acids (methionine, valine, isoleucine, and threonine) have been incorporated into jadomycin derivatives.[24-26] The three jadomycin derivatives produced were purified by flash chromatography and characterized by UV-Vis, IR, LRMS (specifically EPI-MS), HRMS, and NMR (specifically ^{1}H, COSY, 1D TOCSY, 1D nOe, HSQC, and HMBC).

A serine derivative with a terminal alkyne functionality, *O*-propargyl-L-serine, was synthesized for use as the sole nitrogen source in *S. venezuelae* ISP5230 VS1099 growths to produce a jadomycin derivative with a terminal alkyne functionality. The amino acid derivative was synthesized by alkylating *N*-Boc-L-serine using propargyl bromide, then deprotecting the amino group using hydrochloric acid. The hydrochloride salt was produced on a 7.4 g scale, in 80% yield over two steps, with no requirement for chromatography. The optimal concentration for jadomycin production by *S. venezuelae* ISP5230 VS1099 of this particular amino acid derivative was determined by UV-Vis and NMR experiments (30 mM), and *O*-propargyl-L-serine hydrochloride salt was used in large-scale (2 L) growths to produce the corresponding jadomycin derivative, Jadomycin *O*PS. Flash chromatography was found to be ineffective towards purifying this compound, despite working well for jadomycins L, DNV, and DNL. Therefore, prep TLC was used to obtain pure material (12 mg/L) for characterization by UV-Vis, IR, LRMS, HRMS, and NMR.

Eight azides were synthesized for use in copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) reactions with the new jadomycin derivative **28**. The azides were alkyl, aryl, and sugar (α and β) derivatives and were synthesized straightforwardly from their bromide precursors using

excess sodium azide. In the case of the sugars, the acetyl-protected sugars were brominated using phosphorus tribromide, then the azides were formed. The CuAAC reaction conditions were tested using a selection of the azides synthesized and the Boc-protected amino acid derivative **37**. The reaction conditions proved viable, yielding regioisomerically pure triazole products in high yields (98-99%). Flash chromatography proved ineffective towards purifying the sugar triazoles generated, whereas prep TLC was successful. The cycloaddition reactions were then carried out using all of the azides with crude jadomycin **28**. Complete conversions of starting material to product were observed by TLC, and structures of the products were confirmed by MS and NMR. The products were also characterized by UV-Vis spectroscopy and IR spectroscopy. Prep TLC was the method used for purification of the triazole jadomycin products.

A library of triazole jadomycins was thus successfully generated from a single jadomycin derivative, **28**, via chemical means, specifically CuAAC reactions with a library of synthesized azides (**43**, **45**, **48**, **52**, **53**, **57**, **58**, **59**). This work demonstrates the successful exploitation of a new route to jadomycin production: chemical modification of a jadomycin. This project failed to realize the full benefits of this new route to jadomycin production, as the jadomycin starting material for the cycloadditions was surprisingly difficult to purify: the crude material was used as the starting material for the cycloadditions, and each triazole derivative generated required individual purification by prep TLC. The ideal realization of this novel route to jadomycin libraries would have entailed purifying *one* jadomycin derivative and then using it in a number of chemical reactions to obtain a number of new, pure derivatives. Nevertheless, the goal of generating a library of novel jadomycin derivatives from a single derivative using chemistry was realized.

In total, eleven new jadomycin derivatives were produced, and ten of these were purified and characterized. The water-soluble triazole jadomycin **70** could not be purified. All purified jadomycins (**26**, **28**, **34**, **35**, **63-69**) are currently under investigation for their biological activities. The compounds are being tested for their antibiotic activities at NRC-IMB, Halifax and for their anticancer activities at NCI, Maryland. Anticancer activities studies have already been completed for Jadomycin L. Although it displayed high levels of activity against 60 cancer cell lines in *in vitro* 1-dose and 5-dose screening assays and acute toxicity assays, its potency in the hollow fiber assays was deemed too low for further investigation by the NCI.

3.2 Future Work

The strange result observed in the norvaline and norleucine growths, namely the lack of incorporation of the L-amino acids into jadomycin derivatives, merits further investigation. This could be initially explored by using other, related non-proteogenic amino acid derivatives as the sole nitrogen sources in *S. venezuelae* ISP5230 VS1099 growths. Performing growths using D- and L-amino acid derivatives with n-pentyl, n-hexyl, n-heptyl, etc R-groups would reveal whether or not the trend holds with similar amino acid derivatives. Another approach that could be taken involves using fluorescent or radioactive tags to track the paths taken by the amino acid derivatives upon entering the culture media. The use of these tags on the L-amino acid derivatives may help to reveal where the L-isomers are getting stuck: if they are failing to enter the cells, if they are acting as inhibitors in the jadomycin biosynthetic pathway, or if some other blockage, interaction, or degradation is occurring. Experiments such as these may help to reveal the reason(s) why L-norvaline and L-norleucine culture media failed to yield jadomycin derivatives.

Future work should also involve the production of more/new jadomycin triazoles for drug discovery. The biological results will indicate which derivatives have the highest levels of activity, and this will direct future jadomycin production. If any of the compounds display high enough activities that they are selected for further investigation by NCI, then more of the same jadomycin derivatives should be produced. In addition, new jadomycin derivatives should be produced that have structural similarities to these selected compounds or to any of the jadomycin derivatives that display high levels of activity in the antibiotic studies conducted at the NRC. For example, if the glycosyl-functionalized triazoles are the most potent, they can be further explored by using other acetyl-protected glycosyl azides, such as the galactopyranosyl, xylopyranosyl, fucopyransol, or lactosyl derivatives, in the cycloaddition reactions. Likewise, if the alkyl-functionalized triazole displays the best activity, it can be derivatized by using structurally related alkyl azides, including longer, shorter, or branched derivatives, in the cycloaddition reactions. The structurally similar derivatives can then be tested and those with the best biological activities mimicked again and again until a useful SAR database is generated and can be used to produce a jadomycin triazole with optimal biological activity.

Because of difficulties regarding purification, the water-soluble triazole jadomycin **70** was not tested for antibiotic or anticancer activities. It seems reasonable to predict that **70** would probably have levels of activity similar to its acetylated analogue, **69**, because *in vivo*, compound

69 would likely undergo cleavage by an esterase or simple hydrolysis in acidic or basic environments which would convert it to **70**. Compound **69** may have even higher levels of activity than **70** because the acetyl groups confer extra lipophilicity which could help the compound enter cells more readily. These are speculations however, and compound **70** needs to be resynthesized, purified, and tested for its antibiotic and anticancer activities. As the synthesis used here produced a compound that could not be purified by flash chromatography or prep TLC, it may be worthwhile to explore alternative routes to **70**. The compound could potentially be generated via acetyl deprotection of **69**. Depending on the difference in activities between compounds **69** and **70**, chemical deprotection of acetylated sugar groups after triazole formation may be beneficial for compounds **63**, **64**, **65**, and **66** as well.

Generating **70** from purified **69** would circumvent the challenging purification reported here. It could be attempted using catalytic amounts of NaOMe in MeOH, as demonstrated here for the deprotection of compound **48** to **59**, but these highly basic conditions may be too harsh and may result in breakdown of the jadomycin. Milder deprotection conditions, using the hydroxide rather than methoxide ion as the nucleophile, may prove more effective. A solution of ammonium hydroxide in DMF or THF, for example, may yield the deprotected product. Conditions such as these may prevent jadomycin degradation, however it should be noted that they would likely result in stereochemical interconversion. Jakeman and coworkers have previously shown that at basic pH jadomycins undergo ring-opening to an aldehyde intermediate and inverconversion between the 3a*S* and 3a*R* stereochemistries.[47] When the solution of jadomycin analogues is brought back to neutral or slightly acidic pH, the B and E rings reform to give the original jadomycin structures in the original 3a*S*:3a*R* ratio. As all the jadomycins produced here exist as mixtures of inseparable diastereomers, the basic conditions required for chemical deprotection of the acetyl groups would not be detrimental to the compounds. Thus acetyl deprotection of **63** could conceivably be achieved via chemical means. Finally, acetyl deprotection of **63** may be achieved enzymatically using an esterase or mouse serum bioassay, but these would likely impose further obstacles for purification of **70**.

In addition to synthesizing jadomycin triazole derivatives from **28**, focus should be placed on exploiting this same route to jadomycin production, chemical modification of a jadomycin, but with a different starting material or different reaction type. For example, the cycloaddition reactions could be carried out on a different terminal alkyne-functionalized jadomycin. Jadomycin derivatives of this sort could be produced from *O*-propargyl-L-threonine (**72**) or *O*-propargyl-L-tyrosine (**74**; which could be synthesized in the same way as **41**), L-propargylglycine (**76**; available

commercially), or the D-isomers of any of these amino acid derivatives (**73**, **75**, **77**), including *O*-propargyl-D-serine (**78**; Figure 24). The corresponding jadomycin derivatives could be used in cycloadditions like those reported here to make more triazole jadomycins. Perhaps one of these terminal alkyne-containing jadomycins could be purified by flash chromatography, unlike **28**, and the full benefits of a chemical expansion of the jadomycin library could be realized: the alkyne-functionalized jadomycin could be purified *then* reacted to generate pure triazole derivatives.

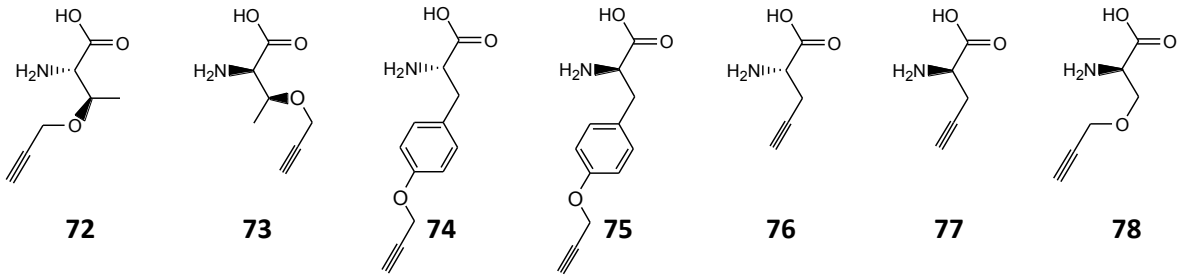
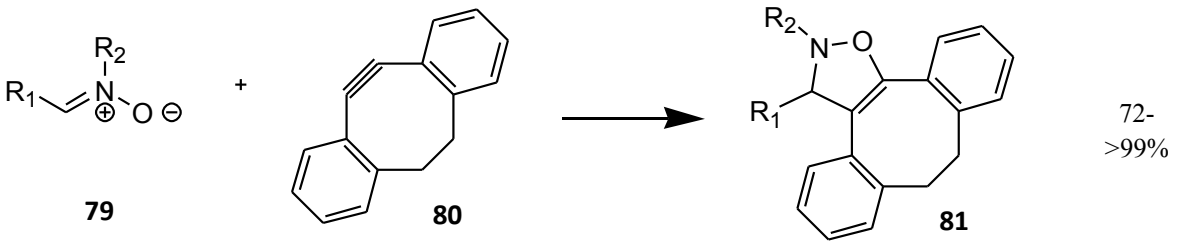


Figure 24. Examples of amino acid derivatives that could be used in *S. venezuelae* ISP5230 VS1099 cultures to generate terminal alkyne-functionalized jadomycin derivatives: *O*-propargyl-L-threonine (**72**), *O*-propargyl-D-threonine (**73**), *O*-propargyl-L-tyrosine (**74**), *O*-propargyl-D-tyrosine (**75**), L-propargylglycine (**76**), D-propargylglycine (**77**), and *O*-propargyl-D-serine (**78**).

Much like alkyne **28** could be replaced with a different alkyne jadomycin, the triazole functionality could also be replaced with a different functionality. Rather than forming triazole derivatives, it may be possible to form derivatives based on other classes of heterocycles, such as isoxazolines which can also be formed via cycloaddition reactions. Isoxazolines have been shown to be active against Gram-positive and Gram-negative bacteria and fungi[48-51] and may be synthesized quickly and easily. A variety of isoxazolines (**81**) have recently been prepared rapidly and at room temperature, without the need for a copper catalyst, from conveniently prepared acyclic and cyclic nitrones (**79**) and benzannulated cyclooctyne (**80**; Scheme 11).[52] If a cyclooctyne based amino acid derivative could be generated, the corresponding jadomycin could be produced and isoxazoline derivatives generated via uncatalyzed cycloaddition reactions with nitrones just as triazoles were generated via CuAAC reactions with azides. Likewise, the uncatalyzed cycloaddition reactions could be attempted using jadomycin **28**, which has already been produced, in place of a strained cyclooctyne-containing jadomycin.



Scheme 11. Synthesis of isoxazolines (**81**) from nitrones (**79**) and benzannulated cyclooctyne (**78**) achieved at ambient temperature, without copper, and in high yields.[52]

Jadomycin derivatives with triazole functionalities formed from different alkyne or azide starting materials or with isoxazoline or related functionalities may possess enhanced biological activities and give new directions towards drug design, but producing derivatives using these functional groups may hold potential for purposes other than drug development, namely investigation of the mechanism of action of jadomycins. The successful addition of an octyl chain to a jadomycin via cycloaddition, described here, suggests it may be possible to attach to a jadomycin a long alkyl chain with a biotin group on the end. Biotin groups are commonly used in protein-binding assays as tags which attach to avidin beads for simple isolation when the assay is complete: the biotinylated molecule binds to a protein, and when the beads are used to bind the biotin, the rest of the molecule and the protein to which it is bound are captured as well.[53,54] Unbound components are rinsed off the beads, and the protein can be eluted and analyzed by mass spectrometry. Such studies using biotin tags are useful in revealing potential protein binding partners for a certain molecule of interest. If a biotin tag could be attached to a jadomycin, with a long, flexible linker chain, it would be possible to determine some jadomycin-protein interactions, and this would help in the greater understanding of how jadomycins work.

In conclusion, this project's demonstration that the jadomycin scaffold is amenable to chemical modification post-biosynthesis has huge implications, and all avenues relevant to drug discovery and discovery of the mechanism of action of jadomycins should be explored.

CHAPTER 4 EXPERIMENTAL

4.1 General Methods

4.1.1 Synthetic Techniques and Instrumentation:

All reagents used were purchased from commercial sources and used without further purification. With the exception of amino acid synthesis and formation of non-jadomycin triazoles, all solvents used were HPLC grade. For the syntheses of **37**, **38**, **41**, and **60-62**, solvents were reagent grade. Thin layer chromatography (TLC) plates used to monitor reactions, assess purity, and run prep TLC were all glass-backed normal phase silica gel plates (SiliCycle®). Prep TLC plates were 20 x 20 cm and 1000 μm in thickness, while all others were 250 μm and cut to the desired size. No visualization was required for jadomycins (**26**, **28**, **34**, **35**, **63-70**), as the bands are highly coloured. For all other compounds, plates were visualized with potassium permanganate dip (1.5 g $KMNO_4$, 10 g K_2CO_3, 125 mg NaOH, 200 mL water) and heat. Flash chromatography was performed on a Biotage SP1™ unit using pre-packaged columns (Biotage®, SiliCycle®).

All purified compounds were characterized by mass spectrometry and NMR spectroscopy. Low resolution mass spectra were recorded using electrospray ionization (ESI) on a 2000Qtrap linear ion trap instrument (Applied Biosystems™). Samples were scanned in positive mode over a range of 300-700 m/z (Q1) and then in MS/MS mode. High resolution mass spectra were recorded using electrospray ionization (ESI) on a Bruker Daltonics® MicroTOF instrument in positive mode from 50-1500 m/z. With the exception of jadomycin *O*PS (**28**) and all triazole jadomycins (**63-70**), NMR spectra were recorded using a Bruker Avance 500 instrument (^{1}H at 500 MHz, ^{13}C at 125 MHz) with broadband observe (BBO) probe at the Nuclear Magnetic Resonance Research Resource (NMR-3), Dalhousie University. NMR spectra of jadomycins **28** and **63-70** were recorded using a 700 MHz Bruker Avance III instrument (^{1}H at 700 MHz, ^{13}C at 150 MHz) with cryoprobe at the National Research Council Canada Institute for Marine Biosciences (NRC-IMB), Halifax. Spectra were recorded in $CDCl_3$, MeOD, or D_2O. Chemical shift values (δ in ppm) were calibrated to residual solvent peak (MeOH at 3.31 ppm in MeOD, $CHCl_3$ at 7.24 ppm in $CDCl_3$, H_2O at 4.71 ppm in D_2O). Peak assignment was achieved using chemical shifts and peak multiplicities from the proton spectra as well as through the use of ^{1}H-^{1}H COSY, and where noted, 1D TOCSY and 1D nOe experiments. Assignment of the ^{13}C spectra was achieved through HSQC, and where noted, HMBC experiments. Not all ^{13}C resonances could be assigned, despite varying

the J value for the HMBC experiments. Stereochemistry at the anomeric centers of all carbohydrates synthesized was determined by measuring $^{1}J_{C1,H1}$ using an HETCOR (^{13}C-^{1}H heteronuclear correlation) experiment.

IR spectra were obtained from solid samples using potassium bromide (KBr) plates and a PerkinElmer Spectrum 100 FT-IR Spectrometer over 16 scans. UV-Vis spectra were recorded using a Varian Cary 50 Bio UV-Visible Spectrophotometer. Samples were run in HPLC grade methanol, and blanked against samples of 100% HPLC grade methanol.

4.1.2 Production of Jadomycins Using *S. venezuelae* ISP5230 VS1099:

Excerpts of this section were taken from Jakeman, D; Dupuis, S; Graham, C. *Pure Appl. Chem.* **2009**, 81, 1041-1050.

Streptomyces venezuelae ISP5230 VS1099[23] colonies were grown on MYM-agar plates [maltose (0.4% w/v), yeast extract (0.4% w/v), malt extract (1% w/v), and agar (1.5% w/v)] for two to four weeks. Single colonies were used to inoculate MYM media [4 x 250 mL in 4-1 L flasks; maltose (0.4% w/v), yeast extract (0.4% w/v), malt extract (1% w/v); pH 7.0] which was then stirred at 30 °C for 20 h. The resulting broth was centrifuged at 3750 rpm for 15 min, and the pellet was washed with MSM medium. MSM medium consisted of the following, per litre: $MgSO_4$ (0.4 g), MOPS (1.9 g), salt solution (9 mL of 1% w/v NaCl and 1% w/v $CaCl_2$), $FeSO_4{\cdot}7H_2O$ (4.5 mL of 0.2% w/v), and trace mineral solution (4.5 mL). The trace mineral solution contained, per litre: $ZnSO_4{\cdot}7H_2O$ (880 mg), $CuSO_4{\cdot}5H_2O$ (39 mg), $MnSO_4{\cdot}4H_2O$ (6.1 mg), H_3BO_3 (5.7 mg), and $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (3.7 mg).

Culture media were prepared by dissolving the selected amino acid in 2 L MSM media to a final concentration of 60 mM (except where specified), adjusting the pH to 7.5 with 5 M NaOH, and autoclaving the solution (8 x 250 mL in 8-1 L flasks). Subsequently, glucose (33 mM) and phosphate (50 μM) were added aseptically, and the *S. venezuelae* pellet slurry was added until the OD_{600} reached 0.6. The culture medium was ethanol shocked using 100% ethanol (3% v/v in the medium) and stirred at 30 °C for 48 h until the A_{526} measured between 0.5 and 1.0. Aliquots were taken and read as described previously.[22]

The cellular debris was removed from production media by suction filtration through No. 5 filter paper, then 0.45 μm and 0.22 μm MF filtration disks. The filtered media was passed through a reversed-phase capture C18 column (6 x 6 cm; Biotage®) which had been preconditioned with

HPLC grade methanol. Water-soluble compounds and other metabolites were eluted using distilled water (until flow-through was colourless, 10-20 L), followed by increasing amounts of methanol in water: 10%, 20%, 30%, and 40% (approx. 250 mL each). The desired secondary metabolite was eluted as a deep purple solution at 60% methanol. Solvent was removed *in vacuo* to yield crude secondary metabolite. Thin layer chromatography using normal phase silica gel plates (10:90 MeOH:DCM as eluant) confirmed the presence of jadomycin derivatives.

4.2 Synthesis of *O*-Propargyl-L-Serine (compounds 37, 38, 41):

***N*-Boc-*O*-Propargyl-L-Serine (37).** *N*-Boc-L-Serine (4.10 g, 20 mmol) was dissolved in anhydrous DMF (180 mL) under a nitrogen atmosphere and cooled in an ice bath to 0°C. NaH (1.6 g, 66 mmol) was added portionwise. After effervescence ceased (15 min), propargyl bromide solution (1.2 mL, 20 mmol; 80% in toluene) was added dropwise over 15 min. The solution was stirred on ice for 1-2 hours until the reaction was determined complete by TLC analysis. The reaction was quenched with dH_2O (50 mL) and evaporated to dryness under vacuo. The crude material was dissolved in dH_2O (100 mL) and washed with diethyl ether (50 mL x 5). The aqueous solution was adjusted to pH 2-3 using $KHSO_4$ (0.5 M), and the product was extracted with DCM (100 mL x 3). The organic layer was washed with $KHSO_4$ solution at pH 2-3 (50 mL x 3), dried with Na_2SO_4, and evaporated to dryness to yield an orange oil (4.18 g, 86% yield). R_f = 0.22 (95:3:1 $CHCl_3$:MeOH:AcOH). $^{\delta}$H ($CDCl_3$, 500 MHz): 1.39 (9H, s, C(C**H**$_3$)$_3$); 2.42 (1H, t, J = 2.4 Hz, $CH_2C{\equiv}C$**H**); 3.75 (1H, dd, J = 9.1 & 2.1 Hz, αCHC**H**$_{2\ a}$); 3.91 (1H, d, J = 8.5 Hz, αCHC**H**$_{2\ b}$); 4.11 (2H, d, J = 2.6 Hz, C**H$_2$**C≡CH); 4.41 (1H, m, αC**H**); 5.48 (1H, d, J = 8.3 Hz, N**H**); 10.97 (1H, bs, COO**H**). $^{\delta}$C ($CDCl_3$, 125 MHz): 28.2 (3C, s, C(**C**H$_3$)$_3$); 53.6 (1C, s, α**C**H); 58.4 (1C, s, **C**$H_2C{\equiv}CH$); 69.6 (1C, s, αCH**C**H_2); 75.1 (1C, s, $CH_2C{\equiv}$**C**H); 78.9 (1C, s, CH_2**C**≡CH); 79.9 (1C, s, **C**(CH$_3$)$_3$); 155.6 (1C, s, **C**OC(CH$_3$)$_3$); 173.1 (1C, s, **C**OOH). HRMS (ESI^+) for $C_{11}H_{17}N_1O_5Na$ $[M+Na]^+$: calcd = 266.0999; found = 266.0990. These data are consistent with literature values.[37]

***O*-Propargyl-L-Serine Trifluoroacetate Salt (38).** Compound **37** (4.18 g, 17.2 mmol) was dissolved in DCM (4 mL), TFA (4 mL, 6 M) was added, and the reaction was monitored until consumption of starting material was observed by TLC analysis (overnight). The reaction mixture was evaporated to dryness and volatiles were removed *in vacuo* overnight. The resulting white crystals were triturated and washed with DCM (30 mL x 3) followed by diethyl ether (30 mL x 3)

to yield a white powder (2.65 g, 60% yield). $^{\delta}$H (D_2O, 500 MHz): 2.79 (1H, t, J = 2.4 Hz, $CH_2C{\equiv}C\mathbf{H}$); 3.86 (1H, dd, J = 10.9 & 3.2 Hz, $\alpha CHC\mathbf{H}_{2\ a}$); 3.94 (1H, dd, J = 10.9 & 4.9 Hz, $\alpha CHC\mathbf{H}_{2\ b}$); 4.09 (1H, dd, J = 4.8 & 3.3 Hz, $\alpha C\mathbf{H}$); 4.14 (2H, d, J = 2.4 Hz, $C\mathbf{H_2}C{\equiv}CH$). $^{\delta}$C (D_2O, 125 MHz): 53.4 (1C, s, $\alpha\mathbf{C}H$); 58.3 (1C, s, $\mathbf{C}H_2C{\equiv}CH$); 66.8 (1C, s, $\alpha CH\mathbf{C}H_2$); 76.4 (1C, s, $CH_2C{\equiv}\mathbf{C}H$); 78.8 (1C, s, $CH_2\mathbf{C}{\equiv}CH$); 116.4 (1C, q, J = 288.7 Hz, $\mathbf{C}F_3$); 162.9 (1C, q, J = 32.1 Hz, $CF_3\mathbf{C}OO^-$); 170.3 (1C, s, $\mathbf{C}OOH$). HRMS (ESI^+) for $C_6H_{10}N_1O_3Na$ $[M+Na]^+$: calcd = 144.0655; found = 144.0660.

***O*-Propargyl-L-Serine Hydrochloride Salt (41).** Compound **37** (4.18 g, 17.2 mmol) was dissolved in EtOAc (8 mL), HCl (12 mL, 10 M) was added, and the reaction was stirred at room temperature. After 10 minutes, all starting material was consumed by TLC analysis, and white crystals had precipitated out of solution. The reaction mixture was evaporated to dryness and volatiles were removed *in vacuo* overnight. The resulting white crystals were triturated and washed with DCM (30 mL x 3) followed by diethyl ether (30 mL x 3) to yield a white powder (2.65 g, 89% yield). $^{\delta}$H (D_2O, 500 MHz): 2.81 (1H, t, J = 2.4 Hz, $CH_2C{\equiv}C\mathbf{H}$); 3.88 (1H, dd, J = 10.9 & 3.2 Hz, $\alpha CHC\mathbf{H}_{2\ a}$); 3.99 (1H, dd, J = 10.9 & 4.9 Hz, $\alpha CHC\mathbf{H}_{2\ b}$); 4.15 (2H, d, J = 2.4 Hz, $C\mathbf{H_2}C{\equiv}CH$); 4.18 (1H, dd, J = 4.8 & 3.3 Hz, $\alpha C\mathbf{H}$). $^{\delta}$C (D_2O, 125 MHz): 53.1 (1C, s, $\alpha\mathbf{C}H$); 58.4 (1C, s, $\mathbf{C}H_2C{\equiv}CH$); 66.7 (1C, s, $\alpha CH\mathbf{C}H_2$); 76.4 (1C, s, $CH_2C{\equiv}\mathbf{C}H$); 78.7 (1C, s, $CH_2\mathbf{C}{\equiv}CH$); 170.0 (1C, s, $\mathbf{C}OOH$). HRMS (ESI^+) for $C_6H_{10}N_1O_3Na$ $[M+Na]^+$: calcd = 144.0655; found = 144.0651.

4.3 Preparation of Acetyl Protected Sugars (compounds 50, 55):

1,2,3,4,6-Penta-*O*-Acetyl-α/β-D-Mannopyranoside (50). α/β-D-Mannose (10.0 g, 55.5 mmol) was dissolved in pyridine (75 mL) and cooled in an ice bath to 0°C. Acetic anhydride (31.5 mL, 334 mmol) was added dropwise, and the reaction was allowed to warm to room temperature. The solution was stirred for 16 hours until the reaction was determined complete by TLC analysis. The reaction was quenched with dH_2O (25 mL) and extracted with DCM (3 x 150 mL). The combined organic layers were washed with water (2 x 150 mL), 1 M HCl (5 x 200 mL), and brine (150 mL), dried with Na_2SO_4, and evaporated to dryness to yield a yellow syrup (19.32 g, 89% yield), as a mixture of α/β anomers. R_f = 0.72 (75:25 EtOAc:Hexanes). $^{\delta}$H ($CDCl_3$, 500 MHz): α/β = 4/1; α-anomer = 2.02, 2.06, 2.10, 2.18, 2.19 (15H, s x 5, $OCOC\mathbf{H_3}$ x 5); 4.06 (1H, ddd, J = 9.4 & 4.7 &

2.4 Hz, C5**H**); 4.10 (1H, dd, J = 12.5 & 2.4 Hz, C6$\mathbf{H_{2b}}$); 4.29 (1H, dd, J = 12.5 & 4.7 Hz, C6$\mathbf{H_{2a}}$); 5.27 (1H, dd, J = 2.4 & 1.8 Hz, C2**H**); 5.34-5.37 (1H, m, C3**H**); 5.34-5.37 (1H, m, C4**H**); 6.09 (1H, d, J = 1.8 Hz, C1**H**); β-anomer = 2.01, 2.06, 2.11, 2.19, 2.22 (15H, s x 5, OCOC$\mathbf{H_3}$ x 5); 3.82 (1H, ddd, J = 9.9 & 5.3 & 2.3 Hz, C5**H**); 4.15 (1H, dd, J = 12.4 & 2.3 Hz, C6$\mathbf{H_{2b}}$); 4.32 (1H, dd, J = 12.4 & 5.3 Hz, C6$\mathbf{H_{2a}}$); 5.15 (1H, dd, J = 10.0 & 3.3 Hz, C3**H**); 5.34-5.38 (1H, m, C4**H**); 5.49 (1H, dd, J = 3.3 & 1.1 Hz, C2**H**); 5.88 (1H, d, J = 1.1 Hz, C1**H**). $^{\delta}$C ($CDCl_3$, 125 MHz): α-anomer = 20.6, 20.6, 20.7, 20.8, 20.9 (5C, s x 5, OCO**C**H_3 x 5); 62.1 (1C, s, **C**6); 65.5 (1C, s, **C**3); 68.3 (1C, s, **C**2); 68.7 (1C, s, **C**4); 70.6 (1C, s, **C**5); 90.6 (1C, s, **C**1); 168.1, 169.5, 169.7, 170.0, 170.7 (5C, s x 5, O**C**OCH_3 x 5); β-anomer = 20.5, 20.6, 20.7, 20.8, 20.8 (5C, s x 5, OCO**C**H_3 x 5); 62.0 (1C, s, **C**6); 65.4 (1C, s, **C**4); 68.2 (1C, s, **C**2); 70.6 (1C, s, **C**3); 73.3 (1C, s, **C**5); 90.4 (1C, s, **C**1); 168.4, 169.6, 169.8, 170.2, 170.6 (5C, s x 5, O**C**OCH_3 x 5). These data are consistent with literature values.[55]

1,2,3,4,-Tetra-*O*-Acetyl-α/β-L-Rhamnopyranoside (55). α/β-L-Rhamnose monohydrate (10.0 g, 54.9 mmol) was dissolved in pyridine (75 mL) and cooled in an ice bath to 0°C. Acetic anhydride (26 mL, 276 mmol) was added dropwise, and the reaction was allowed to warm to room temperature. The solution was stirred for 16 hours until the reaction was determined complete by TLC analysis. The reaction was quenched with dH_2O (25 mL) and extracted with DCM (3 x 150 mL). The combined organic layers were washed with water (2 x 150 mL), 1 M HCl (5 x 200 mL), and brine (150 mL), dried with Na_2SO_4, and evaporated to dryness to yield a yellow syrup (16.90 g, 93% yield), as a mixture of α/β anomers. R_f = 0.73 (75:25 EtOAc:Hexanes). $^{\delta}$H ($CDCl_3$, 500 MHz): α/β = 3/1; α-anomer = 1.26 (3H, d, J = 6.2 Hz, C6$\mathbf{H_3}$); 2.03, 2.09, 2.18, 2.20 (12H, s x 4, OCOC$\mathbf{H_3}$ x 4); 3.96 (1H, dq, J = 10.1 & 6.2 Hz, C5**H**); 5.15 (1H, t, J = 10.1 Hz, C4**H**); 5.28 (1H, dd, J = 3.5 & 1.9 Hz, C2**H**); 5.33 (1H, dd, J = 10.1 & 3.5 Hz, C3**H**); 6.04 (1H, d, J = 1.9 Hz, C1**H**); β-anomer = 1.32 (3H, d, J = 6.2 Hz, C6$\mathbf{H_3}$); 2.03, 2.09, 2.13, 2.24 (12H, s x 4, OCOC$\mathbf{H_3}$ x 4); 3.69 (1H, dq, J = 9.5 & 6.2 Hz, C5**H**); 5.09-5.13 (1H, m, C3**H**); 5.10-5.14 (1H, m, C4**H**); 5.50 (1H, dd, J = 2.6 & 1.1 Hz, C2**H**); 5.86 (1H, d, J = 1.1 Hz, C1**H**). $^{\delta}$C ($CDCl_3$, 125 MHz): α-anomer = 17.5 (1C, s, **C**6); 20.7, 20.7, 20.8, 20.9 (4C, s x 4, OCO**C**H_3 x 4); 68.6 (1C, s, **C**3); 68.7 (1C, s, **C**2); 68.8 (1C, s, **C**5); 70.5 (1C, s, **C**4); 90.6 (1C, s, **C**1); 168.4, 169.9, 169.9, 170.1 (4C, s x 4, O**C**OCH_3 x 4); β-anomer = 17.4 (1C, s, **C**6); 20.6, 20.8, 20.8, 20.9 (4C, s x 4, OCO**C**H_3 x 4); 68.5 (1C, s, **C**2); 70.2 (1C, s, **C**3); 70.7 (1C, s, **C**4); 71.5 (1C, s, **C**5); 90.3 (1C, s, **C**1); 168.5, 169.9, 170.3, 170.4 (4C, s x 4, O**C**OCH_3 x 4). These data are consistent with literature values.[56]

4.4 Bromination of Acetyl Protected Sugars (compounds 47, 51, 56):

2,3,4,6-Tetra-*O*-Acetyl-α-D-Glucopyranosyl Bromide (47). 1,2,3,4,6-Penta-*O*-acetyl-β-D-glucopyranoside (19.5 g, 50.0 mmol) was dissolved in DCM (160 mL) and cooled in an ice bath to 0°C. Phosphorus tribromide (8.0 mL, 84.8 mmol) and dH_2O (5.45 mL, 303 mmol) were added dropwise, and the reaction was allowed to warm to room temperature. The solution was stirred for 3 hours until the reaction was determined complete by TLC analysis. The reaction mixture was diluted with DCM (250 mL) and washed with dH_2O (2 x 400 mL), saturated aqueous sodium bicarbonate (400 mL), and brine (400 mL). The organic layer was dried with Na_2SO_4 and evaporated to dryness to yield a pale yellow oil (19.5 g). This product was impure by TLC but was used in the next synthetic step without further purification due to its instability. R_f = 0.88 (50:50 EtOAc:Hexanes).

2,3,4,6-Tetra-*O*-Acetyl-α/β-D-Mannopyranosyl Bromide (51). Compound **50** (19.32 g, 49.5 mmol) was dissolved in DCM (160 mL) and cooled in an ice bath to 0°C. Phosphorus tribromide (8.0 mL, 84.8 mmol) and dH_2O (5.45 mL, 303 mmol) were added dropwise, and the reaction was allowed to warm to room temperature. The solution was stirred for 3 hours until the reaction was determined complete by TLC analysis. The reaction mixture was diluted with DCM (250 mL) and washed with dH_2O (2 x 400 mL), saturated aqueous sodium bicarbonate (400 mL), and brine (400 mL). The organic layer was dried with Na_2SO_4 and evaporated to dryness to yield a pale yellow oil (20.8 g). This product was impure by TLC but was used in the next synthetic step without further purification due to its instability. R_f = 0.74 (50:50 EtOAc:Hexanes).

2,3,4-Tri-*O*-Acetyl-α/β-L-Rhamnopyranosyl Bromide (56). Compound **55** (16.9 g, 50.9 mmol) was dissolved in DCM (160 mL) and cooled in an ice bath to 0°C. Phosphorus tribromide (8.0 mL, 84.8 mmol) and dH_2O (5.45 mL, 303 mmol) were added dropwise, and the reaction was allowed to warm to room temperature. The solution was stirred for 3 hours until the reaction was determined complete by TLC analysis. The reaction mixture was diluted with DCM (250 mL) and washed with dH_2O (2 x 400 mL), saturated aqueous sodium bicarbonate (400 mL), and brine (400 mL). The organic layer was dried with Na_2SO_4 and evaporated to dryness to yield a pale yellow oil (16.8 g). This product was impure by TLC but was used in the next synthetic step without further purification due to its instability. R_f = 0.85 (50:50 EtOAc:Hexanes).

4.5 Formation of Azides (compounds 43, 45, 48, 52, 53, 57, 58):

Octyl Azide (43). Octyl bromide (10.0 mL, 56.7 mmol) was dissolved in acetone (100 mL) and added to a solution of sodium azide (17 g, 262 mmol) in dH_2O (100 mL). The reaction mixture was stirred vigorously at room temperature for 48 hours. The reaction mixture was extracted with DCM (3 x 150 mL), and the combined organic layers were washed with dH_2O (2 x 150 mL) and brine (150 mL), dried with Na_2SO_4, and evaporated to dryness yielding a yellow oil (6.79 g, 77% yield). $^{\delta}$H ($CDCl_3$, 500 MHz): 0.92 (3H, t, J = 6.9Hz, C**H$_3$**); 1.27-1.42 (10H, m, -(C**H$_2$**)$_5$); 1.63 (2H, p, J = 6.9Hz, C**H$_2$**CH_2N_3); 3.29 (2H, t, J = 6.9Hz, CH_2C**H$_2$**N_3). $^{\delta}$C ($CDCl_3$, 125 MHz): 14.2 (1C, s, **C**$8H_3$); 22.8 (1C, s, **C**7); 26.9 (1C, s, **C**6); 29.0 (1C, s, **C**2); 29.2 (1C, s, **C**4); 29.3 (1C, s, **C**3); 31.9 (1C, s, **C**5); 51.7 (1C, s, **C**$1N_3$). These data are consistent with literature values.[57]

Benzyl Azide (45). Benzyl bromide (10.0 mL, 85.0 mmol) was dissolved in acetone (100 mL) and added to a solution of sodium azide (25 g, 385 mmol) in dH_2O (100 mL). The reaction mixture was stirred vigorously at room temperature for 48 hours until the reaction was determined complete by TLC analysis. The reaction mixture was extracted with DCM (3 x 150 mL), and the combined organic layers were washed with dH_2O (2 x 150 mL) and brine (150 mL), dried with Na_2SO_4, and evaporated to dryness yielding a pale yellow oil (9.50 g, 84% yield). R_f = 0.89 (toluene). $^{\delta}$H ($CDCl_3$, 500 MHz): 4.40 (2H, s, C**H$_2$**); 7.40-7.49 (5H, m, Ar-C**H**). $^{\delta}$C ($CDCl_3$, 125 MHz): 54.9 (2C, s, **C**H_2); 128.3 (2C, s, ortho-**C**H); 128.4 (1C, s, para-**C**H); 128.9 (2C, s, meta-**C**H); 135.5 (1C, s, 4°**C**). These data are consistent with literature values.[58]

2,3,4,6-Tetra-*O*-Acetyl-β-D-Glucopyranosyl Azide (48). Compound **47** (19.5 g, ≤50.0 mmol) was dissolved in acetone (100 mL) and added to a solution of sodium azide (15 g, 231 mmol) in dH_2O (100 mL). The reaction mixture was stirred vigorously at room temperature for 18 hours until the reaction was determined complete by TLC analysis. The reaction mixture was extracted with DCM (3 x 150 mL), and the combined organic layers were washed with dH_2O (2 x 150 mL) and brine (150 mL), dried with Na_2SO_4, and evaporated to dryness yielding an off-white powder (17.0 g). The crude material was purified by flash column chromatography using a normal phase silica column (18 cm x 6.5 cm, 75 $mLmin^{-1}$) and an isocratic solvent system (20:80 EtOAc:Hexanes). The purified product took the form of a bright white powder (8.24 g, 44% yield).

R_f = 0.75 (50:50 EtOAc:Hexanes). $^{\delta}$H ($CDCl_3$, 500 MHz): 2.04, 2.06, 2.11, 2.13 (12H, s x 4, OCOC**H$_3$** x 4); 3.83 (1H, ddd, J = 9.6 & 4.7 & 2.0 Hz, C5**H**); 4.20 (1H, dd, J = 12.4 & 2.0 Hz, C6**H$_{2b}$**); 4.30 (1H, dd, J = 12.4 & 4.7 Hz, C6**H$_{2a}$**); 4.68 (1H, d, J = 8.8 Hz, C1**H**); 4.99 (1H, t, J = 9.2 Hz, C2**H**); 5.13 (1H, t, J = 9.6 Hz, C4**H**); 5.25 (1H, t, J = 9.6 Hz, C3**H**). $^{\delta}$C ($CDCl_3$, 125 MHz): 20.7, 20.7, 20.8, 20.9 (4C, s x 4, OCO**C**H$_3$ x 4); 61.8 (1C, s, **C**6); 68.0 (1C, s, **C**4); 70.8 (1C, s, **C**2); 72.8 (1C, s, **C**3); 74.2 (1C, s, **C**5); 88.1 (1C, s, **C**1); 169.4, 169.5, 170.3, 170.8 (4C, s x 4, O**C**OCH$_3$ x 4). $^1J_{C1,H1}$ = 160 Hz. These data are consistent with literature values.[59]

2,3,4,6-Tetra-*O*-Acetyl-α-D-Mannopyranosyl Azide (52) & 2,3,4,6-Tetra-*O*-Acetyl-β-D-Mannopyranosyl Azide (53). Compound **51** (20.8 g, ≤49.5 mmol) was dissolved in acetone (100 mL) and added to a solution of sodium azide (15 g, 231 mmol) in dH_2O (100 mL). The reaction mixture was stirred vigorously at room temperature for 18 hours until the reaction was determined complete by TLC analysis. The reaction mixture was extracted with DCM (3 x 150 mL), and the combined organic layers were washed with dH_2O (2 x 150 mL) and brine (150 mL), dried with Na_2SO_4, and evaporated to dryness yielding a yellow syrup (17.0 g). The crude material was purified by flash column chromatography using a normal phase silica column (18 cm x 6.5 cm, 75 $mLmin^{-1}$) and EtOAc:Hexanes (30:70). This yielded a mixture of **52** and **53** (1.61 g, 10% yield over 2 steps), which were then separated by a further chromatic step using a second normal phase silica column (8.0 cm x 4.0 cm, 40 $mLmin^{-1}$) and EtOAc:Hexanes (10:90). This afforded **52** as a yellow oil (626 mg) and **53** as a white powder (377 mg). R_f : **52** = 0.65, **53** = 0.60 (50:50 EtOAc:Hexanes). $^{\delta}$H ($CDCl_3$, 500 MHz): **52** = 2.03, 2.09, 2.15, 2.20 (12H, s x 4, OCOC**H$_3$** x 4); 4.16-4.20 (1H, m, C5**H**); 4.20 (1H, dd, J = 12.4 & 2.3 Hz, C6**H$_{2b}$**); 4.34 (1H, dd, J = 12.4 & 5.5 Hz, C6**H$_{2a}$**); 5.19 (1H, dd, J = 3.0 & 1.8 Hz, C2**H**); 5.28 (1H, dd, J = 10.0 & 3.0 Hz, C3**H**); 5.32 (1H, t, J = 10.0 Hz, C4**H**); 5.42 (1H, d, J = 1.8 Hz, C1**H**); **53** = 2.03, 2.09, 2.15, 2.25 (12H, s x 4, OCOC**H$_3$** x 4); 3.80 (1H, ddd, J = 10.1 & 5.7 & 2.5Hz, C5**H**); 4.24 (1H, dd, J = 12.3 & 2.5Hz, C6**H$_{2b}$**); 4.32 (1H, dd, J = 12.3 & 5.7Hz, C6**H$_{2a}$**); 4.77 (1H, d, J = 1.1Hz, C1**H**); 5.08 (1H, dd, J = 10.1 & 3.3Hz, C3**H**); 5.30 (1H, t, J = 10.1Hz, C4**H**); 5.49 (1H, dd, J = 3.3 & 1.1Hz, C2**H**). $^{\delta}$C ($CDCl_3$, 125MHz): **52** = 20.6, 20.7, 20.8, 20.9 (4C, s x 4, OCO**C**H$_3$ x 4); 62.2 (1C, s, **C**6); 65.6 (1C, s, **C**4); 68.3 (1C, s, **C**3); 69.2 (1C, s, **C**2); 70.6 (1C, s, **C**5); 87.5 (1C, s, **C**1); 169.7, 169.8, 169.9, 170.7 (4C, s x 4, O**C**OCH$_3$ x 4); **53** = 20.6, 20.7, 20.8, 20.8 (4C, s x 4, OCO**C**H$_3$ x 4); 62.3 (1C, s, **C**6); 65.3 (1C, s, **C**4); 69.2 (1C, s, **C**3); 71.0 (1C, s, **C**2); 74.7 (1C, s, **C**5); 85.1 (1C, s, **C**1); 170.0, 170.0, 170.0, 169.6 (4C, s x 4, O**C**OCH$_3$ x 4). $^1J_{C1,H1}$: **52** = 170 Hz, **53** = 158 Hz. LRMS (ESI^+): **52** = Q1 found 396 m/z $[M+Na]^+$, 769 m/z $[2M+Na]^+$; MS/MS (769) found 396 m/z

$[M+Na]^+$; **53** = Q1 found 396 m/z $[M+Na]^+$, 769 m/z $[2M+Na]^+$; MS/MS (769) found 396 m/z $[M+Na]^+$. HRMS (ESI^+) for $C_{14}H_{19}N_3O_9Na$ $[M+Na]^+$: calcd = 396.1014; **52** found 396.1011; **53** found 396.1006. These data are consistent with literature values.[60]

2,3,4-Tri-*O*-Acetyl-α-L-Rhamnopyranosyl Azide (57) & 2,3,4-Tri-*O*-Acetyl-β-L-Rhamnopyranosyl Azide (58). Compound **56** (16.8 g, ≤50.9 mmol) was dissolved in acetone (100 mL) and added to a solution of sodium azide (15 g, 231 mmol) in dH_2O (100 mL). The reaction mixture was stirred vigorously at room temperature for 18 hours until the reaction was determined complete by TLC analysis. The reaction mixture was extracted with DCM (3 x 150 mL), and the combined organic layers were washed with dH_2O (2 x 150 mL) and brine (150 mL), dried with Na_2SO_4, and evaporated to dryness yielding a yellow syrup (15.5 g). The crude material was purified by flash column chromatography using a normal phase silica column (18 cm x 6.5 cm, 75 $mLmin^{-1}$) and EtOAc:Hexanes (20:80). This yielded a mixture of **57** and **58** (3.79 g, 24% yield over 2 steps), which were then separated by a further chromatic step using a second normal phase silica column (8.0 cm x 4.0 cm, 40 $mLmin^{-1}$) and EtOAc:Hexanes (10:90). This afforded **57** as a yellow oil (45.2 mg) and **58** as a white powder (952 mg). R_f : **57** = 0.76, **58** = 0.70 (50:50 EtOAc:Hexanes). $^{\delta}H$ ($CDCl_3$, 500MHz): **57** = 1.30 (1H, d, J = 6.2Hz, C6**H**); 2.02, 2.09, 2.19 (9H, s x 3, OCOC**H₃** x 3); 4.06 (1H, dq, J = 9.7 & 6.2Hz, C5**H**); 5.11 (1H, t, J = 10.1Hz, C4**H**); 5.17 (1H, dd, J = 3.3 & 1.9Hz, C2**H**); 5.23 (1H, dd, J = 10.1 & 3.3Hz, C3**H**); 5.34 (1H, d, J = 1.9Hz, C1**H**); **58** = 1.35 (1H, d, J = 6.2Hz, C6**H**); 2.02, 2.09, 2.23 (9H, s x 3, OCOC**H₃** x 3); 3.65 (1H, dq, J = 9.6 & 6.2Hz, C5**H**); 4.72 (1H, d, J = 1.2Hz, C1**H**); 5.02 (1H, dd, J = 10.2 & 3.2Hz, C3**H**); 5.11 (1H, t, J = 10.1Hz, C4**H**); 5.46 (1H, dd, J = 3.2 & 1.2Hz, C2**H**). $^{\delta}C$ ($CDCl_3$, 125MHz): **57** = 17.5 (1C, s, **C**6); 20.7, 20.8, 20.9 (3C, s x 3, OCO**C**H_3); 68.3 (1C, s, **C**3); 68.6 (1C, s, **C**5); 69.5 (1C, s, **C**2); 70.5 (1C, s, **C**4); 87.5 (1C, s, **C**1); 169.9, 169.9, 170.0 (3C, s x 3, O**C**OCH_3); **58** = 17.4 (1C, s, **C**6); 20.6, 20.8, 20.8 (3C, s x 3, OCO**C**H_3); 69.6 (1C, s, **C**2); 70.0 (1C, s, **C**4); 71.0 (1C, s, **C**3); 73.0 (1C, s, **C**5); 85.0 (1C, s, **C**1); 169.8, 170.1, 170.1 (3C, s x 3, O**C**OCH_3). $^1J_{C1,H1}$: **57** = 170 Hz, **58** = 158 Hz. LRMS (ESI^+): **57** = Q1 found 338 m/z $[M+Na]^+$, 653 m/z $[2M+Na]^+$; MS/MS (653) found 338 m/z $[M+Na]^+$; **58** = Q1 found 338 m/z $[M+Na]^+$, 653 m/z $[2M+Na]^+$; MS/MS (653) found 338 m/z $[M+Na]^+$. HRMS (ESI^+) for $C_{14}H_{17}N_3O_7Na$ $[M+Na]^+$: calcd = 338.0959; **57** found 338.0970; **58** found 338.0953. These data are consistent with literature values.[61]

4.6 Deprotection of Sugar Azides (compound 59):

β-D-Glucopyranosyl Azide (59). Compound **48** (335 mg, 0.90 mmol) was stirred at room temperature in a solution of elemental sodium (7.5 mg) in methanol (15 mL). After 30 minutes, complete dissolution had occurred and the starting material had disappeared by TLC analysis. The reaction mixture was evaporated to dryness, and volatiles were removed *in vacuo* overnight, affording shiny white crystals (180 mg, 98% yield). $^{\delta}$H (D_2O, 500 MHz): 3.15 (1H, t, J = 8.9 Hz, C2**H**); 3.30 (1H, t, J = 8.9 Hz, C4**H**); 3.40 (1H, t, J = 8.9 Hz, C3**H**); 3.42 (1H, ddd, J = 9.8 & 5.5 & 2.3 Hz, C5**H**); 3.15 (1H, t, J = 8.9 Hz, C2**H**); 3.63 (1H, dd, J = 12.5 & 5.5 Hz, C6**H$_{2a}$**); 3.80 (1H, dd, J = 12.5 & 2.3 Hz, C6**H$_{2b}$**); 4.63 (1H, d, J = 8.8 Hz, C1**H**). $^{\delta}$C (D_2O, 125 MHz): 60.5 (1C, s, **C**6); 69.1 (1C, s, **C**4); 72.8 (1C, s, **C**2); 75.7 (1C, s, **C**3); 77.8 (1C, s, **C**5); 90.1 (1C, s, **C**1). LRMS (ESI^+): Q1 found 228 m/z $[M+Na]^+$, 433 m/z $[2M+Na]^+$; MS/MS (433) found 228 m/z $[M+Na]^+$. HRMS (ESI^+) for $C_6H_{11}N_3O_5Na$ $[M+Na]^+$: calcd = 228.0591; found = 228.0579. These data are consistent with literature values.[62]

4.7 Preparation of Triazoles via Cycloaddition Reactions (compounds 60, 61, 62):

***N*-Boc-*O*-(1-(2,3,4,6-Tetra-*O*-Acetyl-β-D-Glucopyranosyl)-1H-1,2,3-Triazol-4-yl)methyl-L-Serine (60).** Compound **37** (107 mg, 0.44 mmol) was dissolved in ethanol (3 mL). Compound **48** (164 mg, 0.44 mmol) was added, and the mixture was stirred vigorously. To this mixture was added a solution of $CuSO_4 \cdot 5H_2O$ (22 mg, 88 μmol) and L-ascorbic acid (30.5 mg, 0.17 mmol) in dH_2O (3 mL), and the suspension was warmed to 35°C. After 1 hour, complete dissolution had occurred and both starting materials had disappeared by TLC analysis. The reaction was removed from heat and diluted with dH_2O (10 mL), then extracted with ethyl acetate (2 x 50 mL). The combined organic layers were washed with dH_2O (50 mL) and brine (50 mL), dried with Na_2SO_4, and evaporated to dryness yielding a white solid (264 mg, 97% yield). R_f = 0.25 (75:25 EtOAc:Hexanes). $^{\delta}$H ($CDCl_3$, 500 MHz): 1.42 (9H, s, C(C**H$_3$**)$_3$); 1.91, 2.06, 2.11, 2.14 (12H, s x 4, OCOC**H$_3$** x 4); 3.74 (1H, d, J = 8.5 Hz, αCHC**H$_{2a}$**); 3.89 (1H, d, J = 8.5 Hz, αCHC**H$_{2b}$**); 4.04-4.10 (1H, m, Glc-C5**H**); 4.19 (1H, d, J = 12.5 Hz, Glc-C6**H$_{2a}$**); 4.30 (1H, dd, J = 12.5 & 4.7 Hz, Glc-C6**H$_{2b}$**); 4.42-4.48 (1H, m, αC**H**); 4.60 (1H, d, J = 12.5 Hz, αCHCH$_2$OC**H$_{2a}$**); 4.69 (1H, d, J = 12.5 Hz, αCHCH$_2$OC**H$_{2b}$**); 5.27 (1H, t, J = 9.6 Hz, Glc-C4**H**); 5.42-5.50 (1H, m, Glc-C3**H**); 5.42-5.50 (1H, m, Glc-C4**H**); 5.66 (1H, d, J = 7.3 Hz, N**H**); 5.95 (1H, d, J = 8.7 Hz, Glc-C1**H**); 7.42 (1H, bs,

COO**H**); 7.88 (1H, s, C=C**H**N-tetraAcGlc). $^{\delta}$C ($CDCl_3$, 125 MHz): 20.4, 20.7, 20.8, 20.9 (12C, s x 4, OCO**C**H_3 x 4); 28.5 (3C, s, C(**C**$H_3)_3$); 53.9 (1C, s, **αC**H); 61.6 (1C, s, Glc-**C6**H_2); 64.4 (1C, s, αCHCH_2O**C**H_2); 67.9 (1C, s, Glc-**C4**H); 70.4 (1C, s, Glc-**C3**H); 70.6 (1C, s, αCH**C**H_2); 72.7 (1C, s, Glc-**C2**H); 75.4 (1C, s, Glc-**C5**H); 85.9 (1C, s, Glc-**C1**H); 80.4 (1C, s, **C**$(CH_3)_3$); 121.5 (C=**C**HN-tetraAcGlc); 145.6 (1C, s, **C**=CHN-tetraAcGlc); 156.1 (1C, s, **C**OC$(CH_3)_3$); 169.5, 169.6, 169.9, 170.2 (4C, s x 4, O**C**OCH_3 x 4); 171.4 (1C, s, **C**OOH). LRMS (ESI^+): Q1 found 639 m/z $[M+Na]^+$, 617 m/z $[M+H]^+$. HRMS (ESI^+) for $C_{25}H_{36}N_4O_{14}Na$ $[M+Na]^+$: calcd = 639.2120; found = 639.2101.

***N*-Boc-*O*-(1-(2,3,4-Tri-*O*-Acetyl-β-L-Rhamnopyranosyl)-1H-1,2,3-Triazol-4-yl)methyl-L-Serine (61).** Compound **37** (107 mg, 0.44 mmol) was dissolved in ethanol (3 mL). Compound **58** (139 mg, 0.44 mmol) was added, and the mixture was stirred vigorously. To this mixture was added a solution of $CuSO_4 \cdot 5H_2O$ (22 mg, 88 μmol) and L-ascorbic acid (30.5 mg, 0.17 mmol) in dH_2O (3 mL), and the suspension was warmed to 35°C. After 1 hour, complete dissolution had occurred and both starting materials had disappeared by TLC analysis. The reaction was removed from heat and diluted with dH_2O (10 mL), then extracted with ethyl acetate (2 x 50 mL). The combined organic layers were washed with dH_2O (50 mL) and brine (50 mL), dried with Na_2SO_4, and evaporated to dryness yielding a white solid (244 mg, 99% yield). R_f = 0.25 (75:25 EtOAc:Hexanes). $^{\delta}$H ($CDCl_3$, 500 MHz): 1.37 (3H, d, J = 6.2 Hz, Rha-6**H_3**); 1.45 (9H, s, C(C**$H_3)_3$**); 2.01, 2.11, 2.12 (9H, s x 3, OCOC**H_3** x 3); 3.77 (1H, d, J = 8.5 Hz, αCHC**H_{2a}**); 3.88 (1H, dq, J = 9.7 & 6.2 Hz, Rha-C5**H**); 3.96 (1H, d, J = 8.5 Hz, αCHC**H_{2b}**); 4.39-4.46 (1H, m, αC**H**); 4.62 (1H, d, J = 12.5 Hz, αCHCH_2OC**H_{2a}**); 4.70 (1H, d, J = 12.5 Hz, αCHCH_2OC**H_{2b}**); 5.20 (1H, t, J = 10.0 Hz, Rha-C4**H**); 5.29 (1H, dd, J = 10.2 & 3.1 Hz, Rha-C3**H**); 5.60 (1H, d, J = 7.3 Hz, N**H**); 5.74 (1H, dd, J = 3.0 & 0.6 Hz, Rha-C2**H**); 6.19 (1H, s, Rha-C1**H**); 7.37 (1H, bs, COO**H**); 7.81 (1H, s, C=C**H**N-triAcRha). $^{\delta}$C ($CDCl_3$, 125 MHz): 17.5 (1C, s, Rha-**C6**H_3); 20.6, 20.6, 20.8 (9C, s x 3, OCO**C**H_3 x 3); 23.3 (3C, s, C(**C**$H_3)_3$); 54.0 (1C, s, **αC**H); 64.3 (1C, s, αCHCH_2O**C**H_2); 69.2 (1C, s, Rha-**C2**H); 69.7 (1C, s, Rha-**C4**H); 70.4 (1C, s, αCH**C**H_2); 70.8 (1C, s, Rha-**C3**H); 73.9 (1C, s, Rha-**C5**H); 80.2 (1C, s, **C**$(CH_3)_3$); 84.7 (1C, s, Rha-**C1**H); 121.7 (C=**C**HN-triAcRha); 144.5 (1C, s, **C**=CHN-triAcRha); 156.0 (1C, s, **C**OC$(CH_3)_3$); 179.8, 169.9, 170.0 (3C, s x 3, O**C**OCH_3 x 3); 176.9 (1C, s, **C**OOH). LRMS (ESI^+): Q1 found 581 m/z $[M+Na]^+$, 559 m/z $[M+H]^+$. HRMS (ESI^+) for $C_{23}H_{34}N_4O_{12}Na$ $[M+Na]^+$: calcd = 581.2065; found = 581.2042.

***N*-Boc-*O*-(1-Octyl-1H-1,2,3-Triazol-4-yl)methyl-L-Serine (62).** Compound **37** (107 mg, 0.44 mmol) was dissolved in ethanol (3 mL). Compound **43** (150 mg, 0.97 mmol) was added, and the mixture was stirred vigorously. To this mixture was added a solution of $CuSO_4 \cdot 5H_2O$ (22 mg, 88 μmol) and L-ascorbic acid (30.5 mg, 0.17 mmol) in dH_2O (3 mL), and the suspension was warmed to 35°C. After 1 hour, complete dissolution had occurred and the reaction was complete by TLC analysis. The reaction was removed from heat and diluted with dH_2O (10 mL), then extracted with ethyl acetate (2 x 50 mL). The combined organic layers were washed with dH_2O (50 mL) and brine (50 mL), dried with Na_2SO_4, and evaporated to dryness. Volatiles were removed *in vacuo* overnight, affording a clear oil (174 mg, 99% yield). R_f = 0.29 (75:25 EtOAc:Hexanes). $^{\delta}H$ ($CDCl_3$, 500 MHz): 0.89 (3H, t, J = 7.0 Hz, octyl C8**H$_3$**); 1.25-1.36 (10H, m, octyl C3**H$_2$**,C4**H$_2$**,C5**H$_2$**,C6**H$_2$**,C7**H$_2$**); 1.45 (9H, s, C(C**H**$_3$)$_3$); 1.91 (2H, q, J = 7.3 Hz, octyl C2**H$_2$**); 3.80 (1H, d, J = 8.0 Hz, αCHC**H**$_{2a}$); 4.0 (1H, d, J = 8.0 Hz, αCHC**H**$_{2b}$); 4.35 (2H, t, J = 7.3 Hz, octyl C1**H$_2$**); 4.43-4.49 (1H, m, αC**H**); 4.69 (2H, bs, αCHCH$_2$OC**H$_2$**); 5.63 (1H, d, J = 6.4 Hz, N**H**); 7.60 (1H, s, C=C**H**NC$_8$H$_{17}$); 8.33 (1H, bs, COO**H**). $^{\delta}C$ ($CDCl_3$, 125 MHz): 14.1 (1C, s, octyl **C8**H$_3$); 23.3 (3C, s, C(**C**H$_3$)$_3$); 30.3 (1C, s, **C2**H$_2$); 31.7, 29.0, 29.1, 26.5, 22.6 (5C, s x 5, octyl **C3**H$_2$**C4**H$_2$**C5**H$_2$**C6**H$_2$**C7**H$_2$); 50.6 (1C, s, octyl **C1**H$_2$); 50.6 (1C, s, **αC**H); 64.6 (1C, s, αCHCH$_2$O**C**H$_2$); 70.5 (1C, s, αCH**C**H$_2$); 80.2 (1C, s, **C**(CH$_3$)$_3$); 122.8 (1C, s, C=**C**HNC$_8$H$_{17}$); 144.5 (1C, s, **C**=CHNC$_8$H$_{17}$); 156.0 (1C, s, **C**OC(CH$_3$)$_3$); 173.9 (1C, s, **C**OOH). LRMS (ESI^+): Q1 found 421 m/z $[M+Na]^+$, 399 m/z $[M+H]^+$. HRMS (ESI^+) for $C_{19}H_{34}N_4O_5Na$ $[M+Na]^+$: calcd = 421.2421; found = 421.2412.

4.8 Production of Jadomycins (compounds 26, 34, 35, 28):

Jadomycin L (26). *S. venezuelae* shaker growths were performed according to the procedure outlined above using L-leucine MSM culture media (4 x 2 L). The crude material obtained (718 mg) was loaded onto minimal ISOLEUTE®HM-N sorbant using DCM and purified by automated normal phase silica gel flash chromatography (18 cm x 6.5 cm) using a gradient of DCM to MeOH (30%) at a flow rate of 75 $mLmin^{-1}$. Relevant jadomycin fractions were combined, dried (336 mg), applied to a reversed-phase C18 column (18 cm x 6.5 cm), and eluted using a gradient of water to acetonitrile (50%) at a flow rate of 45 $mLmin^{-1}$. Relevant fractions were combined, dried (259 mg), applied to a normal phase column (22 cm x 4.0 cm), and eluted using a gradient of DCM to MeOH (30%) at a rate of 30 $mLmin^{-1}$. Relevant fractions were combined and solvent removed *in vacuo* to afford the pure secondary metabolite (95 mg, 12 mg/L), as a mixture of diastereomers

(3a*S*/3a*R* = 44/36). R_f = 0.40 (10:90 MeOH:DCM). NMR data follows. For labeling of various protons, see Figure 25. UV-Vis (1.82 x 10^{-5} M, MeOH): λ_{max} (ε) = 289 (17490), 315 (18095), 492 (3135), 528 (2530), 667 (990), 754 (550). LRMS (ESI^+): Q1 found 572 m/z $[M+Na]^+$, 550 m/z $[M+H]^+$; MS/MS (550) found 420 $[M+H\text{-digitoxose}]^+$, 306 $[M+H\text{-}C_6H_{10}O_2]^+$. HRMS ($ESI^+$) for $C_{30}H_{31}N_1O_9Na$ $[M+Na]^+$: calcd = 572.1891; found = 572.1870.

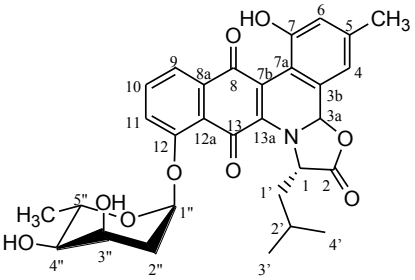


Figure 25. Atom-labeled structure of compound **26**.

Table 3. Jadomycin L 3a*S* NMR data.

Position	**δ ^{1}H** (ppm)	**Multiplicity (J**(Hz))	**δ ^{13}C** (ppm)	**COSY**	**TOCSY**
1	5.47-5.51	m	60.7	1'	
2	-	-		-	
3a	5.66	s	88.4	-	
3b	-	-		-	
4	6.71	d (1.2)	118.6	5-CH_3	
5	-	-	139.6	-	
5-CH_3	2.36	d (7.3)	19.6	4, 6	
6	6.84	q (0.7)	118.8	5-CH_3	
7	-	-	155.7	-	
7a	-	-		-	
7b	-	-		-	
8	-	-	183.0	-	
8a	-	-	137.4	-	
9	7.80	dd (8.6, 1.0)	119.7	10	
10	7.68	t (8.6)	134.9	9, 11	
11	7.54	dd (8.6, 1.0)	119.4	10	
12	-	-	155.1	-	
12a	-	-		-	
13	-	-		-	
13a	-	-		-	
1'	2.03-2.10	m	41.7	1, 2'	
2'	1.63-1.69	m	24.9	1', 3', 4'	
3'	0.97	d (6.6)	21.3	2'	4', 2', 1', 1
4'	0.93	d (6.6)	22.5	2'	3', 2', 1', 1
1"	5.91	d (3.1)	95.3	2"ax, 2"eq	
2"ax	2.20-2.24	m	34.3	2"eq, 1", 3"	
2"eq	2.41-2.45	m	34.4	2"ax, 3", 1"	
3"	4.03-4.07	m	67.1	2"ax, 2"eq, 4"	
4"	3.26	dd (9.9, 3.3)	72.6	5", 3"	
5"	3.91-3.95	m	64.9	5"-CH_3, 4"	
5"-CH_3	1.21	d (6.2)	16.8	5"	5", 4", 3"
water	4.86	s	-	-	
MeOH	3.35	s	48.2	-	
MeOD	3.31	p	47.9	-	

Table 4. Jadomycin L 3a*R* NMR data.

Position	δ ^{1}H (ppm)	Multiplicity (J(Hz))	δ ^{13}C (ppm)	COSY	TOCSY
1	5.56-5.60	m	62.9	1'	
2	-	-		-	
3a	5.69	s	84.5	-	
3b	-	-		-	
4	6.75	d (1.2)	119.0	5-CH_3	
5	-	-	139.6	-	
5-CH_3	2.36	d (7.3)	19.6	4, 6	
6	6.82	q (0.7)	119.1	5-CH_3	
7	-	-	155.7	-	
7a	-	-		-	
7b	-	-		-	
8	-	-	183.0	-	
8a	-	-	137.4	-	
9	7.82	dd (8.6, 1.0)	119.7	10	
10	7.70	t (8.6)	134.9	9, 11	
11	7.54	dd (8.6, 1.0)	119.4	10	
12	-	-	155.1	-	
12a	-	-		-	
13	-	-		-	
13a	-	-		-	
1'	1.88-1.96	m	38.3	1, 2'	
2'	1.19-1.26	m	24.5	1', 3', 4'	
3'	0.86	d (6.6)	22.2	2'	4', 2', 1', 1
4'	0.84	d (6.6)	20.0	2'	3', 2', 1', 1
1"	5.94	d (3.1)	94.7	2"ax, 2"eq	
2"ax	2.16-2.22	m	34.3	2"eq, 1", 3"	
2"eq	2.43-2.49	m	34.4	2"ax, 3", 1"	
3"	4.10-4.16	m	67.1	2"ax, 2"eq, 4"	
4"	3.34	dd (9.9, 3.3)	72.6	5", 3"	
5"	3.94-4.00	m	64.9	5"-CH_3, 4"	
5"-CH_3	1.18	d (6.2)	16.8	5"	5", 4", 3"
Water	4.86	s	-	-	
MeOH	3.35	s	48.2	-	
MeOD	3.31	p	47.9	-	

Jadomycin DNV (34). A shaker growth using D-norvaline MSM medium (2 L) was performed according to the procedure described previously, yielding crude jadomycin (56 mg). This was loaded onto minimal ISOLEUTE®HM-N sorbant using DCM and purified by automated normal phase silica gel flash chromatography (22 cm x 4.0 cm) using a gradient of DCM to MeOH (50%) at a flow rate of 45 $mLmin^{-1}$. Relevant jadomycin fractions were combined, dried (25 mg), applied to a reversed-phase C18 column (15 cm x 3.0 cm), and eluted using a gradient of water to acetonitrile (50%) at a flow rate of 25 $mLmin^{-1}$. Relevant fractions were combined, dried (13 mg), and further purified by prep TLC (5:95 MeOH:DCM), affording the pure secondary metabolite as a deep purple powder (8.5 mg, 4.3 mg/L), as a mixture of diastereomers (3a*S*/3a*R* = 55/33). R_f = 0.42 (10:90 MeOH:DCM). NMR data follows. For labeling of various protons, see Figure 26. UV-Vis (1.87×10^{-5} M, MeOH): λ_{max} (ε) = 291 (15812), 315 (16562), 492 (2680), 529 (2037), 605 (1340), 754 (429). LRMS (ESI^+): Q1 found 538 m/z $[M+Na]^+$, 536 m/z $[M+H]^+$; MS/MS (536)

found 406 [M+H-digitoxose]$^+$, 306 [M+H-$C_6H_{10}O_2$]$^+$. HRMS (ESI$^+$) for $C_{29}H_{29}N_1O_9Na$ [M+Na]$^+$: calcd = 558.1735; found = 558.1706.

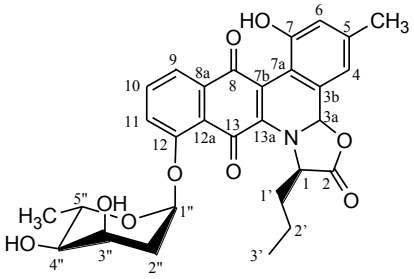


Figure 26. Atom-labeled structure of compound **34**.

Table 5. Jadomycin DNV 3a*S* NMR data.

Position	**δ ^{1}H** (ppm)	**Multiplicity (J**(Hz)**)**	**δ ^{13}C** (ppm)	**COSY**	**TOCSY**
1	5.21-5.26	m	59.3	1'	
2	-	-		-	
3a	6.10	s	87.2	-	
3b	-	-	110.1	-	
4	6.78-6.80	m	114.0	5-CH_3	
5	-	-	142.9	-	
5-CH_3	2.35	s	21.2	4, 6	
6	6.88-6.90	m	120.9	5-CH_3	
7	-	-	153.5	-	
7-OH	10.29	s	-	-	
7a	-	-		-	
7b	-	-		-	
8	-	-		-	
8a	-	-		-	
9	7.98	dd (8.1, 1.1)	121.8	10	
10	7.72	t (8.1)	136.4	9, 11	
11	7.509	dd (8.1, 1.1)	119.9	10	
12	-	-	155.5	-	
12a	-	-		-	
13	-	-		-	
13a	-	-		-	
1'	1.85-1.91	m	35.3	1, 2'	
2'	0.84-1.09	m	17.4	1', 3'	
3'	0.74	t (7.3)	13.5	2'	3', 2', 1', 1
1"	5.88	d (3.1)	94.7	2"ax, 2"eq	
2"ax	2.22	ddd (15.3, 8.1, 4.0)	35.0	2"eq, 1", 3"	
2"eq	2.49	ddd (15.3, 3.0, 1.1)	35.0	2"ax, 3", 1"	
3"	4.10-4.15	m	65.7	2"ax, 2"eq, 4', 3"OH	
3"OH	4.93	d (10.0)	-	3"	
4"	3.25	dd (10.0, 3.5)	72.2	5", 3"	
5"	3.60-3.66	m	66.1	5"-CH_3, 4"	
5"-CH_3	1.24	d (6.2)	17.7	5"	5'', 4'', 3''
$CDCl_3$	7.24	s	77.2	-	

Table 6. Jadomycin DNV 3a*R* NMR data.

Position	δ ^{1}H (ppm)	Multiplicity (J(Hz))	δ ^{13}C (ppm)	COSY	TOCSY
1	5.21-5.26	m	59.3	1'	
2	-	-		-	
3a	6.47	s	87.3	-	
3b	-	-		-	
4	6.84-6.86	m	115.9	5-CH_3	
5	-	-	141.5	-	
5-CH_3	2.34	s	21.2	4, 6	
6	6.87-6.90	m	120.9	5-CH_3	
7	-	-		-	
7-OH	10.49	s	-	-	
7a	-	-		-	
7b	-	-		-	
8	-	-		-	
8a	-	-		-	
9	7.94	dd (8.1, 1.1)	121.6	10	
10	7.71	t (8.1)	136.4	9, 11	
11	7.44	dd (8.1, 1.1)	119.2	10	
12	-	-	155.5	-	
12a	-	-		-	
13	-	-		-	
13a	-	-		-	
1'	1.91-2.05	m	33.1	1, 2'	
2'	1.57-1.65	m	18.4	1', 3'	
3'	0.98	t (7.3)	14.0	2'	2', 1', 1
1"	5.91	d (3.1)	94.7	2"ax, 2"eq	
2"ax	2.20	ddd (15.3, 8.1, 4.0)	35.0	2"eq, 1", 3"	
2"eq	2.52	ddd (15.3, 3.0, 1.1)	35.0	2"ax, 3", 1"	
3"	4.10-4.15	m	65.7	2"ax, 2"eq, 4', 3"OH	
3"OH	4.72	d (10.0)	-	3"	
4"	3.25	dd (10.0, 3.5)	72.2	5", 3"	
5"	3.67-3.74	m	66.1	5"-CH_3, 4"	
5"-CH_3	1.25	d (6.2)	17.7	5"	5", 4", 3''
$CDCl_3$	7.24	s	77.2	-	

Jadomycin DNL (35). A shaker growth using D-norleucine MSM medium (2 L) was performed according to the procedure described previously, yielding crude jadomycin (84 mg). This was loaded onto minimal ISOLEUTE®HM-N sorbant using DCM and purified by automated normal phase silica gel flash chromatography (22 cm x 4.0 cm) using a gradient of DCM to MeOH (50%) at a flow rate of 45 mLmin^{-1}. Relevant jadomycin fractions were combined, dried (47 mg), applied to a reversed-phase C18 column (15 cm x 3.0 cm), and eluted using a gradient of water to acetonitrile (50%) at a flow rate of 25 mLmin^{-1}. Relevant fractions were combined, dried (29 mg), and further purified by prep TLC (5:95 MeOH:DCM), affording the pure secondary metabolite as a deep purple powder (17 mg, 8.5 mg/L), as a mixture of diastereomers (3a*S*/3a*R* = 56/34). R_f = 0.45 (10:90 MeOH:DCM). NMR data follows. For labeling of various protons, see Figure 27. UV-Vis

(1.82 x 10^{-5} M, MeOH): λ_{max} (ε) = 291 (18425), 315 (20570), 492 (3025), 529 (2475), 669 (715), 754 (275). LRMS (ESI^+): Q1 found 572 m/z $[M+Na]^+$, 550 m/z $[M+H]^+$; MS/MS (550) found 420 $[M+H\text{-digitoxose}]^+$, 306 $[M+H\text{-}C_6H_{10}O_2]^+$. HRMS ($ESI^+$) for $C_{30}H_{31}N_1O_9Na$ $[M+Na]^+$: calcd = 572.1891; found = 572.1884.

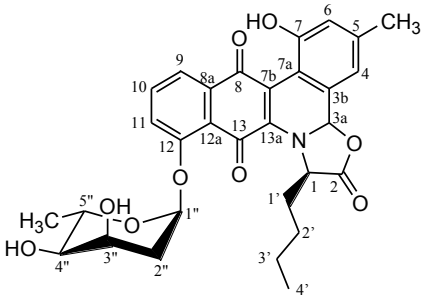


Figure 27. Atom-labeled structure of compound **35**.

Table 7. Jadomycin DNL 3a*S* NMR data.

Position	δ ^{1}H (ppm)	Multiplicity (J(Hz))	δ ^{13}C (ppm)	COSY	TOCSY
1	5.26-5.30	m	59.3	1'	
2	-	-		-	
3a	6.10	s	87.2	-	
3b	-	-	110.2	-	
4	6.83-6.86	m	114.2	5-CH_3	
5	-	-	142.9	-	
5-CH_3	2.34	d (7.9)	21.1	4, 6	
6	6.92-6.94	m	120.9	5-CH_3	
7	-	-	153.5	-	
7-OH	10.27	s	-	-	
7a	-	-		-	
7b	-	-		-	
8	-	-	183.2	-	
8a	-	-		-	
9	7.98	dd (8.1, 1.1)	121.8	10	
10	7.72	t (8.1)	136.5	9, 11	
11	7.49	dd (8.1, 1.1)	119.9	10	
12	-	-	155.4	-	
12a	-	-		-	
13	-	-		-	
13a	-	-		-	
1'	1.88-1.98	m	32.9	1, 2'	
2'	0.85-1.07	m	26.0	1', 3'	
3'	1.09-1.22	m	21.8	2', 4’	4', 2', 1', 1
4'	0.65	t (7.3)	13.5	3’	3', 2', 1', 1
1"	5.88	d (3.1)	94.7	2"ax, 2"eq	
2"ax	2.22	ddd (15.3, 8.1, 4.0)	35.0	2"eq, 1", 3"	
2"eq	2.49	ddd (15.3, 3.0, 1.1)	35.0	2"ax, 3", 1"	
3"	4.14-4.20	m	65.7	2"ax, 2"eq, 4', 3"OH'	
3"OH	4.94	d (10.0)	-	3"	
4"	3.24	dd (9.9, 3.5)	72.2	5", 3"	
5"	3.71-3.77	m	66.3	5"-CH_3, 4"	
5"-CH_3	1.24	d (6.3)	17.7	5"	5’’, 4’’
$CDCl_3$	7.24	s	77.2	-	

Table 8. Jadomycin DNL 3a*R* NMR data.

Position	δ ^{1}H (ppm)	Multiplicity (J(Hz))	δ ^{13}C (ppm)	COSY	TOCSY
1	5.26-5.30	m	59.3	1'	
2	-	-		-	
3a	6.47	s	87.4	-	
3b	-	-		-	
4	6.88-6.9	m	116.1	5-CH_3	
5	-	-	141.5	-	
5-CH_3	2.34	d (7.9)	21.1	4, 6	
6	6.92-6.93	m	120.9	5-CH_3	
7	-	-		-	
7-OH	10.46	s	-	-	
7a	-	-		-	
7b	-	-		-	
8	-	-		-	
8a	-	-		-	
9	7.93	dd (8.1, 1.1)	121.3	10	
10	7.71	t (8.1)	136.5	9, 11	
11	7.44	dd (8.1, 1.1)	119.2	10	
12	-	-	155.4	-	
12a	-	-		-	
13	-	-		-	
13a	-	-		-	
1'	2.00-2.13	m	30.7	1, 2'	
2'	1.54-1.67	m	26.9	1', 3'	
3'	1.36-1.46	m	22.7	2', 4'	4', 2', 1', 1
4'	0.91	t (7.3)	13.7	3'	3', 2', 1', 1
1"	5.90	d (3.1)	94.7	2"ax, 2"eq	
2"ax	2.20	ddd (15.3, 8.1, 4.0)	35.0	2"eq, 1", 3"	
2"eq	2.52	ddd (15.3, 3.0, 1.1)	35.0	2"ax, 3", 1"	
3"	4.14-4.20	m	65.7	2"ax, 2"eq, 4', 3"OH'	
3"OH	4.72	d (10.0)	-	3"	
4"	3.24	dd (9.9, 3.5)	72.2	5", 3"	
5"	3.64-3.70	m	66.3	5"-CH_3, 4"	
5"-CH_3	1.25	d (6.3)	17.7	5"	5", 4"
$CDCl_3$	7.24	s	77.2	-	

Jadomycin *O*PS (28). A shaker growth was performed according to the procedure described previously, using **41** culture medium (2 L; 30 mM). The resulting crude material (120 mg) was used without further purification for reaction with azides. For the purposes of characterization and biological testing, crude material was purified by prep TLC (5:95 MeOH:DCM), affording the pure secondary metabolite as a deep purple powder (6.0 mg from 30 mg crude, 12 mg/L), as a mixture of diastereomers (3a*S*/3a*R* = 64/20). R_f = 0.54 (10:90 MeOH:DCM). NMR data follows. For labeling of various protons, see Figure 28. UV-Vis (1.78 x 10^{-5} M, MeOH): λ_{max} (ε) = 278 (19710), 376 (7525), 447 (3257), 528 (1516), 634 (898), 755 (337). LRMS (ESI^+): Q1 found 562 m/z

$[M+H]^+$; MS/MS (562) found 432 $[M+H\text{-digitoxose}]^+$, 306 $[M+H\text{-}C_6H_{10}O_2]^+$. HRMS ($ESI^+$) for $C_{30}H_{27}N_1O_{10}Na$ $[M+Na]^+$: calcd = 584.1527; found = 584.1539.

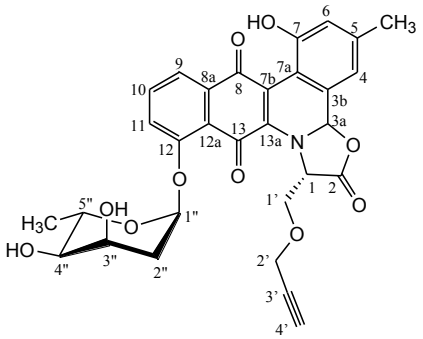


Figure 28. Atom-labeled structure of compound **28**.

Table 9. Jadomycin *O*PS 3a*S* NMR data.

Position	**δ ^{1}H** (ppm)	**Multiplicity (J**(Hz)**)**	**δ ^{13}C** (ppm)	**COSY**
1	5.41	t (2.1)	59.4	1'
2	-	-	170.1	-
3a	6.51	s	88.8	-
3b	-	-		-
4	6.88	s	115.2	-
5	-	-	143.6	-
5-CH_3	2.39	s	21.3	-
6	6.93	s	120.8	-
7	-	-	155.3	-
7-OH	10.62	s	-	-
7a	-	-	111.5	-
7b	-	-	136.5	-
8	-	-	183.3	-
8a	-	-		-
9	7.99	d (7.6)	120.3	10
10	7.75	t (7.6)	136.6	9, 11
11	7.50	d (7.6)	119.2	10
12	-	-	156.1	-
12a	-	-	119.0	-
13	-	-		-
13a	-	-		-
1' CH_{2a}	4.26	dd (10.5, 2.1)	68.9	1
1' CH_{2b}	4.13-4.16	m	68.9	1
2' CH_{2a}	4.21	dd (16.3, 2.4)	58.6	4'
2' CH_{2b}	4.10	dd (16.3, 2.4)	58.6	4'
3'	-	-	79.6	-
4'	2.20	t (2.4)	74.8	2'
1''	5.90	d (3.1)	94.6	2''ax, 2''eq
2''ax	2.25-2.27	m	35.1	2''eq, 1'', 3''
2''eq	2.46-2.49	m	35.0	2''ax, 3'', 1''
3''	4.10-4.14	m	66.0	2''ax, 2''eq, 4', 3''OH
3''OH	4.91	d (10.5)	-	3''
4''	3.30-3.34	m	72.2	5'', 3''
5''	3.70-3.74	m	66.0	5''-CH_3, 4''
5''-CH_3	1.28	d (6.3)	17.8	5''
$CDCl_3$	7.24	s	77.2	-

Table 10. Jadomycin *O*PS 3a*R* NMR data.

Position	δ ^{1}H (ppm)	Multiplicity (J(Hz))	δ ^{13}C (ppm)	COSY
1	5.45	t (2.1)	65.0	1’
2	-	-	170.1	-
3a	6.29	s	87.8	-
3b	-	-		-
4	6.84	s	114.2	-
5	-	-	143.6	-
5-CH_3	2.39	s	21.3	-
6	6.91	s	120.8	-
7	-	-	155.3	-
7-OH	10.04	s	-	-
7a	-	-	111.5	-
7b	-	-		-
8	-	-	183.0	-
8a	-	-	136.4	-
9	8.02	d (7.6)	118.4	10
10	7.77	t (7.6)	136.6	9, 11
11	7.54	d (7.6)	126.8	10
12	-	-	156.6	-
12a	-	-	118.9	-
13	-	-		-
13a	-	-		-
1' CH_{2a}	4.04	dd (10.5, 2.1)	57.9	1
1' CH_{2b}	3.84-3.87	m	57.9	1
2' CH_{2a}	3.78-3.81	m	68.0	4'
2' CH_{2b}	3.75-3.78	m	68.0	4'
3'	-	-	79.6	-
4'	2.18	t (2.4)	76.7	2'
1"	5.94	d (3.1)	98.1	2"ax, 2"eq
2"ax	2.23-2.25	m	35.1	2"eq, 1", 3"
2"eq	2.49-2.51	m	35.0	2"ax, 3", 1"
3"	4.11-4.15	m	66.0	2"ax, 2"eq, 4', 3"OH
3"OH	5.08	d (10.5)	-	3"
4"	3.28-3.32	m	73.6	5", 3"
5"	3.74-3.79	m	66.0	5"-CH_3, 4"
5"-CH_3	1.29	d (6.3)	17.8	5"
$CDCl_3$	7.24	s	77.2	-

4.9 Preparation of Jadomycin Triazoles via Cycloaddition Reactions (compounds 63, 64, 65, 66, 67, 68, 69, 70):

Jadomycin *O*-(1-(2,3,4,6,-Tetra-*O*-Acetyl-α-D-Mannopyranosyl)-1H-1,2,3-Triazol-4-yl)methyl-S (63). Crude **28** (34 mg, <60 μmol) was dissolved in ethanol (1 mL). Compound **52** (35 mg, 94 μmol) was added, and the mixture was stirred vigorously. To this mixture was added a solution of $CuSO_4 \cdot 5H_2O$ (7 mg, 28 μmol) and L-ascorbic acid (10 mg, 57 μmol) in dH_2O (1 mL). At 10 minutes, the reaction was determined complete by TLC analysis. The reaction was diluted

with dH_2O (5 mL), then extracted with ethyl acetate (2 x 25 mL). The combined organic layers were washed with dH_2O (25 mL) and brine (25 mL), dried with Na_2SO_4, and evaporated to dryness. Volatiles were removed *in vacuo*, affording a purple solid (67 mg). The product was purified by prep TLC (5:95 MeOH:DCM), affording a shiny purple solid (5.0 mg, >9.0% yield), as a mixture of diastereomers (3a*S*/3a*R* = 41/35). R_f = 0.54 (10:90 MeOH:DCM). NMR data follows. For labeling of various protons, see Figure 29. UV-Vis (1.07×10^{-5} M, MeOH): λ_{max} (ε) = 282 (26830), 371 (10844), 442 (4394), 528 (2337), 667 (935), 756 (374). LRMS (ESI^+): Q1 found 957 m/z $[M+Na]^+$, 935 m/z $[M+H]^+$; MS/MS (935) found 805 [M+H-digitoxose]$^+$, 306 $[M+H-C_6H_{10}O_2]^+$. HRMS (ESI^+) for $C_{44}H_{46}N_4O_{19}Na$ $[M+Na]^+$: calcd = 957.2648; found = 957.2632.

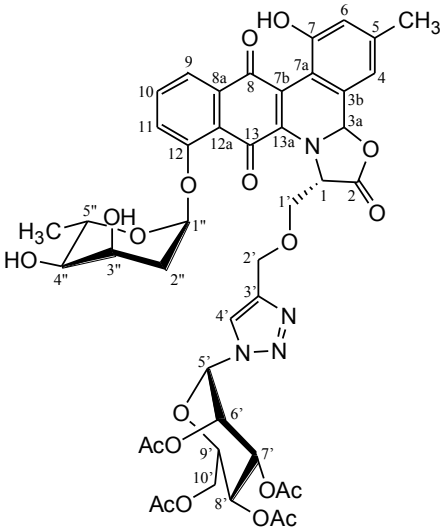


Figure 29. Atom-labeled structure of compound **63**.

Table 11. Jadomycin *O*-(1-(2,3,4,6,-tetra-*O*-acetyl-α-D-mannopyranosyl)-1H-1,2,3-triazol-4-yl)methyl-S 3a*S* NMR data.

Position	**δ ^{1}H** (ppm)	**Multiplicity (J**(Hz)**)**	**δ ^{13}C** (ppm)	**COSY**
1	5.30-5.32	m	59.6	1'
2	-	-	171.2	-
3a	6.19	s	87.4	-
3b	-	-		-
4	6.75	s	115.9	-
5	-	-	143.6	-
5-CH_3	2.28	s	21.2	-
6	6.81	s	120.8	-
7	-	-	155.4	-
7-OH	10.57	s	-	-
7a	-	-	111.8	-
7b	-	-		-
8	-	-	183.3	-
8a	-	-	136.3	-
9	7.84	d (7.6)	121.0	10
10	7.65	t (7.6)	136.7	9, 11
11	7.63	d (7.6)	119.9	10
12	-	-	156.5	-
12a	-	-	119.2	-
13	-	-		-
13a	-	-		-
1' CH_{2a}	4.15	dd (10.4, 2.2)	69.6	1
1' CH_{2b}	4.04	dd (10.4, 2.2)	69.6	1
2' CH_{2a}	4.61	d (12.9)	64.2	-
2' CH_{2b}	4.57	d (12.9)	64.2	-
3'	-	-	146.0	-
4'	7.60	s	122.8	-
5'	5.79	d (1.9)	83.9	-
6'	5.30-5.31	m	67.8	7'
7'	5.66	dd (10.1, 3.7)	68.8	6', 8'
8'	5.28	t (10.1)	65.6	7', 9'
9'	3.61	dd (10.1, 5.0, 2.1)	71.7	8', 10' CH_{2a}, 10' CH_{2b}
10' CH_{2a}	4.28	dd (12.6, 4.9)	61.5	9', 10' CH_{2b}
10' CH_{2b}	4.16	dd (12.6, 4.9)	61.5	9', 10' CH_{2a}
4 (OAc CH_3)	1.98, 1.98, 2.03, 2.13	s x 4	20.7	-
4 (OAc C=O)	-	-	171.2	-
1"	5.86-5.88	m	94.8	2"ax, 2"eq
2"ax	2.14-2.16	m	34.9	2"eq, 1", 3"
2"eq	2.48-2.51	m	34.9	2"ax, 3", 1"
3"	4.05-4.10	m	65.9	2"ax, 2"eq, 4', 3"OH
3"OH	4.83	d (10.5)	-	3"
4"	3.19-3.22	m	72.1	5", 3"
5"	3.54-3.59	m	66.0	5"-CH_3, 4"
5"-CH_3	1.19	d (6.3)	17.8	5"
$CDCl_3$	7.24	s	77.2	-

Table 12. Jadomycin *O*-(1-(2,3,4,6,-tetra-*O*-acetyl-α-D-mannopyranosyl)-1H-1,2,3-triazol-4-yl)methyl-S 3a*R* NMR data.

Position	**δ ^{1}H** (ppm)	**Multiplicity (J**(Hz)**)**	**δ ^{13}C** (ppm)	**COSY**
1	5.37	t (2.2)	60.2	1'
2	-	-	169.4	-
3a	6.33	s	88.7	-
3b	-	-		-
4	6.78	s	115.9	-
5	-	-	144.2	-
5-CH_3	2.31	s	21.2	-
6	6.81	s	120.8	-
7	-	-	155.0	-
7-OH	9.90	s	-	-
7a	-	-	111.8	-
7b	-	-		-
8	-	-	185.3	-
8a	-	-	136.3	-
9	7.91	d (7.6)	121.6	10
10	7.63	t (7.6)	136.7	9, 11
11	7.44	d (7.6)	119.9	10
12	-	-	157.0	-
12a	-	-	118.4	-
13	-	-		-
13a	-	-		-
1' CH_{2a}	4.00	dd (12.4, 2.2)	69.0	1
1' CH_{2b}	3.91	dd (12.4, 2.2)	69.0	1
2' CH_{2a}	4.33	d (12.9)	63.9	-
2' CH_{2b}	4.21	d (12.9)	63.9	-
3'	-	-	146.3	-
4'	6.55	s	122.2	-
5'	5.77	d (1.9)	83.9	-
6'	5.25-5.26	m	67.8	7'
7'	5.71	dd (10.1, 3.3)	68.8	6', 8'
8'	5.24	t (10.1)	65.5	7', 9'
9'	3.47	dd (10.1, 5.0, 2.1)	71.3	8', 10' CH_{2a}, 10' CH_{2b}
10' CH_{2a}	3.99	dd (12.6, 4.9)	61.4	9', 10' CH_{2b}
10' CH_{2b}	3.91	dd (12.6, 4.9)	61.4	9', 10' CH_{2a}
4 (OAc CH_3)	1.93, 1.94, 2.01, 2.18	s x 4	20.7	-
4 (OAc C=O)	-	-	170.8	-
1"	5.88-5.91	m	94.8	2"ax, 2"eq
2"ax	2.12-2.14	m	34.9	2"eq, 1", 3"
2"eq	2.50-2.53	m	34.9	2"ax, 3", 1"
3"	4.05-4.10	m	65.9	2"ax, 2"eq, 4', 3"OH
3"OH	5.02	d (10.5)	-	3"
4"	3.20-3.23	m	72.1	5", 3"
5"	3.65-3.71	m	66.0	5"-CH_3, 4"
5"-CH_3	1.21	d (6.3)	17.8	5"
$CDCl_3$	7.24	s	77.2	-

Jadomycin *O*-(1-(2,3,4,6,-Tetra-*O*-Acetyl-β-D-Mannopyranosyl)-1H-1,2,3-Triazol-4-yl)methyl-S (64). Crude **28** (33 mg, <59 μmol) was dissolved in ethanol (1 mL). Compound **53** (35 mg, 94 μmol) was added, and the mixture was stirred vigorously. To this mixture was added a solution of $CuSO_4 \cdot 5H_2O$ (7 mg, 28 μmol) and L-ascorbic acid (10 mg, 57 μmol) in dH_2O (1 mL). At 10 minutes, the reaction was determined complete by TLC analysis. The reaction was diluted with dH_2O (5 mL), then extracted with ethyl acetate (2 x 25 mL). The combined organic layers were washed with dH_2O (25 mL) and brine (25 mL), dried with Na_2SO_4, and evaporated to dryness. Volatiles were removed *in vacuo*, affording a purple solid (66 mg). The product was purified by prep TLC (5:95 MeOH:DCM), affording a shiny purple solid (4.8 mg, >9.1% yield), as a mixture of diastereomers (3a*S*/3a*R* = 65/22). R_f = 0.49 (10:90 MeOH:DCM). NMR data follows. For labeling of various protons, see Figure 30. UV-Vis (1.07 x 10^{-5} M, MeOH): λ_{max} (ε) = 280 (36459), 371 (10751), 442 (5422), 528 (3459), 669 (1589), 758 (841). LRMS (ESI^+): Q1 found 957 m/z $[M+Na]^+$, 935 m/z $[M+H]^+$; MS/MS (935) found 805 $[M+H\text{-digitoxose}]^+$, 306 $[M+H\text{-}C_6H_{10}O_2]^+$. HRMS ($ESI^+$) for $C_{44}H_{46}N_4O_{19}Na$ $[M+Na]^+$: calcd = 957.2648; found = 957.2641.

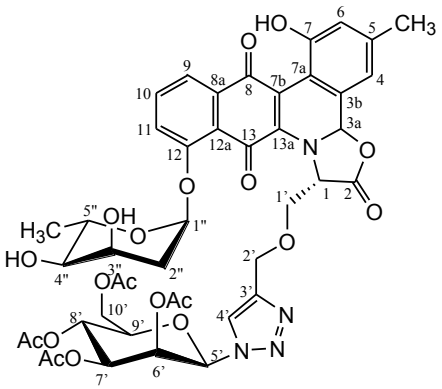


Figure 30. Atom-labeled structure of compound **64**.

Table 13. Jadomycin *O*-(1-(2,3,4,6,-tetra-*O*-acetyl-β-D-mannopyranosyl)-1H-1,2,3-triazol-4-yl)methyl-S 3a*S* NMR data.

Position	**δ ^{1}H** (ppm)	**Multiplicity (J**(Hz)**)**	**δ ^{13}C** (ppm)	**COSY**
1	5.34	t (2.2)	59.9	1'
2	-	-	171.4	-
3a	6.34	s	88.8	-
3b	-	-		-
4	6.73	s	115.1	-
5	-	-	143.7	-
5-CH_3	2.28	s	21.2	-
6	6.82	s	120.9	-
7	-	-	155.3	-
7-OH	10.60	s	-	-
7a	-	-	112.1	-
7b	-	-		-
8	-	-	183.0	-
8a	-	-	136.3	-
9	7.87	d (7.6)	121.2	10
10	7.66	t (7.6)	136.7	9, 11
11	7.43	d (7.6)	119.3	10
12	-	-	156.3	-
12a	-	-	119.2	-
13	-	-		-
13a	-	-		-
1' CH_{2a}	4.17	dd (12.4, 2.2)	70.1	1
1' CH_{2b}	4.03	dd (12.4, 2.2)	70.1	1
2' CH_{2a}	4.61	d (12.7)	64.8	-
2' CH_{2b}	4.51	d (12.7)	64.8	-
3'	-	-	145.2	-
4'	7.66	s	121.2	-
5'	5.98	d (1.1)	84.6	6'
6'	5.54	dd (3.3, 1.1)	68.6	5', 7'
7'	5.17	dd (10.3, 3.3)	70.7	6', 8'
8'	5.28	t (10.3)	64.7	7', 9'
9'	3.87	dd (10.1, 5.0, 2.1)	75.7	8', 10' CH_{2a}, 10' CH_{2b}
10' CH_{2a}	4.13-4.27	m	61.9	9', 10' CH_{2b}
10' CH_{2b}	4.13-4.27	m	61.9	9', 10' CH_{2a}
4 (OAc CH_3)	1.93, 1.99, 2.03, 2.04	s x 4	20.6	-
4 (OAc C=O)	-	-	170.9	-
1"	5.82-5.85	m	94.7	2"ax, 2"eq
2"ax	2.15-2.17	m	35.0	2"eq, 1", 3"
2"eq	2.41-2.45	m	35.0	2"ax, 3", 1"
3"	4.03-4.08	m	65.9	2"ax, 2"eq, 4', 3"OH
3"OH	4.80	d (10.5)	-	3"
4"	3.18-3.23	m	72.2	5", 3"
5"	3.67	dq (9.8, 6.2)	66.0	5"-CH_3, 4"
5"-CH_3	1.19	d (6.3)	17.8	5"
$CDCl_3$	7.24	s	77.2	-

Table 14. Jadomycin *O*-(1-(2,3,4,6,-tetra-*O*-acetyl-β-D-mannopyranosyl)-1H-1,2,3-triazol-4-yl)methyl-S 3a*R* NMR data.

Position	**δ ^{1}H** (ppm)	**Multiplicity (J**(Hz)**)**	**δ ^{13}C** (ppm)	**COSY**
1	5.36	t (2.2)	60.3	1'
2	-	-	171.0	-
3a	6.18	s	87.5	-
3b	-	-		-
4	6.71	s	115.1	-
5	-	-	143.7	-
5-CH_3	2.28	s	21.2	-
6	6.84	s	120.9	-
7	-	-	155.1	-
7-OH	10.07	s	-	-
7a	-	-	112.1	-
7b	-	-		-
8	-	-	183.4	-
8a	-	-	136.5	-
9	7.92	d (7.6)	121.5	10
10	7.68	t (7.6)	136.7	9, 11
11	7.46	d (7.6)	119.7	10
12	-	-	156.3	-
12a	-	-	118.5	-
13	-	-		-
13a	-	-		-
1' CH_{2a}	3.99	dd (12.4, 2.2)	69.4	1
1' CH_{2b}	3.73	dd (12.4, 2.2)	69.4	1
2' CH_{2a}	4.22	d (12.7)	64.5	-
2' CH_{2b}	4.17	d (12.7)	64.5	-
3'	-	-	145.5	-
4'	7.11	s	118.3	-
5'	5.90	d (1.1)	84.4	6'
6'	5.59	dd (3.3, 1.1)	68.3	5', 7'
7'	5.18	dd (10.3, 3.3)	70.5	6', 8'
8'	5.30	t (10.3)	64.7	7', 9'
9'	3.89	dd (10.1, 5.0, 2.1)	75.7	8', 10' CH_{2a}, 10' CH_{2b}
10' CH_{2a}	4.13-4.27	m	61.9	9', 10' CH_{2b}
10' CH_{2b}	4.13-4.27	m	61.9	9', 10' CH_{2a}
4 (OAc CH_3)	1.93, 1.98, 2.03, 2.04	s x 4	20.6	-
4 (OAc C=O)	-	-	170.9	-
1"	5.82-5.85	m	94.7	2"ax, 2"eq
2"ax	2.13-2.15	m	35.0	2"eq, 1", 3"
2"eq	2.45-2.48	m	35.0	2"ax, 3", 1"
3"	4.03-4.08	m	65.9	2"ax, 2"eq, 4', 3"OH
3"OH	4.94	d (10.5)	-	3"
4"	3.18-3.23	m	72.2	5", 3"
5"	3.61	dq (9.8, 6.2)	65.9	5"-CH_3, 4"
5"-CH_3	1.20	d (6.3)	17.8	5"
$CDCl_3$	7.24	s	77.2	-

Jadomycin *O*-(1-(2,3,4-Tri-*O*-Acetyl-α-L-Rhamnopyranosyl)-1H-1,2,3-Triazol-4-yl)methyl-S (65). Crude **28** (30 mg, <53 μmol) was dissolved in ethanol (1 mL). Compound **57** (26 mg, 82 μmol) was added, and the mixture was stirred vigorously. To this mixture was added a solution of $CuSO_4 \cdot 5H_2O$ (7 mg, 28 μmol) and L-ascorbic acid (10 mg, 57 μmol) in dH_2O (1 mL). At 10 minutes, the reaction was determined complete by TLC analysis. The reaction was diluted with dH_2O (5 mL), then extracted with ethyl acetate (2 x 25 mL). The combined organic layers were washed with dH_2O (25 mL) and brine (25 mL), dried with Na_2SO_4, and evaporated to dryness. Volatiles were removed *in vacuo*, affording a purple solid (60 mg). The product was purified by prep TLC (5:95 MeOH:DCM), affording a shiny purple solid (7.1 mg, >15% yield), as a mixture of diastereomers (3a*S*/3a*R* = 52/30). R_f = 0.45 (10:90 MeOH:DCM). NMR data follows. For labeling of various protons, see Figure 31. UV-Vis (1.14 x 10^{-5} M, MeOH): λ_{max} (ε) = 280 (29421), 369 (10311), 438 (5025), 528 (2826), 666 (1214), 768 (614). LRMS (ESI^+): Q1 found 899 m/z $[M+Na]^+$, 877 m/z $[M+H]^+$; MS/MS (877) found 747 [M+H-digitoxose]$^+$, 306 $[M+H-C_6H_{10}O_2]^+$. HRMS (ESI^+) for $C_{42}H_{45}N_4O_{17}$ $[M+H]^+$: calcd = 877.2774; found = 877.2770.

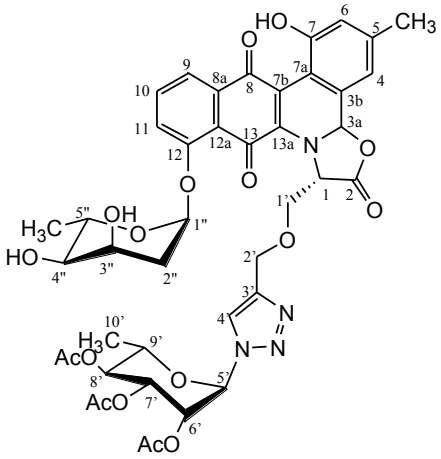


Figure 31. Atom-labeled structure of compound **65**.

Table 15. Jadomycin *O*-(1-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-1H-1,2,3-triazol-4-yl)methyl-S 3a*S* NMR data.

Position	**δ ^{1}H** (ppm)	**Multiplicity (J**(Hz)**)**	**δ ^{13}C** (ppm)	**COSY**
1	5.34	t (2.2)	59.8	1'
2	-	-	171.4	-
3a	6.33	s	88.6	-
3b	-	-		-
4	6.76	s	114.9	-
5	-	-	143.7	-
5-CH_3	2.28	s	21.1	-
6	6.82	s	120.8	-
7	-	-	155.3	-
7-OH	10.63	s	-	-
7a	-	-	111.7	-
7b	-	-		-
8	-	-	183.5	-
8a	-	-	136.3	-
9	7.88	d (7.6)	121.1	10
10	7.65	t (7.6)	136.8	9, 11
11	7.44	d (7.6)	119.5	10
12	-	-	155.9	-
12a	-	-	119.1	-
13	-	-		-
13a	-	-		-
1' CH_{2a}	4.19	dd (12.4, 2.2)	70.1	1
1' CH_{2b}	4.14	dd (12.4, 2.2)	70.1	1
2' CH_{2a}	4.59	d (12.9)	64.7	-
2' CH_{2b}	4.61	d (12.9)	64.7	-
3'	-	-	145.7	-
4'	7.58	s	121.3	-
5'	5.77	d (1.9)	83.6	-
6'	5.78	dd (3.3, 1.9)	68.4	7'
7'	5.62	dd (9.3, 3.3)	68.6	6', 8'
8'	5.07	t (9.3)	69.3	7', 9'
9'	3.48	dq (9.1, 6.3)	69.8	8', 10'
10'	1.10	d (6.3)	17.9	9'
3 (OAc CH_3)	1.97, 2.02, 2.09	s x 3	20.8	-
3 (OAc C=O)	-	-	171.3	-
1"	5.86-5.88	m	94.6	2"ax, 2"eq
2"ax	2.16-2.18	m	35.1	2"eq, 1", 3"
2"eq	2.39-2.42	m	35.1	2"ax, 3", 1"
3"	4.03-4.09	m	65.9	2"ax, 2"eq, 4', 3"OH
3"OH	4.82	d (10.5)	-	3"
4"	3.18-3.23	m	72.1	5", 3"
5"	3.66-3.73	m	66.0	5"-CH_3, 4"
5"-CH_3	1.18-1.20	m	17.6	5"
$CDCl_3$	7.24	s	77.2	-

Table 16. Jadomycin *O*-(1-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-1H-1,2,3-triazol-4-yl)methyl-S 3a*R* NMR data.

Position	**δ ^{1}H** (ppm)	**Multiplicity (J**(Hz)**)**	**δ ^{13}C** (ppm)	**COSY**
1	5.37	t (2.2)	60.3	1'
2	-	-	171.4	-
3a	6.20	s	87.5	-
3b	-	-		-
4	6.74	s	114.9	-
5	-	-	144.3	-
5-CH_3	2.28	s	21.0	-
6	6.80	s	120.8	-
7	-	-	155.2	-
7-OH	10.00	s	-	-
7a	-	-	111.7	-
7b	-	-		-
8	-	-	183.5	-
8a	-	-	136.1	-
9	7.93	d (7.6)	121.1	10
10	7.68	t (7.6)	136.8	9, 11
11	7.49	d (7.6)	120.6	10
12	-	-	155.8	-
12a	-	-	118.5	-
13	-	-		-
13a	-	-		-
1' CH_{2a}	3.98	dd (11.5, 2.2)	69.3	1
1' CH_{2b}	3.80	dd (11.5, 2.2)	69.3	1
2' CH_{2a}	4.37	d (12.9)	64.9	-
2' CH_{2b}	4.23	d (12.9)	64.9	-
3'	-	-	146.2	-
4'	6.65	s	121.6	-
5'	5.70	d (1.9)	83.4	-
6'	5.80	dd (3.3, 1.9)	68.8	7'
7'	5.70	dd (9.3, 3.3)	69.0	6', 8'
8'	5.05	t (10.3)	69.3	7', 9'
9'	3.53	dq (9.1, 6.3)	69.8	8', 10'
10'	1.04	d (6.3)	17.9	9'
3 (OAc CH_3)	1.96, 2.03, 2.15	s x 3	20.8	-
3 (OAc C=O)	-	-	171.0	-
1"	5.84-5.86	m	94.5	2"ax, 2"eq
2"ax	2.14-2.16	m	34.9	2"eq, 1", 3"
2"eq	2.45-2.48	m	34.9	2"ax, 3", 1"
3"	4.03-4.09	m	65.9	2"ax, 2"eq, 4', 3"OH
3"OH	4.95	d (10.5)	-	3"
4"	3.18-3.23	m	73.5	5", 3"
5"	3.59-3.64	m	66.0	5"-CH_3, 4"
5"-CH_3	1.18-1.20	m	17.6	5"
$CDCl_3$	7.24	s	77.2	-

Jadomycin *O*-(1-(2,3,4-Tri-*O*-Acetyl-β-L-Rhamnopyranosyl)-1H-1,2,3-Triazol-4-yl)methyl-S (66). Crude **28** (27 mg, <48 μmol) was dissolved in ethanol (1 mL). Compound **58** (18 mg, 57 μmol) was added, and the mixture was stirred vigorously. To this mixture was added a solution of $CuSO_4 \cdot 5H_2O$ (7 mg, 28 μmol) and L-ascorbic acid (10 mg, 57 μmol) in dH_2O (1 mL). At 10 minutes, the reaction was determined complete by TLC analysis. The reaction was diluted with dH_2O (5 mL), then extracted with ethyl acetate (2 x 25 mL). The combined organic layers were washed with dH_2O (25 mL) and brine (25 mL), dried with Na_2SO_4, and evaporated to dryness. Volatiles were removed *in vacuo*, affording a purple solid (52 mg). The product was purified by prep TLC (5:95 MeOH:DCM), affording a shiny purple solid (5.8 mg, >14% yield), as a mixture of diastereomers (3a*S*/3a*R* = 63/20). R_f = 0.43 (10:90 MeOH:DCM). NMR data follows. For labeling of various protons, see Figure 32. UV-Vis (1.14 x 10^{-5} M, MeOH): λ_{max} (ε) = 282 (27883), 369 (9294), 438 (4823), 529 (2630), 667 (1228), 771 (701). LRMS (ESI^+): Q1 found 899 m/z $[M+Na]^+$, 877 m/z $[M+H]^+$; MS/MS (877) found 747 [M+H-digitoxose]$^+$, 305 $[M+H-C_6H_{10}O_2]^+$. HRMS (ESI^+) for $C_{42}H_{44}N_4O_{17}Na$ $[M+Na]^+$: calcd = 899.2594; found = 899.2540.

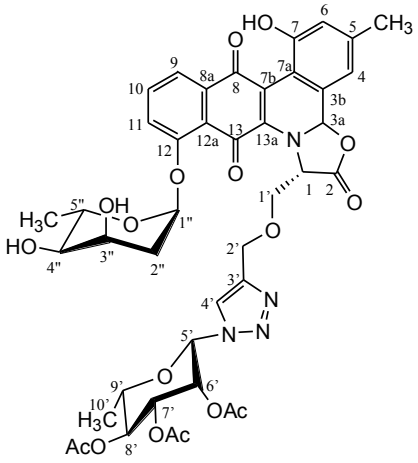


Figure 32. Atom-labeled structure of compound **66**.

Table 17. Jadomycin *O*-(1-(2,3,4-tri-*O*-acetyl-β-L-rhamnopyranosyl)-1H-1,2,3-triazol-4-yl)methyl-S 3a*S* NMR data.

Position	**δ ^{1}H** (ppm)	**Multiplicity (J**(Hz)**)**	**δ ^{13}C** (ppm)	**COSY**
1	5.25	t (2.2)	59.6	1'
2	-	-	171.1	-
3a	6.20	s	88.8	-
3b	-	-		-
4	6.74	s	114.9	-
5	-	-	143.4	-
5-CH_3	2.27	s	21.1	-
6	6.80	s	120.8	-
7	-	-	155.2	-
7-OH	10.50	s	-	-
7a	-	-	111.7	-
7b	-	-		-
8	-	-	183.4	-
8a	-	-	136.4	-
9	7.86	d (7.6)	120.7	10
10	7.72	t (7.6)	136.5	9, 11
11	7.49	d (7.6)	119.7	10
12	-	-	156.8	-
12a	-	-	119.1	-
13	-	-		-
13a	-	-		-
1' CH_{2a}	4.23	dd (12.1, 2.2)	68.9	1
1' CH_{2b}	4.11	dd (12.1, 2.2)	68.9	1
2' CH_{2a}	4.55	d (12.9)	63.6	-
2' CH_{2b}	4.45	d (12.9)	63.6	-
3'	-	-	145.2	-
4'	7.57	s	121.2	-
5'	5.44	d (1.1)	84.5	-
6'	5.50	dd (3.3, 1.9)	68.5	7'
7'	5.03-5.04	m	70.6	6', 8'
8'	5.00-5.04	m	69.3	7', 9'
9'	3.70-3.75	m	73.9	8', 10'
10'	1.16	d (6.3)	17.8	9'
3 (OAc CH_3)	1.92, 1.95, 2.03	s x 3	20.7	-
3 (OAc C=O)	-	-	170.9	-
1"	5.86-5.88	m	95.3	2"ax, 2"eq
2"ax	2.15-2.17	m	35.0	2"eq, 1", 3"
2"eq	2.56-2.59	m	35.0	2"ax, 3", 1"
3"	4.05-4.08	m	66.0	2"ax, 2"eq, 4', 3"OH
3"OH	4.76	d (10.5)	-	3"
4"	3.20-3.23	m	72.2	5", 3"
5"	3.70	dq (9.9, 6.2)	66.0	5"-CH_3, 4"
5"-CH_3	1.22	d (6.2)	17.5	5"
$CDCl_3$	7.24	s	77.2	-

Table 18. Jadomycin *O*-(1-(2,3,4-tri-*O*-acetyl-β-L-rhamnopyranosyl)-1H-1,2,3-triazol-4-yl)methyl-S 3a*R* NMR data.

Position	**δ ^{1}H** (ppm)	**Multiplicity (J**(Hz)**)**	**δ ^{13}C** (ppm)	**COSY**
1	5.32	t (2.2)	60.2	1'
2	-	-	171.1	-
3a	6.17	s	87.5	-
3b	-	-		-
4	6.74	s	114.2	-
5	-	-	144.0	-
5-CH_3	2.31	s	21.1	-
6	6.79	s	122.0	-
7	-	-	155.0	-
7-OH	9.97	s	-	-
7a	-	-	111.7	-
7b	-	-		-
8	-	-	183.4	-
8a	-	-	136.4	-
9	7.91	d (7.6)	121.1	10
10	7.71	t (7.6)	136.5	9, 11
11	7.49	d (7.6)	119.7	10
12	-	-	156.8	-
12a	-	-	118.6	-
13	-	-		-
13a	-	-		-
1' CH_{2a}	3.98	dd (12.1, 2.2)	68.2	1
1' CH_{2b}	3.86	dd (12.1, 2.2)	68.2	1
2' CH_{2a}	4.16	d (12.9)	63.2	-
2' CH_{2b}	4.13	d (12.9)	63.2	-
3'	-	-	145.1	-
4'	7.08	s	121.6	-
5'	5.55	d (1.1)	84.4	-
6'	5.48	dd (3.3, 1.9)	68.5	7'
7'	5.06-5.07	m	70.6	6', 8'
8'	5.04-5.07	m	69.6	7', 9'
9'	3.62	dq (9.6, 6.3)	73.9	8', 10'
10'	1.19	d (6.3)	17.8	9'
3 (OAc CH_3)	1.89, 1.92, 2.04	s x 3	20.7	-
3 (OAc C=O)	-	-	170.9	-
1"	5.84-5.86	m	94.5	2"ax, 2"eq
2"ax	2.13-2.15	m	35.0	2"eq, 1", 3"
2"eq	2.54-2.56	m	35.0	2"ax, 3", 1"
3"	4.05-4.08	m	66.0	2"ax, 2"eq, 4', 3"OH
3"OH	4.94	d (10.5)	-	3"
4"	3.18-3.21	m	72.2	5", 3"
5"	3.62	dq (9.6, 6.2)	66.0	5"-CH_3, 4"
5"-CH_3	1.33	d (6.2)	17.5	5"
$CDCl_3$	7.24	s	77.2	-

Jadomycin *O*-(1-Octyl-1H-1,2,3-Triazol-4-yl)methyl-S (67). Crude **28** (30 mg, <53 μmol) was dissolved in ethanol (1 mL). Compound **43** (1 g, 6.4 mmol) was added, and the mixture was stirred vigorously. To this mixture was added a solution of $CuSO_4 \cdot 5H_2O$ (7 mg, 28 μmol) and L-ascorbic acid (10 mg, 57 μmol) in dH_2O (1 mL). At 2 minutes, the reaction was determined complete by TLC analysis. The reaction was diluted with dH_2O (5 mL), then extracted with ethyl acetate (2 x 25 mL). The combined organic layers were washed with dH_2O (25 mL) and brine (25 mL), dried with Na_2SO_4, and evaporated to dryness. Volatiles were removed *in vacuo* overnight, affording a purple solid (28 mg). The product was purified by prep TLC (5:95 MeOH:DCM), affording a shiny purple solid (3.5 mg, >10% yield), as a mixture of diastereomers (3a*S*/3a*R* = 55/35). R_f = 0.43 (10:90 MeOH:DCM). NMR data follows. For labeling of various protons, see Figure 33. UV-Vis (1.39 x 10^{-5} M, MeOH): λ_{max} (ε) = 280 (29602), 378 (21091), 442 (6142), 529 (1480), 669 (1184), 759 (444). LRMS (ESI^+): Q1 found 718 m/z $[M+H]^+$; MS/MS (718) found 588 [M+H-digitoxose]$^+$, 306 $[M+H-C_6H_{10}O_2]^+$. HRMS (ESI^+) for $C_{38}H_{44}N_4O_{10}Na$ $[M+Na]^+$: calcd = 739.2950; found = 739.2886.

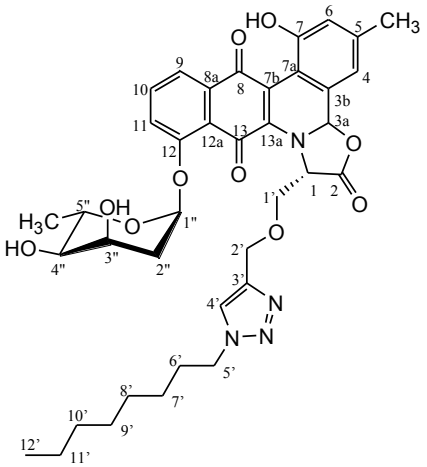


Figure 33. Atom-labeled structure of compound **67**.

Table 19. Jadomycin *O*-(1-octyl-1H-1,2,3-triazol-4-yl)methyl-S 3a*S* NMR data.

Position	**δ ^{1}H** (ppm)	**Multiplicity (J**(Hz)**)**	**δ ^{13}C** (ppm)	**COSY**
1	5.38-5.41	m	59.7	1'
2	-	-	171.1	-
3a	6.40	s	87.4	-
3b	-	-		-
4	6.84	s	115.1	-
5	-	-	143.9	-
5-CH_3	2.37	s	21.2	-
6	6.91	s	120.9	-
7	-	-	155.2	-
7-OH	10.66	s	-	-
7a	-	-	111.8	-
7b	-	-		-
8	-	-	183.4	-
8a	-	-	136.4	-
9	7.97	d (7.6)	121.1	10
10	7.75	t (7.6)	136.5	9, 11
11	7.53	d (7.6)	119.3	10
12	-	-	156.5	-
12a	-	-	119.0	-
13	-	-		-
13a	-	-		-
1' CH_{2a}	4.13-4.17	m	69.4	1
1' CH_{2b}	4.01-4.05	m	69.4	1
2' CH_{2a}	4.65	d (12.4)	64.4	-
2' CH_{2b}	4.62	d (12.4)	64.4	-
3'	-	-	145.2	-
4'	7.43	s	122.0	-
5'	3.74-3.80	m	50.2	6'
6'	1.68-1.74	m	30.0	5', 7'
7'	1.60-1.65	m	30.4	6', 8'
8'	1.52-1.59	m	24.5	7', 9'
9'	1.11-1.24	m	22.5-25.9	8', 10'
10'	1.11-1.24	m	22.5-25.9	9', 11'
11'	1.11-1.24	m	22.5-25.9	10', 12'
12'	0.87-0.91	m	14.1	11'
1''	5.92-5.94	m	94.9	2''ax, 2''eq
2''ax	2.25-2.27	m	34.9	2''eq, 1'', 3''
2''eq	2.56-2.58	m	34.9	2''ax, 3'', 1''
3''	4.11-4.17	m	66.0	2''ax, 2''eq, 4', 3''OH
3''OH	4.87	d (10.5)	-	3''
4''	3.27-3.31	m	72.2	5'', 3''
5''	3.64-3.68	m	66.0	5''-CH_3, 4''
5''-CH_3	1.11-1.24	m	17.8	5''
$CDCl_3$	7.24	s	77.2	-

Table 20. Jadomycin *O*-(1-octyl-1H-1,2,3-triazol-4-yl)methyl-S 3a*R* NMR data.

Position	**δ ^{1}H** (ppm)	**Multiplicity (J**(Hz)**)**	**δ ^{13}C** (ppm)	**COSY**
1	5.43-5.45	m	60.3	1’
2	-	-	171.1	-
3a	6.29	s	88.8	-
3b	-	-		-
4	6.88	s	114.9	-
5	-	-	143.5	-
5-CH_3	2.41	s	21.2	-
6	6.93	s	120.6	-
7	-	-	155.0	-
7-OH	10.19	s	-	-
7a	-	-	111.8	-
7b	-	-		-
8	-	-	184.8	-
8a	-	-	136.4	-
9	8.00	d (7.6)	121.4	10
10	7.77	t (7.6)	136.5	9, 11
11	7.55	d (7.6)	120.0	10
12	-	-	157.1	-
12a	-	-	118.5	-
13	-	-		-
13a	-	-		-
1' CH_{2a}	3.95-3.99	m	68.5	1
1' CH_{2b}	3.90	dd (10.4, 2.7)	68.5	1
2' CH_{2a}	4.37	d (12.4)	64.3	-
2' CH_{2b}	4.27	d (12.4)	64.3	-
3'	-	-	145.2	-
4'	6.50	s	121.3	-
5'	3.74-3.80	m	50.2	6’
6'	1.68-1.74	m	30.0	5’, 7’
7'	1.60-1.65	m	30.4	6’, 8’
8'	1.52-1.59	m	24.5	7’, 9’
9'	1.11-1.24	m	22.5-25.9	8’, 10’
10'	1.11-1.24	m	22.5-25.9	9’, 11’
11'	1.11-1.24	m	22.5-25.9	10’, 12’
12'	0.87-0.91	m	14.1	11’
1"	5.92-5.94	m	94.9	2"ax, 2"eq
2"ax	2.22-2.25	m	34.9	2"eq, 1", 3"
2"eq	2.59-2.61	m	34.9	2"ax, 3", 1"
3"	4.07-4.12	m	66.0	2"ax, 2"eq, 4', 3"OH
3"OH	5.04	d (10.5)	-	3"
4"	3.27-3.31	m	72.2	5", 3"
5"	3.67-3.72	m	66.0	5"-CH_3, 4"
5"-CH_3	1.11-1.24	m	17.8	5"
$CDCl_3$	7.24	s	77.2	-

Jadomycin *O*-(1-Benzyl-1H-1,2,3-Triazol-4-yl)methyl-S (68). Crude **28** (29 mg, <52 μmol) was dissolved in ethanol (1 mL). Compound **45** (1 g, 7.5 mmol) was added, and the mixture was stirred vigorously. To this mixture was added a solution of $CuSO_4 \cdot 5H_2O$ (7 mg, 28 μmol) and L-ascorbic acid (10 mg, 57 μmol) in dH_2O (1 mL). At 2 minutes, the reaction was determined complete by TLC analysis. The reaction was diluted with dH_2O (5 mL), then extracted with ethyl acetate (2 x 25 mL). The combined organic layers were washed with dH_2O (25 mL) and brine (25 mL), dried with Na_2SO_4, and evaporated to dryness. Volatiles were removed *in vacuo* overnight, affording a purple solid (31 mg). The product was purified by prep TLC (5:95 MeOH:DCM), affording a shiny purple solid (3.7 mg, >11% yield), as a mixture of diastereomers (3a*S*/3a*R* = 60/36). R_f = 0.46 (10:90 MeOH:DCM). NMR data follows. For labeling of various protons, see Figure 34. UV-Vis (1.44 x 10^{-5} M, MeOH): λ_{max} (ε) = 280 (31122), 378 (23133), 443 (6322), 529 (1459), 665 (1181), 756 (417). LRMS (ESI^+): Q1 found 717 m/z $[M+Na]^+$, 695 m/z $[M+H]^+$; MS/MS (665) found 565 $[M+H\text{-digitoxose}]^+$, 306 $[M+H\text{-}C_6H_{10}O_2]^+$. HRMS ($ESI^+$) for $C_{37}H_{35}N_4O_{10}$ $[M+H]^+$: calcd = 695.2348; found = 695.2328.

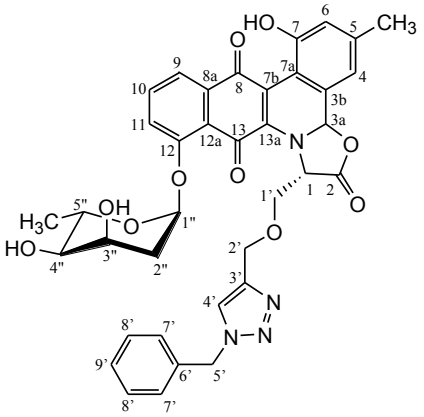


Figure 34. Atom-labeled structure of compound **68**.

Table 21. Jadomycin *O*-(1-benzyl-1H-1,2,3-triazol-4-yl)methyl-S 3a*S* NMR data.

Position	**δ ^{1}H** (ppm)	**Multiplicity (J**(Hz)**)**	**δ ^{13}C** (ppm)	**COSY**
1	5.29-5.31	m	61.0	1'
2	-	-	172.2	-
3a	6.24	s	89.1	-
3b	-	-		-
4	6.73	s	113.9	-
5	-	-	143.2	-
5-CH_3	2.29	s	21.4	-
6	6.82	s	120.8	-
7	-	-	155.3	-
7-OH	10.57	s	-	-
7a	-	-	111.0	-
7b	-	-		-
8	-	-	183.4	-
8a	-	-	136.5	-
9	7.89	d (7.6)	119.2	10
10	7.67	t (7.6)	136.7	9, 11
11	7.45	d (7.6)	124.2	10
12	-	-	156.5	-
12a	-	-	119.1	-
13	-	-		-
13a	-	-		-
1' CH_{2a}	4.16	dd (10.4, 2.2)	69.7	1
1' CH_{2b}	4.05	dd (10.4, 2.2)	69.7	1
2' CH_{2a}	4.52	d (12.4)	64.4	-
2' CH_{2b}	4.49	d (12.4)	64.4	-
3'	-	-	145.9	-
4'	7.24	s	124.3	-
5'	5.22	s	53.5	-
6'	-	-	115.8	-
7'	7.20-7.27	m	127.5	8', 9'
8'	7.07-7.10	m	132.7	7', 9'
9'	7.20-7.27	m	128.1	7', 8'
1"	5.84	d (3.1)	95.5	2"ax, 2"eq
2"ax	2.15-2.17	m	35.3	2"eq, 1", 3"
2"eq	2.49-2.52	m	35.3	2"ax, 3", 1"
3"	4.03-4.07	m	65.8	2"ax, 2"eq, 4', 3"OH
3"OH	4.78	d (10.5)	-	3"
4"	3.20-3.22	m	72.3	5", 3"
5"	3.56-3.63	m	63.9	5"-CH_3, 4"
5"-CH_3	1.19	d (6.3)	17.9	5"
$CDCl_3$	7.24	s	77.2	-

Table 22. Jadomycin *O*-(1-benzyl-1H-1,2,3-triazol-4-yl)methyl-S 3a*R* NMR data.

Position	**δ ^{1}H** (ppm)	**Multiplicity (J**(Hz)**)**	**δ ^{13}C** (ppm)	**COSY**
1	5.34	t (2.2)	60.2	1'
2	-	-	172.2	-
3a	6.18	s	87.1	-
3b	-	-		-
4	6.74	s	114.8	-
5	-	-	143.9	-
5-CH_3	2.22	s	21.1	-
6	6.78	s	120.8	-
7	-	-	155.4	-
7-OH	10.14	s	-	-
7a	-	-	111.4	-
7b	-	-		-
8	-	-	183.9	-
8a	-	-	136.8	-
9	7.92	d (7.6)	123.9	10
10	7.69	t (7.6)	138.1	9, 11
11	7.47	d (7.6)	126.2	10
12	-	-	156.2	-
12a	-	-	118.5	-
13	-	-		-
13a	-	-		-
1' CH_{2a}	3.99	dd (10.4, 2.2)	69.7	1
1' CH_{2b}	3.84	dd (10.4, 2.2)	69.7	1
2' CH_{2a}	4.24	d (12.4)	64.1	-
2' CH_{2b}	4.15	d (12.4)	64.1	-
3'	-	-	146.4	-
4'	6.47	s	124.3	-
5'	5.28	s	53.6	
6'	-	-	116.0	-
7'	7.20-7.27	m	127.9	8', 9'
8'	7.04-7.06	m	132.7	7', 9'
9'	7.20-7.27	m	128.4	7', 8'
1"	5.84	d (3.1)	94.5	2"ax, 2"eq
2"ax	2.13-2.15	m	35.0	2"eq, 1", 3"
2"eq	2.47-2.49	m	35.0	2"ax, 3", 1"
3"	4.03-4.07	m	66.1	2"ax, 2"eq, 4', 3"OH
3"OH	4.95	d (10.5)	-	3"
4"	3.18-3.20	m	72.6	5", 3"
5"	3.63-3.70	m	63.6	5"-CH_3, 4"
5"-CH_3	1.20	d (6.3)	17.9	5"
$CDCl_3$	7.24	s	77.2	-

Jadomycin *O*-(1-(2,3,4,6,-Tetra-*O*-Acetyl-β-D-Glucopyranosyl)-1H-1,2,3-Triazol-4-yl)methyl-S (69). Crude **28** (35 mg, <62 μmol) was dissolved in ethanol (1 mL). Compound **48** (22 mg, 59 μmol) was added, and the mixture was stirred vigorously. To this mixture was added a solution of $CuSO_4 \cdot 5H_2O$ (7 mg, 28 μmol) and L-ascorbic acid (10 mg, 57 μmol) in dH_2O (1 mL). At 10 minutes, the reaction was determined complete by TLC analysis. The reaction was diluted with dH_2O (5 mL), then extracted with ethyl acetate (2 x 25 mL). The combined organic layers were washed with dH_2O (25 mL) and brine (25 mL), dried with Na_2SO_4, and evaporated to dryness. Volatiles were removed *in vacuo*, affording a purple solid (59 mg). The product was purified by prep TLC (5:95 MeOH:DCM), affording a shiny purple solid (9.0 mg, >16% yield), as a mixture of diastereomers (3a*S*/3a*R* = 60/31). R_f = 0.50 (10:90 MeOH:DCM). NMR data follows. For labeling of various protons, see Figure 35. UV-Vis (1.07×10^{-5} M, MeOH): λ_{max} (ε) = 285 (31785), 371 (11031), 442 (5235), 529 (2898), 670 (935), 758 (374). LRMS (ESI^+): Q1 found 957 m/z $[M+Na]^+$, 935 m/z $[M+H]^+$; MS/MS (935) found 805 $[M+H\text{-digitoxose}]^+$, 306 $[M+H\text{-}C_6H_{10}O_2]^+$. HRMS ($ESI^+$) for $C_{44}H_{46}N_4O_{19}Na$ $[M+Na]^+$: calcd = 957.2648; found = 957.2651.

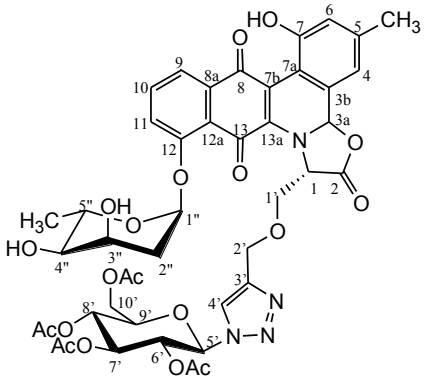


Figure 35. Atom-labeled structure of compound **69**.

Table 23. Jadomycin *O*-(1-(2,3,4,6,-tetra-*O*-acetyl-β-D-glucopyranosyl)-1H-1,2,3-triazol-4-yl)methyl-S 3a*S* NMR data.

Position	**δ ^{1}H** (ppm)	**Multiplicity (J**(Hz)**)**	**δ ^{13}C** (ppm)	**COSY**
1	5.33	t (2.2)	59.8	1'
2	-	-	171.5	-
3a	6.37	s	88.9	-
3b	-	-		-
4	6.78	s	115.3	-
5	-	-	143.5	-
5-CH_3	2.28	s	21.2	-
6	6.81	s	120.9	-
7	-	-	155.1	-
7-OH	10.53	s	-	-
7a	-	-	111.9	-
7b	-	-		-
8	-	-	183.5	-
8a	-	-	136.4	-
9	7.88	d (7.6)	121.2	10
10	7.66	t (7.6)	136.4	9, 11
11	7.42	d (7.6)	119.3	10
12	-	-	156.4	-
12a	-	-	119.4	-
13	-	-		-
13a	-	-		-
1' CH_{2a}	4.15-4.18	m	70.0	1
1' CH_{2b}	4.03-4.06	m	70.0	1
2' CH_{2a}	4.59	d (12.4)	64.7	-
2' CH_{2b}	4.54	d (12.4)	64.7	-
3'	-	-	145.9	-
4'	7.65	s	120.9	-
5'	5.69	d (8.9)	85.6	6'
6'	5.28-5.31	m	70.0	5', 7'
7'	5.30-5.33	m	72.4	6', 8'
8'	5.16	t (9.5)	67.5	7', 9'
9'	3.90	ddd (10.1, 5.0, 2.1)	75.0	8', 10' CH_{2a}, 10' CH_{2b}
10' CH_{2a}	4.20	dd (12.7, 4.9)	61.4	9', 10' CH_{2b}
10' CH_{2b}	4.29	dd (12.7, 4.9)	61.4	9', 10' CH_{2a}
4 (OAc CH_3)	1.66, 1.95, 2.00, 2.01	s x 4	20.6	-
4 (OAc C=O)	-	-	171.1	-
1"	5.82-5.85	m	94.6	2"ax, 2"eq
2"ax	2.15-2.17	m	35.0	2"eq, 1", 3"
2"eq	2.39-2.42	m	35.0	2"ax, 3", 1"
3"	4.03-4.07	m	65.9	2"ax, 2"eq, 4', 3"OH
3"OH	4.83	d (10.5)	-	3"
4"	3.18-3.20	m	72.2	5", 3"
5"	3.56-3.62	m	66.0	5"-CH_3, 4"
5"-CH_3	1.18	d (6.3)	17.8	5"
$CDCl_3$	7.24	s	77.2	-

Table 24. Jadomycin *O*-(1-(2,3,4,6,-tetra-*O*-acetyl-β-D-glucopyranosyl)-1H-1,2,3-triazol-4-yl)methyl-S 3a*R* NMR data.

Position	**δ ^{1}H** (ppm)	**Multiplicity (J**(Hz)**)**	**δ ^{13}C** (ppm)	**COSY**
1	5.39	t (2.2)	60.2	1'
2	-	-	171.3	-
3a	6.17	s	87.5	-
3b	-	-		-
4	6.90	s	114.8	-
5	-	-	144.1	-
5-CH_3	2.33	s	21.2	-
6	6.81	s	120.5	-
7	-	-	154.8	-
7-OH	10.16	s	-	-
7a	-	-	111.9	-
7b	-	-		-
8	-	-	184.7	-
8a	-	-	136.4	-
9	7.88	d (7.6)	121.2	10
10	7.65	t (7.6)	136.4	9, 11
11	7.42	d (7.6)	119.3	10
12	-	-	157.1	-
12a	-	-	118.5	-
13	-	-		-
13a	-	-		-
1' CH_{2a}	3.92	dd (10.6, 2.3)	69.3	1
1' CH_{2b}	3.67	dd (10.6, 2.3)	69.3	1
2' CH_{2a}	4.33	d (12.4)	64.4	-
2' CH_{2b}	4.19	d (12.4)	64.4	-
3'	-	-	146.3	-
4'	6.65	s	121.0	-
5'	5.53	d (8.9)	85.4	6'
6'	5.25	-	70.2	5', 7'
7'	5.24-5.28	m	72.5	6', 8'
8'	5.26-5.30	m	67.5	7', 9'
9'	3.91	dd (10.1, 5.0, 2.1)	75.0	8', 10' CH_{2a}, 10' CH_{2b}
10' CH_{2a}	4.20	dd (12.7, 4.9)	61.4	9', 10' CH_{2b}
10' CH_{2b}	4.29	dd (12.7, 4.9)	61.4	9', 10' CH_{2a}
4 (OAc CH_3)	1.59, 1.96, 2.01, 2.05	s x 4	20.6	-
4 (OAc C=O)	-	-	171.1	-
1"	5.80-5.82	m	94.6	2"ax, 2"eq
2"ax	2.12-2.15	m	35.0	2"eq, 1", 3"
2"eq	2.41-2.45	m	35.0	2"ax, 3", 1"
3"	4.04-4.08	m	65.9	2"ax, 2"eq, 4', 3"OH'
3"OH	4.95	d (10.5)	-	3"
4"	3.19-3.22	m	72.2	5", 3"
5"	3.65-3.70	m	65.9	5"-CH_3, 4"
5"-CH_3	1.20	d (6.3)	17.8	5"
$CDCl_3$	7.24	s	77.2	-

Jadomycin *O*-(1-β-D-Glucopyranosyl-1H-1,2,3-Triazol-4-yl)methyl-S (70). Pure **28** (5.5 mg, 9.8 μmol) was dissolved in ethanol (1 mL). Compound **59** (5.0 mg, 24 μmol) was added, and the mixture was stirred vigorously. To this mixture was added a solution of $CuSO_4 \cdot 5H_2O$ (0.7 mg, 2.8 μmol) and L-ascorbic acid (1.0 mg, 5.7 μmol) in dH_2O (1 mL). At 10 minutes, the reaction was determined complete by TLC analysis. The reaction was diluted with dH_2O (10 mL), then washed with ethyl acetate (10 mL). The aqueous layer was evaporated to dryness and volatiles were removed *in vacuo*, affording a purple solid (9.0 mg). The crude material was purified by automated reversed-phase C18 flash chromatography (8.0 x 1.5 cm) using a gradient of water to MeOH (50%) at a flow rate of 12 $mLmin^{-1}$. Relevant fractions were combined and solvent removed *in vacuo* to afford a shiny purple solid (4.0 mg, impure by NMR). This material was further purified by prep TLC (5:95 MeOH:DCM), affording a shiny purple solid (0.7 mg, impure by NMR). R_f = 0.20 (20:80 MeOH:DCM). Because the material was not pure, NMR data could not be unambiguously assigned. LRMS (ESI^+): Q1 found 789 m/z $[M+Na]^+$, 767 m/z $[M+H]^+$; MS/MS (767) found 637 $[M+H\text{-digitoxose}]^+$, 306 $[M+H\text{-}C_6H_{10}O_2]^+$. HRMS ($ESI^+$) for $C_{36}H_{38}N_4O_{15}Na_1$ $[M+Na]^+$: calcd = 789.2226; found = 789.2218.

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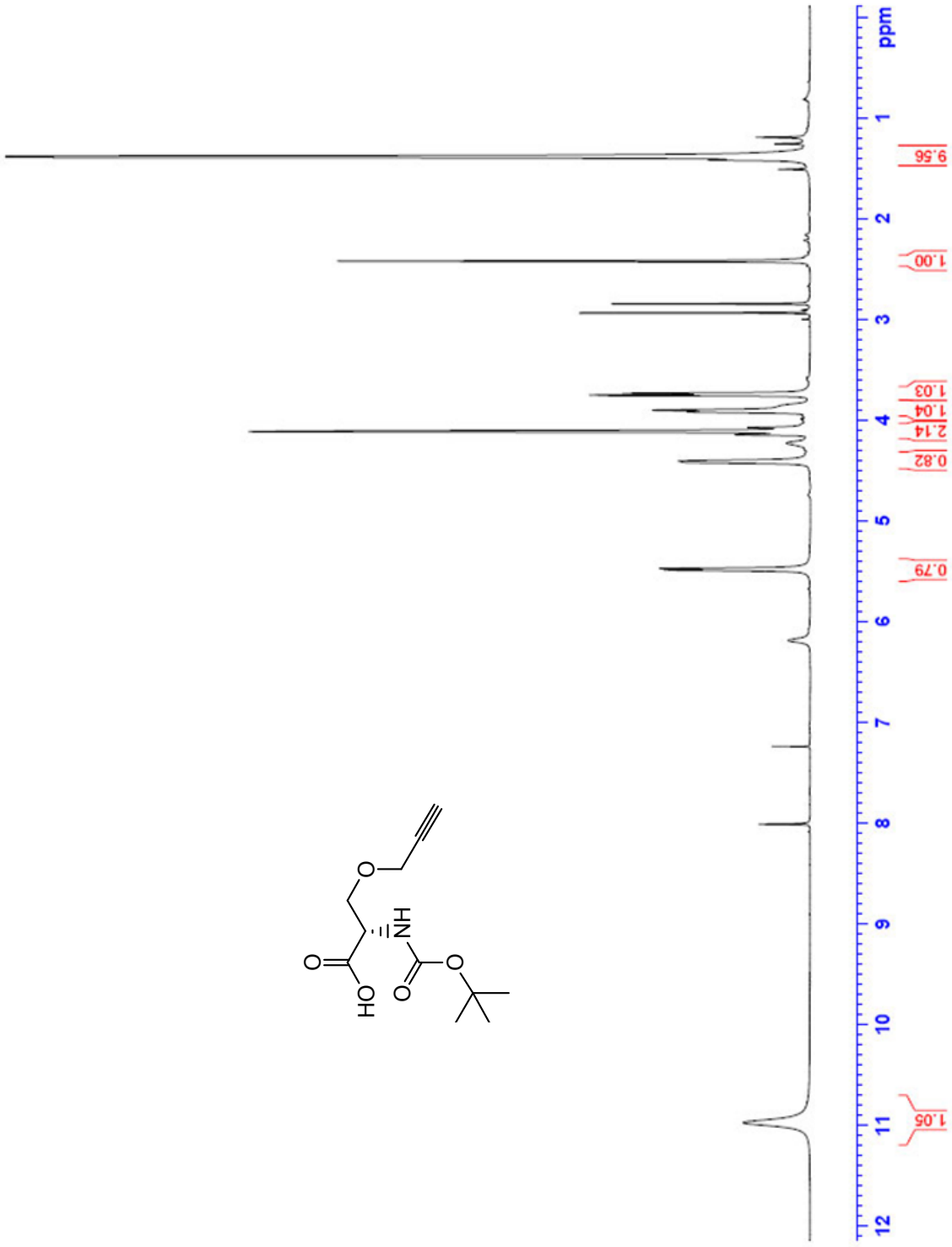
APPENDIX A: Spectral Data for Synthesized Serine Derivatives (Compounds 37, 39, 40)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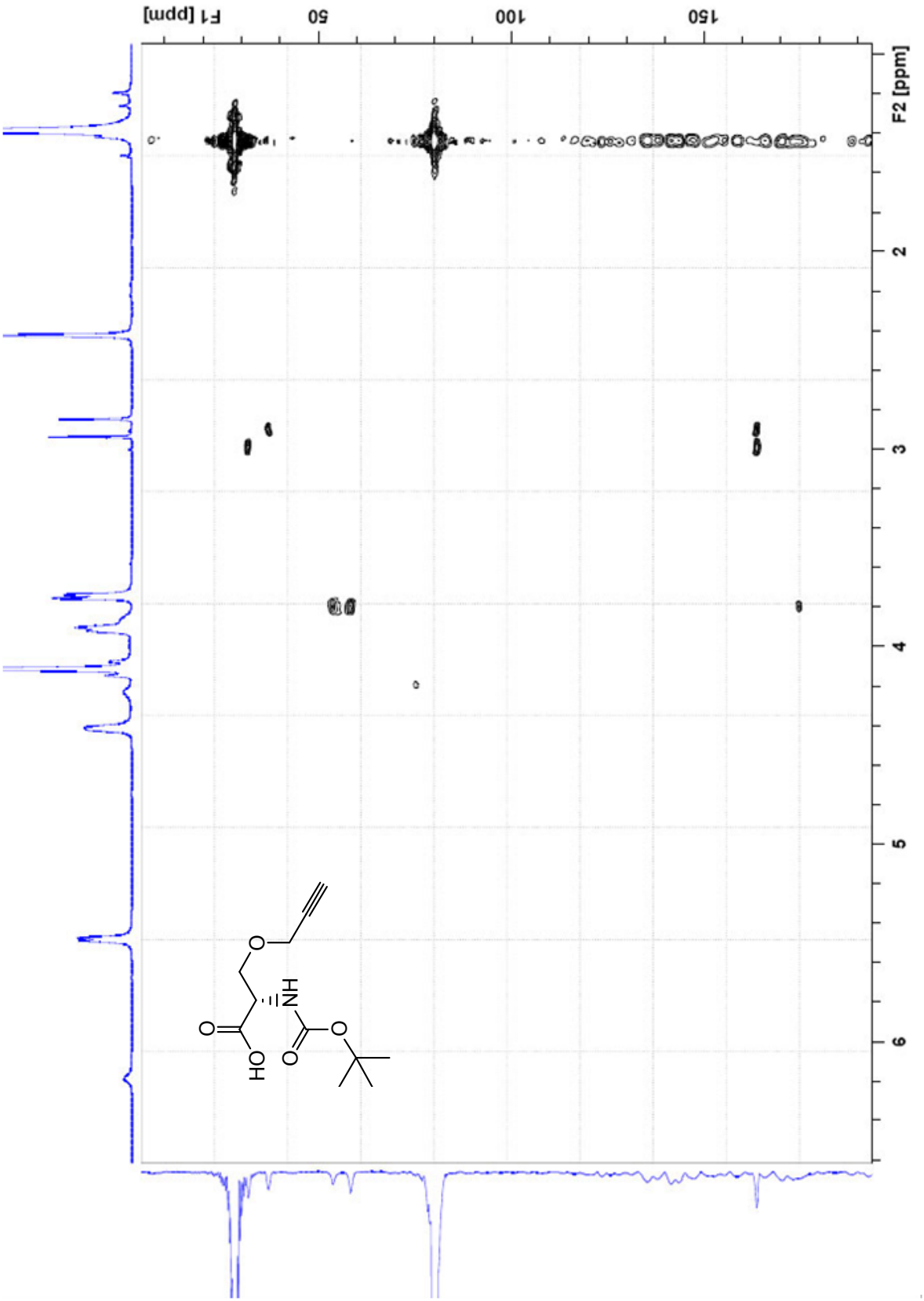
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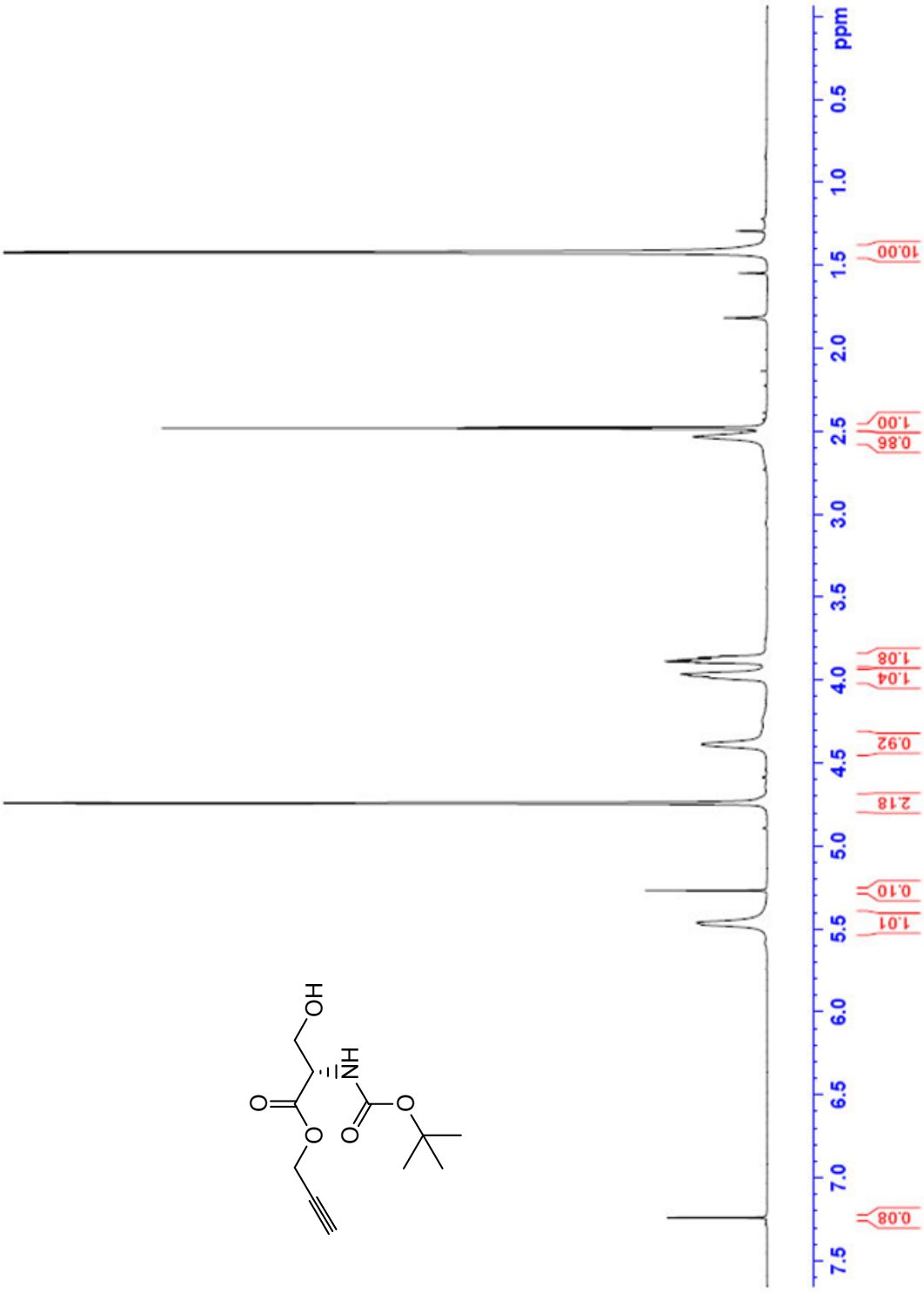
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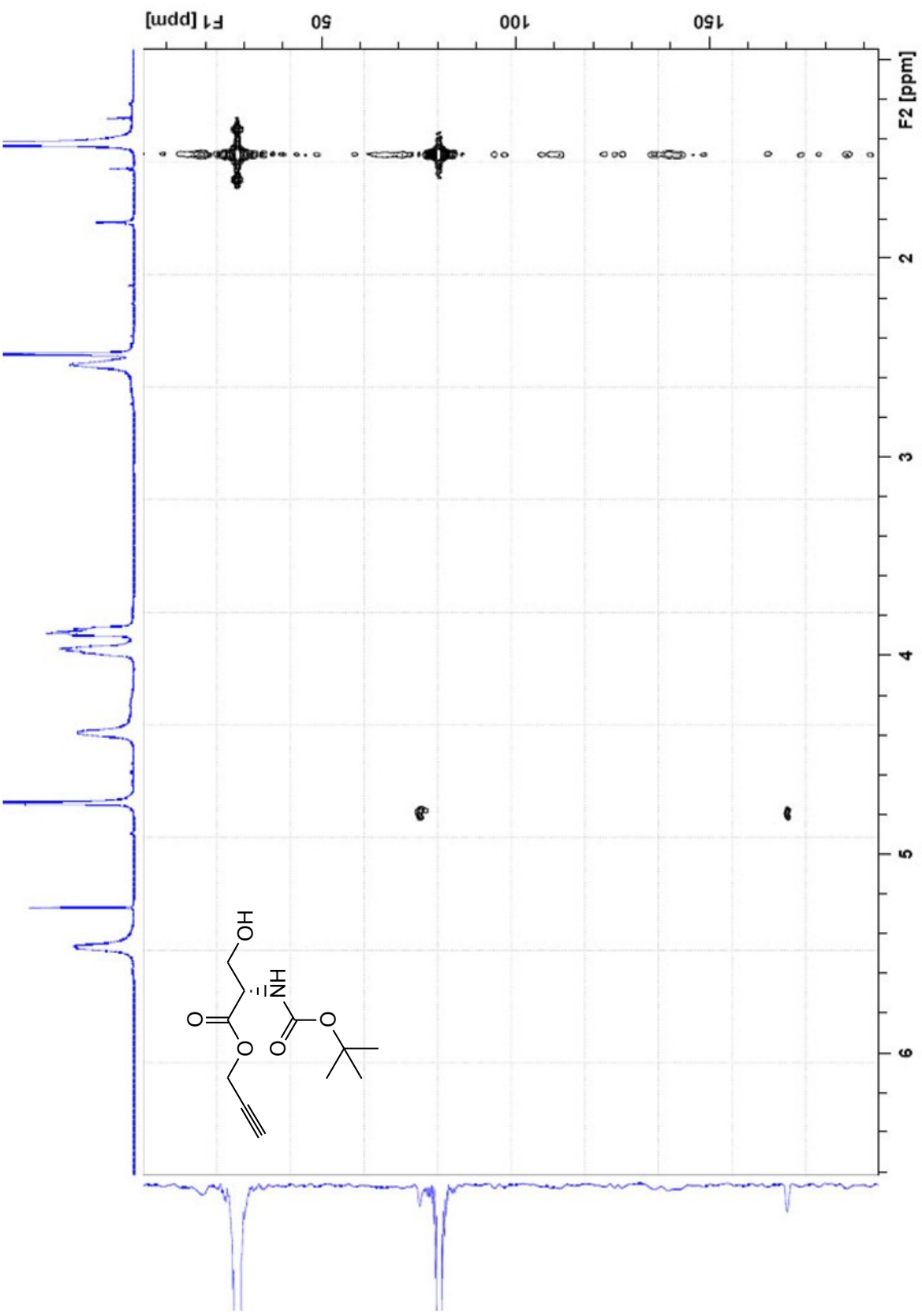
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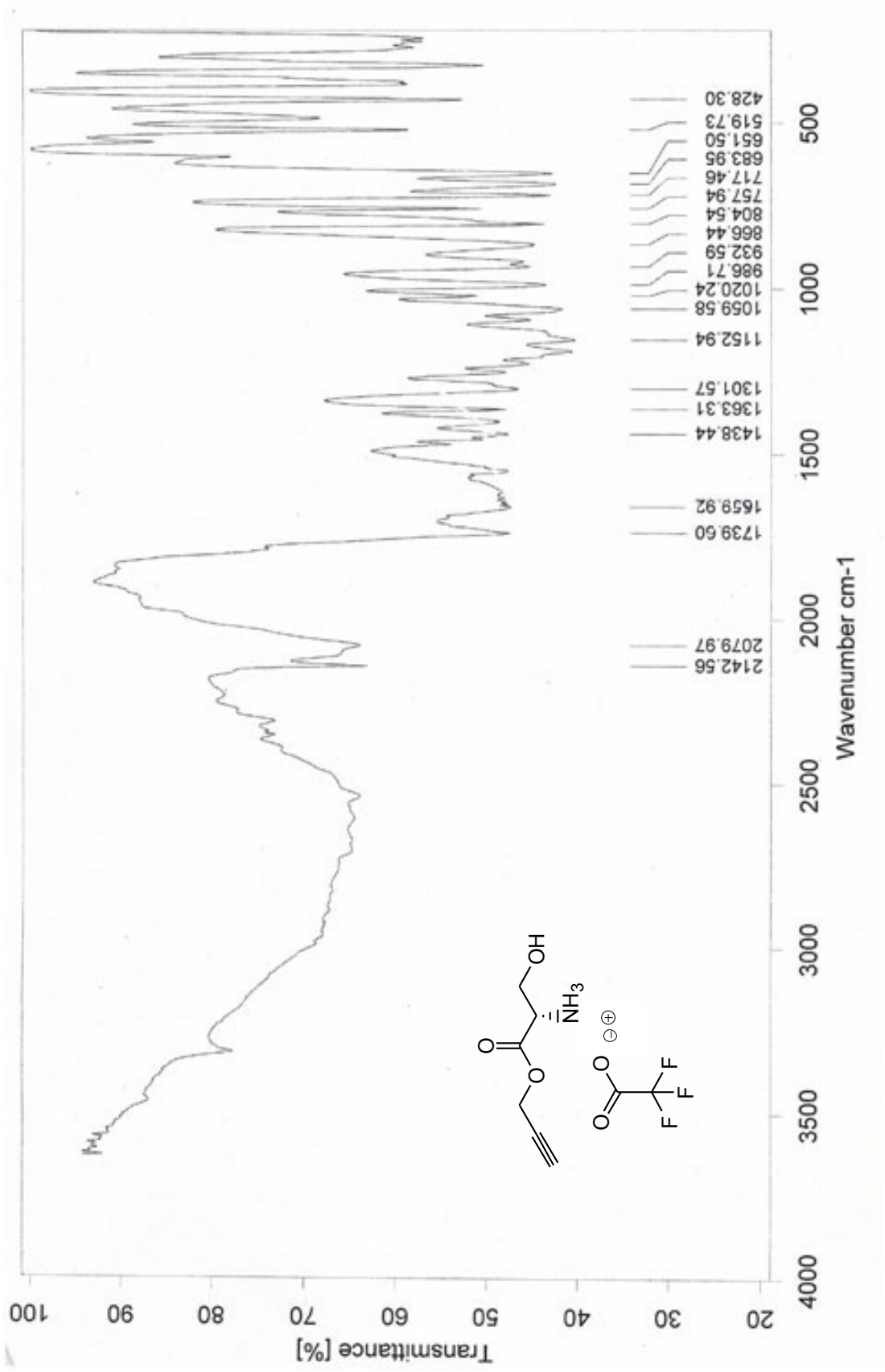
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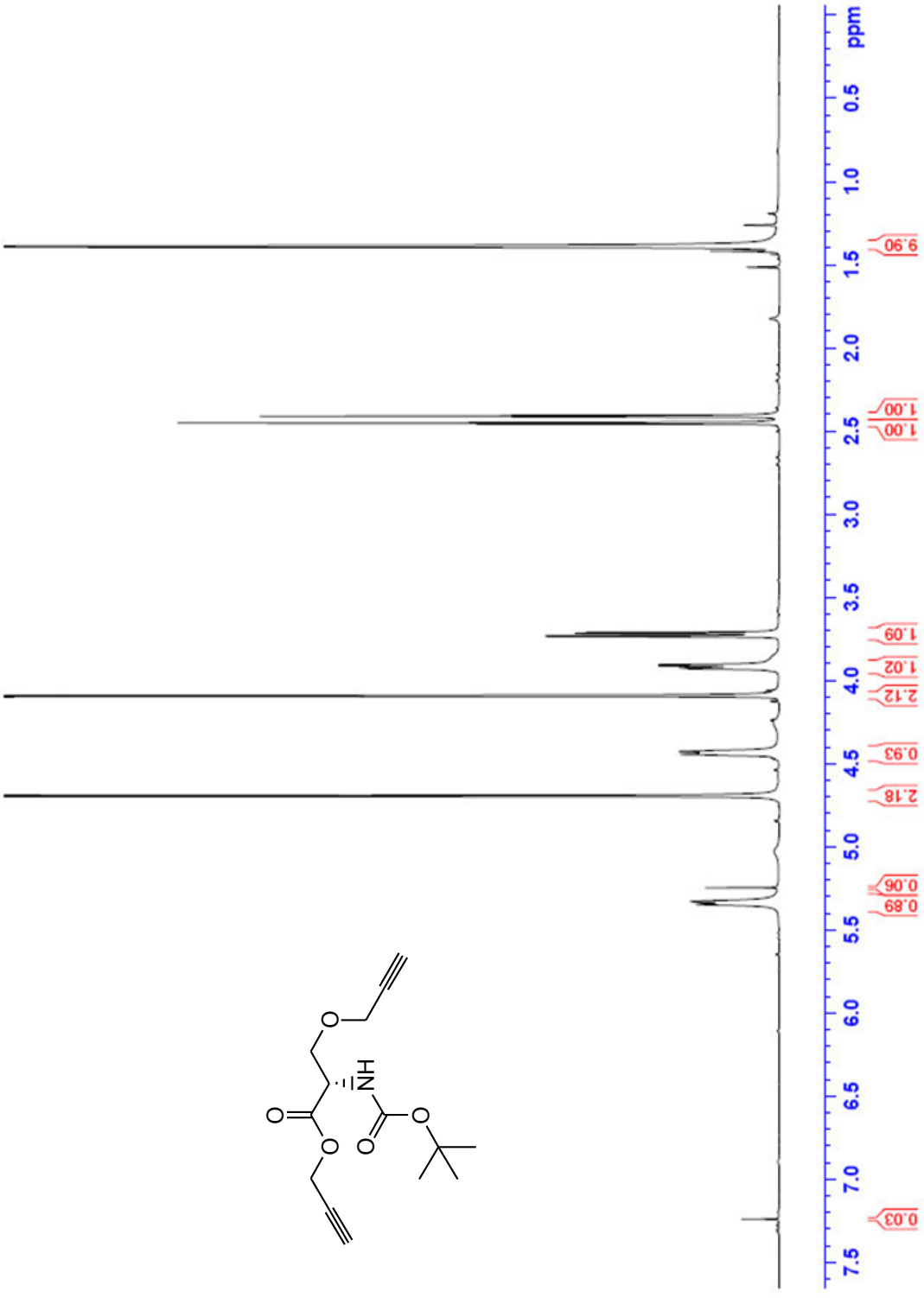
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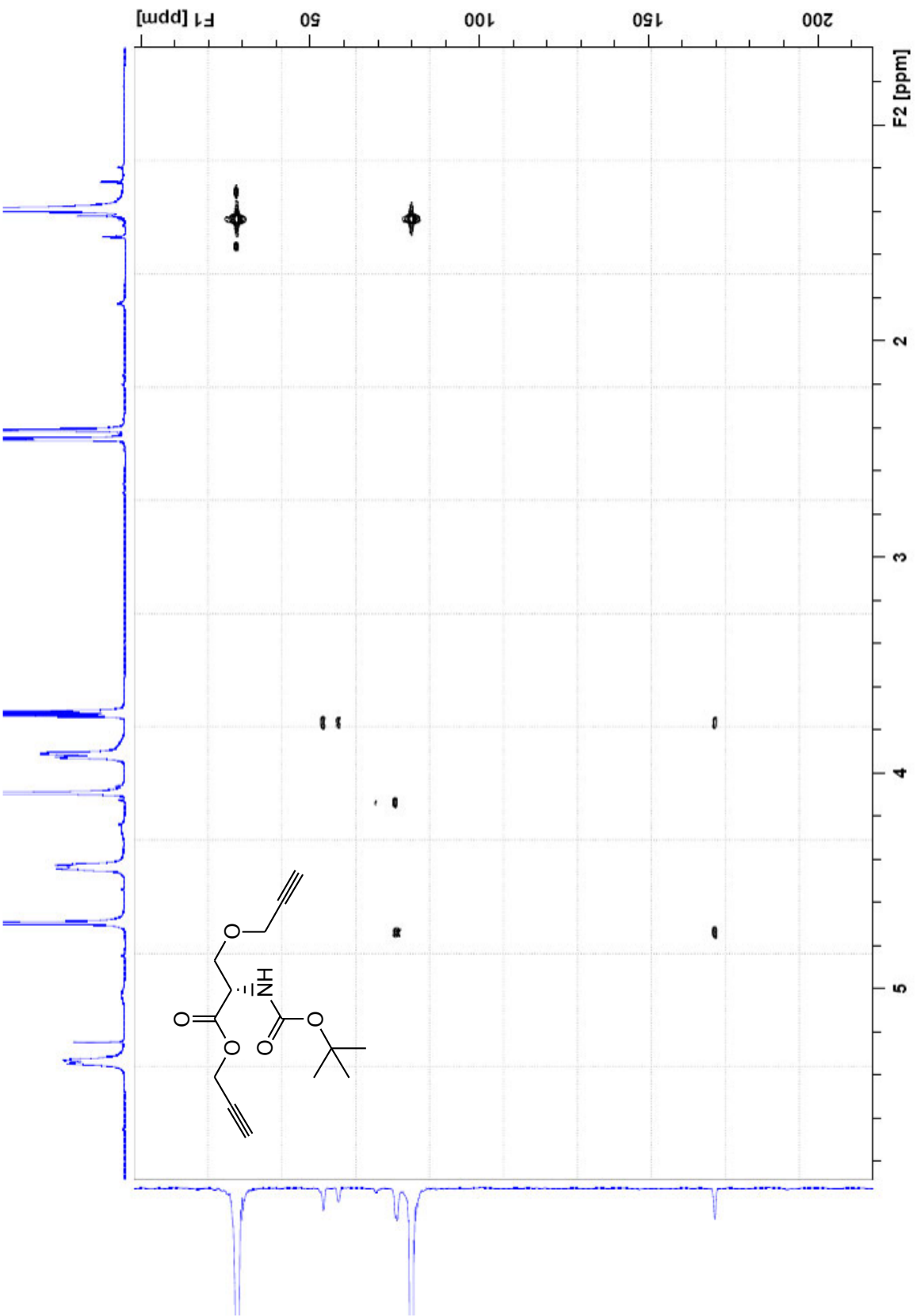
O
HN
O
O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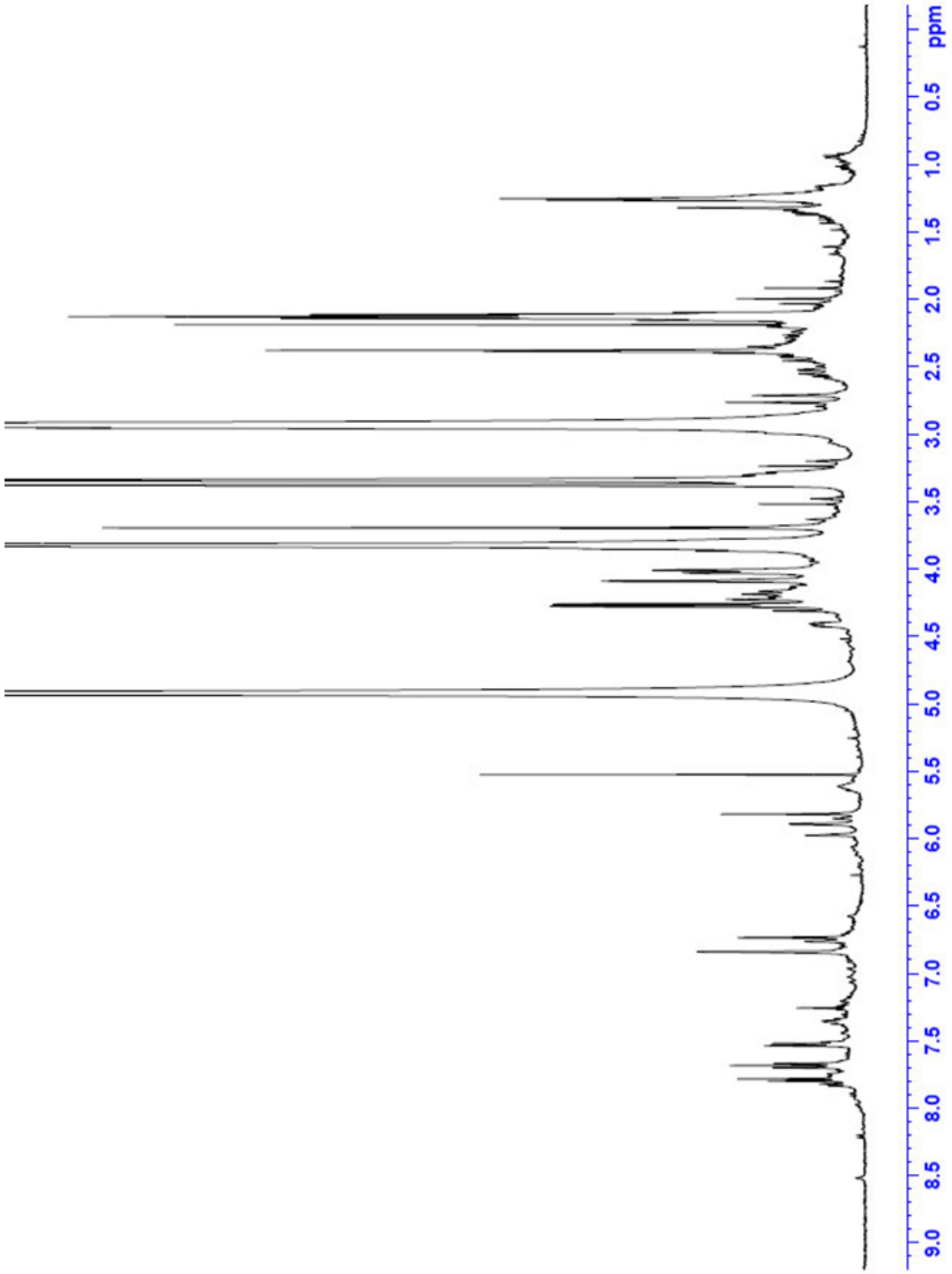
100
150
200
F2 [ppm]
2
3
4
5
HN
O
O
O
O
O
O

APPENDIX B: Comparative NMR Data for Samples of Crude 28 Obtained from 15 mM, 30 mM, 45 mM, and 60 mM 41 Culture Media

^{1}H-NMR (MeOD, 500 MHz) spectra of organic products obtained from culture media containing 15 mM, 30 mM, 45 mM, and 60 mM **41**. All samples were prepared by passing the culture media (25 mL) through a reversed-phase capture C18 column (6.0 cm x 2.5 cm; Biotage®), washing away aqueous-soluble components using distilled water (200 mL), eluting the bound organic material using methanol (50 mL), removing the methanol *in vacuo*, and dissolving the resulting solid material in MeOD (600 μL). All samples were run for 256 scans using identical NMR parameters, and the processed spectra were amplified by a factor of four.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8.0
7.5
7.0
6.5
6.0
5.5
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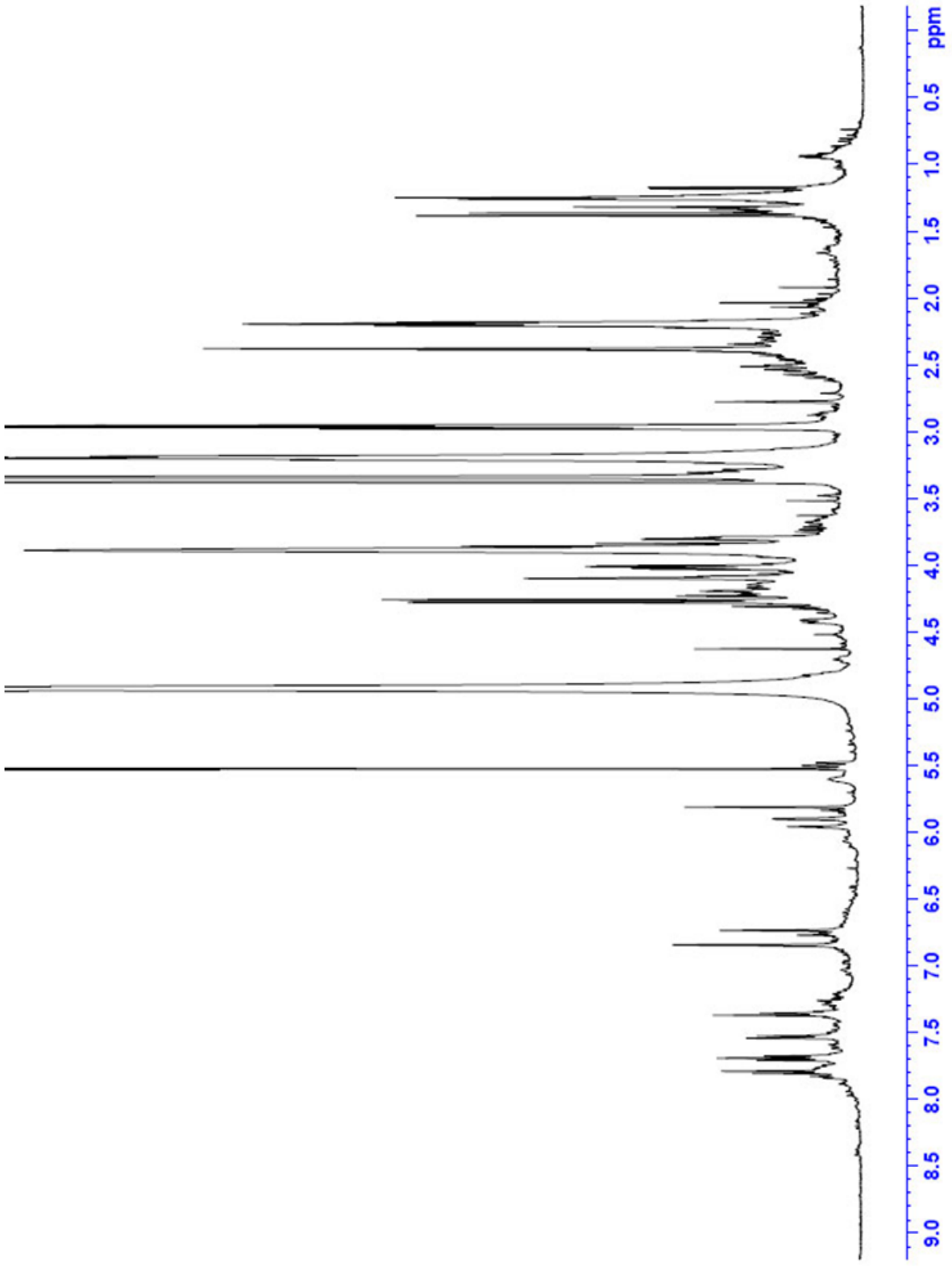
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm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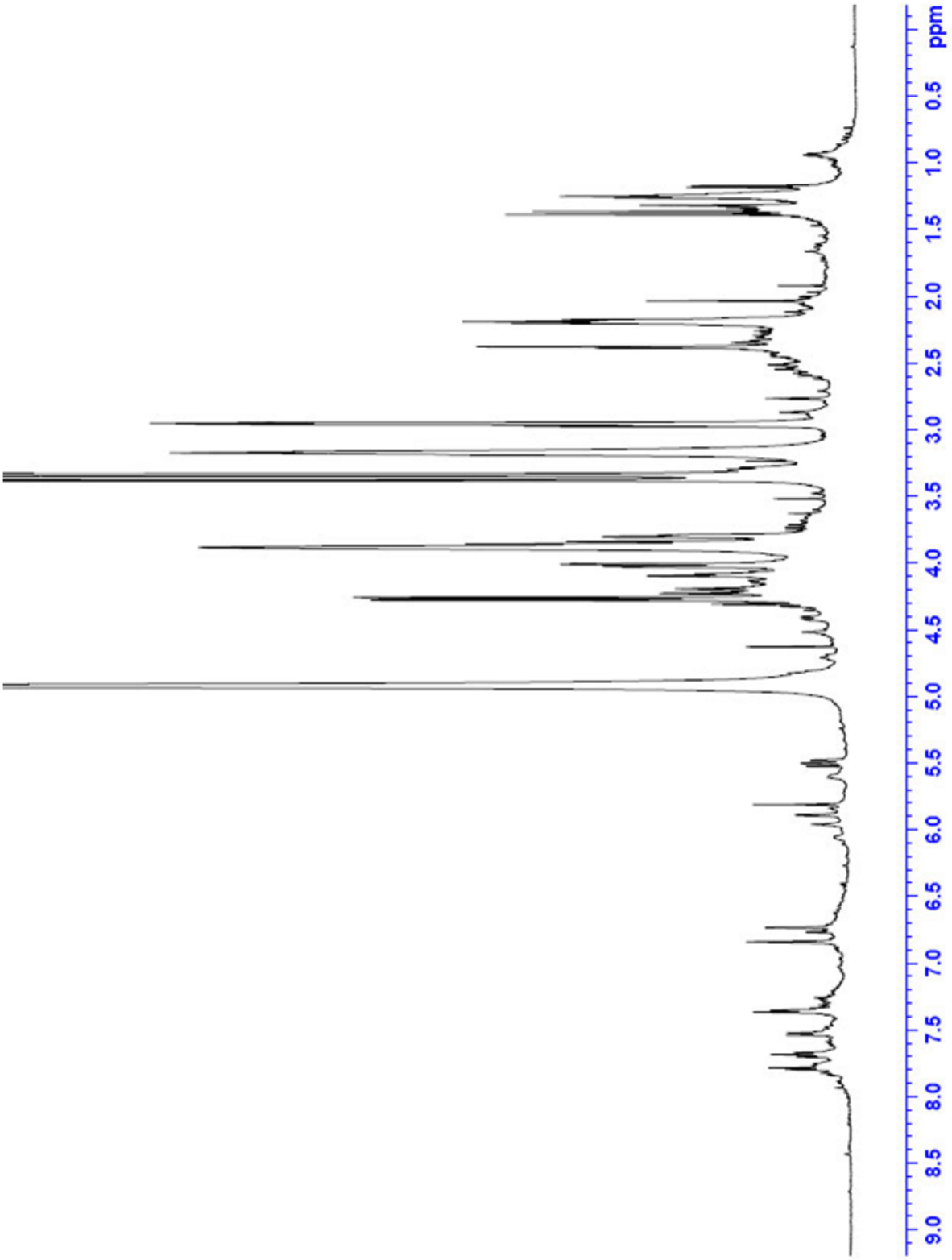
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm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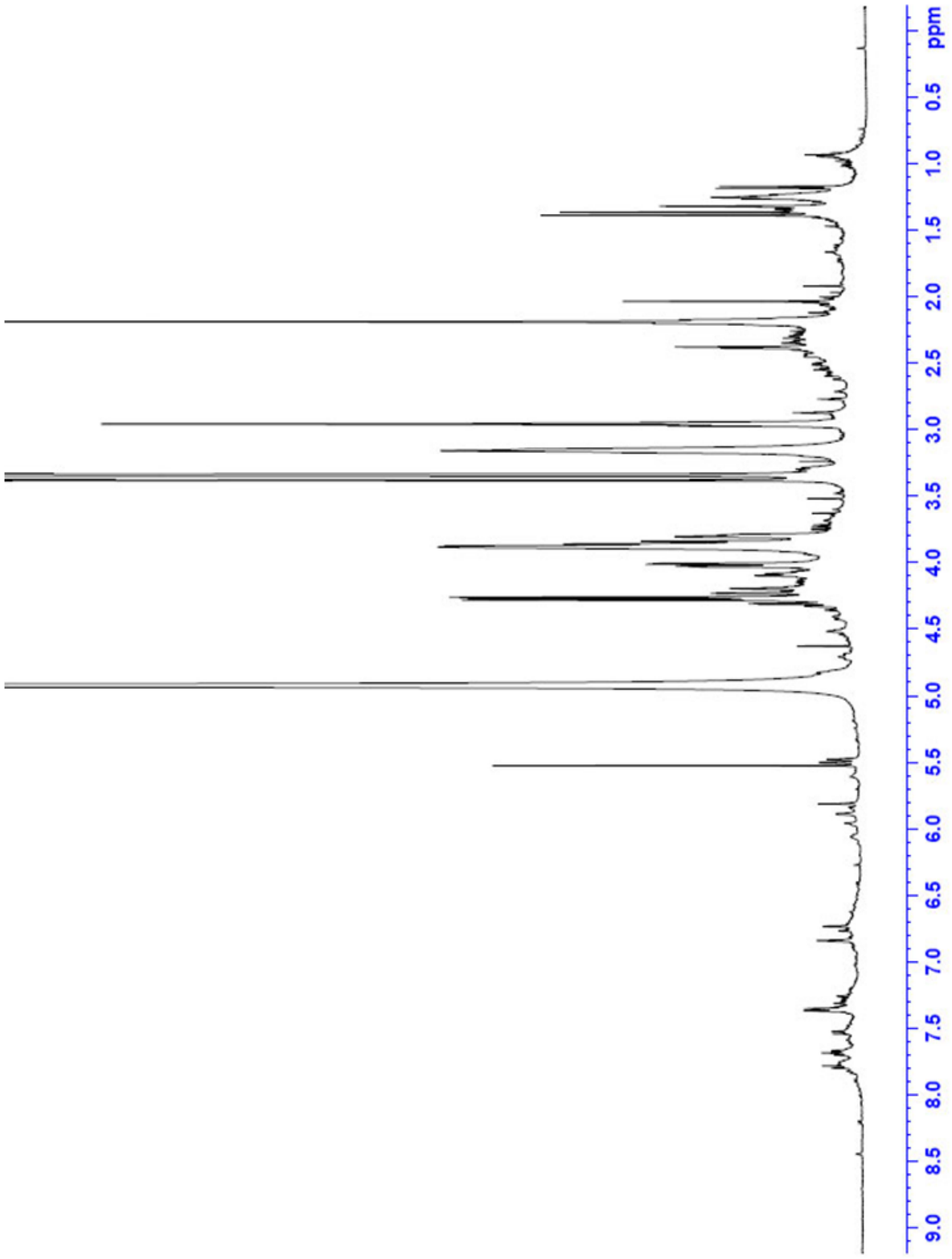
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm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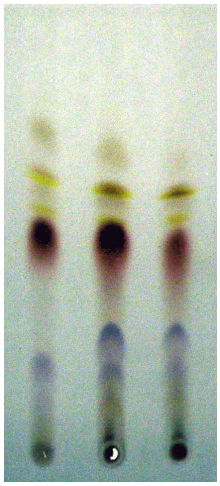
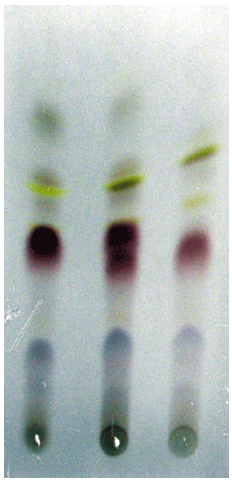
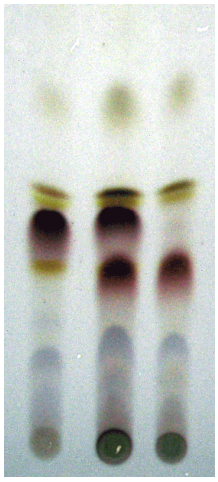
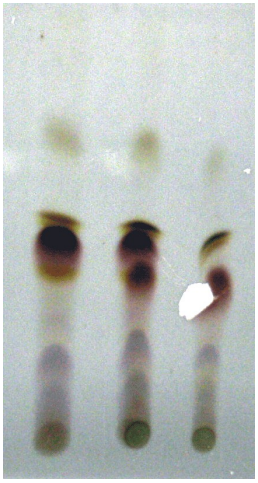
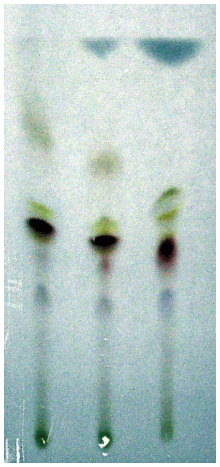
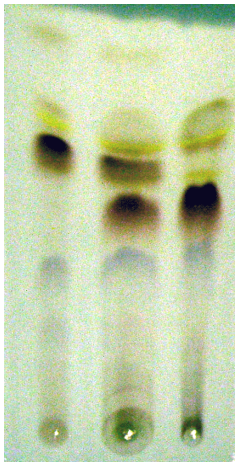
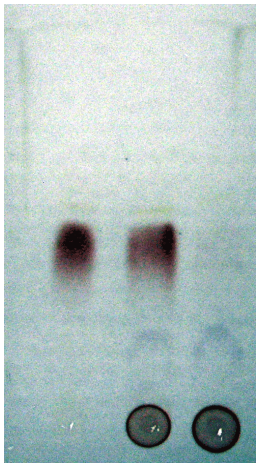
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

APPENDIX C: TLC Images from CuAAC Reactions with 28

TLC analyses (10:90 MeOH:DCM) for cycloaddition reactions using **28** and eight azides: left lane = crude **28**; middle lane = cospot; right lane = reaction mixture.

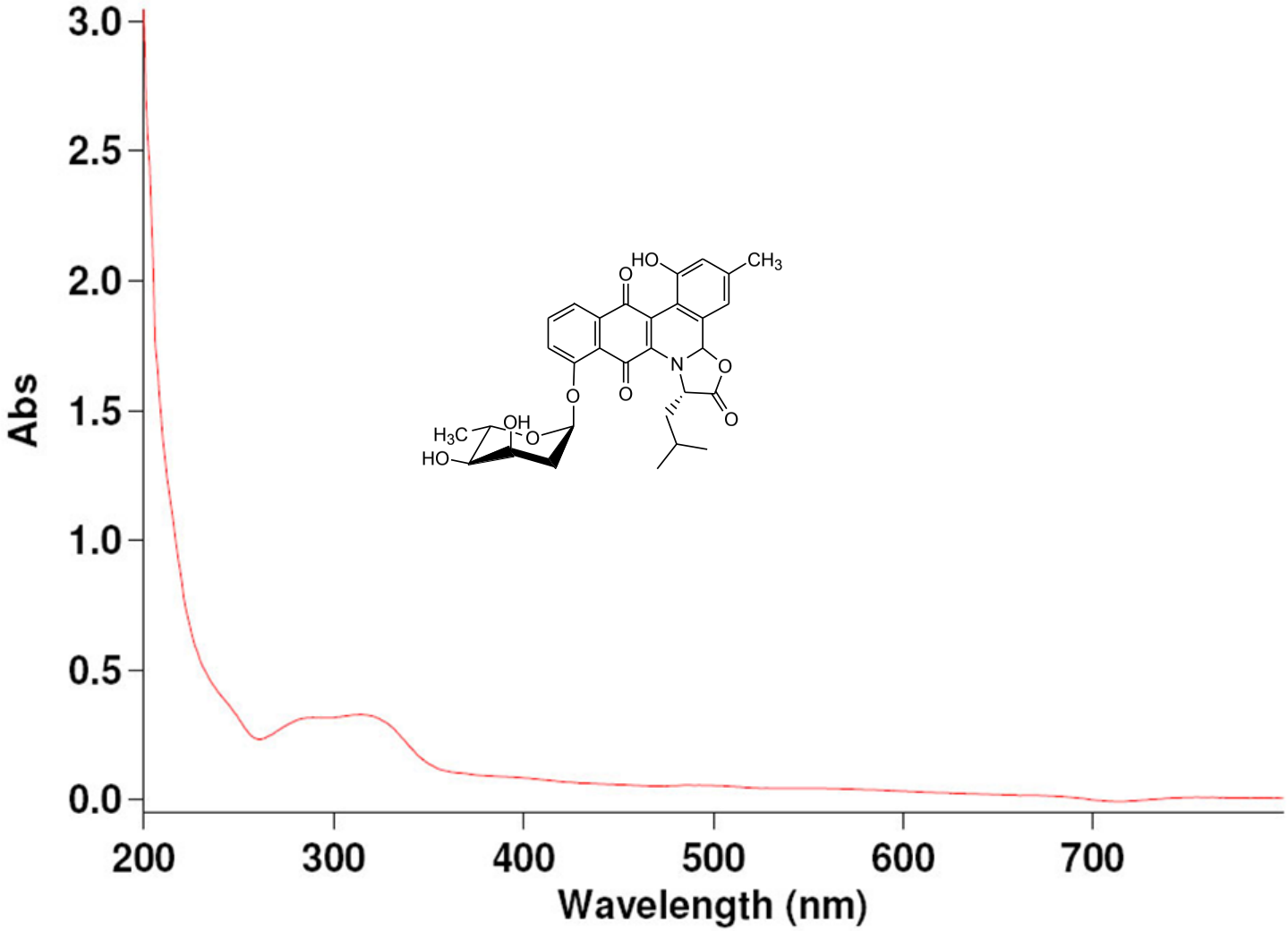
a) Reaction of crude **28** with azide **52** to produce triazole jadomycin **63**
b) Reaction of crude **28** with azide **53** to produce triazole jadomycin **64**
c) Reaction of crude **28** with azide **57** to produce triazole jadomycin **65**
d) Reaction of crude **28** with azide **58** to produce triazole jadomycin **66**
e) Reaction of crude **28** with azide **43** to produce triazole jadomycin **66**
f) Reaction of crude **28** with azide **45** to produce triazole jadomycin **68**
g) Reaction of crude **28** with azide **48** to produce triazole jadomycin **69**
h) Reaction of pure **28** with azide **59** to produce triazole jadomycin **70**

a)  b)  c)  d) 

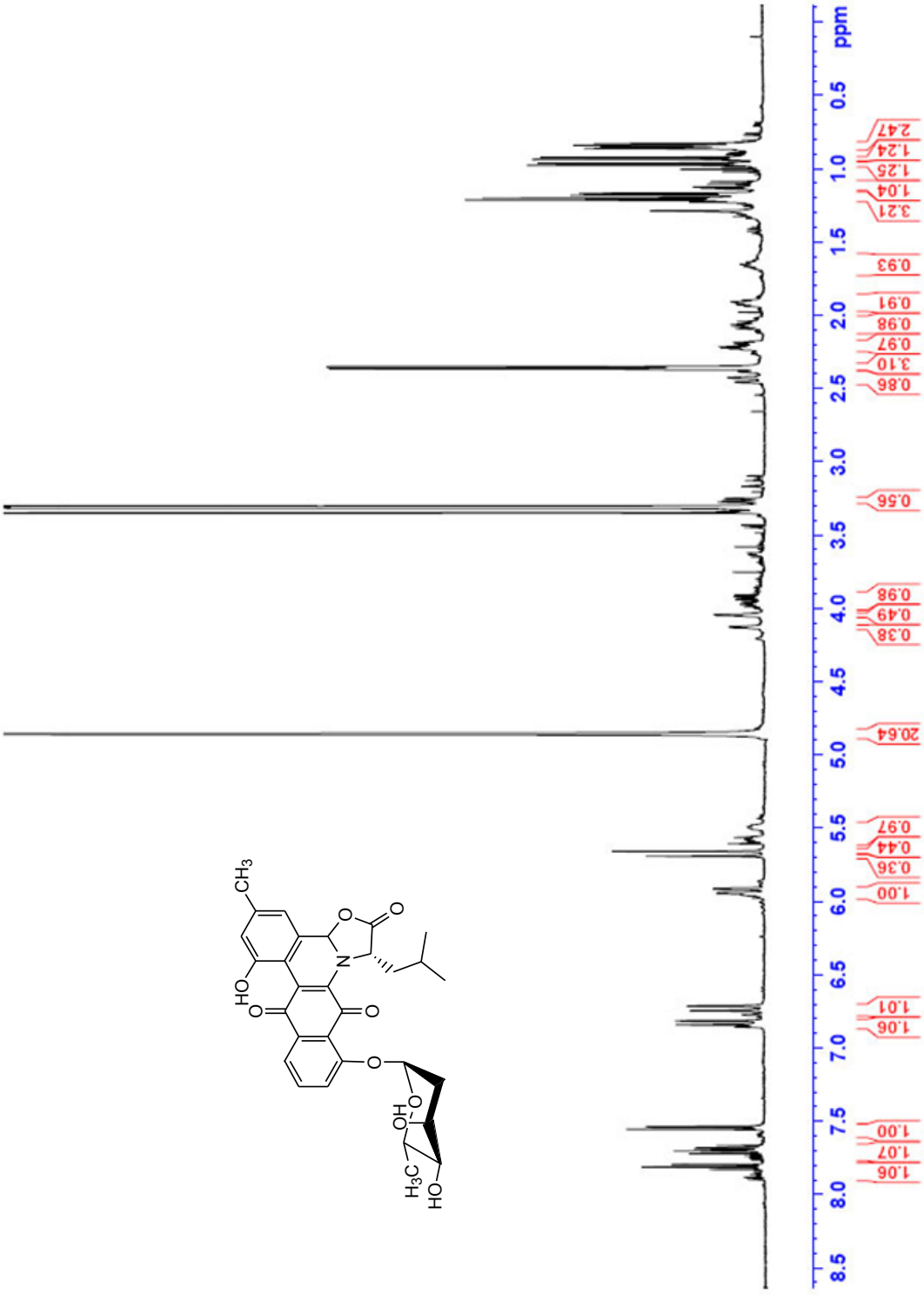
e)  f)  g)  h) 

APPENDIX D: Spectral Data for Purified Jadomycins

(Compounds **26**, **28**, **34**, **35**, **63-69**)


3.0
2.5
2.0
1.5
1.0
0.5
0.0
Abs
200
300
400
500
600
700
Wavelength (nm)
HO
CH3
O
N
O
O
O
O
H3C
OH
HO

%T
cm-1
4000
3500
3000
2500
2000
1500
1000
600
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
CH3
HO
O
O
O
O
N
O
OH
O
H3C
HO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2.5
3.0
3.5
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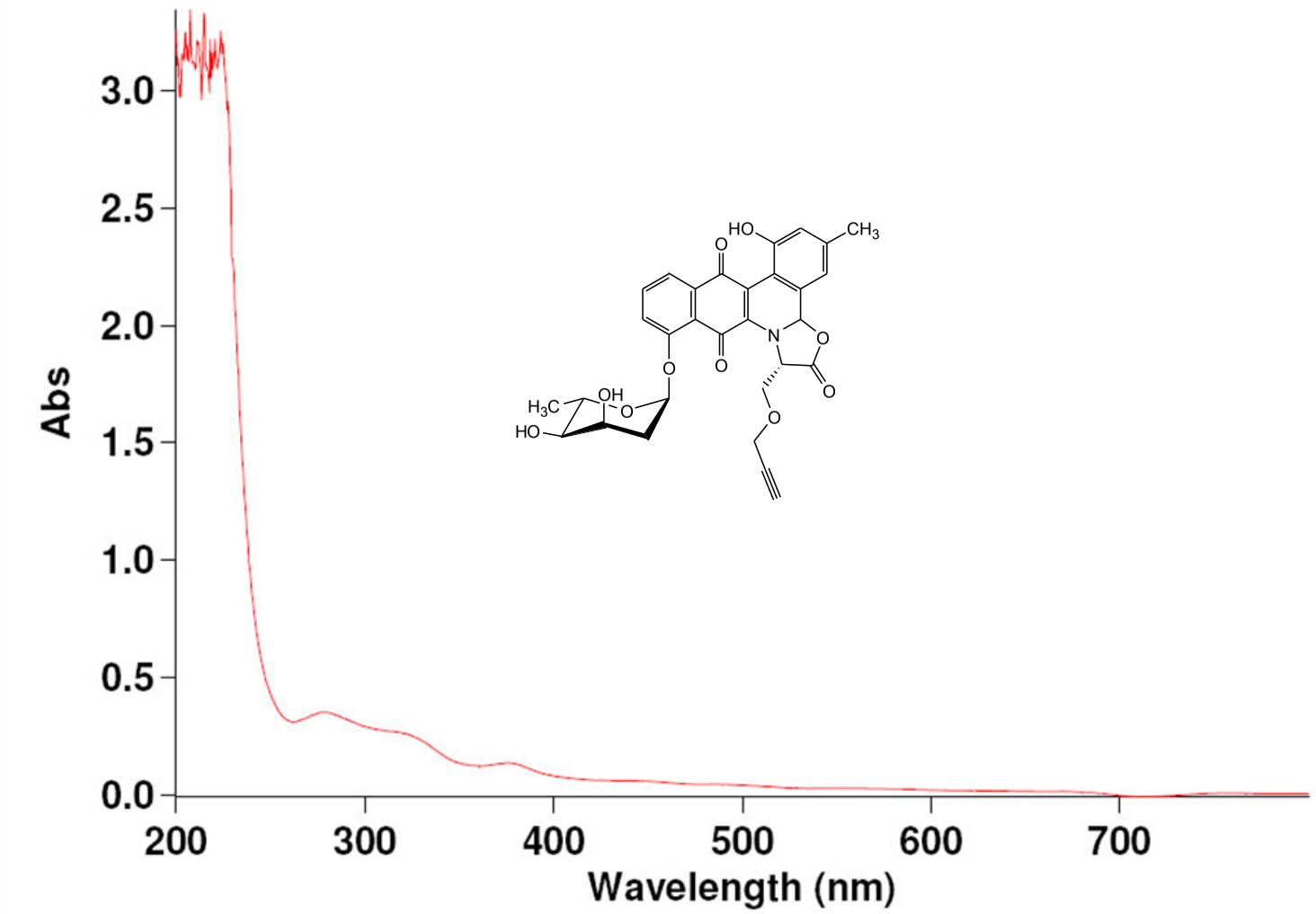
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
1.06
1.07
1.00
1.06
1.01
1.00
0.97
0.44
0.36
20.64
0.38
0.49
0.98
0.56
0.86
3.10
0.97
0.98
0.91
0.93
3.21
1.04
1.25
1.24
2.47
CH3
HO
O
O
O
O
N
O
OH
H3C
HO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2.0
1.5
1.0
0.5
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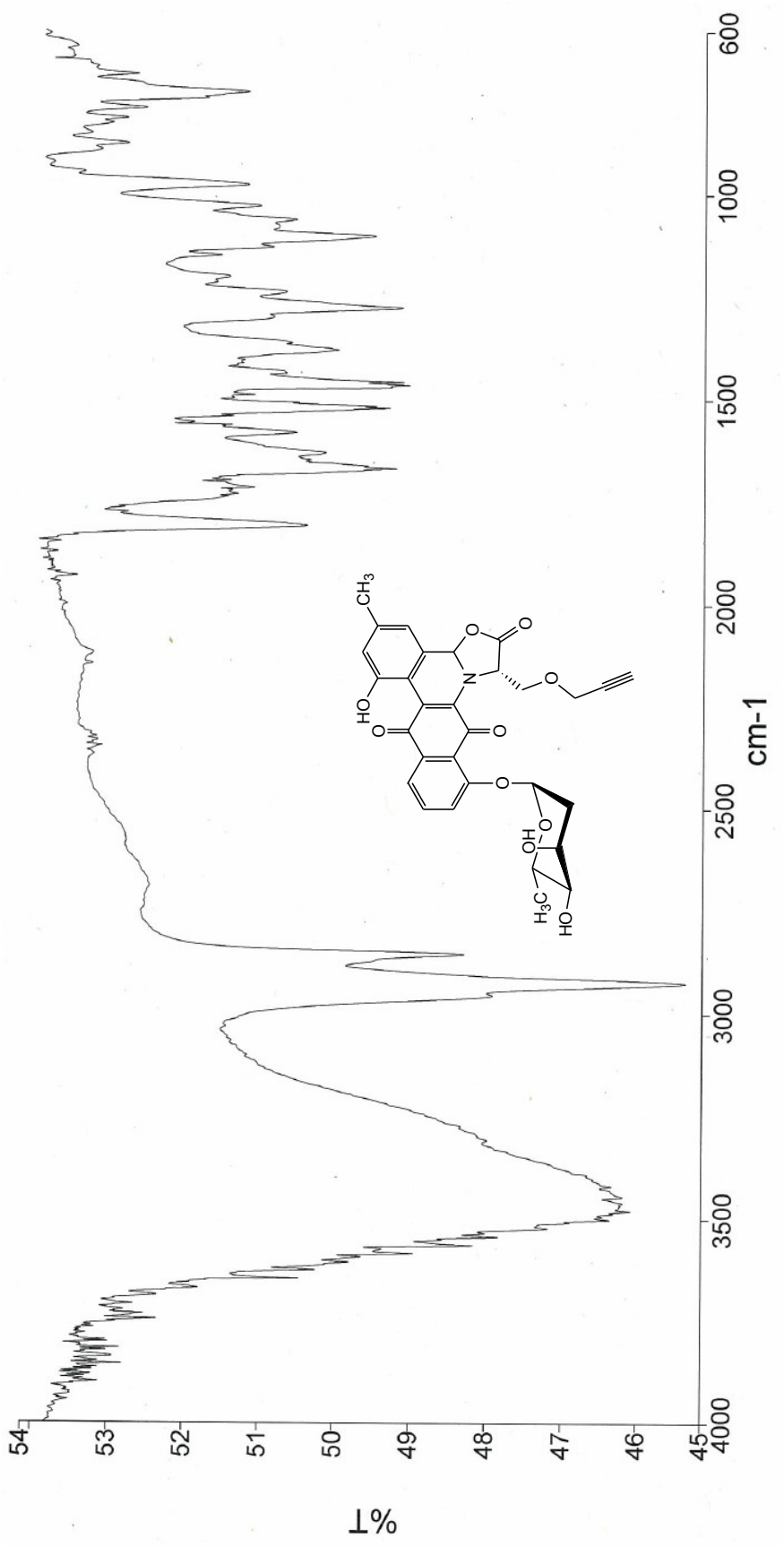
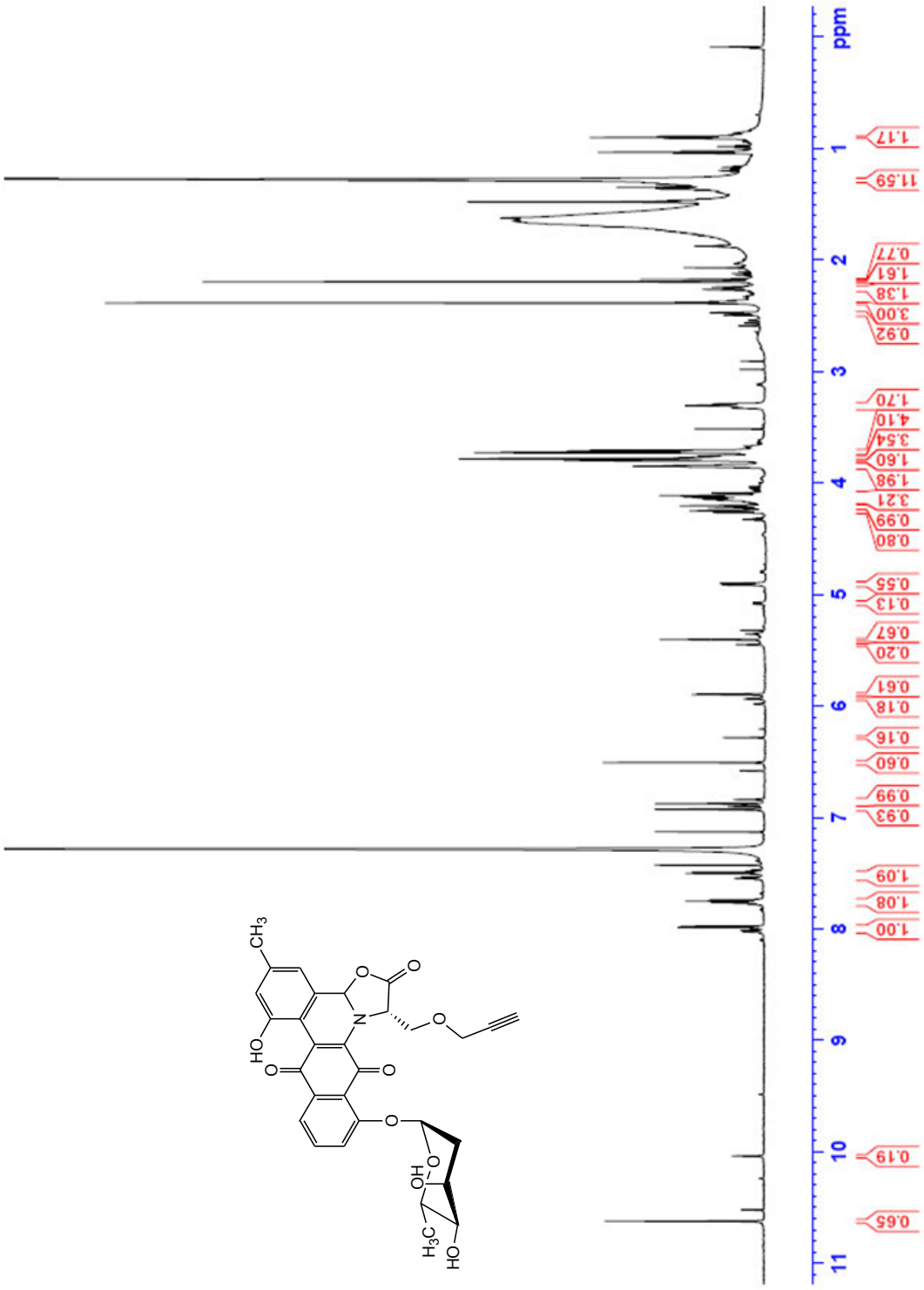
200
300
400
500
600
700
Wavelength (nm)
HO
CH3
O
O
O
N
O
O
O
H3C
OH
O
HO
O


%T
54
53
52
51
50
49
48
47
46
45
4000
3500
3000
2500
2000
1500
1000
600
cm-1
CH3
HO
O
O
O
N
O
O
OH
O
H3C
HO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1.70
4.10
3.54
1.60
1.98
3.21
0.99
0.80
0.55
0.13
0.67
0.20
0.61
0.18
0.16
0.60
0.99
0.93
1.09
1.08
1.00
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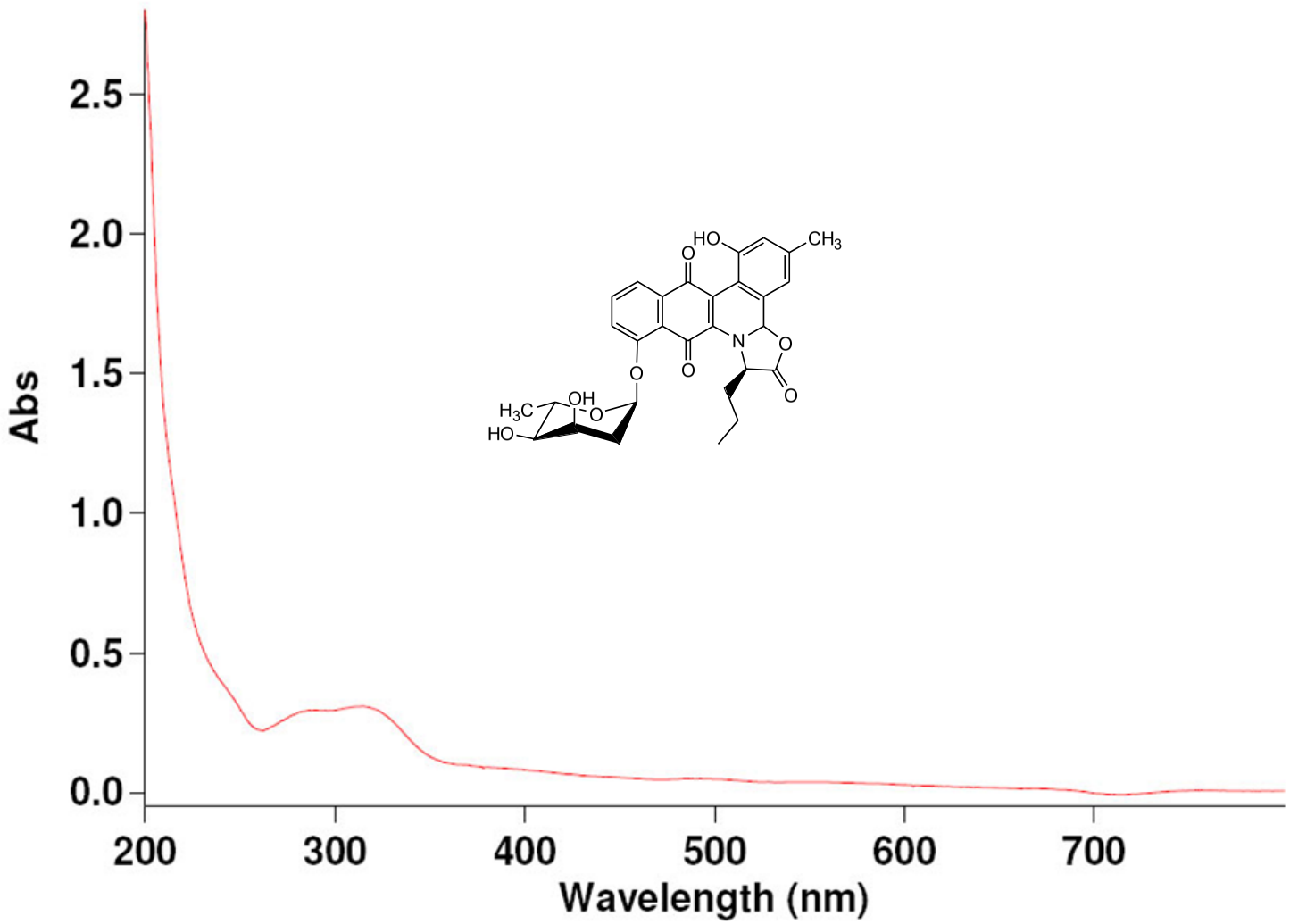
0.65
CH3
HO
O
O
O
O
N
O
O
OH
O
H3C
HO


Abs
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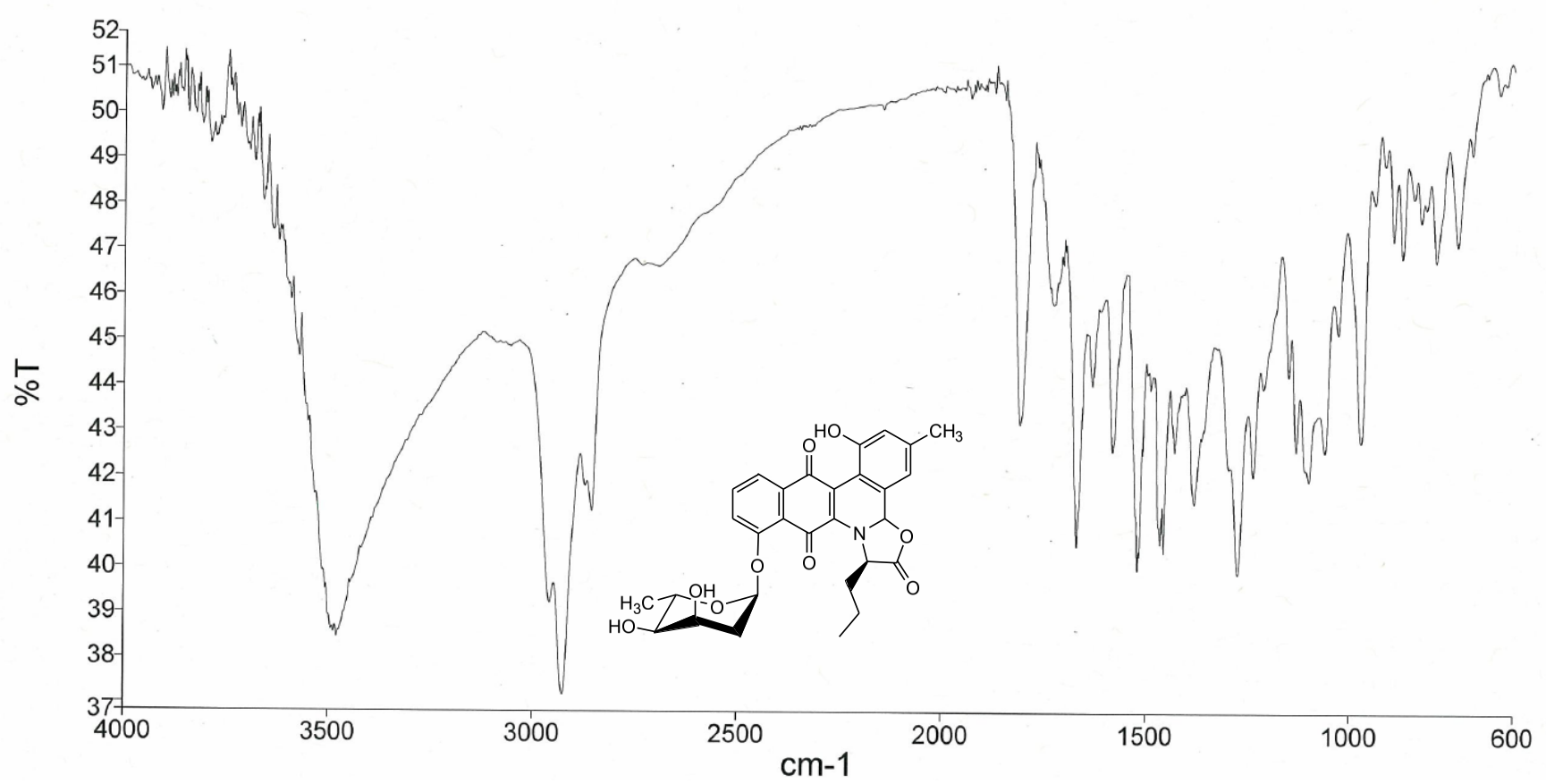
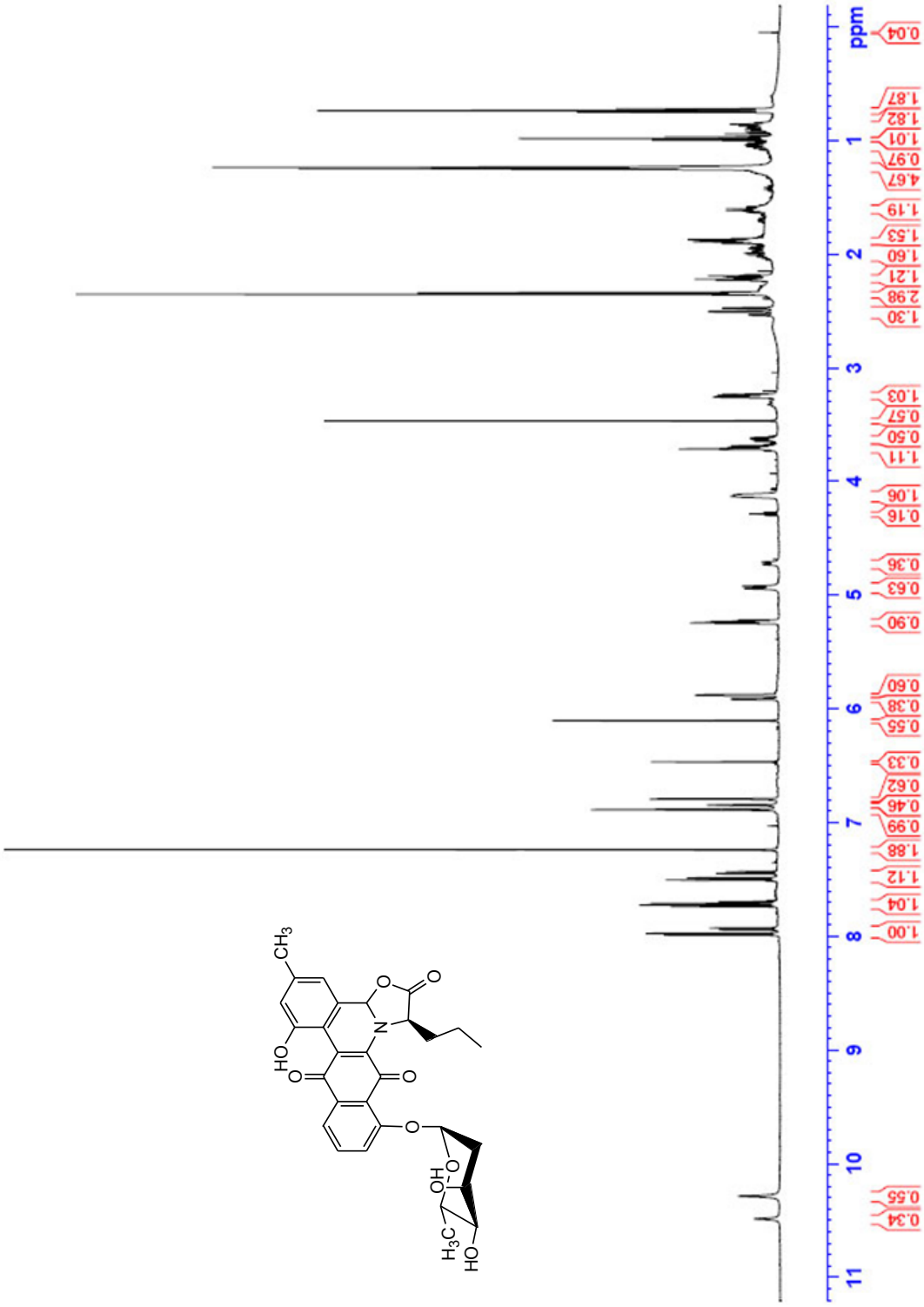
2.0
1.5
1.0
0.5
0.0
200
300
400
500
600
700
Wavelength (nm)
HO
CH3
O
O
N
O
O
O
O
H3C
OH
HO


%T
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
4000
3500
3000
2500
2000
1500
1000
600
cm-1
HO
CH3
O
O
N
O
O
O
H3C
OH
O
HO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1.21
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1.03
0.57
0.50
1.11
1.06
0.16
0.36
0.63
0.90
0.60
0.38
0.55
0.33
0.62
0.46
0.99
1.86
1.12
1.04
1.00
0.34
0.55
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HO
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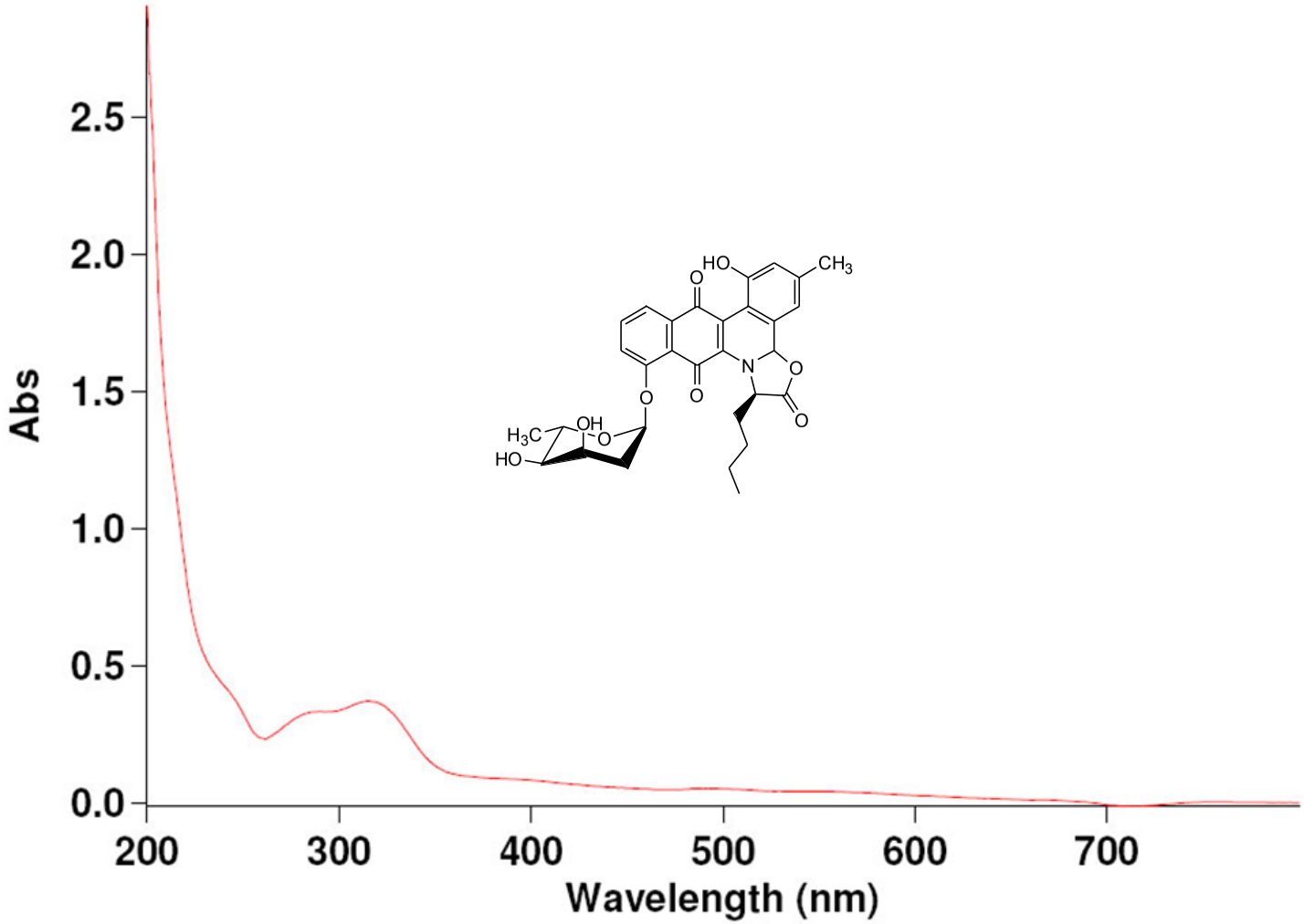
O
O
O
N
O
O
OH
HO
H3C


Abs
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1.0
0.5
0.0
200
300
400
500
600
700
Wavelength (nm)
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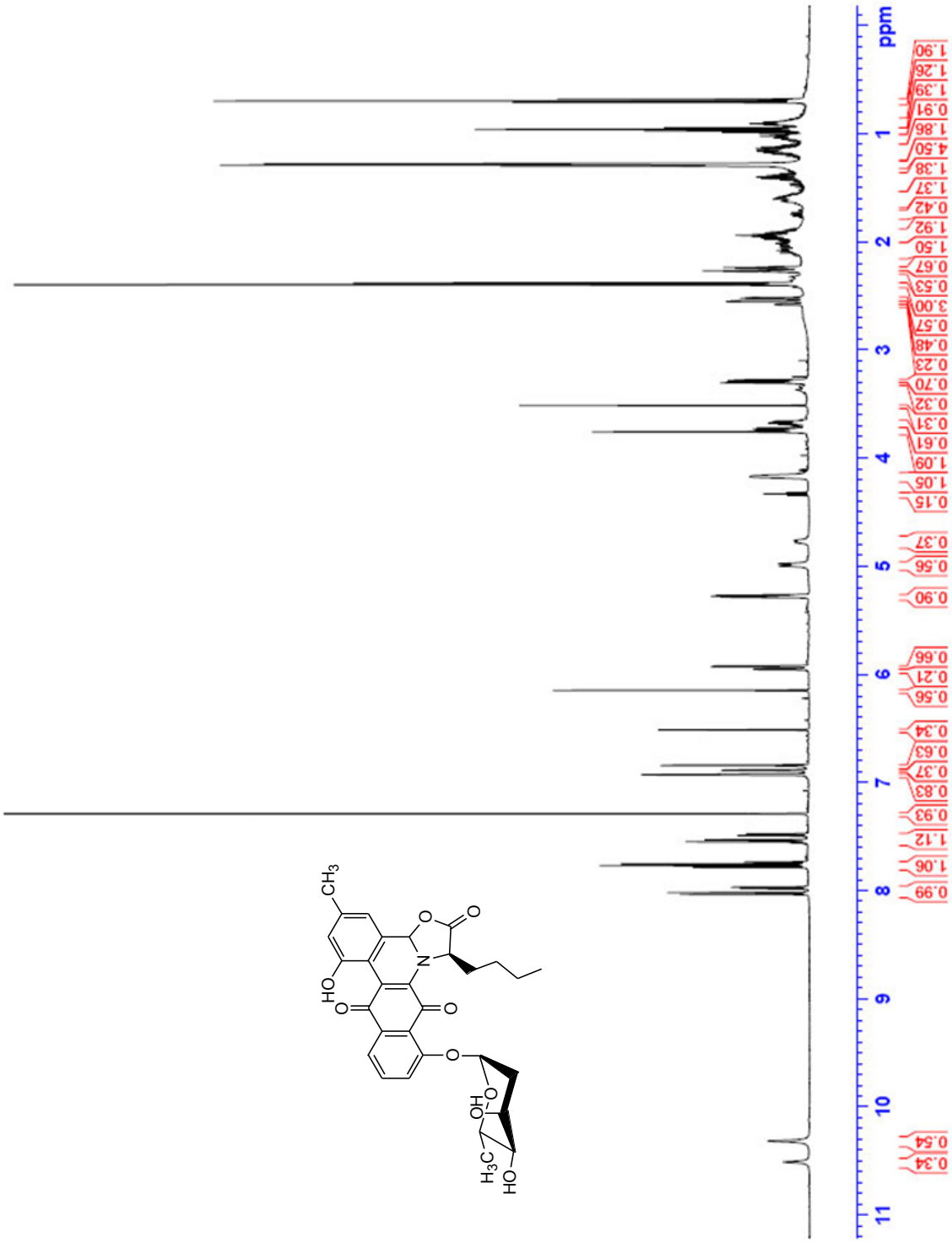
CH3
O
O
N
O
O
O
O
H3C
OH
O
HO

%T
cm-1
4000
3500
3000
2500
2000
1500
1000
600
57
56
55
54
53
52
51
50
49
48
47
CH3
HO
O
N
OH
H3C
HO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1.12
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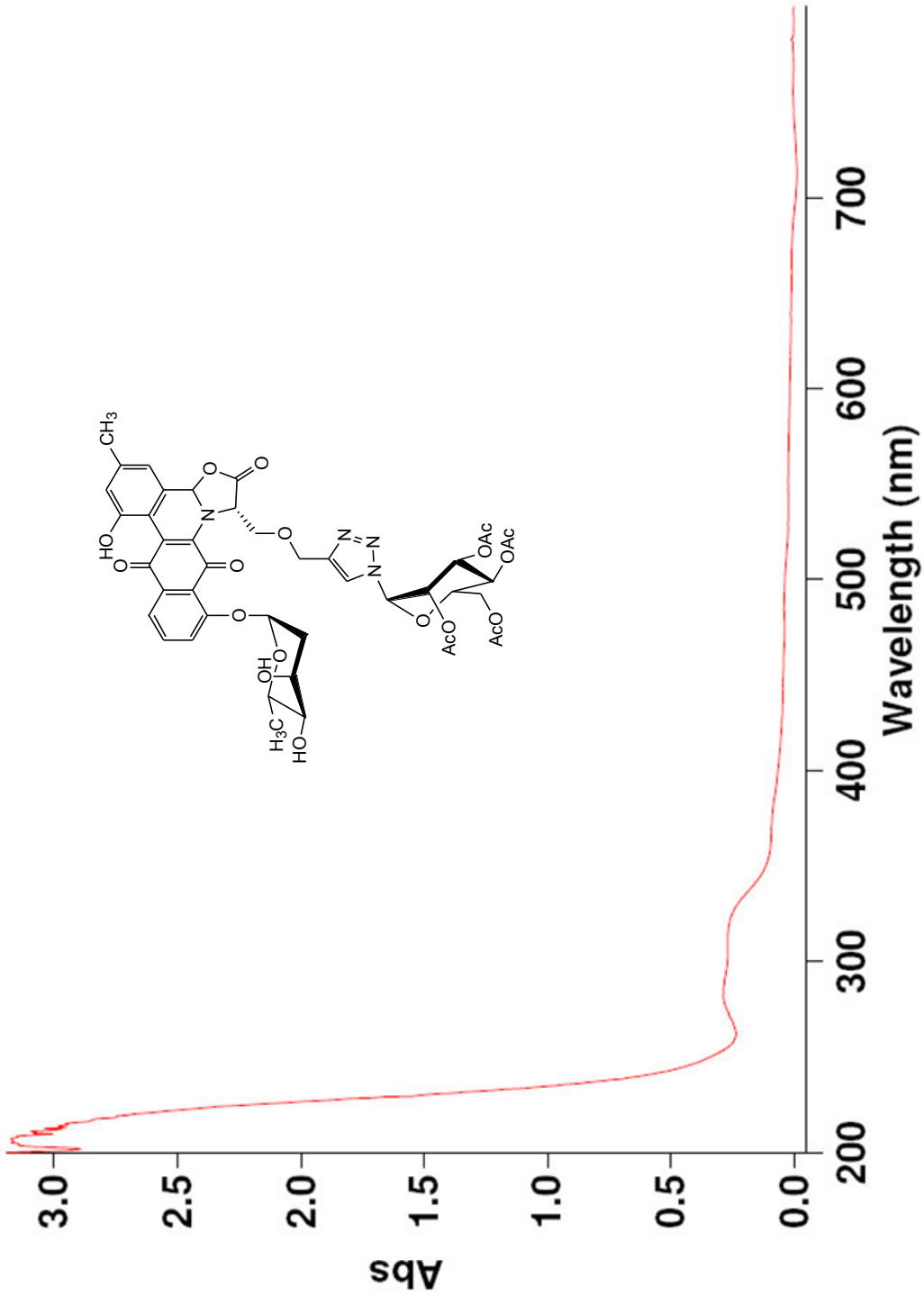
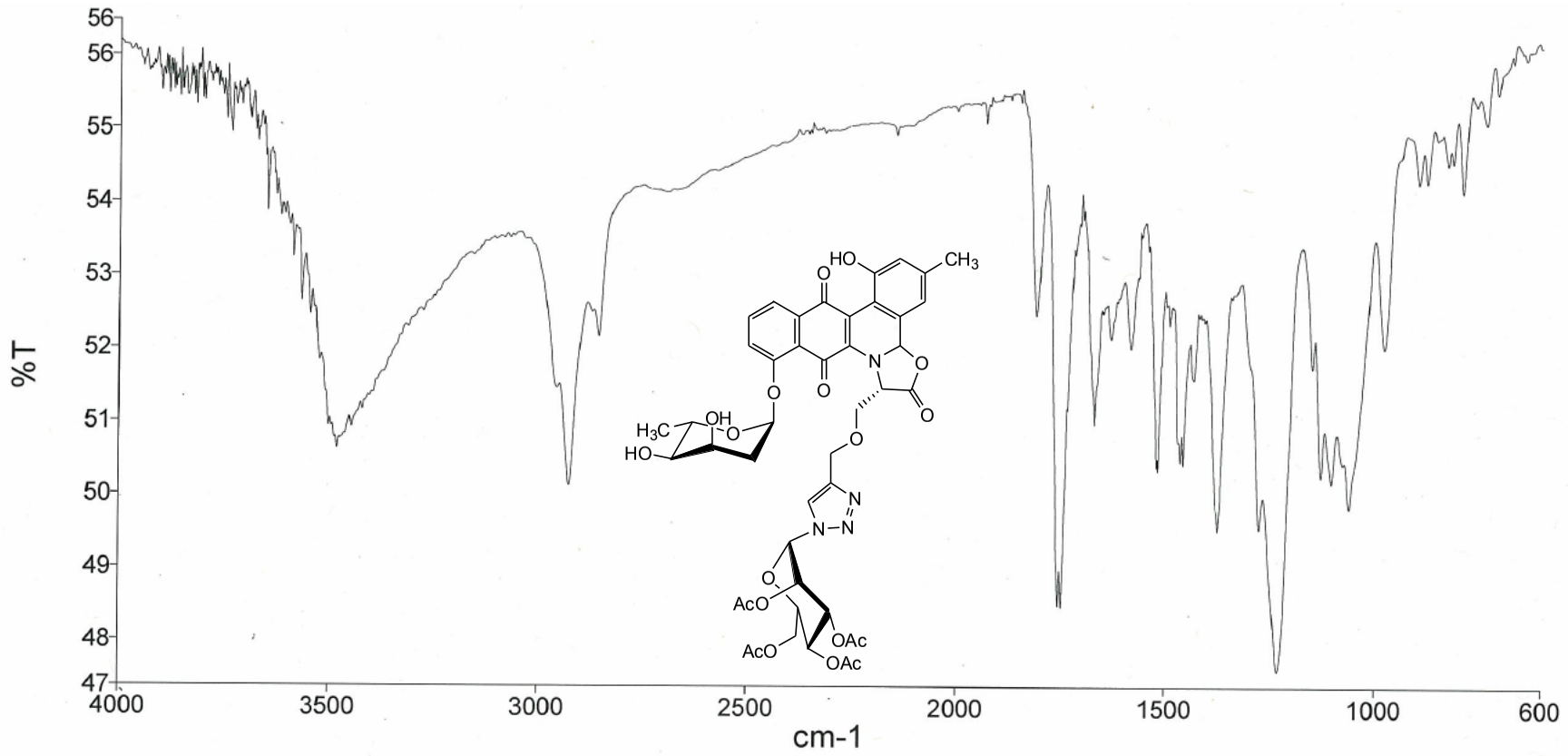
0.37
0.63
0.34
0.66
0.21
0.56
0.90
0.37
0.56
0.15
1.05
1.09
0.61
0.31
0.32
0.70
0.23
0.48
0.57
3.00
0.53
0.67
1.50
1.92
0.42
1.37
1.38
4.50
1.86
0.91
1.39
1.26
1.90
0.34
0.54
CH3
HO
O
O
O
N
O
O
O
OH
O
H3C
HO


Abs
3.0
2.5
2.0
1.5
1.0
0.5
0.0
200
300
400
500
600
700
Wavelength (nm)
CH3
HO
O
O
O
N
O
O
O
N
N
N
OAc
OAc
AcO
AcO
O
OH
O
H3C
HO


%T
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55
54
53
52
51
50
49
48
47
4000
3500
3000
2500
2000
1500
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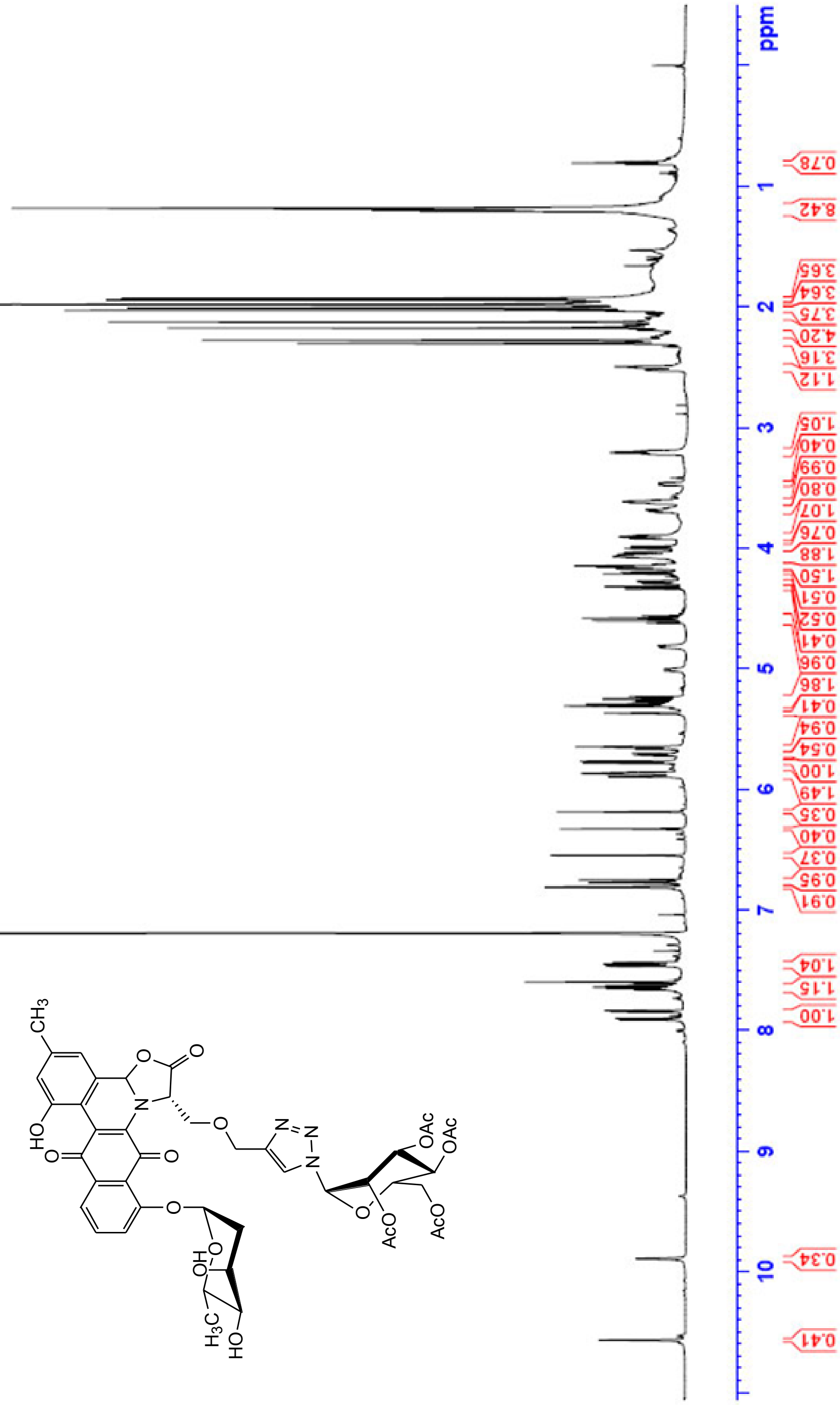
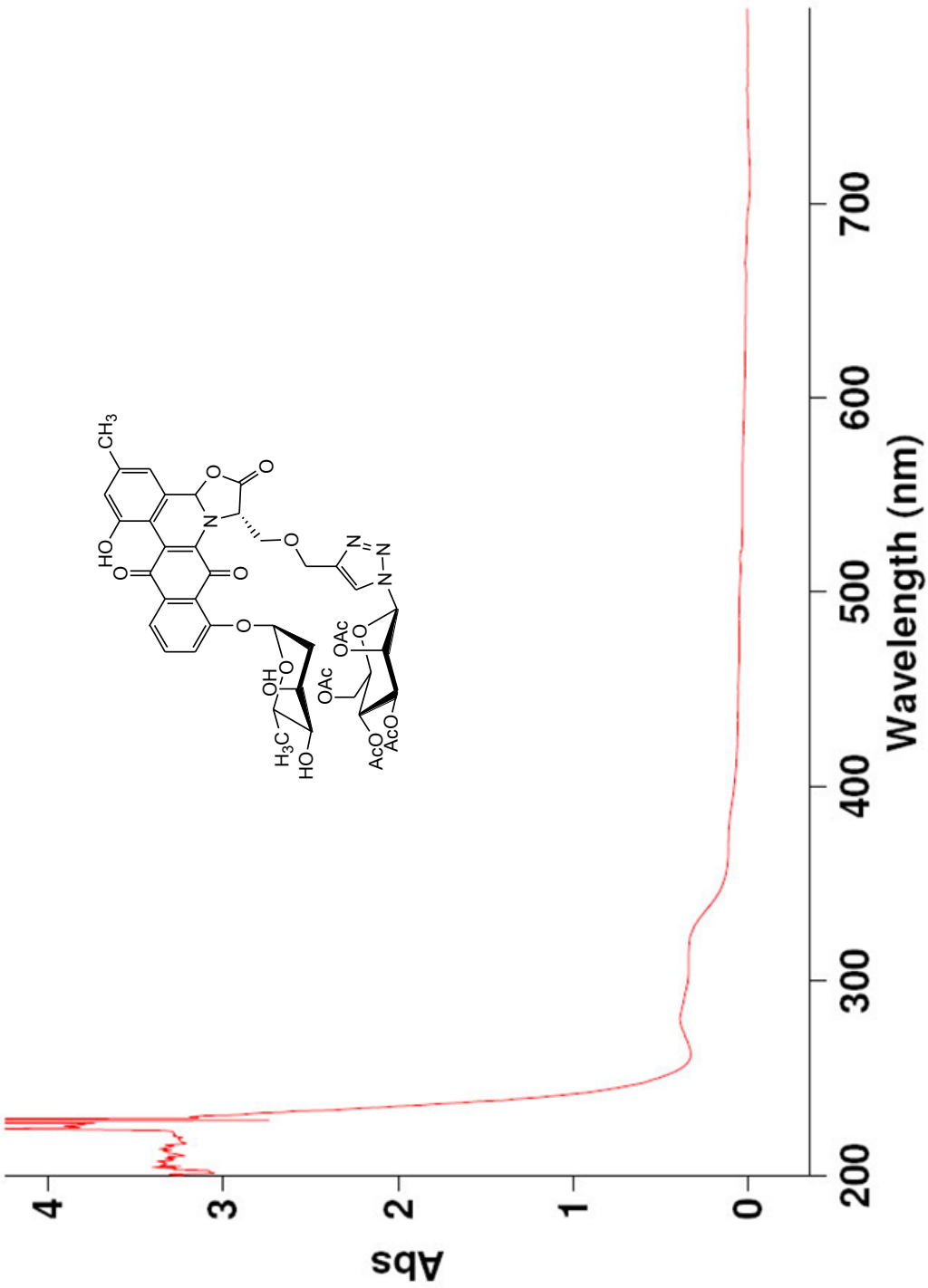
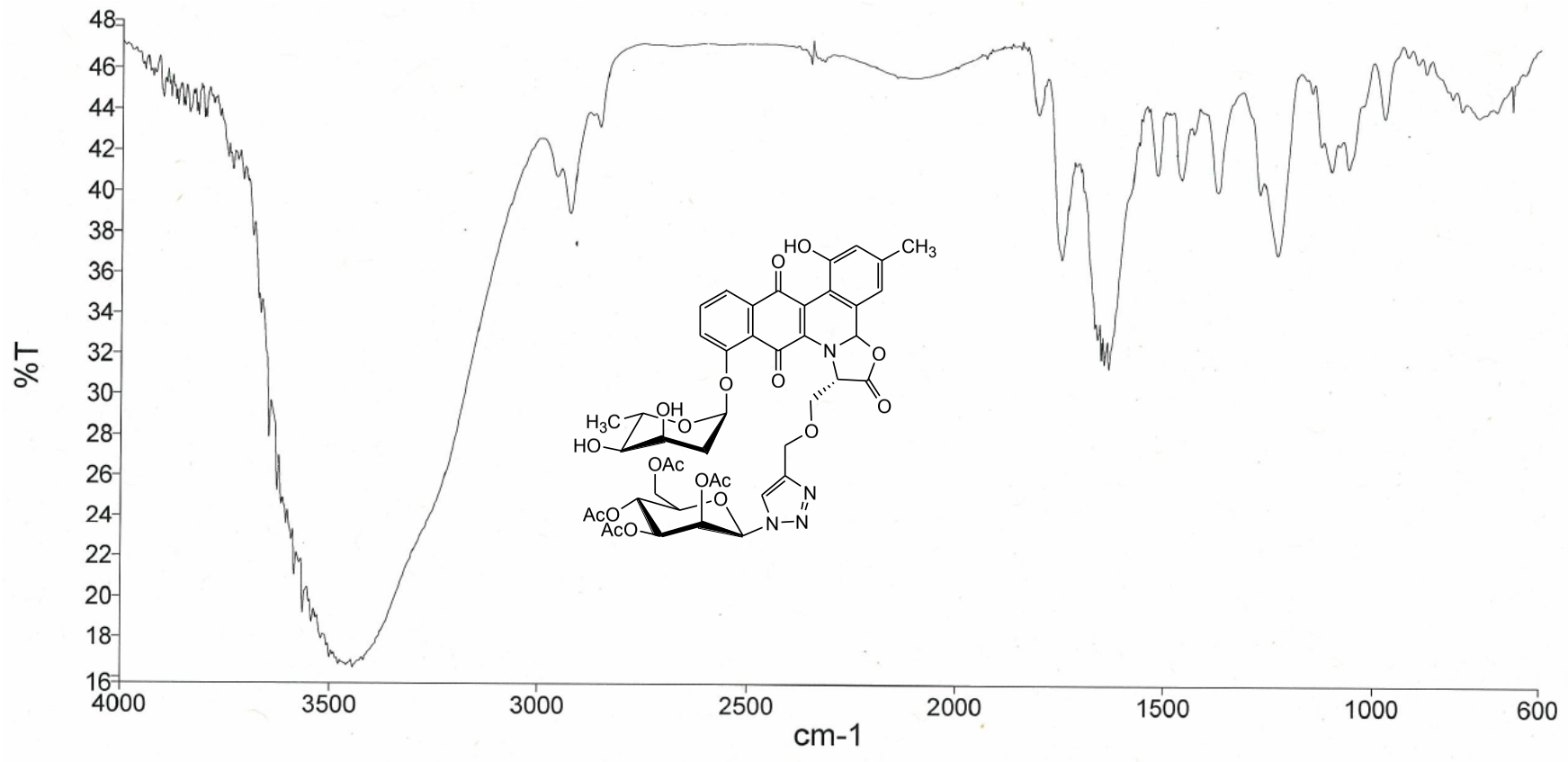
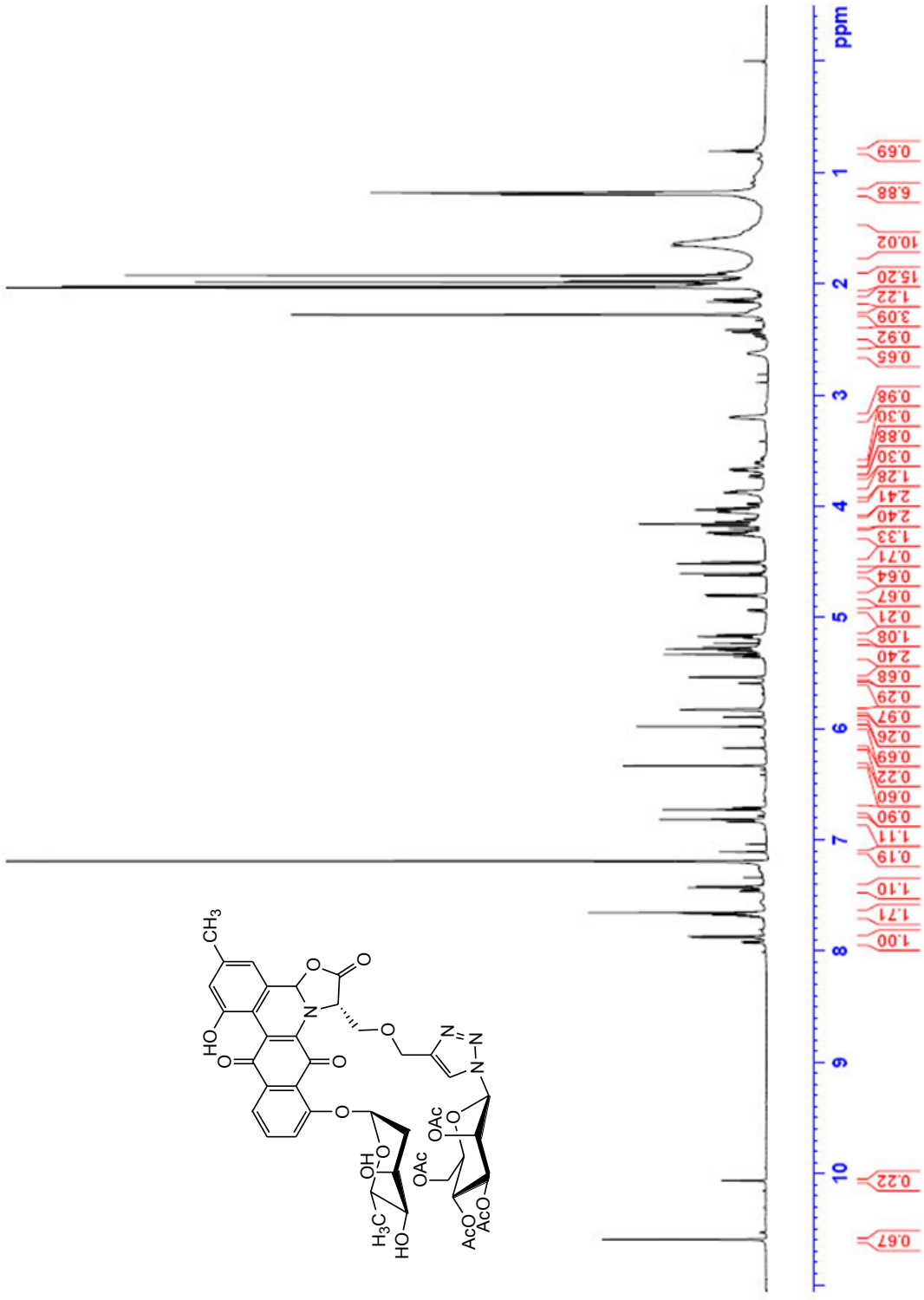
cm-1
HO
CH3
O
O
N
O
O
O
H3C
OH
HO
O
O
N
N
N
O
AcO
AcO
OAc
OAc




Abs
4
3
2
1
0
200
300
400
500
600
700
Wavelength (nm)


%T
48
46
44
42
40
38
36
34
32
30
28
26
24
22
20
18
16
4000
3500
3000
2500
2000
1500
1000
600
cm-1
HO
CH3
O
O
N
O
O
O
O
H3C
OH
HO
OAc
OAc
AcO
AcO
N
N
N


ppm
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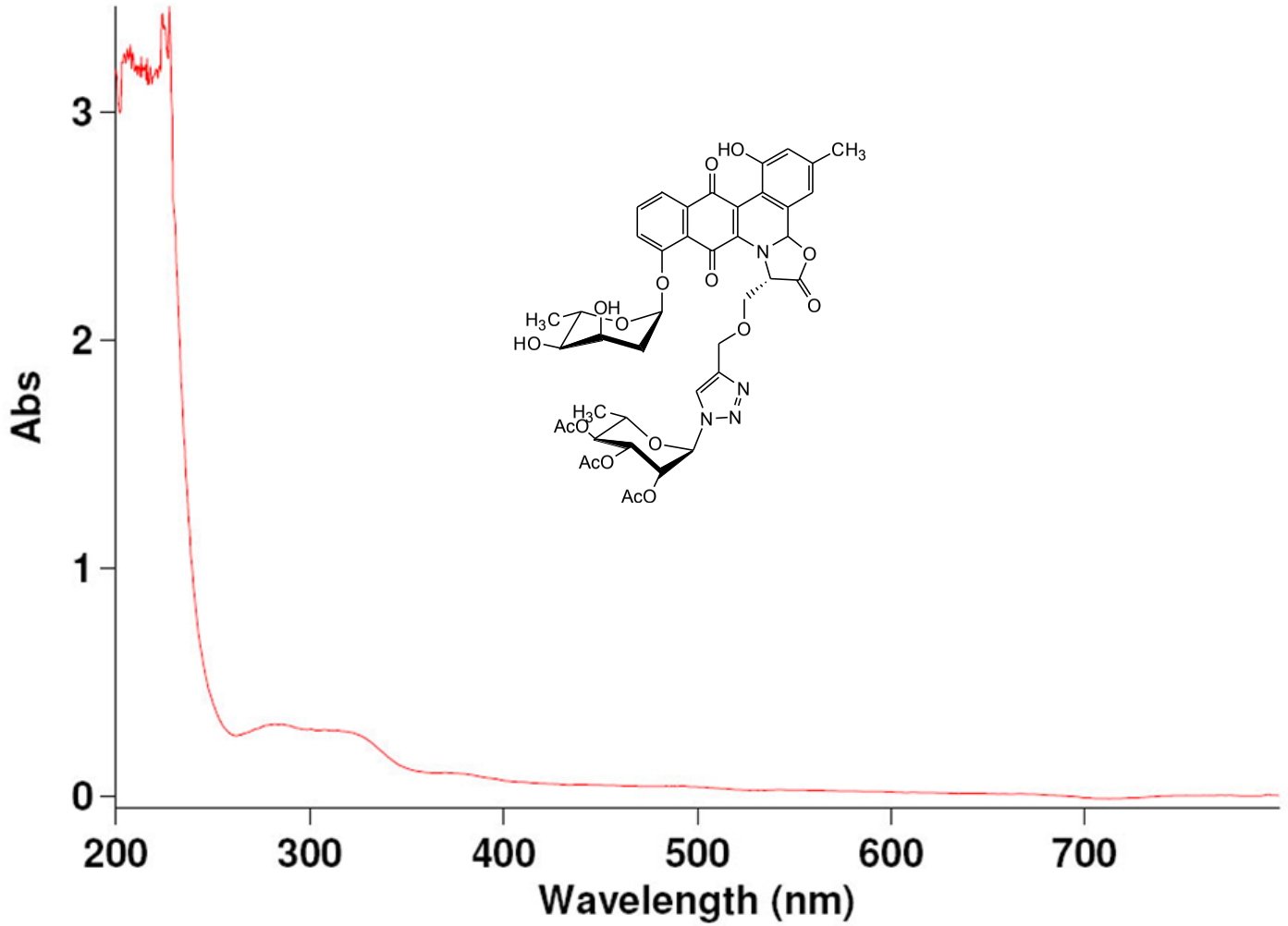
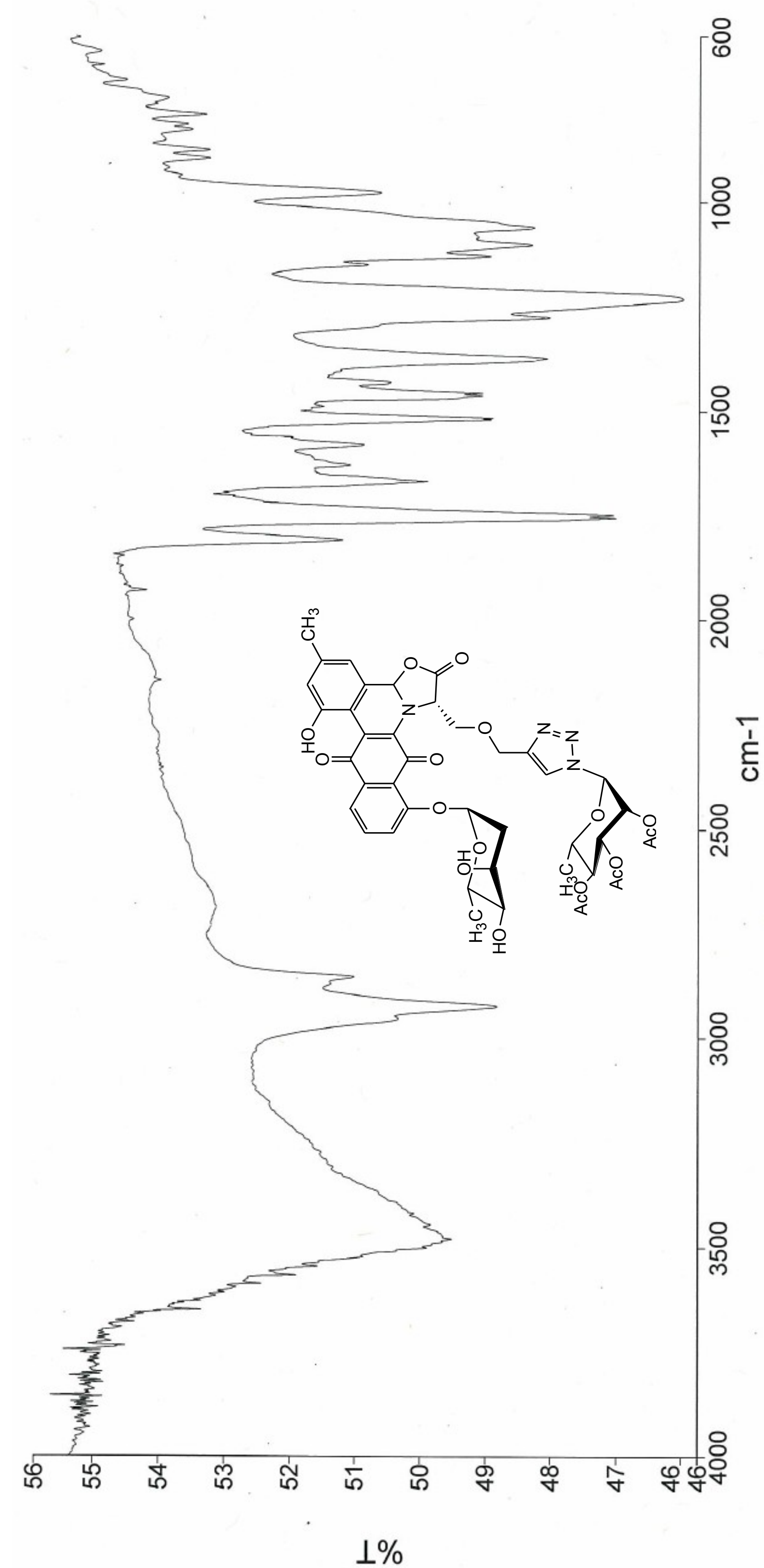
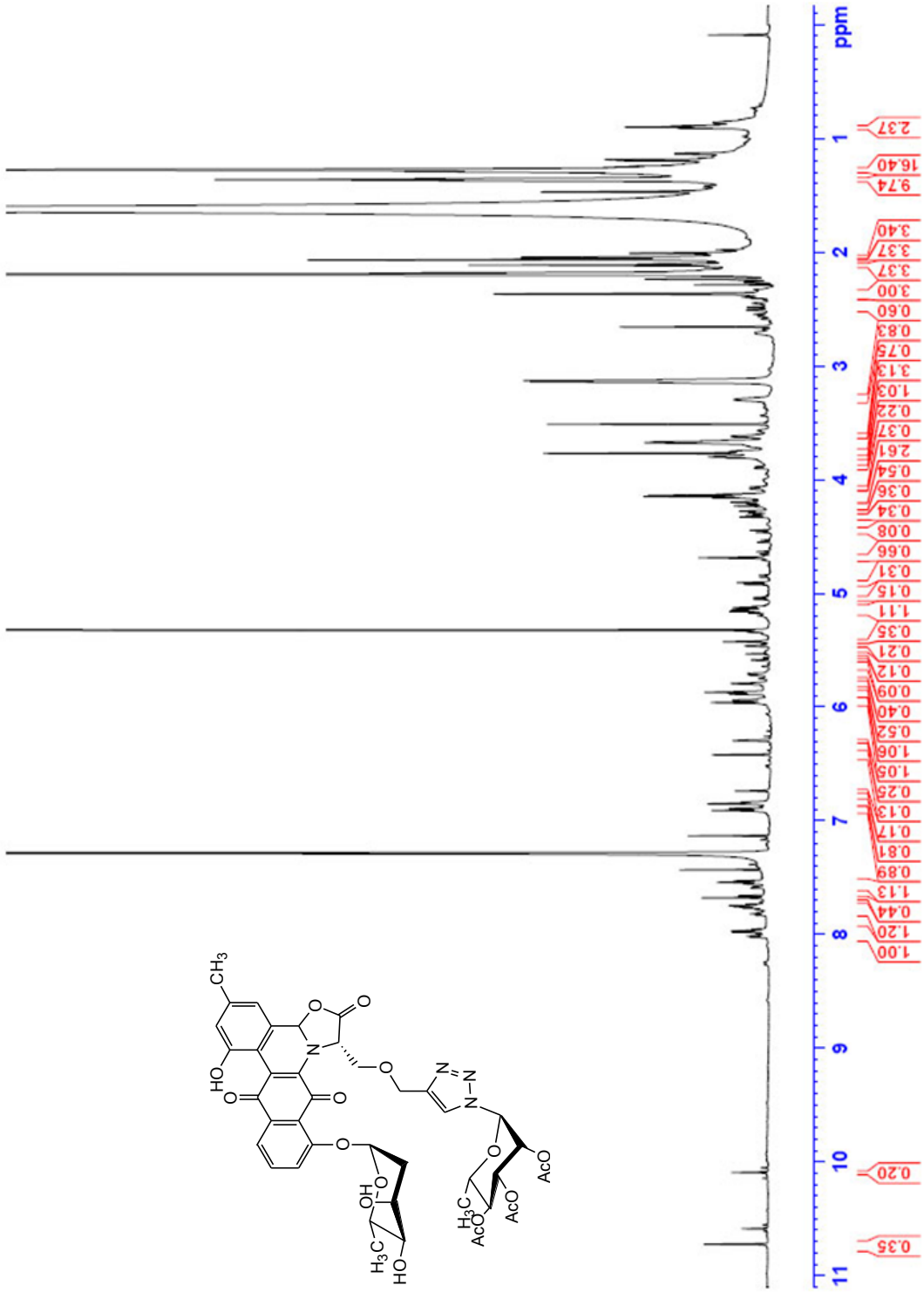
2
3
4
5
6
7
8
9
10
0.69
6.88
10.02
15.20
1.22
3.09
0.92
0.65
0.98
0.30
0.88
0.30
1.28
2.41
2.40
1.33
0.71
0.64
0.67
0.21
1.08
2.40
0.68
0.29
0.97
0.26
0.69
0.22
0.60
0.90
1.11
0.19
1.10
1.71
1.00
0.22
0.67
CH3
HO
O
N
N
N
OAc
OAc
OH
H3C
HO
AcO
AcO


Abs
0
1
2
3
200
300
400
500
600
700
Wavelength (nm)
HO
CH3
O
N
O
O
O
O
H3C
OH
HO
O
N
N
H3C
AcO
AcO
AcO
O


%T
cm-1
56
55
54
53
52
51
50
49
48
47
46
4000
3500
3000
2500
2000
1500
1000
600


ppm
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2
3
4
5
6
7
8
9
10
11
2.37
16.40
9.74
3.40
3.37
3.37
3.00
0.60
0.83
0.75
3.13
1.03
0.22
0.37
2.61
0.54
0.36
0.34
0.08
0.66
0.31
0.15
1.11
0.35
0.21
0.12
0.09
0.40
0.52
1.06
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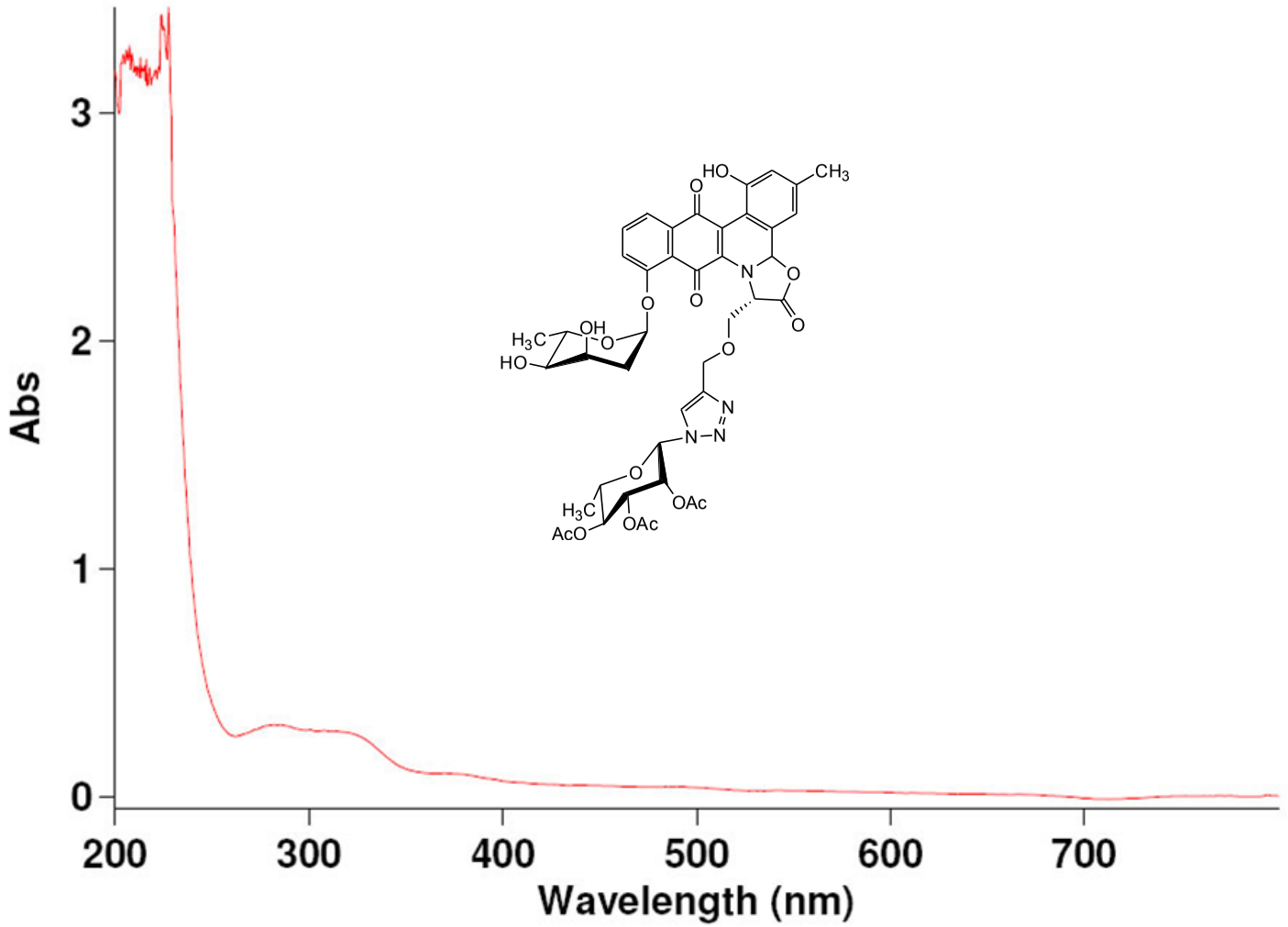
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2
3
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CH3
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OH
O
HO
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N
N
O
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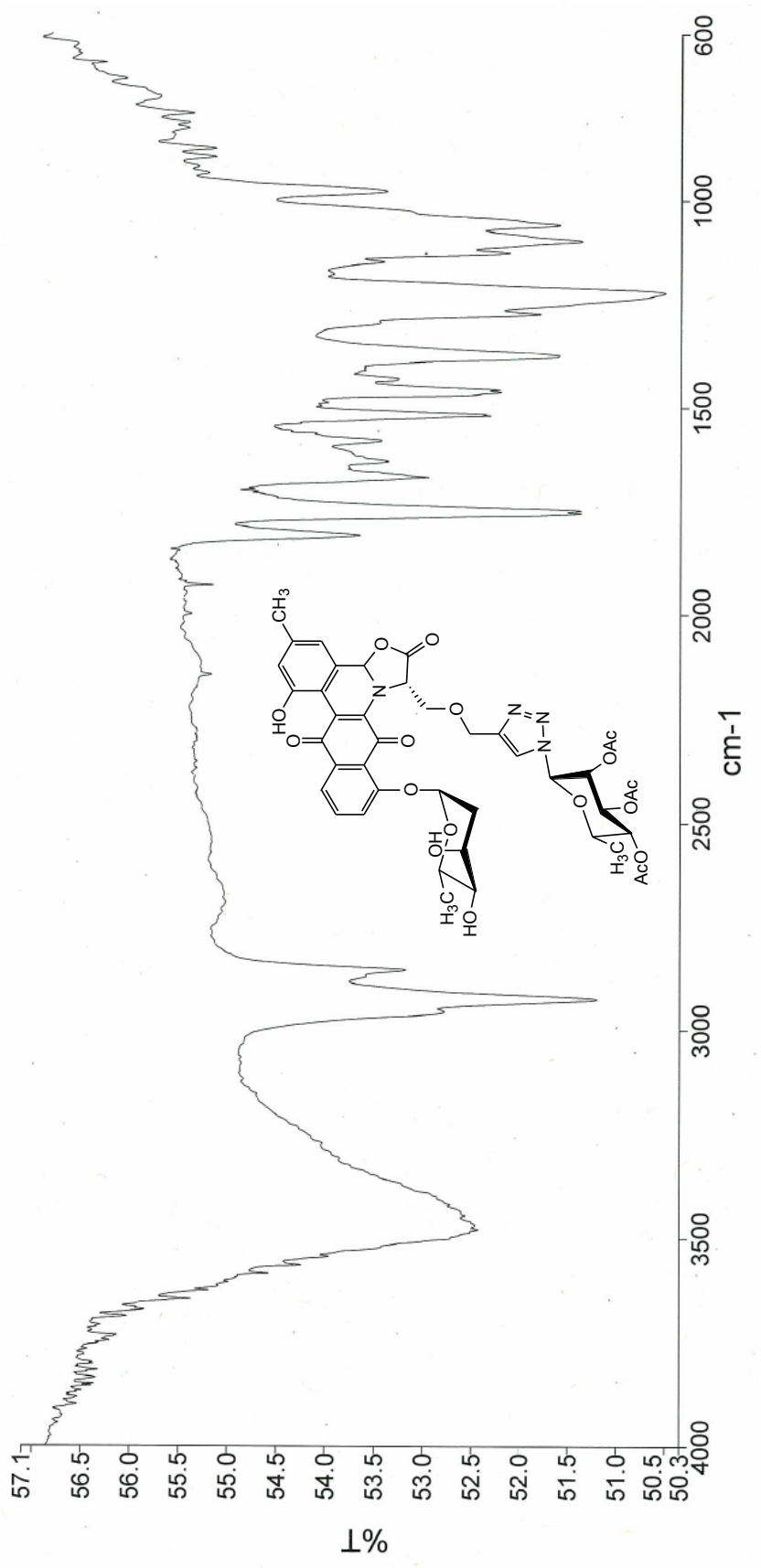
H3C
AcO
OAc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50.3
4000
3500
3000
2500
2000
1500
1000
600
CH3
HO
O
N
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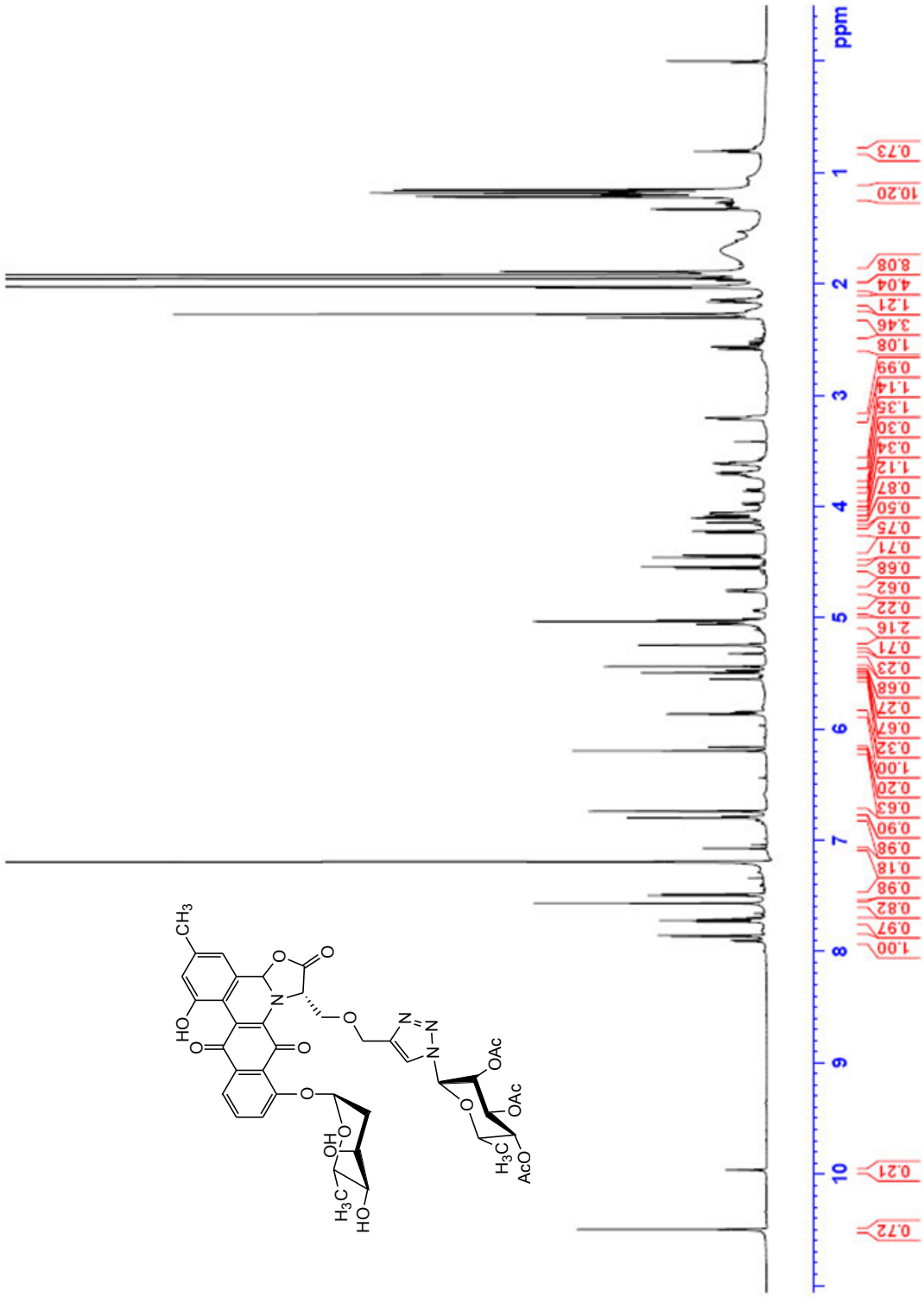
N
OAc
OAc
H3C
AcO
OH
H3C
HO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1.08
0.99
1.14
1.35
0.30
0.34
1.12
0.87
0.50
0.75
0.71
0.68
0.62
0.22
2.16
0.71
0.23
0.68
0.27
0.67
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1.00
0.20
0.63
0.90
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0.18
0.98
0.82
0.97
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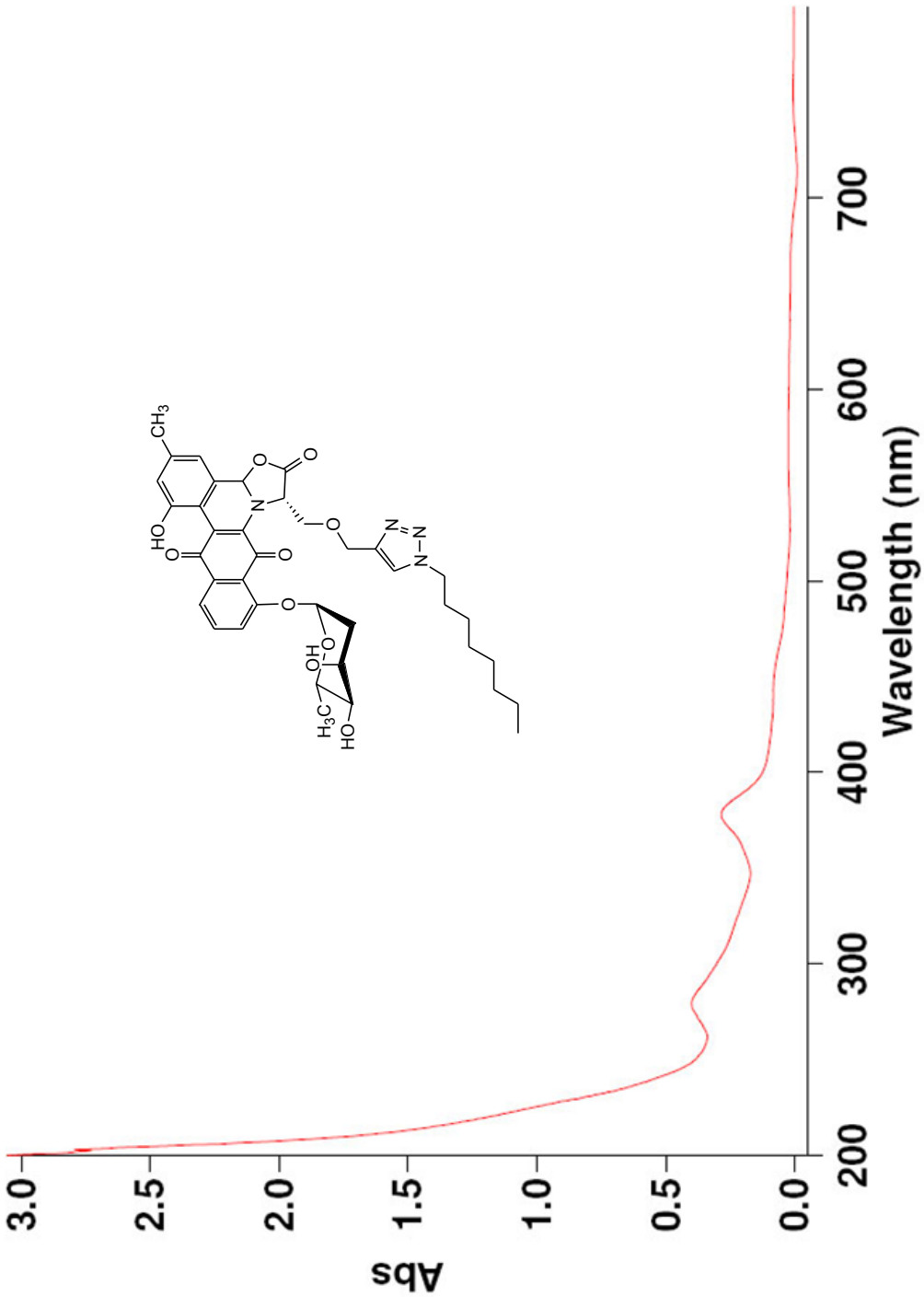
OAc
OAc
H3C
AcO
OH
H3C
HO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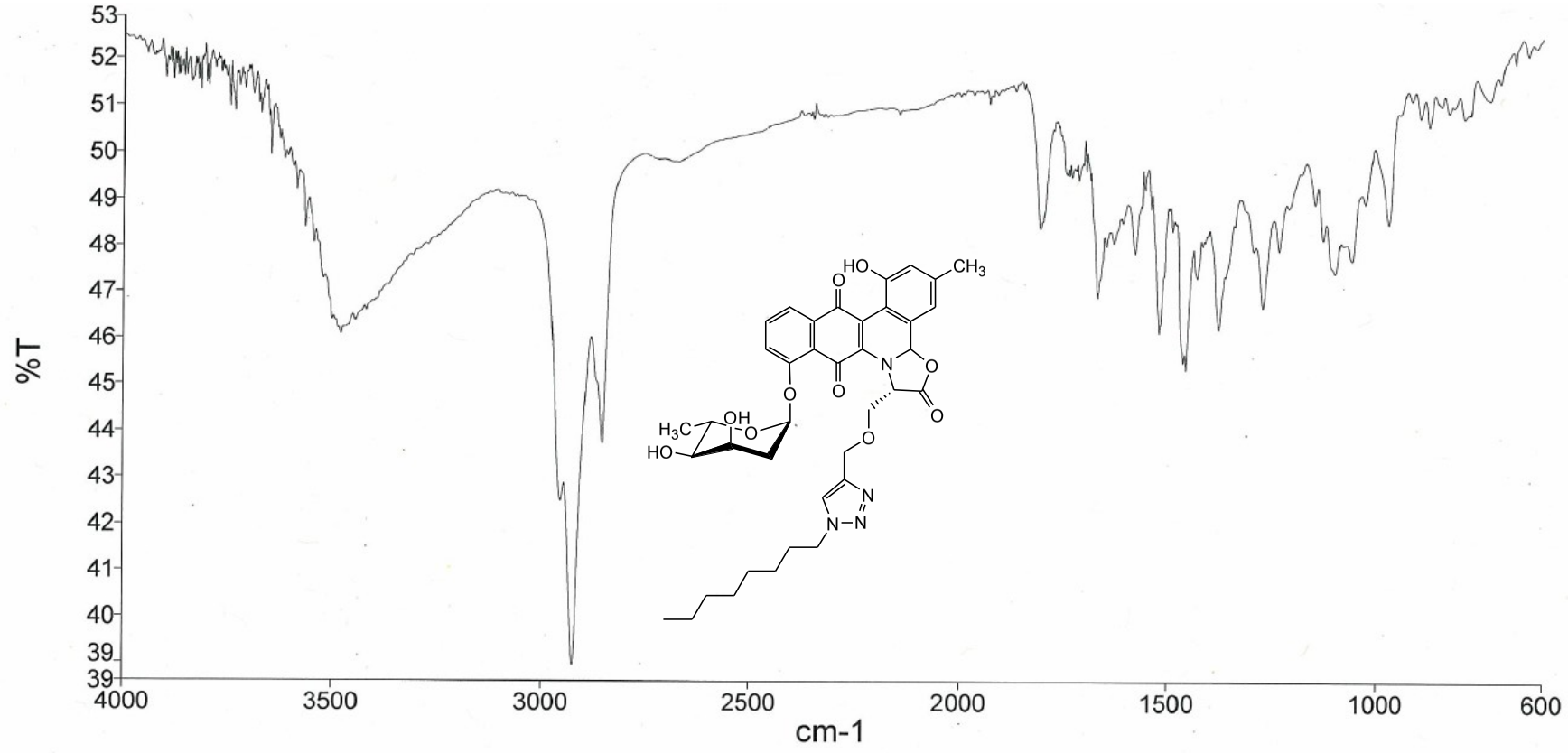
1.5
1.0
0.5
0.0
200
300
400
500
600
700
Wavelength (nm)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2500
2000
1500
1000
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cm-1
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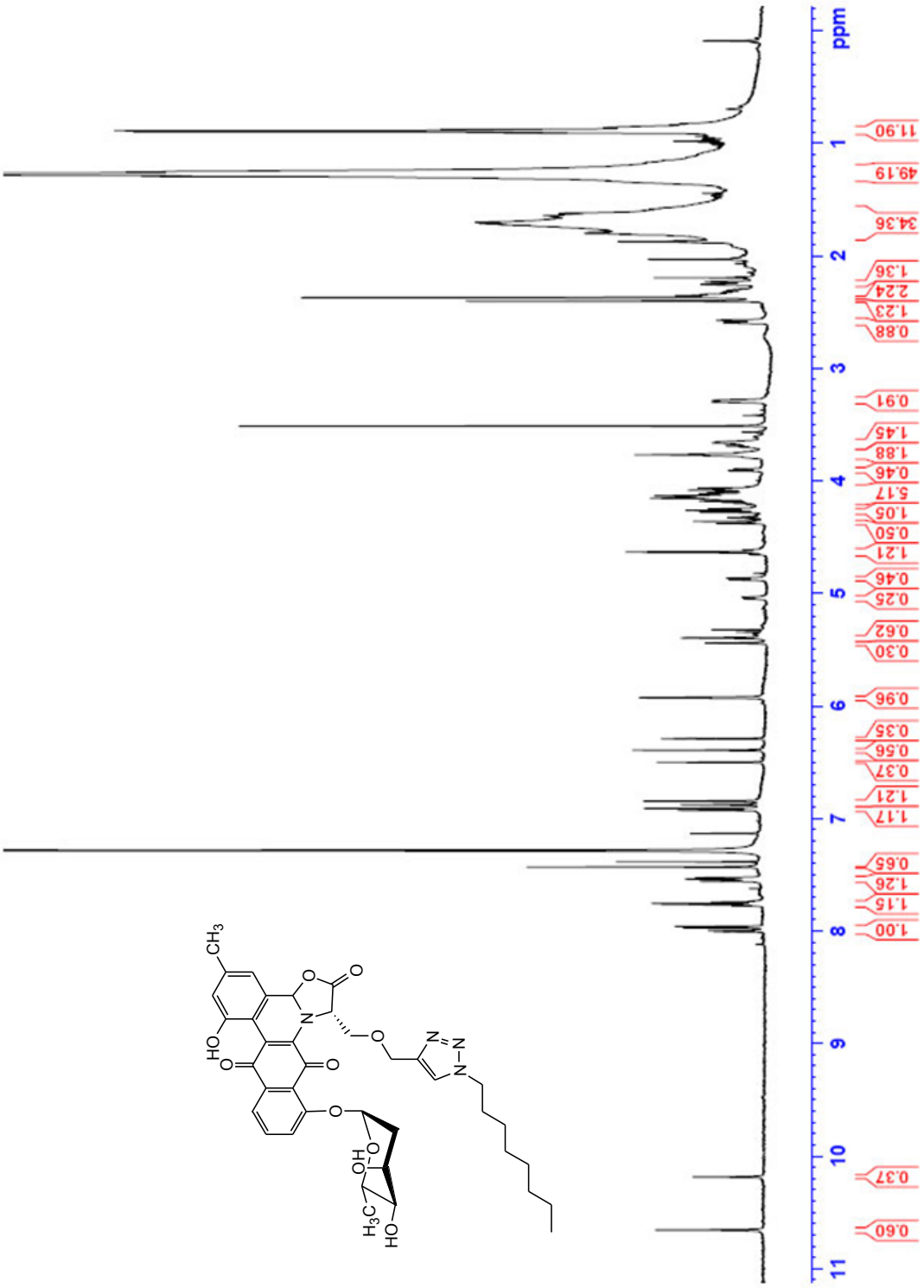
CH3
O
O
O
N
O
O
O
H3C
OH
O
HO
O
N
N
N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0.88
0.91
1.45
1.88
0.46
5.17
1.05
0.50
1.21
0.46
0.25
0.62
0.30
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0.37
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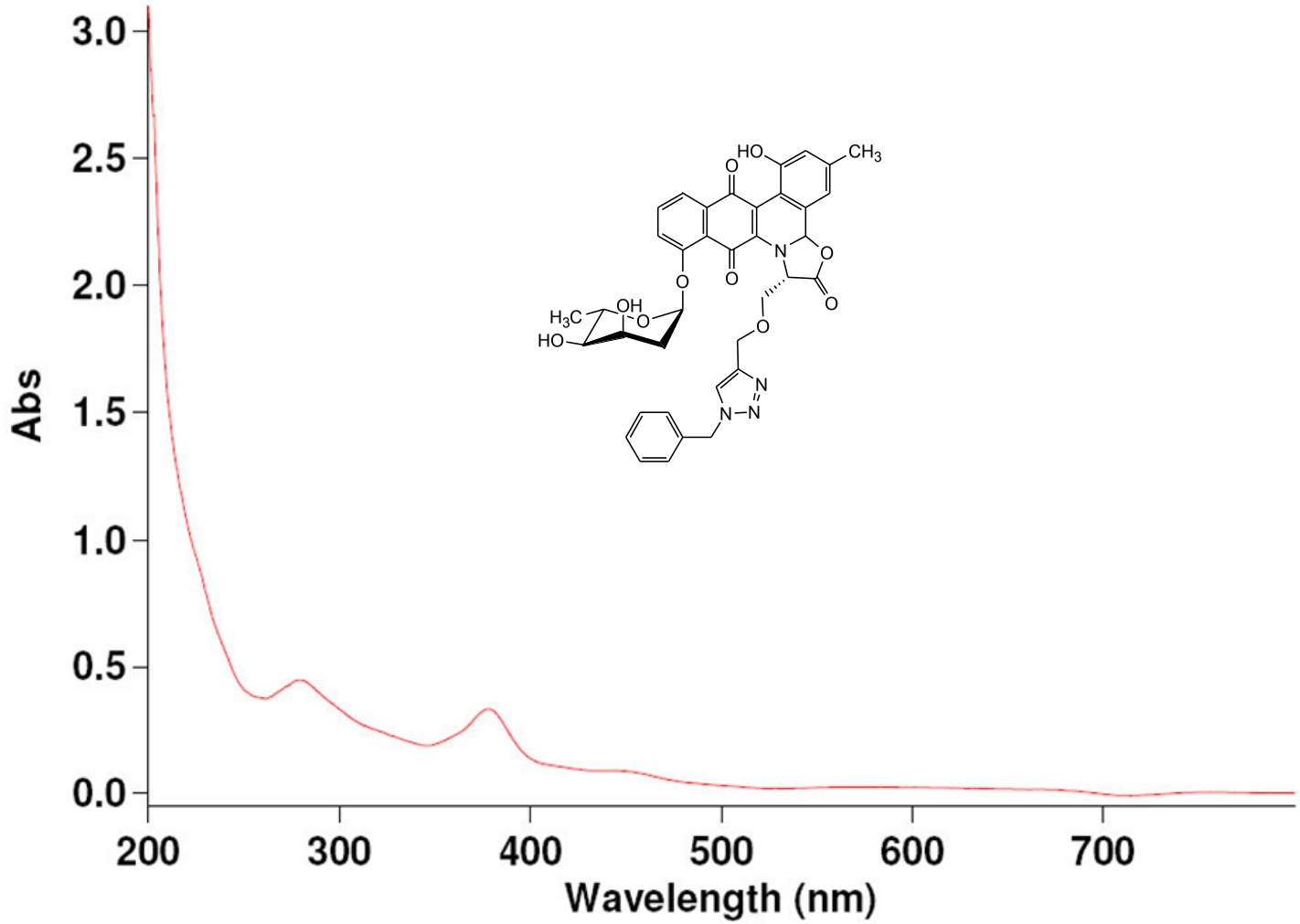
Abs
3.0
2.5
2.0
1.5
1.0
0.5
0.0
200
300
400
500
600
700
Wavelength (nm)
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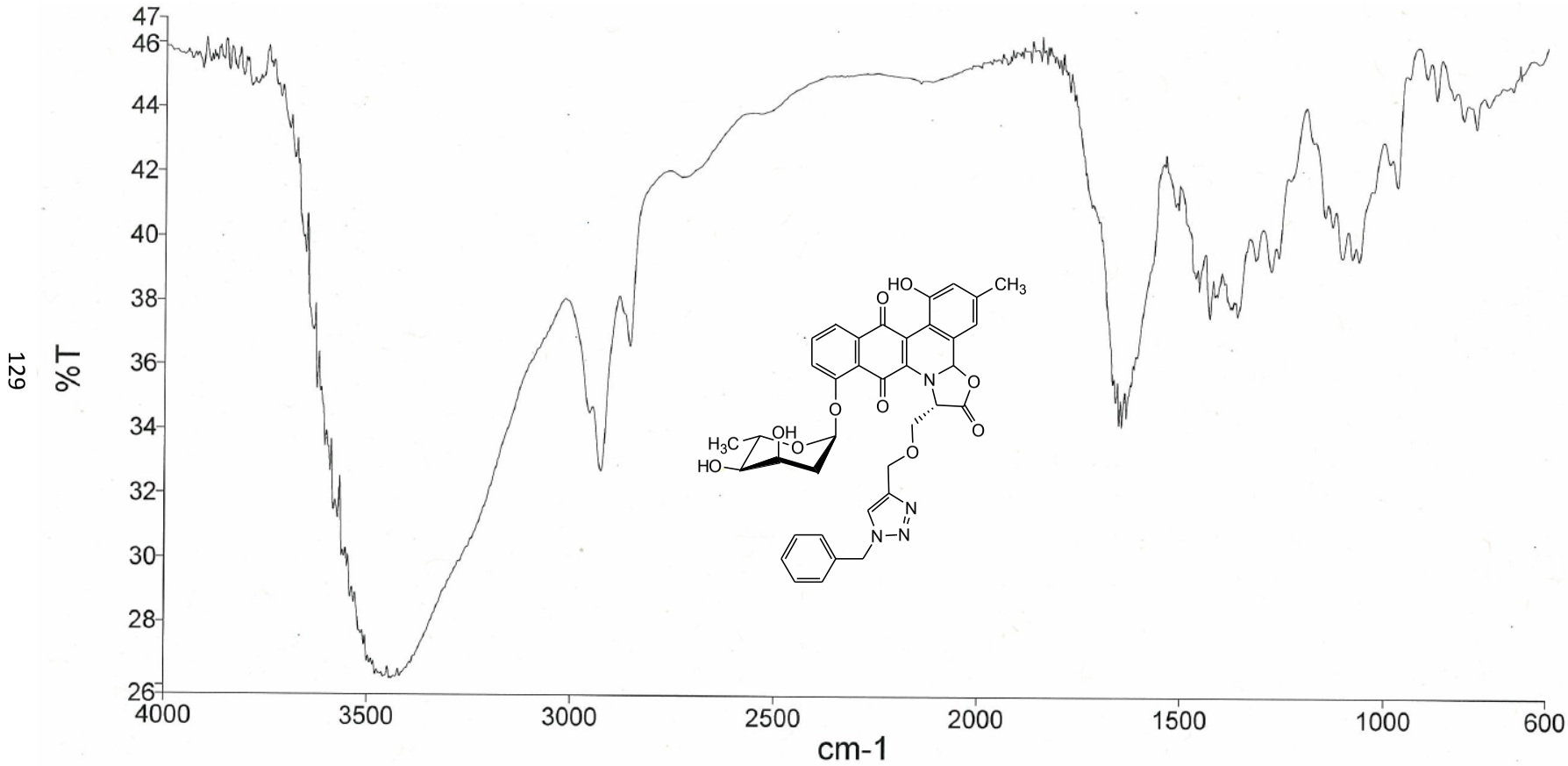
CH3
O
O
N
O
O
O
O
H3C
OH
O
HO
O
N
N
N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26
%T
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3500
3000
2500
2000
1500
1000
600
cm-1
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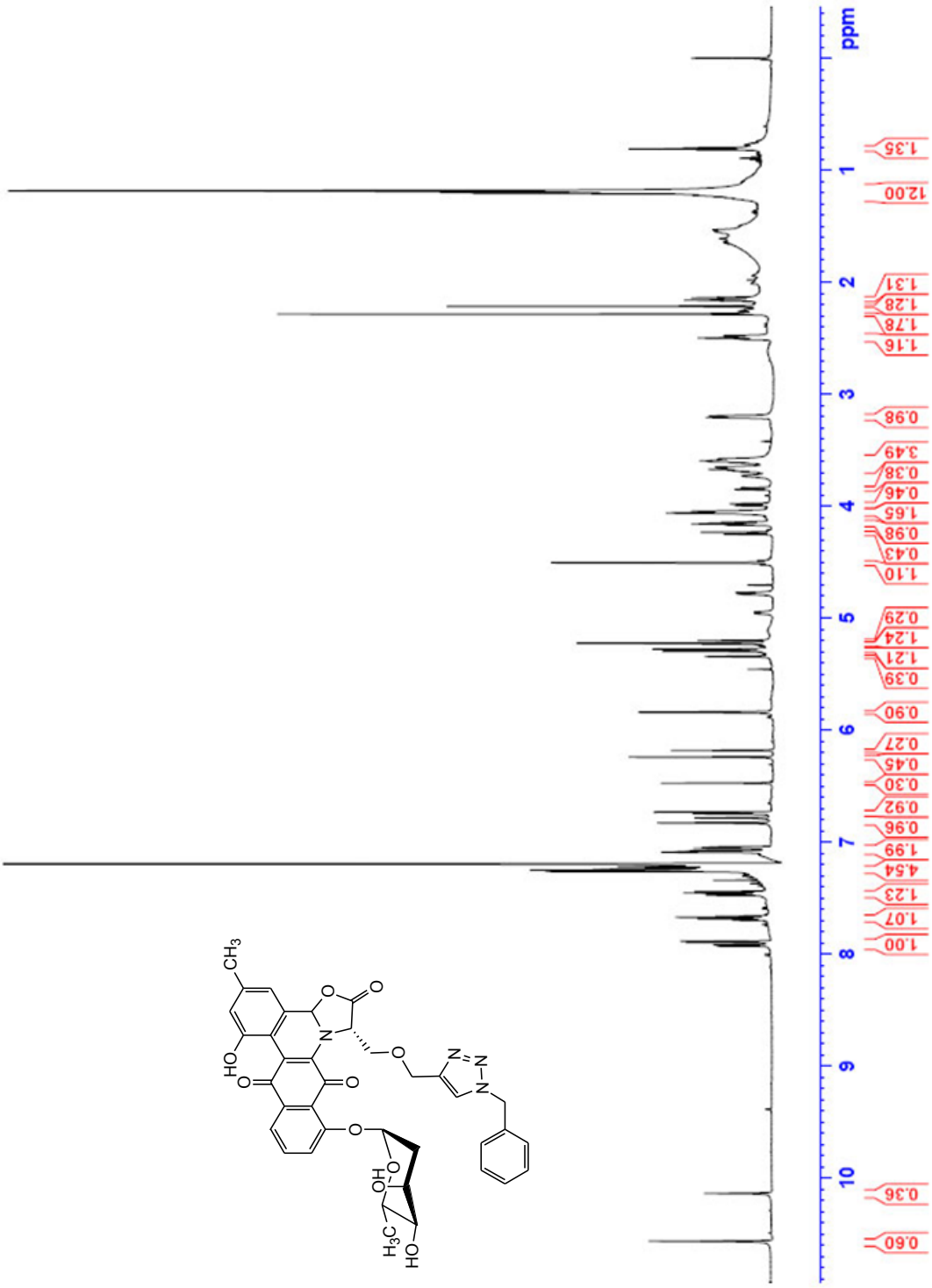
CH3
O
O
O
O
N
O
O
H3C
OH
O
HO
O
N
N
N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1.10
0.29
1.24
1.21
0.39
0.90
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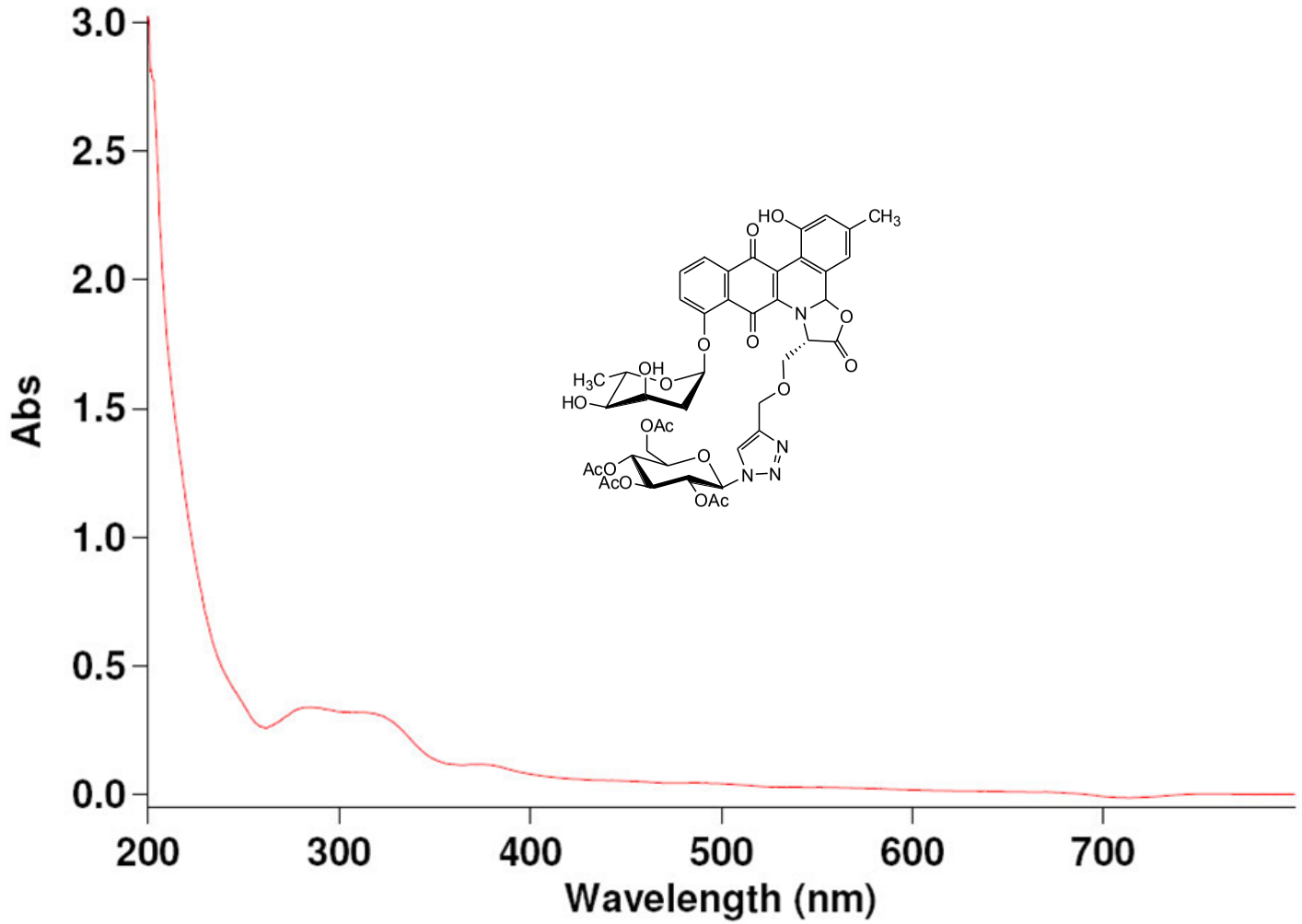
0.45
0.30
0.92
0.96
1.99
4.54
1.23
1.07
1.00
0.36
0.60


Abs
3.0
2.5
2.0
1.5
1.0
0.5
0.0
200
300
400
500
600
700
Wavelength (nm)
HO
CH3
O
O
O
N
O
O
O
H3C
OH
O
HO
O
OAc
AcO
AcO
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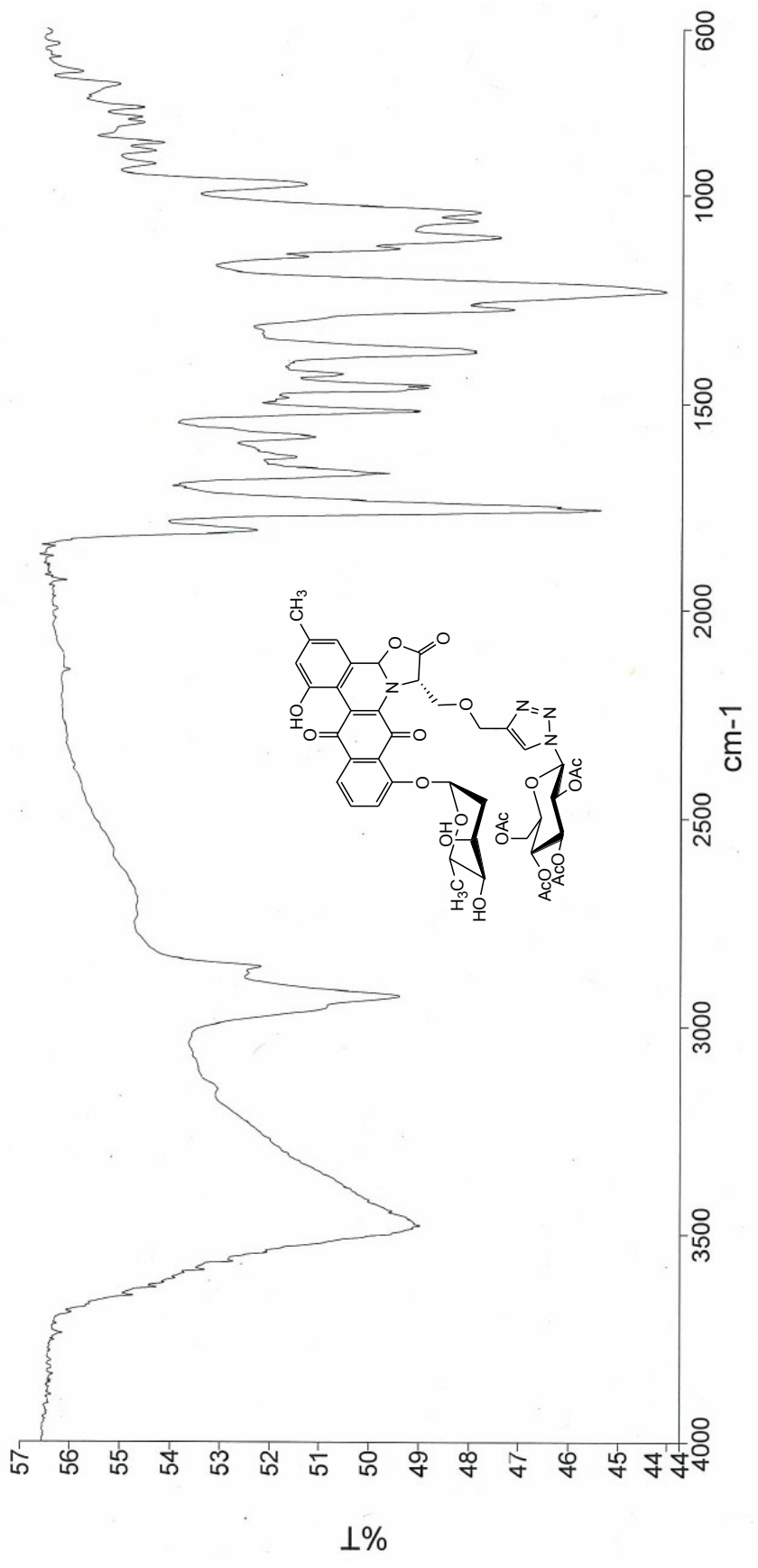
N
N
N
OAc


%T
cm-1
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3500
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2500
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1000
600
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50
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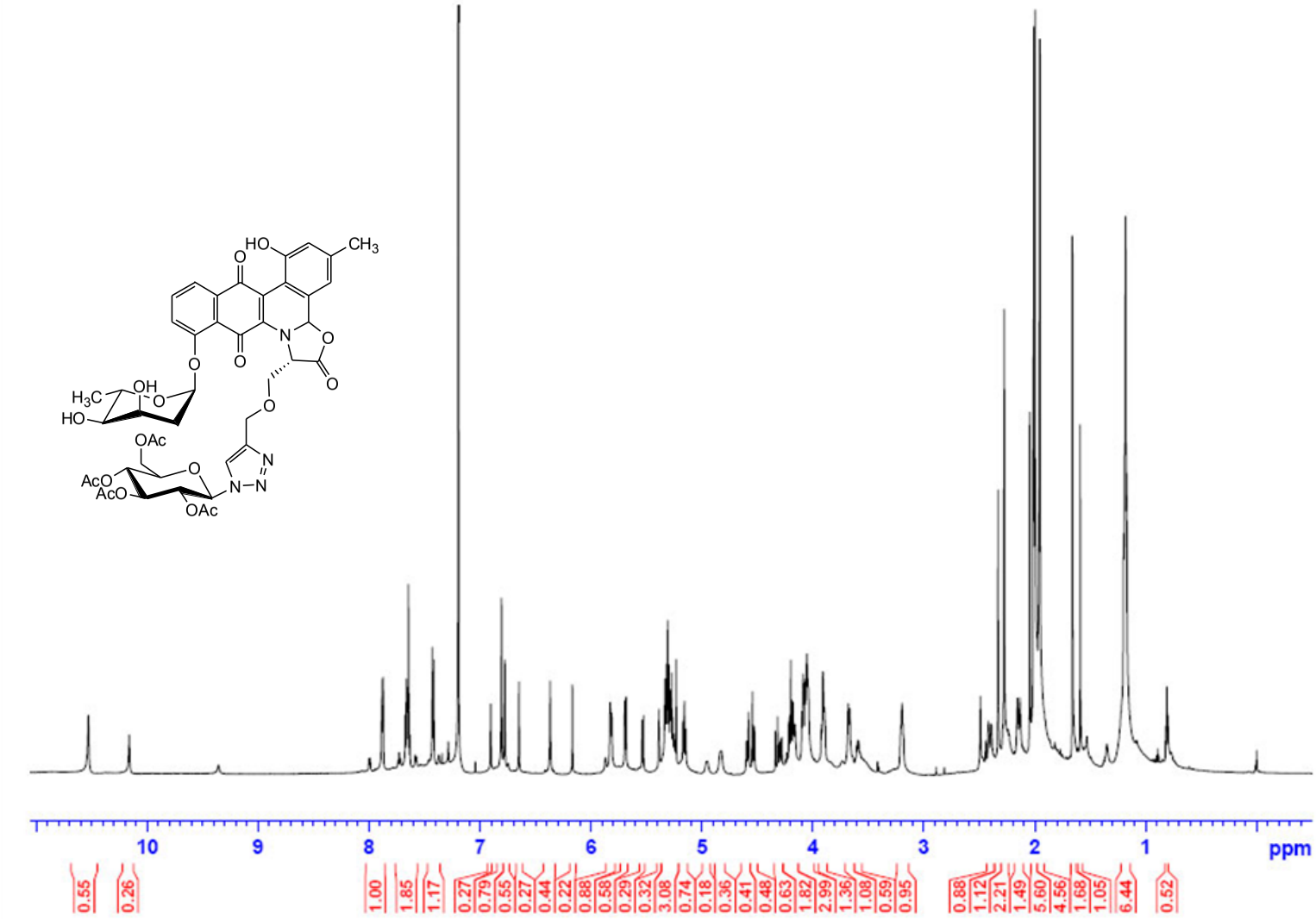
48
47
46
45
44


HO
CH3
O
O
N
O
O
O
H3C
OH
O
HO
O
OAc
AcO
AcO
O
N
N
N
OAc
10
9
8
7
6
5
4
3
2
1
ppm
0.55
0.26
1.00
1.85
1.17
0.27
0.79
0.55
0.27
0.44
0.22
0.88
0.58
0.29
0.32
3.08
0.74
0.18
0.36
0.41
0.48
0.63
1.82
2.99
1.36
1.08
0.59
0.95
0.88
1.12
2.21
1.49
5.60
4.56
1.68
1.05
6.44
0.52

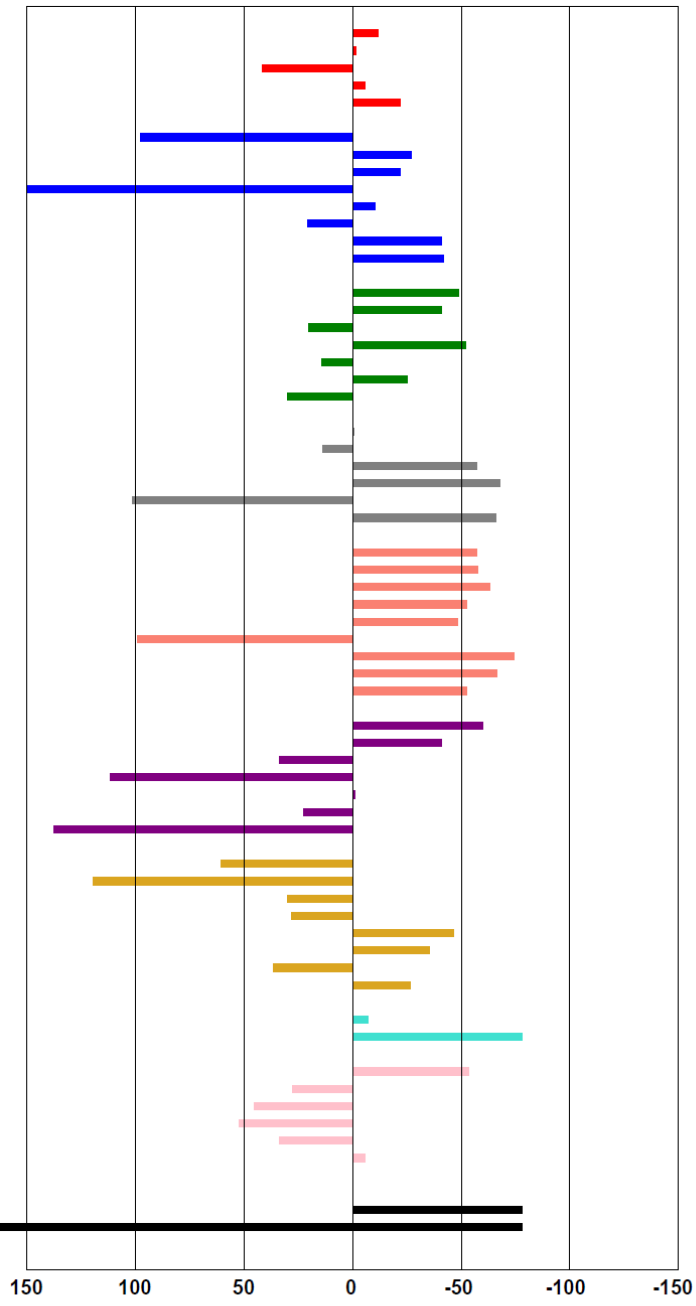
APPENDIX E: Anticancer Activity Data for Jadomycin L

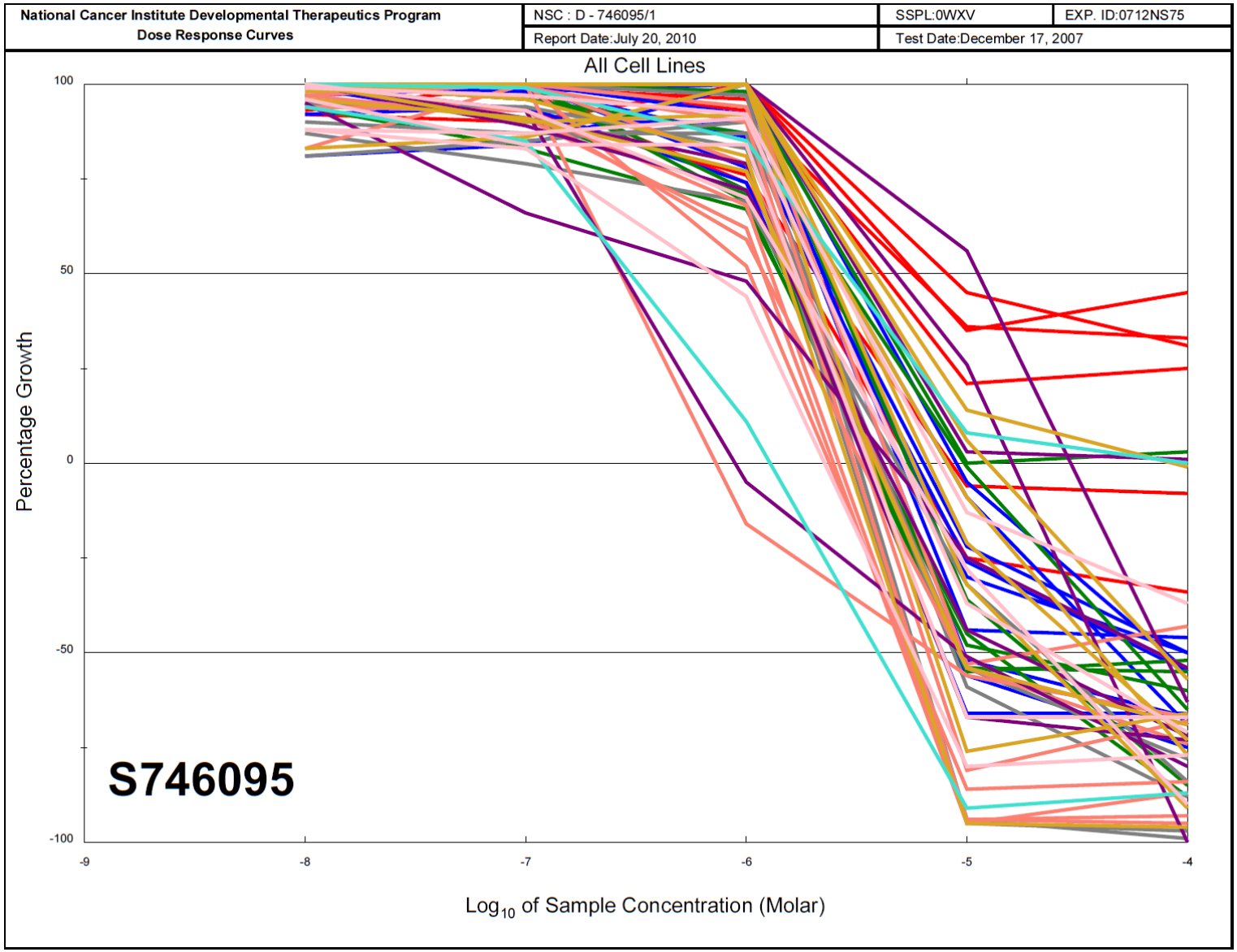
Developmental Therapeutics Program

One Dose Mean Graph

NSC: D-746095 / 1	Conc: 1.00E-5 Molar	Test Date: Sep 24, 2007
Experiment ID: 0709OS12		Report Date: Jul 20, 2010

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	-33.98
HL-60(TB)	-23.71
K-562	19.48
MOLT-4	-27.76
RPMI-8226	-44.17
Non-Small Cell Lung Cancer	
EKVX	75.63
HOP-62	-49.14
HOP-92	-44.01
NCI-H226	170.52
NCI-H23	-32.51
NCI-H322M	-1.66
NCI-H460	-63.19
NCI-H522	-63.98
Colon Cancer	
COLO 205	-71.01
HCC-2998	-63.27
HCT-116	-2.16
HCT-15	-74.04
HT29	-7.97
KM12	-47.40
SW-620	7.99
CNS Cancer	
SF-268	-22.56
SF-295	-8.65
SF-539	-79.39
SNB-19	-89.79
SNB-75	79.06
U251	-87.98
Melanoma	
LOX IMVI	-79.31
MALME-3M	-79.91
M14	-85.31
MDA-MB-435	-74.47
SK-MEL-2	-70.62
SK-MEL-28	76.94
SK-MEL-5	-96.49
UACC-257	-88.46
UACC-62	-74.72
Ovarian Cancer	
IGROV1	-82.31
OVCAR-3	-63.14
OVCAR-4	11.67
OVCAR-5	89.20
OVCAR-8	-23.40
NCI/ADR-RES	0.22
SK-OV-3	115.26
Renal Cancer	
786-0	38.39
A498	97.00
ACHN	7.62
CAKI-1	5.94
RXF 393	-68.58
SN12C	-57.40
TK-10	14.25
UO-31	-48.92
Prostate Cancer	
PC-3	-29.28
DU-145	-100.00
Breast Cancer	
MCF7	-75.50
MDA-MB-231/ATCC	5.27
HS 578T	23.00
BT-549	30.19
T-47D	11.62
MDA-MB-468	-28.03
Mean	-22.15
Delta	77.85
Range	270.52




National Cancer Institute Developmental Therapeutics Program
Dose Response Curves
NSC : D - 746095/1
Report Date:July 20, 2010
SSPL:0WXV
Test Date:December 17, 2007
EXP. ID:0712NS75
All Cell Lines
Percentage Growth
100
50
0
-50
-100
-9
-8
-7
-6
-5
-4
S746095
Log10 of Sample Concentration (Molar)

Nontumored Animal Toxicity Assay for S746095

Report generated on 20-Jul-2010

EXPERIMENT: AAZ-219 / 0 / 8B	**TUMOR: 0**	**HOST: Athymic Nudes**	**IMPLANT DATE: 28-AUG-2008**
MEMO NO:	**SOURCE/LINE: 0**	**SOURCE: APA**	**STAGING DATE: 28-AUG-2008**
BOOK NO:	**IMPLANT SITE:**	**SEX: F**	**EVALUATION DATE: 15-SEP-2008**

	TREATMENT					
Grp	**NSC**	**Dose/Units**	**Rt.**	**Schedule**	**Death Days**	**Surv/Total Day 18**
7	D-S746095	100.00 mg/kg/dose	IP	QD X 1, Day 0	3	0/1
8	D-S746095	200.00 mg/kg/dose	IP	QD X 1, Day 0	3	0/1
9	D-S746095	400.00 mg/kg/dose	IP	QD X 1, Day 0	3	0/1

VEHICLES

Grp	7 ->	NSC # S746095 / 2 (Dose = 100.00)	: in 10% DMSO in Saline/Tween 80	(Soluble - no visible particles)	Inj. Vol.: 0.05 ml/10gm body wt
Grp	8 ->	NSC # S746095 / 2 (Dose = 200.00)	: in 10% DMSO in Saline/Tween 80	(Soluble - no visible particles)	Inj. Vol.: 0.1 ml/10gm body wt
Grp	9 ->	NSC # S746095 / 2 (Dose = 400.00)	: in 10% DMSO in Saline/Tween 80	(Soluble - no visible particles)	Inj. Vol.: 0.2 ml/10gm body wt

NOTE: All treatment was administered according to exact body weight.

NSC: 746095

It was tested against the following disease types and cell lines:

Experiment ID: HF1506

Panel Name	Cell Name	Schedule	Route	High Dose
Breast Cancer	MDA-MB-231	QD X 4	IP	18.75 mg/kg/dose
Non-Small Cell Lung Cancer	NCI-H23	QD X 4	IP	18.75 mg/kg/dose
Colon Cancer	SW-620	QD X 4	IP	18.75 mg/kg/dose

Experiment ID: HF1507

Panel Name	Cell Name	Schedule	Route	High Dose
Colon Cancer	COLO 205	QD X 4	IP	18.75 mg/kg/dose
Melanoma	LOX IMVI	QD X 4	IP	18.75 mg/kg/dose
Ovarian Cancer	OVCAR-3	QD X 4	IP	18.75 mg/kg/dose

Experiment ID: HF1508

Panel Name	Cell Name	Schedule	Route	High Dose
Non-Small Cell Lung Cancer	NCI-H522	QD X 4	IP	18.75 mg/kg/dose
CNS Cancer	U251	QD X 4	IP	18.75 mg/kg/dose
Melanoma	UACC-62	QD X 4	IP	18.75 mg/kg/dose

Experiment ID: HF1509

Panel Name	Cell Name	Schedule	Route	High Dose
Melanoma	MDA-MB-435	QD X 4	IP	18.75 mg/kg/dose
Ovarian Cancer	OVCAR-5	QD X 4	IP	18.75 mg/kg/dose
CNS Cancer	SF-295	QD X 4	IP	18.75 mg/kg/dose

Your compound was scored as follows:

IP Score	4 out of 48
SC Score	0 out of 48
Total	4 out of 96
Cell Kill	N